VOL. 31, NO. 4 December, 1956

THE QUARTERLY REVIEW of BIOLOGY

CELL DIVISION

II. A THEORETICAL APPROACH TO CHROMOSOMAL MOVEMENTS AND THE DIVISION OF THE CELL*

BY NORMAN G. ANDERSON

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

"It is only in the light of some theoretical scheme that we can decide what are the most important questions to ask, and in what directions we should look for further insight into the fundamental nature of the mechanisms with which we have to deal." C. H. Waddington, 1954.

INTRODUCTION

IN THE first paper in this series it was shown that the early development of the surface of the earth, as it is thought to have occurred, would have favored the development of colloidal systems bearing a net negative charge, and that the compounds available for primitive metabolism would have been predominantly organic acids, amino acids, and nonvolatile organic bases. The origin of a division mechanism in primitive living systems was considered to lie in cyclical changes in the rate of formation of nucleic acids and proteins resulting from differences in the diffusion rates of the precursors of the two types of macromolecules. Cyclical changes in the ratio of charges on cellular colloids were proposed as the fundamental mechanism of cell division. Further considerations showed that a great part of the experimental data on the initiation of cell division was compatible with this idea. The condensation of chromosomes at prophase was shown to be most easily explained in terms of a masking or neutralization of negatively charged groups, i.e., a change in the balance of positive and negative charges associated with macromolecules.

In this paper the proposed original mechanism will be related to other aspects of cell division. As in the first article (Anderson, 1956), no attempt is made to present either a review on cell division or an exhaustive bibliographical presentation. For these, the reader is referred to Schrader (1953), Hughes (1952), Ris (1949, 1955), and Mazia (1956).

STRUCTURE AND MOVEMENT

It is self-evident that the physical properties of most material substances are related to the degree of interaction of their constituent molecules. Movement may therefore be considered as evidence of changes in the degree or type of interaction. Although a case has been made for the movement of microscopic structures and for the alteration of cell shape by diffusion drag forces (Rashevsky, 1938, 1939; Landahl, 1942a, b, c, d), the movements observed during cell division are most easily explained on the basis of the physicochemical properties of gels and the formation of contractile fibrils of colloidal dimensions. The latter concept, which originated in van Beneden's suggestion in 1883 that chromosomal movements are paramuscular, has been extended with each advance in knowledge of the nature of muscular contraction. A discussion of

*Under USAEC Contract No. W-7405-eng-26.

some of the possible mechanisms by which contractile systems may be formed in cells is of interest. Although protein will be considered as the chief constituent, other substances, such as nucleic acids and acid polysaccharides, may also be involved.

Artificial protein fibers, which have been given the generic name *Azlon*, have been widely investigated because of their commercial significance (see review by Lundgren, 1949). The essential steps necessary to form such fibers are (1) dissociation of the native corpuscular configuration to form dispersed flexible chains, and (2) alignment of the chains in quasiparallel fashion under conditions favoring the formation of linkages between the side chains to give a fibrous protein characterized by free chain segments interspersed between crystallite regions and regions of mesomorphic order. A wide variety of denaturing agents, including heat, formaldehyde, urea, and alkali, has been used. Alignment of the unrolled molecules is generally achieved by laminar flow through small orifices and by stretching of the wet fibers. It should be emphasized that energy must be supplied for the molecules to be aligned.

Numerous attempts have been made to apply similar principles to fibril formation in cells. Frey-Wyssling (1953), for example, suggested that "the globular macromolecules must simply denature to give expanded chains which aggregate laterally." The native, rolled up, folded, or coiled configuration of a polypeptide chain is much more probable than the unrolled or extended configuration. The energy required to unfold a protein partially may be small, but no mechanism is known that will supply this energy in the cytoplasm and hold the unrolled polypeptide chains extended during lateral aggregation. The unrolling or denaturation of protein at air-water interfaces, which is often mentioned, has no counterpart in the fluid cytoplasm. It does not appear likely, therefore, that the sol-gel changes in the cell are an expression of the formation of contractile fibrils formed from interlinked unrolled polypeptide chains.

In my opinion, emphasis upon the application of the work of Waugh, of Joly and coworkers, and of Zbarskii and Perevoshchikova to cellular physiology is long overdue. In contrast to the drastic alterations necessary to produce artificial protein fibers from denatured proteins, these workers have demonstrated the formation of thread-like particles, often many thousands of Angstroms long, by the end-to-end aggregation of globular molecules without gross denaturation. This has been shown to occur in the first stages of sol-gel transformation of gelatin (Joly, 1949a), in the thermal denaturation of horse serum albumin (Joly, 1949b; Joly and Barbu, 1949, 1951; Barbu and Joly, 1950), human serum albumin (Kleczkowski, 1949) and egg albumin (Foster and Samsa, 1950, 1951a, b; Jaggi and Waugh, 1950), in insulin gelation (Waugh, 1941, 1946; Farrant and Mercer, 1952), in the association of fibrinogen induced by thrombin (Waugh and Livingstone, 1951), and in the formation of contractile fibers by the combination of basic and acidic proteins (Zbarskii and Perevoshchikova, 1948, 1951a, b). Excellent agreement between data obtained by flow birefringence, electrophoresis, ultracentrifugation, and electron microscopy has been obtained by Barbu and Joly (1953), demonstrating the reality of the aggregation of globular proteins.

Factors governing the stability and the aggregation of colloidal particles have been examined in considerable detail (Verwey and Overbeek, 1948; Kruyt, 1949), chiefly in lyophobic systems. The distinction between lyophobic and lyophilic colloids is not a sharp one, however, and all gradations between the classic lyophilic and lyophobic colloids exist. Many of the properties of each may exist in the same colloid particle.

Rees (1951) has developed a theory of directed aggregation in colloidal systems based on the observation of Henri in 1906 (cited by Rees, 1951) that rubber latex particles aggregated in linear arrays during gelation and on the suggestion of Usher (1929) that a single particle would aggregate with a close pair of particles to form a linear rather than a triangular arrangement, since a linear configuration required the approaching particle to overcome the residual forces of only one particle of the pair. Rees calculated the contours for equal potential energy of interaction for a system in which there is a substantial potential energy maximum for the interaction of two particles (a stable suspension). It was assumed that the interaction forces could be treated as the vector sum of the interactions of single particles with each component of the biparticle. The results for such a system are shown in Fig. 1. It is evident that the interacting particle encounters a lower potential barrier for an approach along the axis of the two particles than normal to it. In such a system, a comparatively slow aggregation of an initially stable suspension may be produced by a reduction in the surface potential or by an increase in the thermal energy of the particles themselves.

Barbu and Joly (1953) have presented a detailed mathematical treatment of the linear aggregation of molecules the size of proteins. They concluded that in aggregation after heating or other mild denaturation, denaturation proceeds by the releasing of groups initially coupled inside the molecule. This allows the formation (on particular areas on each molecule) of weak intermolecular bonds such as hydrogen bonds, salt bridges, or weak van der Waals forces.

The principle of linear aggregation of colloids finds numerous applications in biological systems. The polymerization of globular actin (G-actin) has been studied by many workers. It again appears to involve the direct aggregation of globular molecules (Rozsa, Szent-Györgyi, and Wyckoff, 1949). The linear aggregates or fibrils often appear to reach a characteristic length (Barbu and Joly, 1953), perhaps illustrating a self-limiting property suggested by Rees (1951).

The possible steps in the formation of large structures from simple molecules, as given by Rees, are: (1) the formation of small macromolecular aggregates of specific size and shape (in this case, the synthesis of proteins); (2) the linear aggregation of small macromolecular units to form microfibrils; (3) the lateral association of microfibrils to give so-called "tactoid" regions; (4) the consolidation of these regions to give closely packed "tactoids"; and (5) the lateral association of these "tactoids" to give larger fibrous units.

Much has been written about the possible relation between tactoids observed in virus suspensions and the formation of the spindle (Bernal, 1940). It was originally thought that in such suspensions the ratio between attractive and repulsive forces varied with the distance from the particle in such a manner that an optimum of attractive forces existed at some distance from each particle. Repulsive forces predominating closer to the particle prevented particles from a closer approach (Bernal and Fankuchen, 1941). In dried tobacco mosaic viruses, the distances between parallel particles was 152 A. With the addition of increasing amounts of water, the particles moved farther and farther apart, until at 87 per cent water they were separated by 450 A. This was taken as proof of the existence of long-range forces.

The theories that attempt to show that two charged colloidal particles will attract each other at large interparticle distances owing to an overlapping of ionic atmospheres have been shown to be unlikely (Oster, 1950); and, furthermore, it

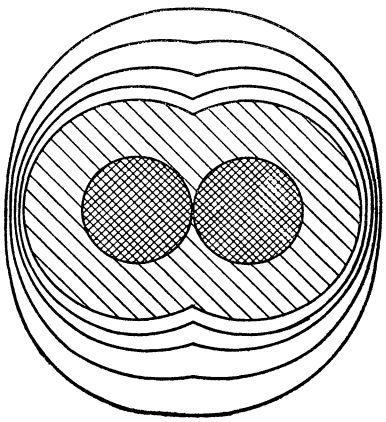

FIG. 1. POTENTIAL-ENERGY CONTOURS WHERE THE SURFACE POTENTIAL IS SMALL

The potential energy is negative throughout. (From Rees, 1951).

does not appear that the conclusions of Bernal are justified by the experimental data that he cites. Bernal does not stress that tactoids, like coacervates generally, exist in equilibrium with a dilute phase. The tactoid goes back into "solution" in the presence of pure solvent. This characteristic alone renders the whole concept of long-range attractive forces based on virus studies suspect, since it implies that the most probable condition is a random distribution, and not long-range orientation.

In an excellent study and discussion of phase formation in virus solutions and of the problem of long-range forces, Oster (1950) concluded that the forces responsible for tactoid formation are *repulsive*, in direct opposition to the concept of Bernal. Oster pointed out that if rod-shaped particles are crowed, i.e., do not have a free sphere of rotation, the orientation of any one particle will determine the orientation of its neighbors so that ordering will set in. In a virus suspension, such ordering results in an area of more efficient packing that, because of its increased density, will fall to the bottom of the solution. Although the tactoid phase may have decreased entropy, this is offset by the increase in entropy in the random or non-ordered phase. No added energy or long-range forces are required. As a first approximation, it would be expected that two phases would be formed when the thermodynamically effective volume of each particle exceeds the volume of solution available per particle. This Oster finds to be true.

The repulsive forces per square centimeter

between rods carrying a similar charge are found to be a function of the squares of the zeta potentials and $e^{-2\kappa R}$, where κ is the reciprocal of the thickness of the ionic double layer and R is the distance between the particles. The important conclusion to be drawn is that with the tactoid-forming viruses the force *is* repulsive and that it decreases rapidly with distance. The range of repulsion becomes even shorter as κ becomes larger. As a result, the addition of electrolytes, which decreases the thickness of the ionic double layer, also shortens the range of the repulsive forces. Although the repulsive forces may be decreased to the point where they no longer prevent aggregation, owing to van der Waals–London attractions or other forces, no interplay of forces was found that allowed attractive forces to predominate at intermediate distances while repulsive forces were dominant at shorter ranges. Oster has, therefore, been able to explain the formation of tactoids in terms of geometrical considerations without recourse to the concept of long-range attractive forces.

Quite apart from the forces involved in tactoid formation, the point of interest here is simply a question of whether the spindle resembles a tactoid. Electronmicrographs of dividing cells indicate that the spindle is not composed of particles the size and shape of viruses (Selby, 1953). The question is then whether the principles that result in the formation of tactoids in virus suspensions also produce similar configurations when the anisotropic particles have a cross section in the range of certain globular proteins. For the purpose of this treatment, it is assumed that beaded chains produced by the alignment of globular proteins will, when a critical concentration is exceeded, separate into a soluble dilute phase and a relatively condensed phase showing some of the properties ascribed to tactoids. These include parallel orientation of the fibrils and variable interfibril distance, depending on the ionic atmosphere or surface charge of the fibril. Note that nothing has been said about the mechanism that would tend to keep the fibrils straight instead of randomly coiled.

The reason for including tactoid formation here is simply that it is a possible mechanism for decreasing the entropy in a system of anisotropic fibrils or chains to produce parallel orientation. Such orientation is a necessary step in the formation of long, contractile fibrils. Once a predominantly parallel orientation is obtained, formation of regions of mesomorphic order could contribute to the formation of gel fibrils. The application of the colloid chemistry of tactoids to the spindle will be described in a subsequent section.

If it is assumed that mechanisms for producing fibrous systems exist, there is a question whether they will contract. The preparation and contractility of artificial actinomyosin fibers is well known, but the problem of a similar contractile mechanism in cells has received less attention. Zbarskii and Perevoshchikova (1948) observed that proteins isolated from nuclei with 0.24 *M* HCl and precipitated by saturation with NaCl yielded a viscous solution when redissolved in very dilute HCl. When the solution was injected into water, fibers were formed that contracted sharply with adenosine triphosphate (ATP) (Table 1). The proteins were originally described as histones. However, further work (Zbarskii and Debov, 1948, 1951; Zbarskii and Perevoshchikova, 1951a, b) demonstrated that the acid extract of freshly isolated nuclei from many sources contained trytophan. Repurified histones prepared from 1 *M* NaCl extracts of the same nuclei contained no tryptophan. The contractile material was a combination of histone and an acidic protein. A number of acidic proteins could be substituted for those found in the nucleus. Zbarskii and Perevoshchikova (1951a, p. 123) concluded that "the contractile properties of nuclear proteins play a very important role in the mechanism of mitosis and, in particular, in such of

TABLE 1

Contraction of "histone fraction" fibers of liver cell nuclei under the influence of varying concentrations of Na_4ATP

	Final concentration of ATP (molarity)						
	7.7×10^{-3}	5.0×10^{-3}	2.5×10^{-3}	10^{-3}	5×10^{-4}	2.5×10^{-4}	10^{-4}
Contraction of fibers (per cent of original length)	48*	50*	45*	43†	37†	34†	22‡

* Very rapid contraction.

† Contraction rapid, but with preliminary stirring of mixtures for more even distribution of ATP.

‡ Contraction after 5 minutes.

(From Zbarskii and Perevoshchikova, 1951a, p. 117).

its phases as the formation of metaphase chromosomes and the contraction of the stretched fibers of the spindle. It is also possible that in the cell nucleus there form or become detached from the nucleic acids (probably by an enzymatic process) polyphosphoric nucleotides whose action on proteins of the cell nucleus may be the same as that of ATP. Such an effect of nucleoproteins can be connected with the actuating mechanism of mitosis."

The author first became interested in this field when it was observed that the addition of basic dyes to rat liver breis produced microscopically observable fibrils (Fig. 17 in Anderson, 1949). Further work showed that protamine and other basic substances also produced numerous fibrils, many at the limits of resolution of the light microscope (Anderson, 1951), direct evidence thus being provided that substances are available in tissues for forming fibrils in the presence of polybases.

Rybak (1950) and Joly and Rybak (1950) have also suggested that basic proteins may combine with other proteins to form the achromatic spindle. Evidence based on birefringence of flow was presented to show that microfibrils could indeed be formed in model systems. The fundamental notion of this mechanism for producing fibrils in the cytoplasm appears to have arisen independently in three different laboratories. The formation of linear aggregates of cytoplasmic proteins, however, does not in itself mean that the aggregate will contract.

The molecular basis of contractile mechanisms is under intensive investigation. The evidence for electrostatic systems has been reviewed by Katchalsky (1954), who states, "the driving mechanisms of living matter are always biogels of pronounced polyelectrolyte nature, varying in type and organization. Different organisms utilize various gel mechanisms for their motility; the amoeboid movement is based on progressive sol-gel transformation, the movement of plants is founded on reversible swelling and shrinkage of protoplasm while the great majority of higher animals and many protista use an intricate and very effective contractile-gel mechanism." It is interesting that myosin in the presence of monovalent salts is isoelectric at *p*H 5.4; whereas in the presence of Ca^{++} or Mg^{++} in very low concentrations, the molecule behaves as a basic protein, remaining positively charged up to *p*H 9. The actinomyosin complex in the presence of divalent cations, therefore, may be considered as a complex of oppositely charged molecules.

The picture, on a molecular level, of the majority of current theories is one of a long-chain molecule or series of molecules held in an extended or stretched condition by virtue of the net charge on the chain. If the charge is altered in such a manner as to decrease the repulsive forces along the chain, Brownian movement returns the extended molecule to a coiled, kindled, or contracted and also more probable state. Theories pertaining to this field have been reviewed by Wilkie (1954), Weber (1955), and by Morales et al. (1955). The difficulty appears to center around the manner in which ATP or some other high-energy compound is involved in alteration of the net charge on the extended molecule. There is no doubt, however, that polyelectrolyte gels can be made to function as mechano-chemical engines of high efficiency (Katchalsky, 1954).

In the production of fibrils composed of globular molecules linearly arranged as the ultimate contractile elements in the cell, emphasis has been laid on mechanisms that would involve only small changes in cell colloids, such as changes in the nature of the surface charge. This is in keeping with the concept that cell function is carried on with an economy of materials and mechanisms. Alternative mechanisms, such as the blood-clotting-like mechanism proposed by Heilbrunn, would require (1) activation of a proteolytic enzyme, (2) degradation of soluble molecules to form gels or "clots," and (3) the eventual digestion of the clot.

A number of doubts have arisen about the electrostatic theory (Weber, 1955; Flory, 1957). Changes in *p*H that should alter surface charges enough to produce contraction do not appear to do so. Other objections, based on the difficulties produced by the salts present in cells and the fact that the repulsive forces would not only affect segments of the molecular fibril but would force different fibrils apart and produce a nondirectional swelling, have been put forth (Flory, 1957). Flory proposes that the extended state of molecular fibrils is maintained by crystalline areas involving parallel strands. If a strongly charged substance is attached to the strands, crystallinity can no longer be maintained; and the strands revert to a more random, amorphous condition (contract). This concept avoids a number of difficulties inherent in electrostatic theories and possesses an appealing simplicity.

Weber (1955) has summarized the evidence for the belief that all vital contraction of cell parts is

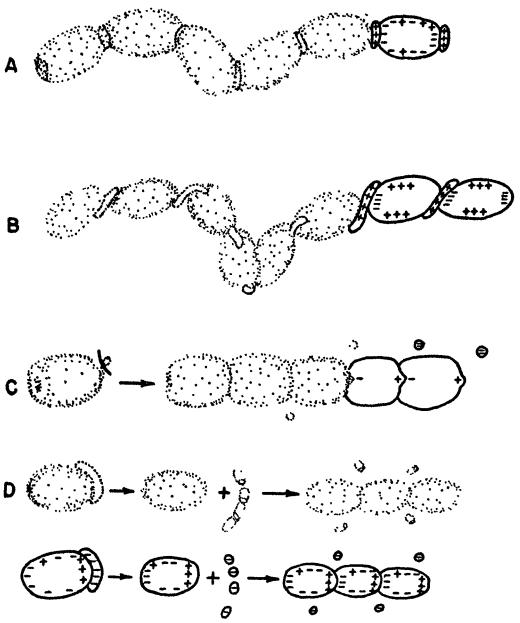

FIG. 2. MECHANISMS FOR ALTERING THE SURFACE CHARGE ON NEGATIVELY CHARGED PROTEIN MOLECULES TO FORM LINEAR AGGREGATES

In A, protein molecules with a fairly symmetric distribution of charge are interlinked with polycations (shown as elongated particles) to give a chain that does not have a pronounced predominance of either positive or negative charges on its surface. In B, polycations interlink proteins having a highly asymmetric distribution of charge so as to give a positively charged chain. In C, a positively charged area on one end of the structure-forming molecule is prevented from interacting with oppositely charged areas on the same species of molecule by the presence of an additional charged group. Removal of this group allows the interaction to occur. In D, interaction between oppositely charged areas on the same species of molecule proceeds after a prosthetic group such as RNA is removed from a negative area on the molecule by depolymerization.

similar, whether in dividing cells, smooth and straited muscle cells, or flagella. Glycerol-extracted models have been used to demonstrate movement in all these, upon the addition of proper concentrations of ATP. Expanded fibroblast models contract (Hoffmann-Berling, 1954a; Hoffmann-Berling and Weber, 1953), and the equator of telophase models constricts into a very narrow bridge (Hoffmann-Berling, 1954b, c, 1955).

Unfortunately, the state of molecular muscle physiology does not now provide a satisfactory molecular model or an isolated actinomyosin particle that exhibits an unequivocal active change in length solution (Weber, 1955). In attempting to apply concepts derived from muscle physiology to the mitotic apparatus, one is faced with the further problem of either assembling this apparatus during prophase, providing for its proper function, and then dissembling and storing it in its entirety for the next division, or else of synthesizing and destroying it.

The mechanism proposed for intracellular formation of fibrils, and several forms that it may take, are summarized as follows. In the simplest instance (Fig. 2, A), a small polycation, of which protamine will serve as a general example, binds other protein molecules to form a beaded chain. The charge on the chain may be predominantly positive or negative in this model, depending on the distribution of charge between the two molecular species. If the distribution of charge is highly asymmetric on the proteins interlinked by polycations, then the over-all charge may be strongly positive (Fig. 2, B). The chain shown in A could form fibers by virtue of crystallization of segments of chains parallel to one another. The second, or B type, could form electrostatically extended fibrils (Kuhn et al., 1950; Kirkwood and Monroe, 1941) that could be cross-linked by the small amount of ribonucleic acid (RNA) or acid polysaccharide present. The absorption of sufficient ATP on the type-A fibrils could lead to contraction of the type discussed by Flory (1957), and the type-B fibrils could contract when sufficient ATP had been absorbed to discharge the chain.

Further refinements are shown in C and D of Fig. 2. In C, the chain is pictured as being formed by complementary molecules that have a single positive and a single negative charge at adjacent points in the complementary areas. (Other charged groups would be present but would not be important for formation of the complex.) A single additional charged group, such as a phosphate group, in one of these areas could prevent polymerization; but removal of the group would allow polymerization to proceed. Similar effects may be obtained if one of the charged groups necessary to the interaction is masked. The mechanism in Fig. 2, D allows opposing charged areas on the fibril-forming proteins to be unmasked by the depolymerization of a prosthetic molecule such as RNA.

All these mechanisms are similar in that they result in fibril formation through the interaction of oppositely charged areas. The forms of the mechanism shown in Fig. 2, A and B are considered more likely to be the original forms of which C and D are considered as possible refinements. It is of interest in connection with model D that negative

charges are removed when fibrils are formed from fibrinogen, the removal thus altering the electrostatic charge pattern on the molecule in the manner postulated here (Lorand, 1954).

Examples of structure formation mediated through weak coulombic forces are available in a number of biological materials. For example, in the vitreous humor, hyaluronic acid and protein, neither of which can fully account for the high viscosity, have been shown to interact electrostatically to produce the observed viscosity (Woodin and Boruchoff, 1955).

THE LIABILITY OF CELLULAR ORGANIZATION

Evidence will now be reviewed for the idea that the cell is composed of a series of colloidal aggregates that, though held together by a wide spectrum of forces, are nevertheless sufficiently liable to be considered as in equilibrium with either smaller complexes or free colloidal particles in solution. The implications of this concept for cell division will also be discussed.

The classical work of Bungenburg de Jong and his coworkers on coacervates (Kruyt, 1949) has emphasized that a coacervate exists in equilibrium with a more dilute solution of the same constituents. The equilibrium may be established only very slowly, and the equilibrium concentration of the dilute phase may be very low. In application of the concept to cellular structures, the objection has been that these are fairly stable in solution. Closer examination, however, suggests that this may not be so. Actually, it appears that the cell contains a wide variety of aggregates that slowly disaggregate when placed in large volumes of solution.

Soluble aggregates

Perhaps the first evidence for a soluble tissue molecular aggregate was the finding by Sorof and Cohen (1951) that material could be reversibly shifted between two electrophoretic peaks in rabbit liver soluble protein preparations by alternate aerobic and anaerobic incubation. Work in this laboratory with rat liver soluble proteins has shown that, in veronal buffers at *p*H 8.5, a complex disaggregates in the electrophoresis cell, with precipitation of one component (Anderson, 1957). Further evidence for formation of a complex is afforded by the marked changes observed when similar preparations are studied at various *p*H's. The enzymes of the pentose cycle also appear to form a soluble complex or cluster, since all the enzymes involved sediment together (Newburgh and Cheldelin, 1956). During the isolation of enzymes from crude extracts, it is not infrequently observed that the enzyme activity is widely distributed among various electrophoretic fractions. However, when preliminary salt precipitation or other procedures that may break up soluble complexes have been performed, the activity is then often found in one peak. It is of interest also that myosin in dilute solutions breaks up into several fragments (Joly, Schapira, and Dreyfus, 1955). The well-known instability of many purified enzymes in dilute solution may mean either that they are stabilized by formation of a complex or that they are composed of subunits that dissociate upon dilution.

Soluble complexes are believed to be of central importance in the economy of the cell, for the following reasons. First, the activities of soluble enzymes may be regulated by the degree to which they are aggregated with other enzymes to form complexes. If the complex contains the enzymes that catalyze a chain of reactions, these enzymes will be able to function more efficiently in the presence of enzymes catalyzing the destruction of intermediates than if the enzymes of the complex were free and separate in solution. Since the formation of complexes may be responsive to a whole spectrum of changes (temperature, *p*H, electric currents, ionic strength, specific ions, hydrostatic pressure, dielectric constant), the mechanism can serve as an effective transducer (in the sense used in electronics) to produce chemical, electrical, and ultimately mechanical responses to these variables.

Second, soluble complexes may serve to solubilize and transport molecules of limited solubility. The importance of this lies in a possible mechanism for cell-membrane formation (Anderson, 1957). If the solubilizing molecule has a different electrophoretic mobility from that of the molecule that it helps to keep in solution, the former may move away from the latter in an electrical field. Since the complexes are labile, the solubilizing molecules remaining attached to the relatively insoluble ones will be swept away as soon as they dissociate. The result will be that a precipitate, possibly in the form of a membrane, will occur wherever a potential gradient exists. It has been shown (Anderson, 1957) that the so-called "current of injury" that occurs in torn cells flows in the proper direction to account for the formation of a new membrane where the damage occurs.

Third, the soluble complexes, if in the form of

linear aggregates, will have a considerable effect on the physical properties of the cytoplasm. Since changes in viscosity occur with little change in the total concentration of substances in the cell, it is evident that aggregation of particles already there must account for the changes in consistency observed. Although particles up to the size of microsomes may well become involved (or entangled), the fundamental change is more likely to be on the molecular level. Monné (1948) and Runnström (1949, 1955) have demonstrated the reversible formation of cytoplasmic fibrils in response to hypertonic solutions and azide. They consider them to be composed largely of microsomes.

The soluble complexes are also considered as intermediates in the formation of many cellular structures. This is in keeping with the concept that the cytoplasm is a labile system in a state of *dynamic equilibrium.*

Structural complexes

The formed elements of the cell include in their fabric such a variety of intermolecular linkages that we find it hard to decide exactly what constitutes a nucleus or a mitochondrion. From the large literature on isolated cell components, only a few citations are necessary. The amount of protein found in isolated nuclei depends on the method of isolation (Stern and Mirsky, 1955; Hale and Kay, 1956). Since the original conclusion that the nuclear envelope is permeable to large molecules (Anderson, 1953a) has been supported in isolated nuclei (Anderson and Wilbur, 1951; Anderson, 1953a, b; Holtfreter, 1954; Stern and Mirsky, 1953), in electron micrographs (Bahr and Beermann, 1954; Watson, 1955), and in intact cells (Brachet, 1956), it appears that a good share of the proteins present in the nucleus of the intact cell are free to diffuse in and out, and that these may be in equilibrium with molecules adsorbed on the nuclear gel-work. The proteins of the microsomes have not been extensively characterized; but again, some of these are removed by increasing the ionic strength (Dallam, 1955) or by the use of mild detergents (Littlefield, Keller, Gross, and Zamecnic, 1955). Perhaps the most interesting cytoplasmic structures from this point of view are the mitochondria, which in simple salt or sucrose solutions swell and may lose a considerable amount of material. This can be partially prevented by the use of chelating agents, ATP, or by proteins or other high-molecular-weight substances. It appears, therefore, that mitochondria tend to dissolve slowly. The ease with which enzymes or other proteins may be removed from mitochondria varies greatly. Catalase, which has most often been found about evenly distributed between the mitochondria and the freely soluble proteins, is almost entirely on the mitochondria when these are isolated in colloid-rich solutions (Greenfield and Price, 1956). Part of the enzymatic complement of heart mitochondria may be isolated by a simple *p*H change. The enzymes that catalyze the oxidation of succinate and reduced diphosphopyridine nucleotide (DPNH) by molecular oxygen are then found associated in a complex particle that has been termed the "electron transfer particle" by Mackler and Green (1956).

Lability similar to that seen in vitro is widely observed in vivo. Starvation can deplete all cell structures of protein, and pathological changes can produce a wide variety of alterations. Of special interest is the sensitive responsiveness of mitochondria that has been stressed by Cowdry (1918). These structures can exhibit marked changes in form in response to heat and cold, starvation, anesthetics, and numerous poisons, and to vitamin deficiencies, endocrine imbalance, and anoxia (see Roberts, 1949). The results have been considered as caused by "functional hypertrophy" (Guillermond, 1934), changes in cell hydration (Kater, 1937), alterations in the physical state of ground cytoplasm as expressed in the glucose-glycogen equilibrium (Clarke and Hair, 1932), or possibly as expressing the utilization of material stored in the form of mitochondria (Bensley, 1947). Of greatest interest is the idea that mitochondria are complexes precipitated from a saturated cytoplasm (Guthrie, 1925). Roberts (1949) concluded that the granulation of mitochondria in response to various experimental methods can be interpreted as an expression of an alteration of normal cellular metabolism. Neither biochemical studies nor electron micrographs do justice to mitochondrial lability.

Many of the changes observed in vivo are suggestive of those observed in vitro. It is significant that the latter may be partially prevented by the addition of high-molecular-weight substances, which would tend to slow down dissolution and which may even form a complex with the substances of the mitochondrion.

Rhythmic changes in the number of mitochondria have been reported during cell division (Chèvremont and Fréderic, 1951; Agrell, 1955). Many of them appear actually to *dissolve* during this process.

The ability of various cell components to be

isolated and studied biochemically in no way counteracts the concepts of labile unquilibrium systems stressed here. This is true for the following reasons. (1) Equilibria in colloid systems are often established very slowly. For isolation of cell particulates, conditions are generally adjusted to further slow down the establishment of equilibria. During the isolation of miotchondria, for example, work is carried on as rapidly as possible and at low temperatures. Also, the particles are not subjected to extreme dilution, but are handled as fairly concentrated suspensions, thus minimizing their dissolution. (2) The equilibrium may be shifted very far in one direction, for example, toward the formation of structure. One does not generally work with breis or brei fractions for more than an hour or two at 37°C. because of the rapid changes that occur. Although these changes can be lumped under the term "autolysis," the question why the changes did not occur in the living cell is still a mystery. The simplest explanation is that structures once "intact" by virtue of their being in equilibrium with a certain concentration of dissolved constituents under controlled conditions of *p*H, salt concentration, intermediary metabolite concentration, and oxygen tension are, in a brei, approaching a different, possibly more self-destructive, equilibrium.

Since an equilibrium may be shifted very far in one direction, almost all of a particular substance may be found in solution or in a particle. However, in the long-term economy of the cell, the very existence of an equilibrium may be of great importance. It certainly accounts for the finite turnover rates of *all* proteins of the cell yet examined, even those of nondividing cells.

In the numerous reviews on the biochemistry of isolated cell organelles (Claude, 1948, 1949; Bradfield, 1950; Dounce, 1950, 1954; Hogeboom, 1951; Potter, Recknagel, and Hurlbert 1951; Schneider and Hogeboom, 1951, 1956; Holter, 1952; Lang, 1952; Schneider, 1953; de Duve and Berthet, 1954; Hogeboom and Kuff, 1955), the nonuniformity in the distribution of enzymes is usually stressed. Certain enzymes are listed as mitochondrial, others as microsomal or soluble, and a few are considered as nuclear. These findings have contributed greatly to our understanding of the biochemistry of cell function. However, the concept is often tacitly assumed and occasionally stated that there *should be* a specific place for each enzyme. It is not my belief that the cell is "the best possible cell, with a place for everything and everything in its place." Rather, the cell as we now know it represents a solution by evolution of a number of problems *some of which cannot be ideally solved simultaneously*. The more important conclusion to be drawn from studies on isolated cell components is that *few instances of apparent exclusive localization exist*, and even with these the total absence of activity in any particular fraction is difficult to demonstrate. One can only set an upper limit for the activity; i.e., the activity is not greater than about 1 to 5 per cent of that found in another fraction.

Additional evidence for the occurrence of the same building blocks—but in different ratios—in all cytoplasmic fractions is provided by serological studies that uniformly show that extensive cross-reactions occur (Arnesen, Goldsmith, and Dulaney, 1949; Dulaney et al., 1949; Malmgren et al., 1951; Schechtman and Nishihara, 1955; Horn, 1956).

Exclusive localization of substances such as proteins (i.e., a nonequilibrium system) could occur only if the cell were completely panmeristic in the sense that Hertwig used the term, i.e., if every persistent part of the cell is self-perpetuating by the process of growth and division. This is obviously not true, and any model of the cell must allow for the migration, on the basis of the available data, of most large molecules in the cell away from the sites of their synthesis. Some mechanism must therefore exist for moving them and assembling them into various cytoplasmic structures. Although a system can be postulated in which each substance synthesized undergoes a complex series of enzyme-mediated changes resulting in the formation of structure, such a system would not so easily exhibit the salient characteristics of the cell, viz., growth, responsiveness, and extreme lability.

The importance of lability lies in (1) the ability of labile structures to grow or diminish when the supply of their constituents in solution changes; (2) the control or "feed back" inherent in an equilibrium system, which dispenses with any separate mechanism for transmitting "information" about structure to synthetic sites; (3) the ability of new complex-forming molecules to find their way into existing structures altering their form, size, or function (i.e., differentiation can occur); and (4) most important for the present discussion, the possibility that many of the constituents of several cell structures are identical.

It is unnecessary to postulate any new intermolecular specificities to account for the condensation of soluble or solubilized molecules into

structure. Serology has demonstrated a variety of these ranging from exquisite selectivity, which often leads to the formation of complexes that are difficult to dissociate without denaturing the proteins involved, to other complexes that can be demonstrated in solution only by biophysical methods. The cytoplasm appears to be a collection of aggregates formed by the interaction of specific surface configurations—a jigsaw puzzle that solves itself by thermal agitation.

The problem of cellular evolution involves not only the evolution of catalytic specificities (enzymes) but of structure-forming specificities as well. Nature appears to have lavished most of her interest and originality on the latter, since most organisms use very nearly the same fundamental enzyme systems but possess wide structural variations throughout the approximately two million species of animals on the surface of the earth. Since the formation of cell structure, as presented here, involves the formation of numerous complementary molecules, it is interesting that antibody formation, far from being a unique activity, may be merely an adaptation of one of the cell's most characteristic functions.

Many different possibilities arise from the finding of Najjar and Fisher (1956) that not only antigen-specific antibodies may exist, but also antibodies specific for the antibody complex, and further antibodies may exist against this secondary complex, others against the tertiary complex, and so on. In theory, the antigen and the antibodies specific for the secondary and all higher complexes could exist together in solution. Only when the original antigen-specific antibody was added would the complex series of reactions leading to the formation of a definite structure take place. The addition of one molecular species could therefore cause a number of other types to condense with it.

Another point of interest in connection with the formation of structure by specific macromolecular interactants is the effect of an imbalance of interactants. If an "antigen" is present in excess, for example, not only will the antigen fail to precipitate (except in certain horse antisera), but the precipitate (structure) already formed will go into solution.

If a structure is in equilibrium with its dissolved constituents, it is evident that a *small change in the dissolved material or the appearance of new substances that aggregate with it can bring about the dissolution of the original structure.* This appears to be true of the assembly of the entire mitotic system. The soluble, structure-forming molecules are drained off to form a new structure. As a result, part of the previous structure is dissolved. A second solution to the jigsaw puzzle may exist either where several new pieces are added or when a small change is made of the pieces. One solution of the puzzle may be the resting cell, the other the dividing cell.

The interrelationship of structures such as the mitochondria and the cell cortex and the mitotic apparatus has been stressed. The dissolved phases of all of these are probably in equilibrium with their synthetic sites, which are thought to be on the microsomes. The microsomes appear to be much less labile than other cytoplasmic structures and are relatively more stable in solution. They may be considered to have a lability of a different order from that of other cytoplasmic constituents and may be in a very slowly formed equilibrium with the genetic mechanism of the nucleus. We might be tempted to consider the latter as having a lability (or conversely, stability) of still a third order whose gradual approach to equilibrium with the rest of the cell sets a time limit on its life span and inevitably results in degenerative changes (aging) unless reorganization with intercomparison of code systems (as discussed in Anderson, 1956), and the elimination of defects together with restoration of losses can be carried out, as it may be during cell division.

In some instances where the approach to equilibrium is too slow, the process may be accelerated enzymically.

THE FORMATION OF THE ASTERS

In the present view the formation of the astral ray system as a result of the release of a substance produced by the centers is postulated. This substance is thought to be either a polycation or to produce a change similar in effect to that of a polycation. The cytoplasmic changes observed are here interpreted in terms of the gradual production of organized fibrils by the interlinking of soluble proteins or protein clusters.

The reality of astral rays and spindle fibers in the living, dividing cell appears to have been firmly established (Inoué and Dan, 1951; Inoué, 1951; Schrader, 1953; Mazia, 1956). Besides protein, they contain RNA (Pollister and Ris, 1947; Stich, 1951b) and polysaccharide (Monné and Slautterback, 1950; Stich, 1951a). The central role of the mitotic center in the formation of the asters has been postulated almost since their discovery. However, little has been said about how they are

produced. As shown in the previous discussion on the formation of protein fibrils, the release of basic substances or of substrates that alter the change ratios on the cytoplasmic proteins in favor of polycations could initiate a formation of fibrils. The radiating configuration of fibrils is easily explained on the basis of a point source of the factor shifting the charge ratios and on the basis of the theory of Rees (1951). As the first fibril elements are formed in the neighborhood of the centers, they are no longer able to have a free sphere of rotation when a certain average length has been reached. As more of the cytoplasmic and centriole fibril-forming constituents diffuse into this area, the concentration of fibrils continues to increase. The problem of fibril packing is solved as in a tactoid by quasiparallel orientation. Since the aggregate surrounds the centers, the preferred lines of organization would be radial rather than tangential. The diffusion of more polycation or polycation producer from the centers would produce further linear condensation on the distal ends of the radially oriented fibrils, continuing until the astral rays are fully formed. Condensation is not considered as being always in terms of one monomer (protein molecule) condensing on the end of a beaded chain. Rather, a large number of dimers, trimers, and so on, would first be formed. The aggregation of these into long chains would occur in the manner suggested by Rees, since the principles apply equally well to the linking of short chains.

The formation of aster-like structures in solution has been beautifully demonstrated by Waugh (1946). In acid solution, insulin forms highly asymmetric fibrils. These aggregate in solution under certain conditions to form spherites in which the fibrils are *radially oriented*. The rate of formation of these spherites could be changed by varying the protein concentration. It was demonstrated that fibril formation in solution *precedes* the formation of spherites. The appearance of the latter was favored by a high concentration of *short fibrils*. No spherites were formed under those conditions that lead, initially, to low concentrations of *very long* fibrils. Waugh (1941) has suggested that this phenomenon is related to the reversible formation of the spindle and asters in living cells. He suggests that "there is a fundamental similarity between the forces which maintain the directional molecular aggregation in the insulin gel and the forces which maintain many reversible and non-reversible directional aggregates *in vivo*, although the processes by which the aggregations are brought about may be expected to be different."

The gross cytoplasmic viscosity changes observed in such a system would be, first, an increase in over-all viscosity with the formation of low polymers (e.g., dimers, trimers), and then by a general viscosity drop as the chains are organized into astral rays. The reason is simply that the chains in the latter stage are concentrated in a rather small percentage of the cell volume. Experimentally, this is precisely what is observed (Heilbrunn and Wilson, 1948). In the *Chaetopterus* egg, for example, the viscosity rises after early prophase begins. Then there is a sharp drop as the asters and spindle form. Somewhat similar results were reported by Carlson (1946). Mazia (1956) has taken a somewhat different view and considers that gelation and then liquefaction occur throughout the cell as the mitotic apparatus forms. It should be noted that the idea presented here is a restatement in more modern terms of part of the older theory of the "archoplasm" propounded by Boveri.

Material responsible for the changes in cytoplasmic viscosity may be derived in part from the cell cortex, which decreases in viscosity during division (Heilbrunn and Wilson, 1955).

The bonds holding the fibril components together have been stressed as being largely ionic, with the reservation that other forces may also be involved. Molecules held together by ionic forces should tend to be dissociated by high hydrostatic pressure, since H^+ and OH^- occupy a smaller volume when associated with charged groups on a protein than when combined as water. According to LeChatelier's principle, this electrostrictive effect should cause structures such as a spindle formed by aligned globular molecules held together by salt linkages to solate. Experimentally, this has been shown in a large number of cell types (Brown, 1934; Pease, 1941, 1946; Marsland, 1939, 1942, 1951; Marsland and Brown, 1936, 1942; Marsland and Landau, 1954). The spindle and asters solated *reversibly*, the cortical gel decreased in viscosity under pressure, and amoeboid movement ceased.

These effects have been interpreted in terms of the reversible folding and unfolding of protein molecules (Johnson, Eyring, and Polissar, 1954). However, as these authors point out, ". . . we know of no unequivocal evidence that proteins after synthesis ever reversibly unfold, even partially, into a one-dimensional chain or two-dimensional network" (p. 31). The effects of pressure may be explained equally well on the basis of a disaggrega-

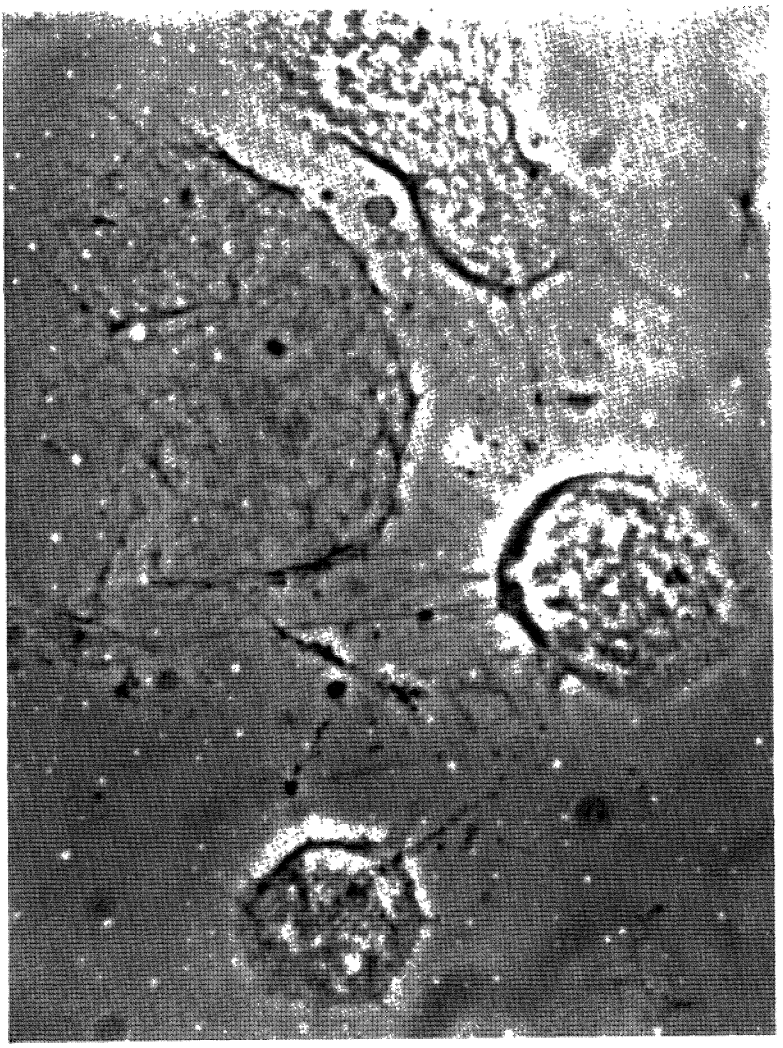

FIG. 3. FIBRIL FORMATION BETWEEN ISOLATED NUCLEI TREATED WITH PROTAMINE SULFATE AT pH 7.1
Nuclei were slightly flattened to allow fibrils that appear to originate from nuclear envelope material to be seen. Note double coiled structures extruding from upper nucleus (Anderson, 1951).

tion of beaded chains as on the basis of the refolding of extended or unfolded protein chains. For reasons previously mentioned, the former view is adopted here.

It should be pointed out that the classical example of specific interaction between large molecules, the antigen-antibody reaction, has been shown in serum albumin to be dependent on the presence of one positively charged group on the antibovine serum albumin antibody and one negatively charged group on the reaction site of the antigen (Singer, 1955). This reaction may, therefore, be considered as being of the general type postulated here. When either of the two ionizing groups was masked, no reaction occurred.

Experimentally, basic dyes or basic proteins added to rat liver breis produced numerous fine, microscopically visible fibrils (Anderson, 1949, 1951). Fig. 3 shows the effect of added protamine sulfate in neutral solution to rat liver nuclei. The chromatin material condenses into fine threads, and the nuclear envelope becomes a series of fine fibrils, many of which are at the limits of resolution of the phase contrast microscope. The substance of the nuclear envelope appears, therefore, to be available for fibril formation and may function in this manner during division.

The actual isolation of the mitotic apparatus by Mazia and Dan (1952) and the partial characterization of the major protein component constitute an important advance in the understanding of the mechanism of cell division. In the sea urchin egg, this apparatus contains about 11.6 per cent of the total protein of the cell (Mazia and Roslansky, 1956). Since calculations showed that the nucleus could contain at the most only about 2 per cent of the material found in the mitotic apparatus, the substance of the apparatus cannot be stored in the nucleus of the cell during interphase. This lends direct support to the concept that the building blocks for the mitotic apparatus—the asters and the spindle—form part of other structures in the resting cell and may also be partly in solution either as single molecules or in the form of soluble complexes. Chèvremont and Fréderic (1951), studying mitosis in muscle cells and fibroblasts from chick embryos and using phase contrast, observed a reduction in size of mitochondria during prophase and metaphase. Certain of the mitochondria were seen to dissolve partially. After mitosis, the mitochondria increased both in size and number. Agrell (1955) observed a sharp drop in the number of mitochondria after the breakdown of the nuclear envelope. Actual counts showed that the mitochondria were dissolved from prophase to metaphase, with a consistent increase in number from metaphase up to the next prophase.

THE SPINDLE

The spindle is here considered to be produced by linear aggregates of structural proteins held together by predominantly ionic forces. The changes in surface properties of these substances necessary to produce linear arrays are thought to be caused by the presence of a polycation or substances capable of producing charge reversal on a part of the surface of the molecule. Possible mechanisms for movement in such systems will be discussed.

The first problem concerns the forces or structures that allow the spindle to exist as a distinct entity. The simplest mechanism would be that of an enclosing membrane, impermeable to the substance of the spindle. The persistence of the nuclear envelope during division has been suggested in the *Tradescantia* stamen hair cells (Wada, 1950). However, certain obvious difficulties arise if such a membrane is to be impermeable to proteins or other colloids. Chief of these is that it cannot persist as an impermeable barrier in the form of the nuclear membrane from generation to generation (Anderson, 1953a). At best, it would provide a mechanism for keeping the spindle material in position but would not explain how it got there in the first place.

Descriptions of the spindle in terms of a gel structure do little better because they generally do not specify the changes in intermolecular bonding necessary to form a gel. The author is reluctant to accept the concept of the spindle as a simple liquid crystal, in view of the multiplicity of compounds known to make up this structure, predominantly proteins, which according to Pfeiffer (1954) include those of the histone or basic type, and RNA and polysaccharide (Monné and Slauterback, 1950; Pollister and Ris, 1947; Stich, 1951b). Adenosine triphosphatase has also been demonstrated on the spindle (Biesele, 1949).

The most plausible description appears to be based on the concept of a separation of colloid phases (Wada, 1955). If this is true, then many of the same molecular species should be found in the interastral ray cytoplasm, in the asters, and in the spindle, but in different proportions. The finding by Mazia and Dan (1952) that the mitotic apparatus consists mostly of a single substance provides strong support for this idea. The test is, of course, the isolation of the same substance for the cytoplasm outside the mitotic apparatus.

The mechanisms described in a previous section as producing the astral rays may serve equally well for the spindle. Such anisodiametric micelles or microfibrils as do not form part of the astral system, either for reasons of length or microcharge distribution, would, if present in sufficient concentration, give rise to ordering of the type described by Oster (1950) as occurring in virus suspensions. The longer fibrils may be found in the spindle since, as was shown by Waugh (1946) with insulin, only the short fibrils tend to form spherites.

If the quasiparallel ordering of the fibrils is due to interaction between fibrils, as was shown with tobacco mosaic virus (Oster, 1950), then the "tactoid" would be expected to fall to the bottom, like virus tactoids. The confinement of these fibrils to the spindle region may be controlled by the presence of a few fibers extending from pole to pole. Free fibrils in the immediate neighborhood of these would tend to be ordered in the same way that particles approaching the edge of a tactoid are ordered. As ordering occurs around a fiber, the concentration of random fibrils in the immediate vicinity is decreased, and other fibrils would tend

to diffuse in. This would continue until the concentration of fibrils in the cytoplasm is lowered to the point where each has approximately a free sphere of rotation. The formation of the original fibers is thought to be caused by the diffusion of polycationic substances from the poles and the kinetochores. The idea that such a substance exists is not new. For example, Wada (1950, 1955), on the basis of cytological studies, has suggested that a secretion from the kinetochore serves to hold or cement the particles of the chromosomal fibers together. If both the kinetochore and the centers elaborate the charge-shifting substances, the formation of fibrils connecting the two is not difficult to explain.

The fundamental mechanism, an association of macromolecules because of changes in the surface charges, would appear to be subject to experimental alteration by both chemical and physical agents. If the masking of acidic groups on the surface of proteins by polycations so as to produce a reversal of charge or the interlinking of such proteins by polycations is essential for spindle formation, then substances capable of interfering with these changes should have profound effects. Specifically, the interfering substance should (1) bear a negative charge, (2) be a fairly large molecule, and (3) bear groups that contribute to its water solubility without markedly altering its charge. Such a substance would be able to occupy sites that would normally bind polycations and effectively prevent the formation of complex structures such as the spindle. The polycation binding area would then be essentially uncharged. As shown in fig. 4, the best spindle poisons, colchicine and N-methyl colchicamide fulfill these requirements. No activity is observed in the absence of the basic group (Lettré, 1952). Another series of active compounds has been built around α,β-diphenylethylamine (stilbylamine), which also contains a basic group. This compound is not active in itself, but addition of various side-chains produces activity. The 4′-ethoxy derivative is the most active of the series.

The specificity of these spindle poisons suggests that the spindle aggregates may be formed through the mechanism described but that specific complementary surfaces are also involved. Colchicine may be specific by virtue of a stereochemical configuration that is more specific for one of the surfaces of the spindle-forming protein than for the components of the chromosome. The net result of the action of colchicine would be that fewer constituents would be available for forming the spindle (i.e., the spindle would be smaller), and polycationic material otherwise used in spindle construction would be free to condense other systems. This would explain why colchicine causes over condensation of the chromosomes (Coleman, 1947; Suomalainen, 1954) in addition to its better-known effects on the spindle. This suggests again that cell division in its entirety may be derived essentially from one basic mechanism.

The spindle is reversibly solated by high hydrostatic pressure. As noted by Pease (1946), the spindle may re-form after pressure solation first around the centers and the kinetochores. This observation again suggests that these organelles serve as centers for organizing spindle miceles.

One of the most formidable difficulties that confronts us when we attempt to treat mitotic mechanisms arises from impressions gained from observing time-lapse motion pictures. The chromosomes on the metaphase plate dance about in an irregular manner and then suddenly move to the poles. The seemingly autonomous movements are

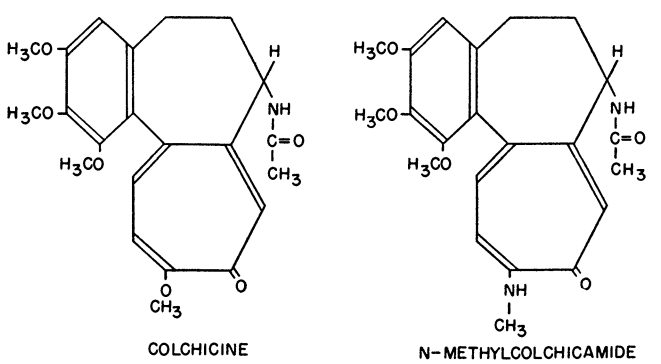

FIG. 4. COLCHICINE (formula of Dewar, 1945) AND N-METHYL COLCHICAMIDE
The latter is four times as active as colchicine.

most puzzling. Similar anomalous behavior has been described by Seifriz (1953). Granules in the streaming protoplasm of plant cells were observed to reverse direction occasionally and move against the stream. Again, parts of the cell appeared to exhibit autonomous behavior.

Much has been said about the extension and contraction of thread-like particles. These do *not* behave like flexible steel rules—either straight or neatly coiled up. Rather, the same molecular agitations that give rise to the now commonplace Brownian movement would be seen to produce similar effects on molecular chains if we could observe small enough segments. A bundle of molecular strands would then appear more like a group of freshly caught eels. Still more violent thrashing about may be expected when the concentration of the contraction-producing substance (ATP?) approaches its critical concentration. Even before it is reached throughout the whole system, the critical value will be exceeded locally in certain segments of the contractile system because of purely statistical fluctuations. Convulsive movements caused by a combination of thermal agitation and localized contractions (which are also ultimately caused by thermal agitation) would be expected to occur. If the chromosomes are attached to such strands, the observed irregular movements should be expected.

Part of the difficulty is that no model of such a system is available, whereas ordinary Brownian movement is easily demonstrated in inanimate particles. The attempt to make a suitable model with very dilute nucleoprotein gels containing visible particles did yield interesting results, however. When the preparation was forced through a capillary tube on a microscope stage, we observed irregular flow, first down the center, then along the edge, and not infrequently in two directions at once! Occasionally a particle would move with the stream, stop, and then reverse direction. The observations are a demonstration of the elastic properties of a relatively few molecules. They illustrate that an anomalous movement of cell structures may find an explanation in the properties of long-chain contractile molecules whose properties are rarely observed outside the cell at the microscopic level. The molecules or molecular strands need not be attached to visible structures at both ends. The movement of a particle by a strand may occur when the other end of the strand is attached to a weak "gel island" that may serve as an effective "sea anchor."

The picture of the spindle that emerges is one of a few long fibrils formed by interlinking protein molecules with polycationic materials produced by the mitotic centers and the kinetochores. These orient fibrillar material occurring in the cytoplasm and produce tactoid areas between the fibrils. (The word "tactoid" is used in the descriptive sense and does not imply the presence of long-range forces.) Such a structure would have the extreme lability characteristic of spindles in living cells, viz., sensitivity to hydrostatic pressure and chemical agents and to agitation with a microneedle. Although disulfide bonds might be important in producing the fibrils that become oriented around the fibers, the lability of the spindle indicates that these bonds are not involved in maintaining the structure as a whole.

THE BREAKDOWN OF THE NUCLEAR ENVELOPE

Very little experimental information is available on the mechanism of the breakdown or dissolution of the nuclear envelope during cell division or in regard to its reformation at the end of division. It has been proposed by Heilbrunn and Wilbur (1937) and more recently by Goldstein (1952) that calcium, released from the cortex of *Nereis* or *Chaetopterus* eggs, activates a proteolytic enzyme of a type described by Gross (1952). Nuclear envelopes of isolated rat liver nuclei are indeed easily digested by proteolytic enzymes (Anderson, 1953a and b), but such enzymes have even more drastic effect on nucleoproteins.

Observations by Monné (1946) and by Baud (1948) suggest the possibility that surface-active substances may be involved. From studies on the stability of nuclei in breis, I prefer to conclude that the structural component of the envelope is not digested in the usual sense, but becomes part of the mitotic apparatus, returning to its former position at the nuclear-cytoplasmic interface at the end of division. In this connection, Schrader (1953) has stressed the probable role of envelope material in mitotic events.

A simple explanation for the dissolution of the nuclear envelope is to be found in precisely those changes that may result in the dissolution of parf of the mitochondrial system. If the substance ot this structure is in equilibrium with its dissolved constituents, then a migration of the latter into the structure of the mitotic apparatus should result in a gradual dissolving of the envelope. Furthermore, if the substance causing the dissolved molecules to link together at dimers and trimers origi-

nated in the nucleus, the disappearance of the nuclear membrane would be expected to be a very early event, which indeed it generally is.

CHROMOSOMAL MOVEMENTS

Chromosomal movements have been reviewed in detail by Schrader (1953). This discussion will be largely limited to a consideration of molecular changes that could account for the movements observed.

Energy is required to keep fibrils such as those that compose spindle fibers and astral rays in an extended condition. In muscle, the extending force is thought by many to be an electrostatic one. Contraction occurs when the electrostatic forces are diminished and allow an "entropic" contraction to occur. The muscle fibrils could be positively charged in the extended state and be discharged by the highly anionic ATP molecule. Whether binding and contraction go together or the splitting of ATP is crucial to the contraction process cannot be decided as yet. Considerable evidence supports the conclusion that the spindle fibers do respond to ATP (Weber, 1955).

The formation of astral and spindle fibrils has been postulated here as being caused by substances that alter the surface charge on the normally negative or anionic cellular proteins, so as to make them at least partially cationic. The result could well be a beaded chain system which is held in an extended state by positive charges, and similar to that proposed by Morales, Botts, Blum, and Hill (1955). Contraction of such a system should occur when the surface charges are neutralized by adsorption of ATP or some similar substance.

The source of the substance producing the positive electrostatic charge on the fibrils has been postulated as being in the centers and the kinetochores. It is interesting that, whereas calcium and magnesium have been implicated in charge reversal in muscle proteins, the organic bases such as spermine, which are widely distributed and which could also serve as a ready and controllable source of positive charges, have been largely overlooked. Fujii's report (1954) that zinc moves from the nucleolus to the spindle during division is of interest, since several organic bases react with the reagent used.

If the contractile model proposed by Flory (1957) is adopted, then the spindle may be considered to be partially crystalline and to be stabilized by the forces holding crystalline segments together. The release of any charged substance such as ATP would, when adsorbed on the fibrils, disrupt the crystalline segments and result in contraction as the fibrils return to an amorphous state. The crystallization could also help to account for the movement of the chromosomes to the spindle plate.

If the fiber-forming substances are produced at the centers and the kinetochores, why are not the kinetochores of sister chromosomes merely bound tightly together? Why should they instead be pulled apart? The answer may indeed be that a considerable amount of fiber-forming material is condensed between them. If this material were somewhat over-condensed (contracted), it might indeed revert to an extended shape at some later time, and thereby cause such an expansion of the spindle area between the chromosomes as occurs in many cells. An alternative, and not necessarily counter-possibility, may lie in the effect of RNA that the chromosomes shed between them (Jacobson and Webb, 1952; Ris and Kleinfeld, 1952). Since RNA is a polyanion, it would have a solating effect on at least part of the spindle between the separating chromosomes. This solated material is in admirable position to diffuse to the cell cortex and creat a band of extra cortical material at the edge of the space occupied by the metaphase plate. Here it is not necessary to postulate a mechanism for reforming this material into a contracting gel-work, since calcium from the surrounding medium could serve to cross-link it and keep it in a constant state of tension.

It may be asked how the mitotic system forms, remains in a state of quasi-stability for a considerable time (metaphase), and then in a very short period goes through anaphase and into telophase. As we know, from observing time-lapse motion pictures of dividing cells, the chromosomes at the metaphase plate move and jostle about. The state of the chromosome fibers at this point may be similar to that described in a theoretical model of a myosin system contraction by adsorbing ATP (Morales et al., 1955). Here very little change occurs as ATP is adsorbed, until a critical point is reached where almost maximal contraction occurs. Addition of more ATP causes little further change. In the metaphase cell, the adsorption and breakdown of ATP on the chromosomal fiber would proceed at a rate dependent on the rate of ATP synthesis maintained by the cell. If no special additional ATP synthesis occurs during division, as appears to be the case experimentally, much time might elapse before the critical concentration is reached on the fibers. All fibers could be very

close to this concentration at the *same* time. Production of ATP by the kinetochore in rather small amounts could produce contraction near the chromosomes. Alternatively, additional polycations produced by the kinetochores might be necessary to produce fibrils that can contract. The metaphase pause and the rapid anaphase movement do not necessarily require a sudden burst of synthetic activity or a special signaling substance or stimulus.

A similarity between the kinetochore and the mitotic center has been suggested on theoretical (Darlington, 1936; Schrader, 1936) and structural grounds (Schrader, 1936). It was pointed out that they possess similar staining reactions and similar functions during mitosis. In meiosis in atypical spermatogenesis in viviparid molluscs, Pollister (1939) found that kinetochores' detached from degenerating chromosomes, became associated with the centriole and mimicked its behavior and appearance accurately. If this similarity holds true biochemically, it may be postulated that both function by producing low-molecular-weight polycations. The movements of the centrioles during spermatogenesis and their close association with the nucleus during its period of condensation suggest that the centrioles may function here to produce the basic subprotein protamine, which, as discussed by Anderson (1956), may serve both for efficient DNA packing and as the cause of sperm aster formation in the fertilized egg. The centrioles are also associated with the formation of fibrils incorporated in the sperm tail. The structural agent proposed by Swann (1951a,b, 1952a,b, 1954) may be either additional polycationic material or ATP. In connection with the assumption that a substance is produced at a site because a physical effect is observed at increasing distances, it is evident that the site may just as well be a destructive center (a "sink"), and that the effect seen may be due to a decrease instead of an increase in the concentration of an agent.

CYTOKINESIS

As stressed by Mazia (1956), the mechanisms by which the cell body divides vary so markedly between plant cells, egg cells, and cells of irregular shape that it is difficult to detect any basic unity; in fact, little may exist. Studies in which hydrostatic pressure was used support the contracting ring theory (Lewis, 1951; Marsland, 1951), which assumes that cleavage occurs as a result of the contraction of an equatorial ring in the furrow, a concept supported by work with cell models (Hoffmann-Berling, 1954b,c, 1955). Mitchison and Swann (Mitchison, 1952; Mitchison and Swann, 1954a,b; Roberts, 1955) have presented evidence for an expanding membrane, produced by the release of a "structural agent," that pushes inward at the equator and causes division. Dan (1948) believes the driving force to be the elongation of the spindle, which, by pushing the asters apart, causes a constriction to appear in the cleavage plane. It is not unlikely that all three mechanisms actually occur, and no decision in favor of any one theory can be made on the basis of the considerations presented here.

However, certain relations between these mechanisms and the general scheme presented here deserve comment. The contracting ring theory has been discussed in a preceding section in which it was proposed that material solated in the presence of RNA shed by the chromosomes diffused to the cortex. If all cortical material is maintained in a state of contraction, then constriction should occur where an excess is present. The elongation of the spindle, necessary in the theory of Dan, could be produced by subtile changes in the packing of the spindle fibrils, due to changes in the average fibril length or in surface charge. The expanding membrane evident in the work of Mitchison and Swann would require an increased tendency for soluble cell constituents to form such membranes. It is of considerable interest that several basic substances, including the basic dye Victoria blue, tend to cause bleb formation (an increase in surface area) in living cells (Lettré, 1951). These mechanisms may therefore ultimately depend on the one proposed here.

THE RECONSTRUCTION OF THE RESTING CELL

Cell division has been considered here as being set in motion by the release of a polycationic material that, adsorbed on cellular colloids, could alter the ratio of positive and negative charges, or by the production of a substance capable of producing an equivalent effect, as discussed in a previous section. The problem of telophase is to return the cell to its original state.

If the mitotic apparatus is in equilibrium with soluble constituents, which are also in equilibrium with the formed elements including the cell cortex, the mitochondria, and the nuclear envelope, then an increase in those molecular species that are characteristic of the latter structures but do not participate in the formation of the mitotic appa-

ratus should shift the balance at least partially back in favor of the resting state.

The synthesis of substances competing with the mitotic apparatus for structural proteins requires that material stimulating the production of protein in the cytoplasm be released from the nuclear apparatus at precisely the right moment. Since a variety of lines of evidence now favor the idea that such a substance would most likely be a nucleoprotein particle (Borsook et al., 1950; Hultin, 1950; Keller, 1951; Allfrey, Daly, and Mirsky 1953), this role may be assigned to the RNA shed by the chromosomes as they leave the equatorial plate. The movement of the chromosomes to the poles should allow enough time for the RNA to become incorporated into a protein-synthesizing particle and for protein synthesis to

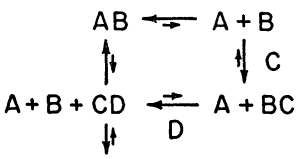

FIG. 5. FORMATION OF STRUCTURE AND OF FIBRILS BY ALTERNATE PRODUCTION OF POLYCATION C AND POLYANION D

The cell structure, AB, is in equilibrium with dissolved A and B. On the addition of C, the dimer unit BC is formed, which polymerizes to form fibrils. Addition of D, which competes for C, results in the formation of the strongly bound complex CD, which essentially removes C from the system. A and B are then free to re-form cell structure AB. A similar system may exist where A is absent and structure is formed by the cross-linking of B, as shown in Fig. 6.

start. Since the dissolution of the apparatus between the poles would then occur first, structural elements sufficient to form increasing amounts of cortex should be available in the plane perpendicular to the spindle axis. This material could form a constricting band that would pinch off the cell.

In the simplest case, the formation of a cell structure may be considered as being caused by the formation of a complex between two molecular species, A and B, which are in equilibirum with a solution containing the same molecules. If a second structure can be formed from B and C, then the following can be expected:

$$AB + C \leftrightarrows A + B + C \rightleftarrows BC + A.$$

If we assume that the over-all equilibrium is shifted to the right, then the addition of C to the mixture of A and B should result in the dissociation of part of AB and the formation of BC. To reverse this reaction, the further addition of a molecule that will compete with B for C will result in the re-formation of AB. The complete scheme is shown in Fig. 5. CD is considered to be more stable than AB or BC. If AB is the interphase structure, BC would be the division structure. The entire cycle may proceed, in a system where all constituents are constantly being synthesized and destroyed, when the synthetic rates of all reactants (A, B, C, and D) vary appropriately.

In this discussion C may be considered to be a polycationic material, and the role of D could be filled by an acid polysaccharide. Experimentally, acid polysaccharides such as heparin will cause rapid swelling of nuclear gels (Anderson and Wilbur, 1951; Roberts and Anderson, 1951), solation of amoeba cytoplasm, and the disaggregation of microsomes (Hoster et al., 1950), thus demonstrating its ability to alter cell structures. A cyclical variation in polycation and polyanion concentration could therefore underlie the whole division mechanism.

Such macromolecular displacements probably play a very important role in cell function. For example, acid polysaccharides could well displace finished protein molecules from synthetic sites. Mucus, which is so widely and easily produced, may well be synthesized in such a process. The protein moiety would be synthesized on a template, whereas the acid polysaccharide may be formed in solution. The polysaccharide would compete with the template for the protein molecule and combine with it to complete the synthesis. In an analogous manner, D could displace C off BC and effectively remove it from the reaction.

The mechanism for reversibly forming fibrils from molecules that also form three-dimensional structures in the cell is shown in Fig. 6. The formation of membrane-like structure is shown as being caused by interaction of oppositely charged areas on the same molecule. The structure is in equilibrium with free molecules that may be also solubilized by complex formation with other molecules indicated for convenience in Fig. 6 as being smaller than the structure-forming molecules. In the presence of polycationic molecules (shown as oblong bodies), the structure-forming molecules are condensed into chains. Schematic drawings of the same structures are shown on the right in Fig. 6.

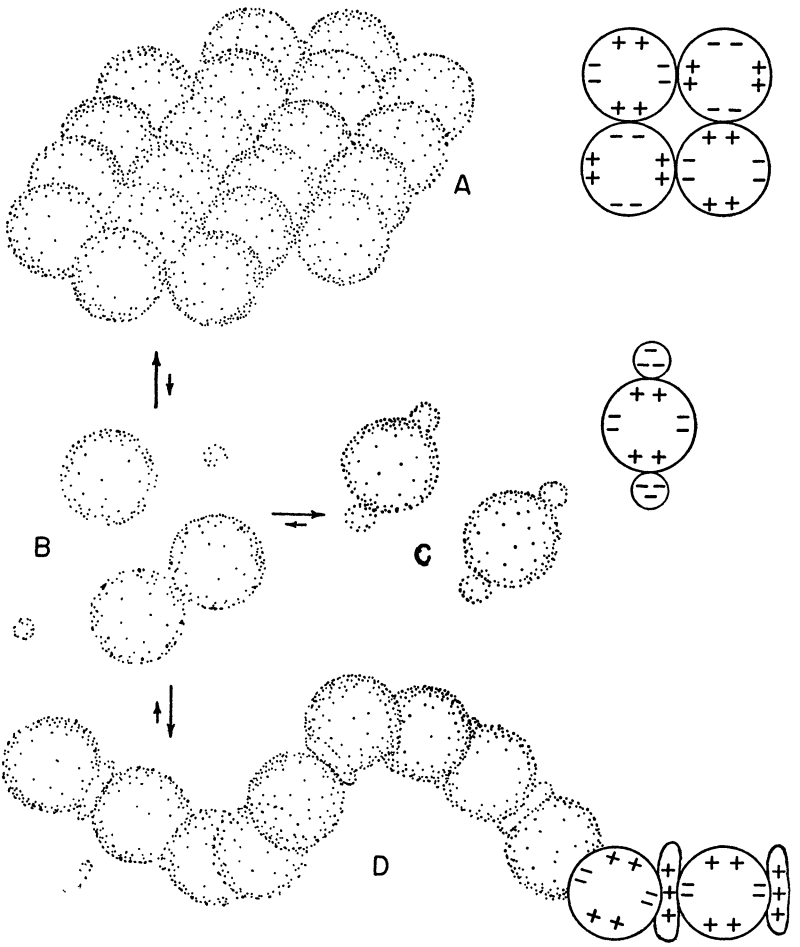

FIG. 6. FORMATION OF TWO TYPES OF STRUCTURE BY STRUCTURE-FORMING PROTEINS

In A, protein condenses to form a sheet that is in equilibrium with protein in solution (B) and with solubilized protein molecules (C). Addition of a polycation (shown as an oblong molecule) results in fibril formation (D). The solubilizing molecules at C are shown, for convenience, as small spheres. The distribution of charges is shown schematically along the right margin.

THE CHARGE OF CYTOPLASMIC COLLOIDS

As has been pointed out (Anderson, 1956), the sign of the predominant charge on cell colloids is fundamental to considerations of the nature of gelation in cells generally and to the primitive cell division mechanism here proposed specifically. This mechanism depends on the production of positively charged groups on otherwise negatively charged particles, causing either condensation or fibril formation. Since there has been considerable disagreement as to whether the predominant charge on cytoplasmic colloids is negative or positive, a somewhat detailed discussion of the subject is necessary. The term "cytoplasmic colloids" includes a variety of colloidal particles and is not properly a generic entity.

Heilbrunn (1928; Heilbrunn and Daugherty, 1939) has summarized the evidence for a predominant positive charge, whereas Chambers and Kao (1952) have presented some of the data supporting the concept that the charge is predominantly negative. The former concept received support initially from elemental analyses of whole *Arbacia* eggs, which demonstrated a high ratio of divalent to monovalent cations. These results are somewhat misleading since the jelly coat contains acid polysaccharides that may be expected to bind calcium as does also the nucleus (Williamson and Gulick, 1942). If the presumed positive charge on cell col-

loids is due to adsorbed calcium ions, substances that bind calcium; e.g., citrate and versene, would be expected to have a precipitating effect. Experimentally, the opposite is true, and the injection of calcium results in coagulation (Chambers and Kao, 1952).

Since the amount of calcium is too small to account for a positive charge by charge reversal, one is at a loss to find suitable groups to which a positive charge may be ascribed. Actually, the great majority of inorganic cations are monovalent, and the inorganic anions such as phosphate and sulfate exist for the most part in the form of polyanions such as ATP, nucleic acids, and acid polysaccharides. No polycation of comparable strength is found in the cytoplasm of the interphase cell. The only organic base strong enough to be at all comparable with phosphate or sulfate is choline, which is not known to exist as the recurring base in any basic cytoplasmic polymer. Furthermore, the majority of tissue proteins have an isoelectric point in the acid region, indicating an excess of available carboxyl groups over available amino groups in neutral solution. Since the charge on the colloidal constituents *is* negative, the typical mammalian cell, for example, contains very little monovalent anion (HCO_3^-, Cl^-), whereas the great preponderance of the cationic charge is monovalent (K^+, Na^+) (see Gamble, 1952, Chart 2-A).

It may be argued that, since the charge on a colloidal particle is determined only by the properties of its surface, a small number of multicationic molecules coating these particles might result in an over-all positive charge excess in spite of the evidence to the contrary already cited. Experimental studies on the surface properties of isolated rat liver mitochondria suggest that some RNA may be located on the surface (Schneider, 1946; Anderson and Wilbur, 1950). Addition of a polycation such as protamine flocculates brei constituents (Weil et al., 1952), as does the basic dye Janus green B (Anderson, 1949). It is difficult to escape the conclusion that the predominant charge is negative.

A similar conclusion may be drawn from the observation of cells broken in various solutions (Heilbrunn, 1928, 1952; Costello, 1933). Citrate, a trivalent anion, *prevents* gelation or flocculation of the extruding cytoplasm, whereas calcium (which in low concentration would be expected to cross-link and precipitate a solution of anionic colloids) produces the familiar surface precipitation reaction. Furthermore, as is well known, anionic colloids tend to flocculate as the pH is lowered toward their isoelectric points, whereas cationic colloids tend to become more soluble. The viscosity-*increasing* effect of lowering cell pH has been repeatedly demonstrated (Loeb and Blanchard, 1922; M. R. Lewis, 1923; Barth, 1929; Minshall and Scarth, 1952) and is consistent with the concept that the colloids are moving closer to their isoelectric point; i.e., they are negatively charged. Above pH 7 solation tends to occur as would be expected (M. R. Lewis, 1923; Barth, 1929). Under very alkaline condition, where highly viscous DNA is dissociated from the proteins of the nucleus, the viscosity increases. It is interesting that nuclear constituents have usually been thought to be negatively charged (Churney and Klein, 1937; Heilbrunn, 1952).

Perhaps the best evidence on cytoplasmic charge comes from the early microinjection studies of Chambers and others (Chambers, 1924), who found that only the basic dyes caused a local coagulation. No pH changes appear to be involved. Protamine, when injected, causes a similar sudden coagulation (N. G. Anderson, 1952, unpublished data), whereas heparin produces liquifaction. If the colloids were positively charged, the opposite results would have been produced.

The assumption that the predominant charge on protoplasmic colloids is positive has introduced a number of difficulties into the colloid-chemical theory of Heilbrunn. Thus immersion of cells in $CaCl_2$ solutions is shown to cause first a solation and then a gelation. The solation is presumed to be due to the effect of adding divalent cations to a polycationic system, although Heilbrunn later noted that the problem of determining the effect of various cations on the internal protoplasm of cells is rather complicated since in cells immersed in solutions of sodium or potassium salts, the sodium or potassium ions may release calcium from the outer cortical protoplasm (Heilbrunn, 1952, page 97). However, in invoking the participation of calcium in stimulation and other cellular responses, Heilbrunn states, "Typically, when calcium in small amounts is released to the interior of a cell, a gelation occurs . . ." In the surface precipitation reaction, calcium is shown to cause a precipitation of cytoplasmic constituents. Clearly, calcium should promote the outflow of the cytoplasm if it is positively charged. Since the actual findings are in such marked opposition to the original assumption, the dilemma was solved by postulating the existence of a process similar to

blood clotting. This was done by assuming that here calcium plays a role, not in the stabilization or destabilization of colloids, but in activating thromboplastic substances. The roles of citrate and oxalate, which sould have profound effects on a polycationic system (especially one whose charge is due to adsorbed calcium), are attributed to their effects on a clotting mechanism. Heparin, the physiological polyanion *par excellence*, which again should wreak havoc in a polycationic system, prevents cytoplasmic gelation. Again, the effect is ascribed to a clotting mechanism similar to that found in vertebrate blood (Heilbrunn, 1952). Lastly, in breis, calcium is not released by a variety of experimental procedures, but is actually taken up as would be expected in a system bearing a predominantly negative charge. In order to demonstrate calcium "release" as the colloid-chemical theory would predict, one must add more calcium and consider a diminished binding of the added calcium as "calcium release" (Weimar, 1953). As is evident, the anomalous aspects of this theory largely disappear if one assumes instead that the predominant charge is negative. The electrophoretic studies that have been cited as conclusive (Heilbrunn and Daugherty, 1939) have not been successfully repeated (Tobias and Solomon, 1950).

The data indicating that calcium decreases and potassium and sodium increase cytoplasmic viscosity, with *opposite* effects on the cortex, have been summarized by Heilbrunn (1952, page 96) and deserve comment here. The precipitation of negatively charged colloids by cations of higher valence, prompted him to interpret these findings as indicating that the dispersed particles of the protoplasmic colloids bear a positive charge. (Certain qualifying statements are added to the effect that this is true only when the ions enter slowly since gelation occurs when free calcium enters rapidly.) The experimental observations are reinterpreted here on the assumption that: (1) the cell cortex is largely composed of a calcium complex, (2) the constituents of the cortex complex (protein, acid polysaccharide, etc.) are normal constituents of the cytoplasm, (3) an equilibrium exists between these substances in the cortex and in the cytoplasm, and (4) the complex-forming substances in the cytoplasm are generally in the form of their potassium or sodium salts. The first assumption is borne out by a number of experiments cited by Heilbrunn. The second assumption is borne out by the many observations of the formation of a surface precipitation in outflowing protoplasm in the presence of calcium, where the precipitated material is considered to be identical with that of the normal cortex. In a cell existing in a calcium-containing medium (as is generally the case), the cortex is thought to represent only part of the complex-forming material, the rest being distributed throughout the cytoplasm. When the calcium content of the surrounding medium is increased, it penetrates the cell slowly and precipitates *more* of the complex-forming material, decreasing its concentration in the cytoplasm and also the viscosity of the cytoplasm. The cortical viscosity, therefore, increases and that of the cytoplasm decreases as is observed. The result is, therefore, very different from that seen when calcium is injected into the cell, where it precipitates at once all cortex-forming materials.

When an excess of sodium or potassium is added, calcium may be expected to be displaced from the cortex and from the cell much as calcium may be eluted from an ion-exchange resin. The result is a softening and dispersion of the cortex-forming materials throughout the cell, resulting in a large decrease in cortical viscosity and a small increase in the viscosity of the rest of the cytoplasm as is observed. This interpretation, which harmonizes the results obtained with both injection and immersion studies, may bring some respite in the long argument that has resulted from these seemingly conflicting findings.

DISCUSSION

Cell division in its entirety—with the erection of the mitotic machinery, with its precise functioning and subsequent disappearance, and with the reorganization of one cell into two complete daughter cells—would seem to lie in the province of the arts rather than the sciences. The special cases, such as spermatogenesis, oogenesis, and the variations and exceptional instances so treasured and emphasized by the cytologist, appear to far exceed the capabilities of any mere molecular assemblages, to say nothing of the shear impossibility of packing the entire mechanism into tiny cells to produce others in their image, complete, with no missing piece, not once, but through long spans of time.

It would seem logical to assume that the division mechanism, which originated in a single primeval particle and which has had at least the possibility of as much experimentation expended on its development as any other product of evolution, would

be characterized by two things: (1) economy of materials, and (2) economy of methods. This is especially true since the same mechanism appears throughout the whole animal kingdom. Put somewhat more naïvely, if it could be done more simply, sooner or later it ought to be. Operationally, a mechanism drawing materials from those readily available in several cell structures and that is triggered by variations in the rate of synthesis of substances normally present is more amenable to attack than one involving a whole complex of special substances synthesized by other suitably controlled mechanisms, to be destroyed when no longer needed by still other special enzymes. This latter idea, as partially stated, assumes that, " . . . during mitosis there must be a need for special enzymes involved in functions such as dissolving and regenerating the nuclear membrane, and synthesizing and dissolving the special protein of the mitotic apparatus" (Monty et al., 1956). Enzymes to destroy G, H, and I, to synthesize J; enzymes to destroy J and resynthesize G, H, and I; enzymes to destroy the enzymes destroying G, H, and I; templates to make the enzymes at a particular time; mechanisms to inhibit template utilization at other times; a sequential timer that senses when each task is complete and signals the next—such complexity, even if it did exist, should not confront the investigator at the outset. Further, some of the peculiar division patterns often reviewed may be based on erroneous observations, unusual fixation artifacts, or incorrect interpretation of stained figures. These "exceptions" should not be treasured, they should be repeated, using living cells if possible.

In the first two papers of this series, the author attempts to coordinate certain facts about cell division in such a manner as to suggest unifying relations and critical experiments to test the validity of these relations. The need for extensive physical and chemical characterization of as many molecular species as can be isolated from cells becomes self-evident from these considerations. It is in terms of the properties of these that the story of cell division must eventually be rewritten.

Other papers in this series will deal with experimental findings that bear on the material discussed here.

LIST OF LITERATURE

AGRELL, I. 1955. A mitotic rhythm in the appearance of mitochondria during the early cleavages of the sea urchin embryo. *Exp. Cell Res.*, 8: 232–234.

ALLFREY, V., M. M. DALY, and A. E. MIRSKY. 1953. Synthesis of protein in the pancreas. II. The role of ribonucleoprotein in protein synthesis. *J. gen. Physiol.*, 37: 157–175.

ANDERSON, N. G. 1949. Master's Dissertation, Duke University.

——. 1951. Doctor's Dissertation, Duke University.

——. 1953a. On the nuclear envelope. *Science*, 117: 517–521.

——. 1953b. Studies on isolated cell components. VI. The effects of nucleases and proteases on rat liver nuclei. *Exp. Cell Res.*, 5: 361–374.

——. 1956. Cell division. I. The primeval mechanism, the initiation of cell division, and chromosomal condensation. *Quart. Rev. Biol.*, 31: 169–199.

——. 1957. Labile colloidal complexes of the cytoplasm. In *Symposium on Biocolloids, J. cell. comp. Physiol.*, Suppl., in press.

——, and K. M. WILBUR. 1950. Release of nucleic acids from cell components by heparin. *Fed. Proc.*, 9: 254.

——, and ——. 1951. Studies on isolated cell components. II. The release of a nuclear gel by heparin. *J. gen. Physiol.*, 34: 647–656.

ARNESEN, K., Y. GOLDSMITH, and A. D. DULANEY. 1949. Antigenic properties of nuclei segregated from spleens of normal and leukemic mice. *Cancer Res.*, 9: 669–671.

BAHR, G. F., and W. BEERMANN. 1954. The fine structure of the nuclear membrane in the larval salivary gland and midgut of *Chironomus*. *Exp. Cell. Res.*, 6: 519–522.

BARBU, E., and M. JOLY. 1950. Mécanismes de gélification de la sérumalbumine. Étude par la biréfringence d'ecoulement. *Bull. Soc. Chim. biol.*, Paris, 32: 116–122.

——, and ——. 1953. The globular-fibrous protein transformation. *Disc. Faraday Soc.*, No. 13: 77–93.

BARTH, L. G. 1929. The effects of acids and alkalies on the viscosity of protoplasm. *Protoplasma*, 7: 505–534.

BAUD, C. A. 1948. Action de divers composés tensio-actifs sur la texture de la membrane nucléaire. *C. R. Soc. Biol., Paris*, 142: 181–182.

BENSLEY, R. R. 1947. On the nature of the pigment of mitochondria and of submicroscopic particles in the hepatic cell of the guinea pig. *Anat. Rec.*, 98: 609–619.

BERNAL, J. D. 1940. Structural units in cellular physiology. In *The Cell and Protoplasm* (F. R. Moulton, ed.), pp. 199–205. A.A.A.S. Pub. No. 14, Washington, D. C.

——, and I. FANKUCHEN. 1941. X ray and crystallographic studies of plant virus preparations. I. Introduction and preparation of specimens. II. Modes of aggregation of the virus particles. *J. gen. Physiol.*, 25: 111–146.

BIESELE, J. J. 1949. Phosphatases of the mitotic apparatus in cultured normal and malignant mouse cells. In *Proc. First Nat. Cancer Conf., Amer. Cancer Soc. and Nat. Cancer Inst.*, pp. 34–41. Washington, D. C.

BORSOOK, H., C. L. DEASY, A. J. HAAGEN-SMIT, G. KEIGHLEY, and P. H. LOWY. 1950. Metabolism of C^{14}-labeled glycine, L-histidine, L-leucine, and L-lysine. *J. biol. Chem.*, 187: 839–848.

BRACHET, J. 1956. Further observations on the action of ribonuclease on living amoeba. *Exp. Cell. Res.*, 10: 255–256.

BRADFIELD, J. R. G. 1950. The localization of enzymes in cells. *Biol. Rev.*, 25: 113–157.

BROWN, D. E. S. 1934. The pressure coefficient of "viscosity" in the eggs of *Arbacia punctulata*. *J. cell. comp. Physiol.*, 5: 335–346.

CARLSON, J. G. 1946. Protoplasmic viscosity changes in different regions of the grasshopper neuroblast during mitosis. *Biol. Bull., Woods Hole*, 90: 109–121.

CHAMBERS, R. A. 1924. The physical structure of protoplasm as determined by microdissection and injection. In *General Cytology* (E. V. Cowdry, ed.), pp. 235–309. University of Chicago Press, Chicago.

——, and C. Y. KAO. 1952. The effect of electrolytes on the physical state of the nerve axon of the squid and of *Stentor*, a protozoon. *Exp. Cell Res.*, 3: 564–573.

CHÈVREMONT, M., and J. FRÉDERIC. 1951. Recherches sur le comportement du chondriome pendant la mitose. *C. R. Soc. Biol., Paris*, 145: 1245–1247.

CHURNEY, L., and H. M. KLEIN. 1937. The electrical charge on nuclear constituents (salivary gland cells of *Sciara coprophila*.) *Biol. Bull., Woods Hole*, 72: 384–388.

CLARK, J. A., and G. E. HAIR. 1932. Observations on the mitochondria of the hepatic cell of the frog in normal and hyperglycemic state. *Z. Zellforsch.*, 15: 123–130.

CLAUDE, A. 1948. Studies on cells: morphology, chemical constitution, and distribution of biochemical functions. *Harvey Lect.*, 43: 121–164.

——. 1949. Proteins, lipids, and nucleic acids in cell structures and functions. *Advanc. Protein Chem.*, 5: 423–440.

COLEMAN, L. 1947. Chromosome abnormalities in an individual of *Chorthippus longicornis* (Acrididae) *Genetics*, 32: 435–447.

CORNMAN, I. 1944. A summary of evidence in favor of the traction fiber in mitosis. *Amer. Nat.*, 78: 410–422.

COSTELLO, D. P. 1933. The surface precipitation reaction in marine eggs. *Protoplasma*, 17: 239–257.

COWDRY, E. V. 1918. The mitochondrial constituents of protoplasm. *Contr. Embryol. Carneg. Instn.*, 8(25): 39–160.

DALLAM, R. D. 1955. Studies on the chemical composition of nuclei and cytoplasmic granules. *Arch. Biochem. Biophys.*, 54: 24–37.

DAN, J. C. 1948. On the mechanism of astral cleavage. *Physiol. Zool.*, 21: 191–218.

DARLINGTON, C. D. 1936. The external mechanics of the chromosome. I. The scope of enquiry. *Proc. roy. Soc.*, B, 121: 264–319.

DEDUVE, C., and J. BERTHET. 1954. The use of differential centrifugation in the study of tissue enzymes. *Int. Rev. Cytol.*, 3: 225–275.

DEWAR, M. J. S. 1945. Structure of colchicine. *Nature, Lond.*, 155: 141–142.

DOUNCE, A. L. 1950. Enzyme systems of isolated cell nuclei. *Ann. N. Y. Acad. Sci.*, 50: 982–997.

——. 1954. The significance of enzyme studies on isolated cell nuclei. *Int. Rev. Cytol.*, 3: 199–223.

DULANEY, A. D., Y. GOLDSMITH, K. ARNESEN, and L. A. BUXTON. 1949. A serological study of cytoplasmic fractions from the spleens of normal and leukemic mice. *Cancer Res.*, 9: 217–221.

FARRANT, J. L., and E. H. MERCER. 1952. Electron microscopical observations of fibrous insulin. *Biochim. biophys. Acta*, 8: 355–359.

FLORY, P. J. 1957. Crystallinity and dimensional changes in fibrous proteins. In *J. cell. comp. Physiol.*, Suppl. (Symposium on Biocolloids), in press.

FOSTER, J. F., and E. G. SAMSA. 1950. Streaming birefringence of denatured ovalbumin. *Science*, 112: 473–474.

——, and ——. 1951a. Streaming orientation studies on denatured proteins. I. Heat denaturation of ovalbumin in acid media. *J. Amer. chem. Soc.*, 73: 3187–3190.

——, and ——. 1951b. Streaming orientation studies on denatured proteins. IV. Denaturation of ovalbumin in the presence of urea. *J. Amer. chem. Soc.*, 73: 5388–5391.

FREY-WYSSLING, A. 1953. *Submicroscopic Morphology of Protoplasm*. Elsevier Press, Houston.

FUJII, T. 1954. Presence of zinc in nucleoli and its possible role in mitosis. *Nature, Lond.*, 174: 1108–1109.

GAMBLE, J. L. 1952. *Chemical Anatomy, Physiology, and Pathology of Extracellular Fluid* (5th ed.). Harvard University Press, Cambridge.

GOLDSTEIN, L. 1952. The mechanism of nuclear breakdown in the Chaetopterus egg. *Biol. Bull., Woods Hole*, 103: 292.

GREENFIELD, R. E., and V. E. PRICE. 1956. Liver catalase. III. Isolation of catalase from mitochondrial fractions of polyvinylpyrrolidone-sucrose homogenates. *J. biol. Chem.*, 220; 607–618.

GROSS, P. R. 1952. A study of colloidal changes in sea urchin egg homogenates. *Biol. Bull., Woods Hole*, 103: 293.

GUILLERMOND, A. 1934. Les constituants morphologiques du cytoplasme: le chondriome. In *Actualités Scientifiques et Industrielles, No. 170.* Hermann & Cie., Paris.

GUTHRIE, M. J. 1925. Cytoplasmic inclusions in cross-activated eggs of teleosts. *Z. Zellforsch.*, 2: 347–381.

HALE, A. J., and E. R. M. KAY. 1956. A comparison of nuclear dry weights determined by chemical and by interferometric methods. *J. biochem. biophys. Cytol.*, 2: 147–158.

HEILBRUNN, L. V. 1928. The colloid chemistry of protoplasm. *Protoplasm—Monogr., Berl., Vol. 1.* Borntraeger, Berlin.

——. 1952. *An Outline of General Physiology*, 3rd Ed. W. B. Saunders Company, Philadelphia.

——, and K. DAUGHERTY. 1939. The electric charge of protoplasmic colloids. *Physiol. Zool.*, 12: 1–12.

——, and K. M. WILBUR. 1937. Stimulation and nuclear breakdown in the *Nereis* egg. *Biol. Bull., Woods Hole*, 73: 557–564.

——, and W. L. WILSON. 1948. Protoplasmic viscosity changes during mitosis in the egg of *Chaetopterus. Biol. Bull., Woods Hole*, 95: 57–68.

——, and ——. 1955. The initiation of cell division in the *Chaetopterus* egg. *Protoplasma*, 44: 377–384.

HOFFMANN-BERLING, H. 1954a. Adenosintriphosphat als Betriebsstoff von Zellbewegungen. *Biochim. biophys. Acta*, 14: 182–194.

——. 1954b. Die Bedeutung des Adenosintriphosphat fur die Zell- und Kernteilungsbewegungen in der Anaphase. *Biochim. biophys. Acta*, 15: 226–236.

——. 1954c. Die Glycerin-wasserextrahierte Telophasezelle als Modell der Zytokinese. *Biochim. biophys. Acta*, 15: 332–339.

——. 1955. Geiselmodelle und Adenosintriphosphat (ATP). *Biochim. biophys. Acta*, 16: 146–154.

——, and H. H. WEBER. 1953. Vergleich der Motilität von Zellmodellen und Muskelmodellen. *Biochim. biophys. Acta*, 10: 629–645.

HOGEBOOM, G. H. 1951. Separation and properties of cell components. *Fed. Proc.*, 10: 640–645.

——, and E. L. KUFF. 1955. Relation between cell structure and cell chemistry. *Fed. Proc.*, 14: 633–638.

HOLTER, H. 1952. Localization of enzymes in cytoplasm. *Advanc. Enzymol.*, 13: 1–20.

HOLTFRETER, J. 1954. Observations on the physicochemical properties of isolated nuclei. *Exp. Cell. Res.*, 7: 95–102.

HORN, E. C. 1956. Ascites tumor development. II. Cytotoxicity of various antisera prepared against Ehrlich ascites tumor cell components. *Cancer Res.*, 16: 595–599.

HOSTER, M. S., B. J. MCBEE, H. A. ROLNICK, Q. VAN WINKLE, and H. A. HOSTER. 1950. Marcomolecular particles obtained from human neoplastic and non-neoplastic lymph nodes. I. Procedure and preliminary results. *Cancer Res.*, 10: 530–538.

HUGHES, A. 1952. *The Mitotic Cycle.* Academic Press, Inc., New York.

HULTIN, T. 1950. Incorporation in vivo of N^{15}-labelled glycine into liver fractions of newly hatched chicks. *Exp. Cell Res.*, 1: 376–381.

INOUÉ, S. 1952. The effect of colchicine on the microscopic and the submicroscopic structure of the mitotic spindle. *Exp. Cell Res.*, Suppl. 2: 305–314.

——, and K. DAN. 1951. Birefringence of the dividing cell. *J. Morph.*, 89: 423–456.

JACOBSON, W., and M. WEBB. 1952. The two types of nucleoproteins during mitosis. *Exp. Cell Res.*, 3: 163–183.

JAGGI, M. P., and D. F. WAUGH. 1950. Structure of gels from ovalbumin and serum albumin. *Fed. Proc.*, 9: 66.

JOHNSON, F. H., H. EYRING, and M. J. POLISSAR. 1954. *Kinetic Basis of Molecular Biology.* John Wiley & Sons, New York.

JOLY, M. 1949a. Étude par la biréfringence d'écoulement de la gélification de la gélatine et de ses interactions avec d'autres substances organiques. *Bull. Soc. Chim. biol. Paris*, 31: 105–107.

——. 1949b. Untersuchungen über die Umwandlungen einiger kolloider Systeme mit Hilfe der Strömungsdoppelbrechung. *Kolloidzschr.*, 115: 83–89.

——, and E. BARBU. 1949. Étude par la biréfringence d'écoulement de la denaturation termique de la sérumalbumine. *Bull. Soc. Chim. biol. Paris*, 31: 1642–1655

——, and ——. 1951. Étude par la biréfringence d'écoulement de l'agrégation thermique de la sérumalbumine. *J. chem. Phys.*, 48: 636–642.

——, and B. RYBAK. 1950. Recherches sur la constitution du fuseau archromatique. II. Études rheologiques de quelques modèles protéiques. *Bull. Soc. Chim. biol. Paris*, 32: 894–903.

——, G. SCHAPIRA, and J. C. DREYFUS. 1955. Length of myosin particles in dilute solutions determined by flow birefringence. *Arch. Biochem. Biophys.*, 59: 165–180.

KATCHALSKY, A. 1954. Polyelectrolyte gels. In *Progress in Biophysics and Biophysical Chemistry*, Vol. IV (J. A. V. Butler and J. T. Randall, eds.), pp. 1–59. Academic Press, New York.

KATER, J. M. 1937. The liver-blood fluid exchange

and the morphology of hepatic cell mitochondria. *J. Morph.*, 61: 473–484.

KELLER, E. B. 1951. Turnover of proteins of cell fractions of adult rat liver *in vivo*. *Fed. Proc.*, 10: 206.

KLECZKOWSKI, A. 1949. A quantitative study of complex formation in heated protein mixtures. *Biochem. J.*, 44: 573–581.

KRUYT, H. R. (ed.). 1949. *Colloid Science*. Vol. II. *Reversible Systems*. Elsevier Press, New York.

KUHN, W., B. HARGITAY, A. KATCHALSKY, and H. EISENBERG. 1950. Reversible dilation and contraction by changing the state of ionization of high-polymer acid networks. *Nature, Lond.*, 165: 514–516.

LANDAHL, H. D. 1942. An analysis of the shapes of a cell during division with particular reference to the role of surface tension. *Bull. math. Biophys.*, 4: 151–154.

——. 1942b. Equilibrium shapes in non-uniform fields of concentration. *Bull. math. Biophys.*, 4: 155–158.

——. 1942c. A kinetic theory of diffusion forces in metabolizing systems. *Bull. math. Biophys.*, 4: 15–26.

——. 1942d. A mathematical analysis of elongation and constriction in cell division. *Bull. math. Biophys.*, 4: 45–62.

LANG, K. 1952. Lokalisation der Fermente und Stoffwechselprozesse in den einzelnen Zellbestandteilen und deren Trennung. In *Mikroskopische und chemische Organization der Zelle*, pp. 24–42 (2. Colloquium der Deutschen Gesellschaft für physiologische Chemie). Springer-Verlag, Berlin.

LETTRÉ, H. 1951. Zellstoffwechsel und Zellteilung. *Naturwissenschaften*, 21: 490–496.

——. 1952. Some investigations on cell behavior under various conditions: A review. *Cancer Res.*, 12: 847–860.

LEWIS, M. R. 1923. Reversible gelation in living cells. *Johns Hopk. Hosp. Bull.*, 34: 373–379.

LEWIS, W. H. 1951. Cell division with special reference to cells in tissue cultures. *Ann. N. Y. Acad. Sci.*, 51: 1287–1294.

LOEB, L., and M. BLANCHARD. 1922. The effect of various salts on the outgrowth from experimental amoebocyte tissue near the isoelectric point and with the addition of acid or alkali. *Amer. J. Physiol.*, 60: 277–307.

LITTLEFIELD, J. W., E. B. KELLER, J. GROSS, and P. C. ZAMECNIK. 1955. Studies on cytoplasmic ribonucleoprotein particles from the liver of the rat. *J. biol. Chem.*, 217: 111–123.

LORAND, L. 1954. Interaction of thrombin and fibrinogen. *Physiol. Rev.*, 34: 742–752.

LUNDGREN, H. P. 1949. Synthetic fibers made from proteins. *Advanc. Protein Chem.*, 5: 305–351.

MACKLER, B., and D. E. GREEN. 1956. Studies on the electron transport system. II. On the "opening" phenomenon. *Biochim. biophys. Acta*, 21: 1–6.

MALMGREN, R. A., B. E. BENNISON, B. F. ANDERSON, and C. C. RISLEY. 1951. A serologic study of the microsome fraction of normal and neoplastic mouse tissues. *J. nat. Cancer Inst.*, 11: 1277–1286.

MARSLAND, D. A. 1939. The mechanism of cell division. Hydrostatic pressure effects upon dividing egg cells. *J. cell. comp. Physiol.*, 13: 15–22.

——. 1942. Protoplasmic streaming in relation to gel structure in the cytoplasm. In *American Society of Plant Physiologists Symposium on Structure of Protoplasm* (W. Seifriz, ed.), pp. 127–161. Iowa State College Press, Ames.

——. 1951. The action of hydrostatic pressure on cell division. *Ann. N. Y. Acad. Sci.*, 51: 1327–1335.

——, and D. E. S. BROWN. 1936. Amoeboid movement at high hydrostatic pressure. *J. cell. comp. Physiol.*, 8: 167–178.

——, and ——. 1942. The effects of pressure on sol-gel equilibria, with special reference to myosin and other protoplasmic gels. *J. cell. comp. Physiol.* 20: 295–305.

——, and J. V. LANDAU. 1954. The mechanism of cytokinesis: Temperature-pressure studies on the cortical gel system in various marine eggs. *J. exp. Zool.*, 125: 507–539.

MAZIA, D. 1956. Materials for the biophysical and biochemical study of cell division. *Advanc. biol. med. Phys.*, 4: 69–118.

——, and K. DAN. 1952. The isolation and biochemical characterization of the mitotic apparatus of dividing cells. *Proc. Nat. Acad. Sci., Wash.*, 38: 826–838.

——, and J. D. ROSLANSKY. 1956. The quantitative relations between total cell proteins and proteins of the mitotic apparatus. *Protoplasma*, 46: 528–534.

MINSHALL, W. H., and G. W. SCARTH. 1952. Effect of growth in acid media on the morphology, hydrogen-ion concentration, viscosity, and permeability of water hyacinth and frogbit root cells. *Canad. J. Bot.*, 30: 188–208.

MITCHISON, J. M. 1952. Cell membranes and cell division. *Symp. Soc. exp. Biol.*, 6: 105–127.

——, and M. M. SWANN. 1954a. The mechanical properties of the cell surface. I. The cell elastimeter. *J. exp. Biol.*, 31: 443–458.

——, and ——. 1954b. The mechanical properties of the cell surfaces. II. The unfertilized sea-urchin egg. *J. exp. Biol.*, 31: 461–472.

MONNÉ, L. 1946. Action of narcotics and of hydration and dehydrating agents on the structure of the cytoplasm. *Ark. Zool.*, 39A (7): 1–32.

——. 1948. Functioning of the cytoplasm. *Advanc. Enzymol.*, 8: 1–69.

——, and D. B. SLAUTTERBACK. 1950. Differential staining of various polysaccharides in sea urchin eggs. *Exp. Cell Res.*, 1: 477–491.

MONTY, K. J., M. LITT, E. R. M. KAY, and A. L. DOUNCE. 1956. Isolation and properties of liver cell nucleoli. *J. biochem. biophys. Cytol.*, 2: 127–145.

MORALES, M. F., J. BOTTS, J. J. BLUM, and T. L. HILL. 1955. Elementary processes in muscle action: An examination of current concepts. *Physiol. Rev.*, 35: 475–505.

NAJJAR, V. A., and J. FISHER. 1956. The mechanism of antibody-antigen reaction. *Biochim. biophys. Acta*, 20: 158–169.

NEWBURGH, R. W., and V. H. CHELDELIN. 1956. The intracellular distribution of pentose cycle activity in rabbit kidney and liver. *J. biol. Chem.*, 218: 89–96.

OSTER, G. 1950. Two-phase formation in solutions of tobacco mosaic virus and the problem of long range forces. *J. gen. Physiol.*, 33: 445–473.

PEASE, D. C. 1941. Hydrostatic pressure effects upon the spindle figure and chromosome movement. I. Experiments on the first mitotic division of *Urechis* eggs. J. Morph., 69: 405–441.

——. 1946. Hydrostatic pressure effects upon the spindle figure and chromosome movement. II. Experiments on the meiotic divisions of *Tradescantia* pollen mother cells. *Biol. Bull., Woods Hole*, 91: 145–169.

PFEIFFER, H. H. 1954. Beiträge zur Cytochemie des isolierten Atraktoplasma von Spermatocyten *in vitro*. *La Cellule*, 56: 243–249.

POLLISTER, A. W. 1939. Centrioles and chromosomes in the atypical spermatogenesis of *Vivipara*. *Proc. Nat. Acad. Sci., Wash.*, 25: 189–195.

——, and H. RIS. 1947. Nucleoprotein determination in cytological preparations. *Cold Spr. Harb. Symp. quant. Biol.*, 12: 147–157.

POTTER, V. R., R. O. RECKNAGEL, and R. B. HURLBERT. 1951. Intracellular enzyme distribution, interpretation and significance. *Fed. Proc.*, 10: 646–653.

RASHEVSKY, N. 1938. *Mathematical Biophysics. Physico-mathematical Foundations of Biology*. The University of Chicago Press, Chicago.

——. 1939. The mechanism of cell division. *Bull. math. Biophys.*, 1: 23–30.

REES, A. L. G. 1951. Directed aggregation in colloidal systems and the formation of protein fibers. *J. phys. Chem.*, 55: 1340–1344.

RIS, H. 1949. The anaphase movement of chromosomes in the spermatocytes of the grasshopper. *Biol. Bull., Woods Hole*, 96: 90–106.

——. 1955. Cell division. In *The Analysis of Development* (B. H. Willier, P. A. Weiss, and V. Hamburger, eds.), pp. 91–125. W. B. Saunders Company, Philadelphia.

——, and R. KLEINFELD. 1952. Cytochemical studies on the chromatin elimination in *Solenobia* (Lepidoptera). *Chromosoma*, 5: 363–371.

RISEMAN, J., and J. G. KIRKWOOD. 1948. Remarks on the physico-chemical mechanism of muscular contraction and relaxation. *J. Amer. chem. Soc.*, 70: 2820–2822.

ROBERTS, H. S. 1949. Changes in mitochondrial form. *Anat. Rec.*, 104: 163–188.

——. 1955. The mechanism of cytokinesis in neuroblasts of *Chortophaga viridifasciata* (De Geer). *J. exp. Zool.*, 130: 83–105.

——, and N. G. ANDERSON. 1951. Studies on isolated cell components. III. A cytological study of the effects of heparin on isolated nuclei. *Exp. Cell Res.*, 2: 224–234.

ROZSA, G., A. SZENT-GYÖRGYI, and R. W. G. WYCKOFF. 1949. The electron microscopy of F-actin. *Biochim. biophys. Acta*, 3: 561–569.

RUNNSTRÖM, J. 1949. The mechanism of fertilization in metazoa. *Advanc. Enzymol.*, 9: 241–327.

——. 1955. Changes in the submicroscopic structure of the cytoplasm attending maturation and activation of the sea urchin egg. *Exp. Cell Res.*, 8: 49–61.

BYBAK, B. 1950. Recherches sur la constitution du fuseau achromatique. I. Étude chimiques de quelques modèles proteiques. *Bull. Soc. Chim. biol.* Paris, 32: 703–718.

SCHECHTMAN, A. M., and T. NISHIHARA. 1955. The cell nucleus in relation to the problem of cellular differentiation. *Ann. N. Y. Acad. Sci.*, 60: 1079–1094.

SCHNEIDER, W. C. 1946. The action of ribonuclease on the succinoxidase system. *J. biol. Chem.*, 164: 241–247.

——. 1953. Biochemical constitution of mammalian mitochondria. *J. Histochem. Cytochem.*, 1: 212–233.

——, and G. H. HOGEBOOM. 1951. Cytochemical studies of mammalian tissues: the isolation of cell components by differential centrifugation. A review. *Cancer Res.*, 11: 1–22.

——, and ——. 1956. Biochemistry of cellular particulates. *Ann. Rev. Biochem.*, 25: 201–224.

SCHRADER, F. 1936. The kinetochore or spindle fiber locus in *Amphiuma tridactylum*. *Biol. Bull., Woods Hole*, 70: 484–498.

——. 1953. *Mitosis. The movements of chromosomes in cell division* (2nd ed.). Columbia University Press, New York.

SEIFRIZ, W. 1953. Mechanism of protoplasmic movement. *Nature, Lond.*, 171: 1136–1138.

SELBY, C. C. 1953. Electron micrographs of mitotic cells of the Ehrlich mouse ascites tumor in thin sections. *Exp. Cell Res.*, 5: 386–393.

SINGER, S. J. 1955. On the chemical structure of the reactive sites of antibody molecules. *Proc. Nat. Acad. Sci., Wash.*, 41: 1041–1045.

SOROF, S., and P. P. COHEN. 1951. Electrophoretic studies of the soluble proteins of rabbit liver. *J. biol. Chem.*, 190: 303–309.

STERN, H., and A. E. MIRSKY. 1953. Soluble enzymes of nuclei isolated in sucrose and non-aqueous media. *J. gen. Physiol.*, 37: 117–187.

STICH, H. 1951a. Das Vorkommen von Kohlenhydraten im Ruhekern und während der Mitose. *Chromosoma*, 4: 429–438.

——. 1951b. Das Vorkommen von Ribonucleinsaüre in Kernsaft und Spindel sich teilender Kerne von *Cyclops strenuus*. *Z. Naturf.*, 6b: 259–261.

SUOMALAINEN, H. O. T. 1954. Effect of colchicine on spermatogenesis in certain grasshoppers. *Exp. Cell Res.*, 7: 563–564.

SWANN, M. M. 1951a. Protoplasmic structure and mitosis. I. The birefringence of the metaphase spindle and asters of the living sea-urchin egg. *J. exp. Biol.*, 28: 417–433.

——. 1951b. Protoplasmic structure and mitosis. II. The nature and cause of birefringence changes in the sea-urchin egg at anaphase. *J. exp. Biol.*, 28: 434–444.

——. 1952a. The nucleus in fertilization mitosis and cell division. *Symp. Soc. exp. Biol.*, 6: 89–104.

——. 1952b. Structural agents in mitosis. *Int. Rev. Cytol.*, 1: 195–210.

——. 1954. The mechanism of cell division: Experiments with ether on the sea urchin egg. *Exp. Cell Res.*, 7: 505–517.

TOBIAS, J. M., and S. SOLOMON. 1950. Electrically induced polar changes in viscosity in the hyaline protoplasm of *Elodea* with observations on streaming and plastid charge. *J. cell. comp. Physiol.*, 35: 1–9.

USHER, F. L. 1929. A mechanism of gelatinization. *Proc. roy. Soc.*, A, 125: 143–151.

VERWEY, E. J. W., and J. T. G. OVERBEEK. 1948. *Theory of the Stability of Lymphobic Colloids.* Elsevier Press, New York.

WADA, B. 1950. The mechanism of mitosis based on studies of the submicroscopic structure and of the living state of the *Tradescantia* cell. *Cytologia (Tokyo)*, 16: 1–26.

——. 1955. Some problems on the mitotic figures studied in the living cell. *Caryologia*, 7: 389–403.

WADDINGTON, C. H. 1954. The cell physiology of early development. In *Recent Developments in Cell Physiology* (J. A. Kitching, ed.). pp. 105–119. [*Proc. Seventh Symposium of the Colston Research Society.*] Butterworths Scientific Publications, London.

WATSON, M. L. 1955. The nuclear envelope. Its structure and relation to cytoplasmic membranes. *J. biophys. biochem. Cytol.*, 1: 257–270.

WAUGH, D. F. 1941. The properties of protein fibers produced reversibly from soluble protein molecules. *Amer. J. Physiol.*, 133: 484–485.

——. 1946. A fibrous modification of insulin. I. The heat precipitate of insulin. *J. Amer. chem. Soc.*, 68: 247–250.

——, and B. J. LIVINGSTONE. 1951. Kinetics of the interactions of bovine fibrinogen and thrombin. *J. phys. Chem.*, 55: 1206–1218.

WEBER, H. H. 1955. Kontraktile Proteins und Motilität der Lebewesen. *Naturwissenschaften*, 42: 270–275.

WEIMAR, V. L. 1953. Calcium binding in frog muscle brei. *Physiol. Zool.*, 26: 231–242.

WEIL, M. L., J. WARREN, S. S. BREESE, S. B. RUSS, and H. JEFFRIES. 1952. Separation of encephalomyocarditis virus from tissue components by means of protamine precipitation and enzymatic digestion. *J. Bact.*, 63: 99–105.

WIERCINSKI, F. J. 1944. An experimental study of protoplasmic pH determination. I. Amoebae and *Arbacia punctulata*. *Biol. Bull., Woods Hole*, 86: 98–112.

WILKIE, D. R. 1954. Facts and Theories about Muscle. In *Progress in Biophysics and Biophysical Chemistry*, Vol. IV (J. A. V. Butler and J. T. Randall, eds.), pp. 288–324. Academic Press, New York.

WILLIAMSON, M. B., and A. GULICK. 1942. The estimation of alkaline earth minerals in thymus nuclei. *J. cell. comp. Physiol.*, 20: 116–118.

WOODIN, A. M., and S. A. BORUCHOFF. 1955. Particle interaction in solutions derived from ox vitreous humor. *J. biophys. biochem. Cytology*, 1: 489–500.

ZBARSKII, I. B., and S. S. DEBOV. 1948. On cell-nucleus proteins. *Dokl. Akad. Nauk SSSR*, 62: 795–798. (In Russian).

——, and ——. 1951. Protein fractions of cell nuclei. *Biokhimii*, 16: 390–395. (In Russian).

——, and K. A. PEREVOSHCHIKOVA. 1948. On certain properties of cell-nucleus albumens. *Dokl. Akad. Nauk SSSR*, 60: 77–80. (In Russian).

——, and ——. 1951a. Contractile properties of cell nucleus albumens. *Biokhimii*, 16: 112–124. (In Russian).

——, and ——. 1951b. The nature of the contractile substance of the cell nucleus. *Biokhimii*, 16: 547–555. (In Russian).