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CELL DIVISION

PART ONE

A THEORETICAL APPROACH TO THE PRIMEVAL MECHANISM, THE INITIATION OF CELL DIVISION, AND CHROMOSOMAL CONDENSATION

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"Regarding fear of hypotheses, all that need be said is this: the only hypotheses we need be afraid of are those which do not have testable consequences."

J. H. Woodger, 1948

INTRODUCTION

THE present series of papers represents a frank attempt made during the past five years to formulate a detailed theoretical approach to the problem of cell division. In the past a number of theories relating to mitotic events have been put forward. The more plausible of these have been critically reviewed by Schrader (1953). None of these appear to have served the functions of a scientific theory, which are first to suggest fruitful experiments that would not otherwise have been done, and second to indicate crucial experiments that will either support or destroy the theory itself. No general review will be presented here.

The point of view to be adopted here is that mitosis, as seen in contemporary plants and animals, includes many different refinements and modifications of one simple ancient mechanism operative in the first living cells. This view is in contrast to that presented by Schrader (1953), who does not consider that one underlying common denominator exists, but rather that ". . . mitosis is comprised of a great complex of different mechanisms." The nature and course of development of a proposed primeval process will be discussed in some detail before attempting to show its role in modern mitotic events, viz., the initiation of cell division, chromosomal condensation, nuclear envelope and nucleolar dissolution and re-formation, spindle and aster production, chromosomal movement, and the reconstruction of the nucleus.

It is necessary to present a number of topics in considerable detail. Since the thread of the argument is thereby easily lost, a brief summary is presented in the first paragraph of each large section of the paper. Initially, certain fundamental aspects of living systems generally will be considered, since these must form the basis for the postulates on which the theory is based. Space, time, and the limitations of the author's interests of necessity set bounds to the literature surveyed, and direct the choice of works cited.

It is evident that the cell is not an infinitely diverging system (Haurowitz, 1950). Thus, although the synthesis of many simple compounds is accomplished in a stepwise manner by an array of enzymes, the enzymes themselves cannot each

be formed by another array of specific enzymes which in turn are each produced by still another set of specific enzymes. The finite size of the cell limits the number of such sets or generations. Therefore, although at the present time new enzymes and new enzyme systems are constantly being described, so as to result in greater apparent complexity (divergence), there must come a point where convergence in biological systems will become evident. In the present context convergence means multiplicity of function. The cell can only be of finite size if it contains a number of functional entities each of which does more than one thing. Thus, on a simple level, phosphate acceptors in the cell serve a number of specific functions in addition to which, by their varying availability, they control the rates of a great number of enzymatic reactions involved in their synthesis (Potter, Recknagel, and Hurlbert, 1951). All the cell solutes, from the micro-ions, such as potassium and magnesium which have profound effects on certain enzymatic reactions, to the macromolecular enzymes with their well-known specificities, may also be considered as a group of substances which dictate by their concentration the tonicity and certain osmotic properties of the cell. The association of enzymes with "insoluble" cell particulate material (Green, 1952; Schneider and Hogeboom, 1951) suggests that enzyme molecules may be linked together to form the fabric of these structures, and may thus serve at one time both catalytic and structural functions. On a higher level, the very nature of life necessitates the existence of molecules which duplicate themselves by one means or another (autocatalysis) and which engage in some second or third highly specific function in addition to self-duplication (heterocatalysis or structure-building). The gene is a case in point, although multiplicity of function should not be considered as being limited to the nucleus.

The multiplicities of function and interaction of a great number of constituents of the cell, which are here considered to be the essence of a living system, are the bases for the delicate, continuous, internal control which characterizes all living cells. For this reason a number of familiar biological entities, such as nucleic acids and enzymes, may be treated both as molecules possessing great individuality and functional specificity, and as classes of substances engaging in certain other important processes where such simple properties as size, charge, or shape may be the only relevant characteristics. It is from these "secondary" characteristics of protoplasmic constituents that the proposed mechanism will be constructed.

An attempt to describe a complex process such as cell division should avoid postulating the existence of a new substance, plasm, or enzyme for each change or effect observed (Woodger, 1948). Therefore, these discussions will be limited to fairly well-characterized cell constituents. These include nucleic acids and certain general classes of proteins including known enzymes, coenzymes, and salts. The mechanism proposed is based on known properties of these substances. Only when it is found impossible to explain the many aspects of cell division with such known substances, is it justifiable to invoke any of the many hypothetical substances which have been postulated. It goes without saying that much of the terminology of cytological literature, which often includes several dozen names for the same morphological entity, is often of little use in a discussion of cell division on a molecular level. This is especially true of the various "plasms" which have been described.

THE PRIMEVAL MECHANISM

It is proposed that the primeval mechanism, which is thought to underlie present-day mitotic processes, involved a change in the number of polymerized positively or negatively charged groups (a shift in polyelectrolyte balance) in a colloidal system composed predominantly of colloids bearing a net negative charge. The nature and origin of such a mechanism will be discussed in terms of contemporary thought concerning early conditions on the surface of the earth and the origin of life. It will be shown that the composition of the primeval atmosphere and the *p*H of the primeval seas dictated the nature of the building blocks available and the sign of the charge on the colloids formed, and laid the basis for metabolic systems extracting energy from organic acids in place of organic bases.

It appears to be generally accepted that a rich variety of organic compounds was formed on the earth's surface prior to the advent of life (Haldane, 1933; Oparin, 1938; Holmes, 1948; Lwoff, 1951; Bernal, 1951; Blum, 1951; Urey, 1952). The original experimental work purporting to demonstrate the formation of a vast variety of organic substances when water, carbon dioxide, and ammonia were irradiated with ultraviolet light, which was cited by Haldane (1933), has never been repeated despite numerous attempts, according to van Niel (1949), although Calvin

(1956) claims positive results. However, Miller (1953, 1955) was able to demonstrate the production of a number of amino acids and other organic compounds, by using an electrical discharge in a closed system. Miller used an atmosphere of methane, ammonia, hydrogen, and water as suggested by Oparin (1938), Urey (1952), and Bernal (1951). This experiment has been repeated (Anderson and Tolbert, 1954, unpub.) and at least fourteen ninhydrin-positive substances demonstrated by paper chromatography. Although much experimental work remains to be done, it does not appear unreasonable to assume that the "soup" of organic substances originally proposed by Haldane actually did exist.

It is evident that an ocean covered by an atmosphere containing ammonia must be alkaline. The synthetic system suggested by Urey and Miller then involved a gigantic refluxing process in which organic materials formed in the atmosphere would be constantly settling into the seas. Substances volatile in alkali would be recycled. As a result, fatty acids would accumulate in the seas, while organic bases would be involved in further reactions. As these volatile organic bases were subjected again and again to the ionizing radiations and electrical discharges of the upper air, they were built into more and more complex but also less volatile substances such as amino acids and purine and pyrimidine bases. The system is thus biased precisely in favor of the accumulation of simple acidic substances familiar to us now as metabolic intermediates, the amino acids, and the complex organic bases which are fundamental to the formation of nucleic acids and metal-containing catalysts—i.e., the building blocks for living cells. Further, in an alkaline medium, the most stable colloids would be those bearing a net negative charge.

The formation of compounds of colloidal dimensions, however, is a quite different matter. One of the major difficulties is that the same factors which are thought to have produced the variety of simple compounds originally, tend to destroy nucleic acids and nucleoproteins as well as other organic compounds. Thus proteins are denatured or inactivated by ultraviolet light (reviewed by Schomer, 1936), heat, or ionizing radiations (Svedberg and Brohult, 1939; Barron, 1954), while nucleic acids are depolymerized by similar treatments (Hollaender, Greenstein, and Jenrette, 1941; Sparrow and Rosenfeld, 1946; Taylor, Greenstein, and Hollaender, 1948). Solar radiation includes not only ultraviolet but x-radiation as well (see review by Sanderson and Hulburt, 1954). In the present-day atmosphere oxygen begins to absorb at 2700 Å and removes a large percentage of radiation below this wave length. As a result of this absorption a small amount of ozone is produced. The ultraviolet absorption of ozone, beginning with the Huggins bands between 3400 and 3100 Å and rising sharply below 3100 Å in the so-called Hartley continuum, accounts for the complete absence of radiation between 2150 and 2915 Å on the surface of the earth. If the primeval atmosphere were low in oxygen, as has been suggested, large amounts of short ultraviolet would have reached the surface of the earth. Under such conditions it is necessary to postulate the polymerization of amino acids or nucleotides as occurring either in sands or clays, or under a water blanket.

A second difficulty is that energy is required for the synthesis of either proteins or nucleic acids, a fact making the spontaneous formation of a large polymer to appear as a very unlikely event. The chances of forming a chain of ten amino acids, for example, is given by Blum (1951) as about 10^{-20}. Lanham (1952) avoided this difficulty by proposing that the original proteins were formed from precursors other than simple amino acids. These precursors were presumed to be energy-rich and to condense spontaneously. Only when the environment became depleted of these substrates does Lanham propose that a mechanism for the synthesis of adenosine triphosphate (ATP) or other high-energy compounds was evolved. Blum, on the other hand, suggests that sources of chemical energy such as ATP were present in the environment originally and were used by the first autocatalytic molecules for protein or nucleic acid synthesis, without stating how the first such ATP-utilizing molecule was formed. The instability of ATP in alkaline solutions makes Blum's suggestion highly unlikely.

Two general theories of the original "living" substance have been proposed. These are the coacervate theory (Oparin, 1938) and the nucleoprotein or reduplicating molecule theory (Alexander, 1948) as developed by van Niel and others (see Lanham, 1952; Madison, 1953).

Certain of the concepts of protein synthesis presented by Haurowitz (1950) deserve consideration here, since they relate to mechanisms for exact reduplication. These may be summarized as follows: (1) Protein expanded to form a monolayer may serve as a template for autoduplication. (2) Specific adsorption of amino

acids to identical amino acids on the expanded protein layer occurs. (3) Through the action of "nonspecific" enzymes on the adsorbed amino acids an expanded protein film is formed which is a duplicate of the original protein template. (4) Folding of the two-dimensional protein film occurs so that a three-dimensional globular protein molecule is formed. The shape of this three-dimensional molecule will depend on the shape and electrostatic field of polar groups of adjacent cellular structures. These ideas may be adapted to the reduplicating-molecule concept by assuming that a wide variety of substances in addition to nucleic acids, such as phosphate gels, clay, and oil droplets, could serve to adsorb and unroll the simple original proteins. The duplication of these would then be presumed to occur as a result of the adsorption of energy-rich amino acid derivatives rather than amino acids, since no "nonspecific" enzymes required in the Haurowitz model would have been available to link amino acids.

The original "protocells" envisioned by Oparin (1938) consisted not of single molecules or of associations of small numbers of molecules, but rather were coacervate droplets. These were condensed from an environment rich in colloidal material. Concerning division, Oparin suggested that the postulated coacervate droplet continued to grow by adsorbing or condensing additional material until it was fragmented by external mechanical forces or by surface tension. He further pointed out that droplets whose properties favor fragmentation would tend to maintain a more favorable relation between surface area and mass, and would thereby increase the adsorption of dissolved substances. Thus a coacervate droplet endowed with the ability to divide was said to have a definite advantage over other droplets.

The attempt by Oparin to apply the concepts of coacervation to the origin of life is open to several criticisms. These apply equally well to several other attempts to use the term in connection with subcellular particles where authors have been more concerned with the morphology of coacervates than with the colloid chemistry involved. A coacervate is considered to be a colloid-rich system in equilibrium with a colloid-poor solution (the equilibrium liquid) (Bungenberg de Jong, 1949a). In contrast to the reduplicating-molecule theory, where one must account for the appearance of one or two special colloidal molecules, the coacervate theory requires a whole sea-full of them. Although it is true that these are not considered to be self-duplicating at the outset, the difficulties in accounting for the production of protein molecules on this scale are insurmountable. To postulate that the coacervation occurred in a small lake or pond does not help greatly, since ultraviolet degradation or a lack of sufficient substrate molecules would soon bring the venture to an end. Since a coacervate is a colloid-rich phase which can be formed only in equilibrium with a more dilute solution of the same colloids, it is evident that all coacervate droplets in the same system will tend to be similar in composition and structure even though very complex. When the droplets begin to differ one from the other, they are no longer simple coacervates. This is true whether the differentiation consists of a smaller average size giving a droplet a higher growth rate, as mentioned by Oparin, or if autocatalysis appears. In the latter instance, new autocatalytic molecules would tend to be lost from the droplet unless certain additional changes such as the appearance of a semipermeable membrane are postulated. Either the coacervate theory should be altered to conform with what is known about coacervates, or the systems postulated by Oparin should be described in other terms.

In the theory of the reduplicating or autocatalytic molecule, it has been proposed that a vast supply of organic compounds present in the environment were used for reduplication and that no special enzymatic activities were present. In the simplest case, division is presumably taken care of by random breakage of long, growing chains.

It appears more likely that the first stages in the transition to what might be called life involved single autocatalytic molecules. These may well have been relatively small compared with present-day protein, nucleic acid, or nucleoprotein molecules. If condensed from relatively energy-rich intermediates, their initial formation would present no insurmountable obstacles. When the quantity of reactants is measured in terms of kilotons, the formation of almost any type of molecule becomes a likely event even if the equilibrium constants suggest otherwise to the chemist accustomed to considering gram quantities.

The difficulty at this point is that there is little experimental knowledge of organic autocatalytic molecules. The first reasonable model of a self-duplicating (autocatalytic) system which

may possibly be capable of other activities (heterocatalysis) is that proposed for deoxyribonucleic acid (DNA) by Watson and Crick (1953; Gamow, 1954). The interesting concept that nucleotides and amino acids are the only building blocks suitable for constructing a complex living system has recently been suggested by Bragg (1954). Certainly the primeval environment provided sufficient time and materials for a thorough experimental investigation of a variety of possibilities.

In the laboratory, autocatalysis by large molecules has not been demonstrated in the absence of life. In a cell, autocatalytic molecules exist in a very special environment which stands little chance of being duplicated outside the cell at present. The possibility exists that autocatalytic molecules have been isolated but that suitable conditions for demonstrating autocatalysis have not been worked out. The temptation is to expect such molecules to reduplicate rapidly. However, under the conditions which may be devised, or in the primeval "soup," autocatalysis may proceed at a very slow rate. Thus a molecule which required one or several years for reduplication could easily be a "life ancestor" but be overlooked in the laboratory. The mechanisms by which the daughter molecules of an autocatalytic duplication could be made to separate will be considered in a subsequent section on chromosomal condensation.

With the formation of specific enzymes necessary to synthesize certain substrates whose concentration in the environmental "soup" has been depleted by "vital" activity (Horowitz, 1945), the protocell becomes more complex. Since it is assumed that these newly developed enzymes remain associated with the original self-duplicating molecule, and in the first instance are probably produced by it, a point of complexity may soon be reached where reduplication of the original molecule and associated protein and its separation into two can no longer be presumed to involve a separation of a variety of associated enzyme molecules (primordial cytoplasm). It is at this stage of development that the origin of some mechanism for separating a constellation of associated colloidal particles into two similar associations must be postulated. In some respects the reduplicating molecule developed to this stage of complexity resembles the coacervate droplet postulated by Oparin. This is also true of systems such as those condensed on oil films (Haldane, 1933) or clay particles (Bernal, 1951) at a later stage in their development. It becomes necessary, therefore, to consider the origin and development of a mechanism for cell division.

Oparin (1938) suggests that mechanical forces, including the actions of waves and currents, account for early divisions. This may have sufficed under certain local conditions in systems containing large numbers of similar molecules. Here no even distribution of reduplicating material is necessary, since all daughter "cells" would contain representatives of all molecular species (primitive endopolyploidy). It does not appear reasonable to assume that this dependence on external forces for division persisted for long. On the other hand, it does not appear justifiable to postulate the sudden appearance of a special mechanism devoted solely to cell division. On the contrary, it appears necessary to propose the nonexistence of any new specific differentiated mechanism to account for division in a protocell and to look for properties which are inherent in such entities and which will result in division automatically.

At this level of complexity many of the theoretical treatments of Rashevsky (1941a, b, 1948, 1952) and Landahl (1942a, b, c, 1943) are pertinent. Certain of these approaches have been recently reevaluated by Rashevsky (1952). The diffusion drag-force theory (Rashevsky, 1938, 1939; Landahl, 1942a), which will probably find an important application in explaining the movements of such actively metabolizing centers as the mitochondria and possibly the centrospheres, does not appear to be applicable to very simple systems with very low metabolic activities. Additional difficulties with this theory have also been presented (Rashevsky, 1952).

Elastic stresses in gels have also been considered by Rashevsky (1948) as possible causes of cell elongation and division. It was concluded from considerations of the theory of plasticity, through the application of Betti's theorem, that elastic forces produced by gelation could not cause division. However, this conclusion has now been shown to hold only in the case of a homogeneous system (Isenberg, 1953). Since living systems generally contain both micro and macro viscosity gradients (Seifriz, 1952), it has been possible to develop a mathematical theory of cell division based on elastic stresses in colloidal systems (Rashevsky, 1952). If we assume the applicability of the theoretical treatments re-

ferred to, it then becomes necessary to consider at some length the nature of biochemical events which alter viscosity and cause sol-gel changes in living systems.

Fundamentally, gelation involves cross linking between adjacent macromolecules to give a semisolid structure exhibiting a yield value (Hermans, 1949).

[A yield value is a property characteristic of the solid state. A solution exhibiting it will resist flow up to a given shearing force. It behaves as an elastic solid below that tension.]

The folding and unfolding of protein chains, so frequently invoked to account for biological phenomena (Frey-Wyssling, 1949; Loewy, 1949; Goldacre and Lorch, 1950; Meyer and Mark, 1951; Goldacre, 1952), is relevant here only to the extent that such folding and unfolding alters the number and availability of cross-linkage sites. Thus the changes occurring when fibrinogen is changed to fibrin would result in only a small viscosity increase in the blood if the only change were an unfolding. As is well known, the extensive cross-linking accounts for the gelation or clotting observed. Although changes in consistency which may be termed gelation are seen in both normal and pathological cells, little is known directly of their true nature. It has often been likened to the clotting of vertebrate blood (Heilbrunn, 1928, 1952; Lettré, 1952). This analogy may have been useful in the past, but its value is questioned here for several reasons.

(1) The blood-clotting mechanism, though far from completely understood, has nevertheless been made to proceed in systems composed of relatively pure substances whose properties are fairly well defined. No similar successful isolation of the *components* of a cytoplasmic "clotting" mechanism has been described. Experiments relating to this point will be presented in a subsequent paper. (2) The thromboplastic substances present in the cell do not appear to be present in an inactive state, as would be expected if they were part of a cell-clotting mechanism. (3) Sol-gel changes in the normal cell are readily and rapidly reversible (Chambers, 1917, 1921; M. R. Lewis, 1923, 1934; Marsland, 1951). This is not the case with the clotting of blood. (4) Changes in *p*H which do not dissolve blood clots can cause wide variations in cytoplasmic viscosity. (5) On purely theoretical grounds, if an analogy is valid, a two-directional flow of information and ideas might be expected. Thus, if blood coagulation resembles the cytoplasmic viscosity changes occurring during cell division and if research on blood coagulation furnishes clues useful in the study of cell division, as has been the case with the effects of certain anticoagulants, then it should be expected that studies on cell division would also suggest ideas and effects which would increase the understanding of blood clotting. This does not appear to have been the case. Furthermore, the analogy creating the impression that more is known than actually is about cytoplasmic behavior, tends to channel thinking and experimentation, and obscures many observations inconsistent with the concept. Although certain details of the two systems may ultimately be found to be similar, the comparison does not appear to be a fruitful one and, for the purposes of this discussion, will be discarded.

With regard to the folding and unfolding of protein chains, it should be noted that such changes are generally studied at air-water interfaces, which obviously do not exist within the cell. The surfaces which may exist within cells which contain few fat droplets probably involve very small surface tension forces. It is evident also that it is not possible for more than a small fraction of the protein molecule population of a cell to be at interfaces at any one time. In a clear, granule-free cytoplasm such as is seen in centrifuged cells, there seems to be no obvious mechanism for the denaturation, unfolding, or the refolding of proteins. Yet fairly large changes in viscosity are thought to occur. The local nature and non-Newtonian character of many of these alterations has been justifiably emphasized (Seifriz, 1952). No adequate treatment of the energy requirements for chain folding and unfolding has been presented (Seifriz, 1953).

It is suggested that the sol-gel transformations in the cytoplasm may be best described in terms of reversible cross-linking, not of unrolled polypeptide chains but of beaded chains formed by the linking of *intact, undenatured* macromolecules. The junction-point energies involved are in general rather similar and low as occurs in the so-called type III gels of Freundlich (1937; Marsland, 1951). These show a slight increase in volume on gelation.

In any colloidal system, particles with opposite charges will tend to flocculate out. It would appear, then, that the colloidal stability of a cell depends on maintaining a state of affairs such

that the vast majority of particles, in the cytoplasm at least, have a *like* charge. The question of the sign of the charge on cytoplasmic particles is therefore an important one, and one which has been the subject of considerable controversy. For the purposes of this discussion the predominant charge on cytoplasmic colloids generally is assumed to be *negative*. The reasons for this conclusion are many and will be discussed in a subsequent section. They may be summarized here as follows: (1) The nucleic acids, most tissue proteins, and the colloidal constituents of protoplasm generally have an *acid* isoelectric point; (2) the cations of the cell are largely monovalent; (3) the cytoplasm and cytoplasmic constituents, both inside (microinjection) and outside (surface precipitation, brei studies) the cell are flocculated or precipitated by multivalent cations; (4) electrophoretically, cytoplasmic constituents pile up at the anode; (5) tissue breis take up added calcium (Weimar, 1953) and flocculate (Gross, 1952); and (6) fresh cytoplasm and cells fixed without removal of nucleic acids are generally basophilic (exceptions will be noted later).

In a colloidal system of negatively charged particles, the simplest approach to viscosity or sol-gel changes is to consider the types of low-energy (reversible) cross-linkages which may occur between macromolecular polyanions to form beaded chains. In a fairly concentrated system such as the cell cytoplasm, changes which could be observed as flocculations when in dilute solutions may appear as gelations or as increases in viscosity. Thus lowering the cell *p*H produces an increase in viscosity or consistency (Loeb and Blanchard, 1922; M. R. Lewis, 1923; Barth, 1929; Minshall and Scarth, 1952), whereas in more dilute systems such as tissue homogenates, lowering the *p*H will result in flocculation (Dounce, 1950; Anderson, 1950). Similarly, cations with more than one charge have a pronounced effect on such systems. The addition of calcium in low concentrations will precipitate nucleoproteins (Taylor, Greenstein, and Hollaender, 1948) and will shrink isolated nuclei (Wilbur, Anderson, and Skeen, 1949; Wilbur and Anderson, 1951; Schneider and Petermann, 1950; Anderson and Wilbur, 1952). If more calcium is added, the familiar reversal of charge occurs, the nucleoproteins go back into solution, and isolated nuclei swell. With cations of increasingly higher valence, a very strong precipitating effect is seen, but there is much less tendency toward re-solution in the presence of excess cation (Bungenberg de Jong, 1949b).

These concepts may be applied to living systems generally by considering the degree of polymerization of positively and negatively charged groups in the cell. Since it is obvious that electrical neutrality must exist, there must be an equal number of cationic and anionic groups or charges. However, insofar as gross changes in colloidal stability are concerned, the effect of divalent and multivalent electrolytes so far outweighs the effects of monovalent ions that the effects of the latter may be neglected. A typical cell therefore consists of a series of highly polymerized negative charges (nucleic acids, sulfated polysaccharides, and proteins with an acid isoelectric point) with only a few polycations of varying sizes (histones, protamines, cytochrome c, the basic polypeptide described by Bloom and Blake (1948), spermine, spermidine, agmatine, and other multiply-charged amines). The greater part of the positive charge resides in potassium, with smaller additional amounts in Na^{+}, Ca^{++}, and Mg^{++} and in the amino groups of otherwise negatively charged proteins. The calcium and magnesium would be expected to be bound to the polyanions present, and this indeed appears to be the case experimentally (Weimar, 1953; Gross, 1952). A small shift in the degree of polymerization of the positive charges will have a profound effect on such a system. This has been demonstrated repeatedly in a wide variety of colloidal solutions. In these considerations the important things are the degree of polymerization of the charges, and their quantitative ratio. These two factors characterize the *balance of polyelectrolytes* in the cell. Experimentally, the polyelectrolyte balance in cells may be altered by injecting polyanions (heparin, sulfated polysaccharides, nucleic acids, and polyacid dyes) with a resulting solation; or by injecting polycations (heavy metals, protamine, and basic dyes) with a resulting gelation. The factors which are thought to cause a shift in polyelectrolyte balance are listed in Table 1.

Cell colloids, with the exception of substances such as dextran or glycogen, may be classified as polycations, polyanions, or polyampholytes. The majority of proteins would probably be included in the last group. In this discussion the term polyampholyte has not been used because it has been considered necessary to stress the slight excess negative charge of most polyampholytes in the cell. It is preferred here to refer to poly-

TABLE 1

Factors causing a shift in the balance of colloid-associated charges

Shift toward polyanions (Solation)	Shift toward polycations (Gelation)
Synthesis of polyanions	Synthesis of polycations
Addition of polyvalent anions	Addition of polyvalent cations
Rise in *p*H	Drop in *p*H
Depolymerization of polycations	Depolymerization of polyanions

ampholytes as either weak polyanions or weak polycations. Instances where the charge distribution over the surface of a colloid is such that it becomes a giant dipole are discussed in a subsequent section on fibril formation.

It has been tacitly assumed that the basis for movement in all cells is ultimately to be found in macromolecular structure of some type. The necessity for such an assumption has been presented by Frey-Wyssling (1953). The changes in viscosity or consistency which occur generally involve little or no change in the concentration of dissolved substances in the cell. The physical alterations observed must therefore involve changes in either the physical state of the macromolecules present, or in their associations.

The unrolling of either globular proteins to form long polypeptide chains, or of rolls of preformed chains of proteins (Kopac, 1951) is considered unlikely. It appears simpler to consider that the submicroscopic structures which are constantly being formed and broken down in the living, moving cytoplasm are formed by the association of cytoplasmic protein molecules *without* either unrolling or denaturation. The resulting chains are beaded and of varying length and with varying degrees of cross-linking between chains. No special proteins need be postulated for this purpose since, as will be shown in a subsequent section of fibrillization, such chains may be formed experimentally from a variety of ordinary proteins. The bonds which hold macromolecules together have been discussed in some detail by Frey-Wyssling (1953) and are the following: (1) a homopolar cohesive bond, which accounts for the mutual attraction of lipidic groups; (2) a heteropolar cohesive bond, which occurs between groups of pronounced dipole character (hydrogen bonds); (3) heteropolar valency bonds, which occur in salt formation; and (4) homopolar valency bonds, such as occur in disulfide linkages.

Frey-Wyssling has pointed out the difficulties involved in attempting to determine experimentally which type of bond is important in any one system. A procedure which is designed to alter one type of linkage in the cell may in fact alter several.

In a living system the important thing is not how many different types of bonds may bind macromolecules together, but rather, which one or ones can be *varied* either in number or strength, and can be *easily broken and reformed.* The temperature dependence of cytoplasmic viscosity between 10° and 18°C. in the amoeba may indicate that homopolar cohesive bonds are involved, as Frey-Wyssling has suggested. However, the problem in cell division is how to explain changes occurring at *one* temperature in a system involving no wide fluctuations in *p*H, redox potential, salt concentration, or pressure. No obvious mechanism for reversibly masking or altering homopolar or heteropolar cohesive bonds between proteins has been postulated. The homopolar valency bonds, such as occur in the disulfide linkages in wool or keratin, are generally ruptured only by relatively drastic means.

[Evidence suggesting that R—SH HS—R $\rightleftarrows$ R—S—S—R bonds may be implicated in certain aspects of mitosis in contemporary cells will be discussed in a subsequent paper.]

The heteropolar valency bonds (salt linkages) remain. These are the bonds which link polyelectrolytes containing areas or groups with opposite charges.

Thus, although several types of bonds may participate in the formation of structure by linking macromolecules together, the salt linkages are believed to be the ones chiefly responsible for the continuously variable affinities of macromolecules in the cytoplasm.

A shift in the balance of polyelectrolyte charges in favor of polycations in a predominantly polyanionic cytoplasm will favor the formation of a large number of salt linkages between the constituent macromolecules. The result is, first, bonding to form dimers and trimers, and then the formation of longer beaded chains with variable degrees of cross-linking. A further shift will lead to contraction of the chain. A shift in the direction of polyanions will return the system to a state of discrete molecules in solution. The concept of

polyelectrolyte balance has been used here as a unifying principle to relate all the various effects listed in Table 1. In the cytoplasm of the nondividing cell the local shifts in polyelectrolyte balance which account for the movements observed (other than those caused by diffusion gradients) are most likely related to changes in the degree of polymerization of phosphoric acid groups associated with adenylic acid, i.e., the synthesis and breakdown of the polyanion, adenosine triphosphate (ATP).

The proposed primeval mechanism consists therefore of a cyclical variation in the relative proportions of polycationic and polyanionic charges occurring during the growth of a protocell. In the growth period (interphase) the cell volume increases and is composed of predominantly polyanionic colloids. At the end of this phase an increase in the number of polymerized cationic groups relative to the number of polymerized polyanionic groups occurs. This is not thought to be necessarily due to the production of any new and different substances, but rather to a change in the *relative rates* of synthesis of two substances normally being produced.

During the course of cellular evolution this mechanism has doubtless undergone numerous alterations and refinements. For example, shifts in the balance of polyelectrolytes may well occur as a result of a small change on the surface of a colloid rather than as a result of synthesis of new ones.

Taking into account the predominant negative charge on cell colloids and the effects of shifts in polyelectrolyte balance on the state of the colloids, one may construct the following picture of the origin of the dividing mechanism. The majority of the complex building blocks necessary for reduplication of the original pseudocoacervate or nucleoprotein-enzyme associates (protocells) are thought to have occurred in the primeval seas (Haldane, 1933; Oparin, 1938; Alexander, 1948; Horowitz, 1945; Blum, 1951; Urey, 1952). As the size of such protocells increased, the rate of synthesis of any particular compound (nucleic acid or protein) would depend on the substrate concentration and the rate of diffusion of the substrate molecule to the site of utilization. For any short period of geologic time the environmental concentration is assumed to have remained constant. Differences in the diffusion rates of nucleotides and amino acids could be expected to favor the synthesis of proteins over nucleic acids in larger cells, since amino acids are generally smaller than nucleotides. Also the concentration of the amino acids was probably higher than that of the nucleotides, since the latter are more complex molecules and would probably not be synthesized at as high a rate. The proteins generally found associated with DNA are rich in diamino acids. If the synthesis of such proteins continues while the production of nucleic acid diminishes, the net effect would be a shift in the balance of polyelectrolytes, resulting in local areas of gelation and contraction throughout the cell. The effect would be to produce a stressed gel system with local variations in viscosity such as has been treated mathematically by Isenberg (1953) and by Rashevsky (1952), and consequently resulting in *cell division.*

THE INITIATION OF CELL DIVISION

The initiation of cell division will be considered in terms of a shift in the balance of polyanions and polycations.

Various substances and treatments have been shown to stimulate or to delay the division of cells (E. N. Harvey, 1910; Loeb, 1913; Lillie, 1926, 1931, 1941; Morgan, 1927; E. B. Harvey, 1940; Tyler, 1941; Marshak and Walker, 1945; Wilson and Leduc, 1947, 1950; Harding, 1951; Shaver, 1953). Danielli (1951) has classified a number of these as acting either on the surface or on the interior of cells. However, it has not been possible to relate all the active agents to one simple biochemical event or chain of events in the cell directly or indirectly. Heilbrunn (1952) has considered that the common denominator is the stimulation of the cell which involves the release of calcium. Danielli, however, has preferred to believe that the release of calcium is a secondary event. Until the biochemistry and physical chemistry of stimulation is better understood, these differing points of view cannot be fully evaluated. In this discussion the effects of several stimulants of cell division will be interpreted in the light of their effects on the balance of polyelectrolytes within the cell.

In the egg cell the most obvious agent for inducing cleavage is the sperm, which characteristically contains an abundance of very basic protein (polycation). Thus, if it is assumed that the genetic pattern or code is carried on the DNA, the stimulus for cell division could reside in these basic proteins which, in the case of many fishes (Miescher, 1897; Kossel, 1928) and of the chicken (Daly, Mirsky, and Ris, 1951), have been shown to consist almost entirely of the extremely simple

subprotein, protamine. This substance may contain over 85 per cent of one diamino acid, arginine (Hamer and Woodhouse, 1949). The chemistry of nucleoprotamines and protamines has been intensively reinvestigated by Felix and coworkers (1951, 1953).

Several observations suggest a causal relation between basic proteins and the initiation of cell division. According to Bataillon (1929), the amphiaster in the anuran egg activated by the puncture method is derived from a cytaster which arises only when the puncture introduces some foreign *nuclear* material in the form of leukocytes or other cells which occur in the egg jelly. It has been shown that isolated nuclei from brook trout sperm (*Salmo fontinales*) can be used to activate eggs (Felix, Hartleib, and Krekels, 1952). The nuclei contained little besides basic protein and DNA. Karyometric studies suggested that the embryos were haploid, however, and that the chromatin material from the isolated nuclei probably was not used (Schneider, 1953). Protamine ". . . is one of the most efficient substances for the causation of artificial parthenogenesis" (Loeb, 1913). Since many eggs possess jelly coats which precipitate with protamines, these substances have received little attention. It appears quite possible that a number of other polycations may have similar effects, since such unphysiologic substances as methylene blue are parthenogenetic. The basic polypeptide described by Bloom and Blake (1948) deserves attention in this respect since it appears to be a normal tissue constituent. Shaver (1953) found cleavage-initiating substances in microsomes, but noted that this negative results with nuclear material were probably due to the injection of too large an amount rather than to the absence of activating substances from the nucleus.

If polycations are causally related to cell division, as here proposed, then polyanions should inhibit the initiation of cell division. In fact, if the experiments had not already been done, they would be listed as being crucial to the present approach. The inhibition of cell division by acid polysaccharides such as heparin has been demonstrated by a number of authors (Goerner, 1930; Zakrzewski, 1932; Fischer, 1936; Balazs and Holmgren, 1949; Heilbrunn and Wilson, 1949, 1950; Harding, 1949; Heilbrunn, Wilson, and Harding, 1951; Chaet, 1952) and is probably a very general phenomenon. The simplest explanation is that, as a large and strongly charged polyanion, heparin shifts the polyelectrolyte balance of the cell by combining with any basic substances present (Anderson and Wilbur, 1950, 1951; Roberts and Anderson, 1951). It would be of interest to know whether the cyclic polyphosphates, which are not broken down in the rat (Gosselin et al., 1952), also inhibit cell division.

In regard to the effects of ATP on cell division, it is difficult to distinguish between the effects of this substance as a polyanion and as a source of energy for the synthesis of numerous other substances. Thus, if ATP furnished the energy required to synthesize basic proteins, the net effect of adding it might be different from that predicted purely on the basis of the number and strength of its charges. Certainly the effects of ATP inside and outside the cells appear to be drastically different (Green and Stoner, 1950). Bullough (1952) has reviewed the data on the role of ATP as an energy source in mitosis.

Harding (1951) has summarized the evidence for the initiation of cell division by a number of *acid* substances obtained from damaged cells. Experiments both cited and presented by her indicate that the active principles were generally among the lower fatty acids and that the effect was observed *only at an acid* pH. The parthenogenetic effect of acids is well known (E. N. Harvey, 1910; Chambers, 1921; Lillie, 1926, 1941; Tyler, 1941). As was noted in the previous section, polyelectrolyte balance may be shifted effectively in favor of polycations by lowering the pH. The effects of acids added to a cell suspension on the pH of the cell interior would appear to depend on the permeability of the cell to the anion in question. As has been known since the early work of Overton, lipid-soluble substances generally penetrate cells more readily than substances which are not lipid-soluble. The lower fatty acids, by virtue of their negative charge and their nonpolar groups—which enable them to penetrate cells—may be expected to influence intracellular pH rather readily and to have a basic protein-mimicking or "sparing" effect in egg cells. The activating effect of heat may find a similar explanation, since it has been interpreted as being due to increased acid formation in the egg (Lillie, 1931).

Considerable emphasis has been placed on the role of calcium in the physiology of the cell (Heilbrunn, 1952). Danielli (1951) has questioned whether this element is involved in the primary phases of cell stimulation and has suggested instead that calcium can be released only by (1) the production of another cation which is preferentially bound in its place, (2) the production of

acid (or some equivalent process) which will reduce the number of ionizing acidic groups of the surfaces of the cell colloids, or (3) the conversion of these colloids into units of smaller molecular weight, for example, the conversion of a protein into amino acids. Thus it would appear that calcium may serve as an *indicator* of shifts in polyelectrolyte balance within the cell, by virtue of its release under conditions which favor an increase in effective polycation concentration. A detailed reinterpretation of much of the experimental data used in support of the central role of calcium in cell stimulation appears feasible but will not be attempted here.

The role of the calcium ion in the initiation of cell division has been stressed by Dalcq, Pasteels, and Heilbrunn (see review by Tyler, 1941). In a wide variety of cells calcium has been shown to be capable of producing activation and to be necessary for the full effectiveness of a number of parthenogenetic agents. From the point of view of the theory presented here, calcium may be expected to assist in the displacement of polycations in the cytoplasm and, like acid, to have a polycation-"sparing" action. In a loaded system, such as a marine egg, it appears that a variety of colloidal substances must be held in readiness in a functionally inactive condition awaiting fertilization. The polycations stressed here, which may well include histones poured into the egg cytoplasm by nurse cells, must be bound or restrained. Calcium or any other salts in concentrations above that normally found in the cytoplasm may be expected to release part of the bound histone or other basic substance much as histone is released from DNA by strong saline. When the egg is returned to sea water, the basic substances are again bound, but at *different sites*. Thus if AB represents the original inactive bound condition (B = basic substance, A = binding site), and BC represents the active condition where C may be a fibril-forming protein or may be DNA ready to be condensed, then the reaction

$$AB + C \xrightarrow[Ca^{++} \text{ or KCl}]{} A + B + C \xrightarrow[\text{sea water}]{} A + BC$$

would lead to the formation of structures characteristic of the BC complex (spindle fibers, condensed chromosomes, etc.). The well-known activation of marine eggs by hypertonic solutions (Loeb, 1913; Morgan, 1927; Tyler, 1941) is thus readily harmonized with the views presented here.

As to the stimulus to cell division in contemporary cells, it is probable that the production of the basic substances stressed here is a highly specialized and well-controlled process, in contrast to the condition postulated for primeval cells where differences in diffusion rates and concentrations of nucleotides and amino acids were effective.

The stimulus to cell division observed after injury to part of a tissue or organ may well be due to the acid "injury substances" studied by Harding (1951). However, a number of aspects of autolysis should not be neglected. The production of amines under such conditions is well known (e.g., cadaverine, putrescine). The possible stimulating effects of such substances remains to be investigated. Little is known concerning nuclear constituents after cell death. The breakdown of nucleic acids by intracellular nucleases (Oes, 1908, 1910 cited by Hughes, 1952) and of protamines by cathepsins (Maver and Greco, 1949a, b; 1950) has been adequately demonstrated. However, the relative rates at which these processes might occur in autolysing cells is not known. The possibility that the nucleic acids may break down sufficiently to release the basic proteins after tissue damage deserves consideration. It should be noted that the injection of isolated liver chromatin material into rabbits apparently increases the mitotic rate in the liver (Marshak and Walker, 1945).

Recent work on the rate of turnover of DNA during cell division suggests that this rate may be twice as high as would be expected from the number of cells produced (Barnum, Huseby, and Vermund, 1953; Stevens, Daoust, and Leblond, 1953; Daoust, Bertalanffy, and Leblond, 1954). Although this work has been questioned (Barton, 1954), it appears possible that twice the amount of nucleoprotein necessary for the normal complement of the two daughter cells is produced, but that half the DNA produced is broken down. This could occur in such a manner that there would be only small changes in the total amount present in the nucleus at any one time. The net result would then appear to be a doubling of the DNA and a quadrupling of the histone.

Bloch and Godman (1955), however, conclude that DNA and histone synthesis proceed simultaneously and that they are present in constant proportions. Nevertheless, the standard error for their histone values was very large; and no source of the variability could be adduced. The possibility exists, therefore, that a small excess of histone may be produced in the nucleus before cell division.

Since histone turns over much more rapidly than DNA (Brunish and Luck, 1952), it is evident that it is either broken down in situ, or that it escapes the nucleus and perhaps serves some other function in the cytoplasm. The rate of histone synthesis may well change markedly during the various stages of cell division, and the amount present could vary considerably without being at variance with the available data. Such variation could effectively shift the balance of polyelectrolytes in the cell and thereby control cell division.

It was considered of interest to postulate, on the basis of the theory presented here, a substance which might fulfill the requirements of a simple physiological polycation and to investigate its effects on dividing cells. Decarboxylated arginine (agmatine) was chosen for trial since it could be readily produced from arginine, and because it possesses a double negative charge. Experiments were performed (St. Amand, Anderson, and Gaulden, 1955) with grasshopper neuroblasts by using the technique and culture medium described by Carlson, Hollaender, and Gaulden (1947). In these cells the duration of several stages of cell division may be accurately timed. In a series of four experiments, 0.005 *M* agmatine shortened the time from the beginning of prometaphase to the end of metaphase by 32 per cent, did not alter the length of anaphase, but shortened early and middle telophase by 37 per cent and 6.3 per cent, respectively. Further observations on six different preparations similarly treated showed that agmatine increased the number of cells going through cell division in *each* preparation, with an average increase of 80.6 per cent over a three-hour period. The increased number of cell divisions is sustained for at least five hours. *Thus, it appears that agmatine enhances the rate of changes normally occurring in all mitotic stages except anaphase.*

Substances which accelerate mitosis are rare. It is rather unusual, therefore, to select with no prior experimental knowledge a single substance which does this effectively. These results constitute the most satisfactory experimental results stimulated by this theory to date.

The recent isolation and synthesis of kinetin (Miller et al., 1955a and b), a factor which stimulates cell division in plants, adds further support to the concept that basic substances are causally related to the initiation of cell division. This substance, which is active in concentrations as low as 0.01 p.p.m., appears to be 6-furfuryl-aminopurine, a weakly basic derivative of adenine.

Histone and protamine have been frequently mentioned in this discussion because they are the only polycations generally available in any quantity in cells. However, another source of polycationic material deserves investigation. In the most general case, cell division may be considered as resulting from an imbalance between the non-growing nucleus and the growing cytoplasm. If a substance X is produced in proportion to the mass of the cytoplasm, but is utilized in proportion to the mass of the nucleus, then it will vary rhythmically in quantity, reaching a peak before each cell division. Quantitatively, the most important cell product is protein. If protein synthesis proceeds by two steps, the first of which is under general cytoplasmic control, with the second under nuclear control, then the products of the first step would tend to accumulate if the cytoplasmic mass were disproportionately larger than the nuclear mass. The detailed steps of protein synthesis are unknown. However, it can be said that polypeptide intermediates *must* exist if for no other reason than that it is inconceivable that a row of amino acids could all be interlinked at precisely the same fraction of a microsecond. Polypeptide intermediates would doubtless include predominantly basic and predominantly acidic molecules. The former would fill admirably the requirements for a polycationic material appearing in excess when the cell has exceeded a certain size. While protein intermediates of this nature have not been found previously, it is suggested that more refined methods will show they exist.

CHROMOSOMAL CONDENSATION

Both the division of the primeval protocells and the initiation of cell division in contemporary cells have been postulated here to be due to a shift in polyelectrolyte balance such as would occur with an increased production of polycations. In this section it will be shown that the same mechanism can serve to account for the condensation of chromosomes into compact bodies such as are observed during metaphase. This mechanism will be treated first as acting on a DNA-protein gelwork and secondly as it may affect the structure of the chromosome as it is thought to exist.

Chromosomal condensation and the control of nuclear volume are viewed as two aspects of the same problem. Here the concept that somatic nuclei generally consist almost entirely of swollen, hydrated, or "extended" chromosomes is followed. Whereas several earlier authors have presented

evidence that the chromosomes swell, forming vesicles which fill the interphase nucleus (Richards, 1917; Kater, 1927, 1928; Lewis, 1947), Ris and Mirsky (1949b) have shown that the swelling is due to a change in the physical state of the DNA and that the chromosomes of the resting nucleus are not merely swollen vesicles. Later work on the enzymatic dissection of the isolated rat liver nucleus gives little evidence of internal nuclear septation or vesicular structure (Anderson, 1953b) and supports the view of Ris and Mirsky. The volume of the interphase nucleus (minus the nucleolar volume) and the combined volumes of the metaphase chromosomes indicate the limits of the swelling and shrinking of the chromosomal material which may occur normally. At the outset, therefore, it is instructive to consider the factors which influence the size of nuclei and may alter their volumes, since the same factors may be important in chromosomal condensation.

Numerous studies have been concerned with the effects of various solutions on nuclei. These include the effects of salts on nuclear structure (Strugger, 1930; Shinke, 1937; Zollinger, 1948; Chambers and Black, 1941; Bank, 1941; Duryee, 1937; Laskowski and Ryerson, 1943; Ris and Mirsky, 1949b; Kassel and Kopac, 1950), changes in structure produced by acids and alkalis (Zollinger, 1948; van Herwerden, 1924; Zeiger, 1935; Dangeard, 1947), and in volume changes accompanying changes in tonicity (Churney, 1941, 1942; Callan, 1949; Goldstein and Harding, 1950; Shinke, 1937; Beck and Shapiro, 1936; Shapiro and Parpart, 1937) which were interpreted as evidence that the nucleus behaved osmotically. These studies raise the possibility that chromatin condensation may be due to changes in salts, *p*H, or tonicity. Sufficiently wide variations in the intracellular concentrations of simple salts or in *p*H to account for the condensation of chromosomes, however, do not appear to occur. But if the nuclei behave osmotically, at least the possibility exists that volume changes observed in chromatin during cell division reflect changes in the concentration of solutes within the cell. This problem has been examined in some detail on isolated nuclei, and it has been found that such volume changes are due, at least in somatic cells, to certain colloidal properties of the nuclear substance (Anderson and Wilbur, 1952) and do not indicate that the nuclear envelope is a semipermeable structure. Although results obtained with isolated cell components must be interpreted with caution—a point the

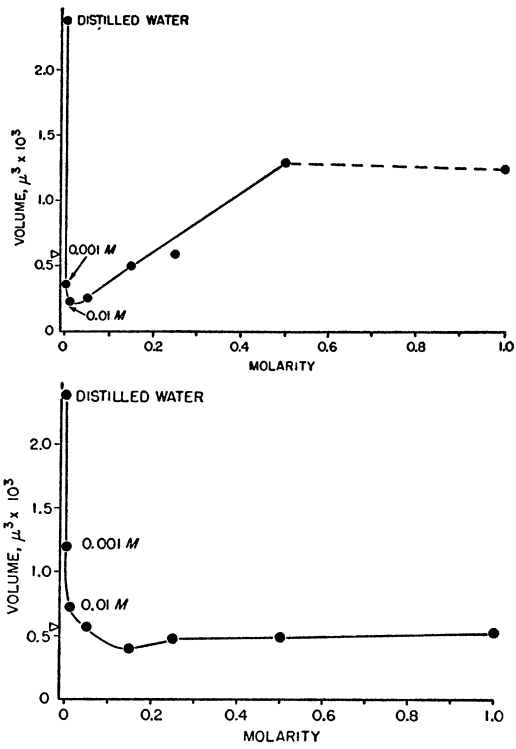

FIG. 1. EFFECT OF VARIOUS CONCENTRATIONS OF $CaCl_2$ (TOP), AND NaCl (BOTTOM) ON THE VOLUMES OF ISOLATED RAT LIVER NUCLEI
(From Anderson and Wilbur, 1952).

author has repeatedly made—*no* evidence is available which indicates that the nucleus of the intact somatic cell differs in its permeability from the isolated nucleus (Anderson, 1953a). A summary of the effects of various solutions on isolated rat liver nuclei adapted from a previous study (Anderson and Wilbur, 1952) is given in Fig. 1. Marked volume changes in response to different salt concentrations are observed, but the non-osmotic character of these changes is apparent from an examination of the curve for calcium chloride. Here maximal shrinkage is obtained with a concentration slightly higher than 0.01 *M* calcium chloride. Swelling is observed at both *higher* and *lower* concentrations. These findings are explicable in terms of a gel-like material composed of strands bearing a negative charge. The major functional component of these strands is believed to be DNA, since extraction of this substance (Ris and Mirsky, 1949b) or its digestion by deoxyribonuclease (Anderson, 1952, 1953c) abolishes the response to salts.

It should be noted that many earlier workers

were aware of the peculiar volume changes exhibited by nuclei in response to various salts. These were generally interpreted as indicating that the nucleus was a complex coacervate (Bank, 1941). Since a coacervate exists in equilibrium with a dilute colloidal solution (Bungenberg de Jong, 1949a), the failure of whole or broken nuclei or isolated chromosomes to dissolve readily in solutions resembling the intracellular fluid in ionic composition suggests that either they are not coacervates, that the equilibrium is shifted very far in favor of the complex, or that the equilibrium between the complex and the solution is established very slowly.

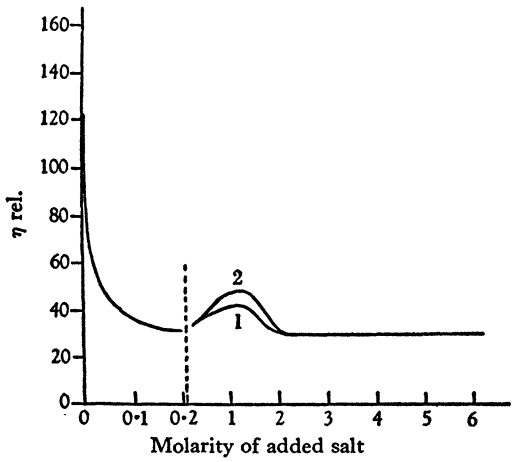

FIG. 2. EFFECT OF SODIUM CHLORIDE ON THE VISCOSITY OF DEOXYRIBONUCLEIC ACID
(From Gulland and Jordan, 1947).

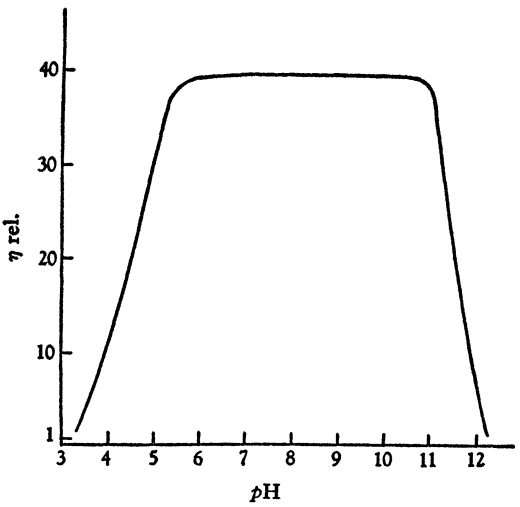

FIG. 3. VISCOSITY OF THYMUS DEOXYPENTOSE NUCLEIC ACID SOLUTIONS AS A FUNCTION OF *p*H
(From Gulland and Jordan, 1947).

The behavior of nuclei and chromosomes, as described above, finds striking parallels in a number of properties which the DNA molecule exhibits in solution. The high non-Newtonian viscosity of DNA is markedly affected by the concentration and type of salts present (Jordan, 1950, 1952; Basu, 1951). In the absence of salts, the ionization of the phosphate groups gives rise to mutually repulsive charges along the chain, and results in a stiffening of the molecule and a high anomalous viscosity (Alexander and Hitch, 1952). With small increments of sodium chloride, a large drop in viscosity is seen (Fig. 2). After a concentration of about 0.15 *M* is reached, little further change in viscosity is observed with added salt. These changes are believed to be due to repression or neutralization of the repulsive forces along the length of the molecule, so as to result in a folding or coiling of the previously extended structure. The sensitive manner in which the shape of ionizable polymeric molecules depends on the degree of ionization has been stressed by Kuhn et al. (1950). *A striking similarity is noted between the volume changes observed in isolated nuclei in sodium chloride solutions and the molecular changes observed in DNA in similar solutions by viscosimetric methods* (cf. Figs. 1 and 2).

The viscosity (and therefore the degree of coiling) of DNA is also affected markedly by the hydrogen ion concentration. Over the range of approximately *p*H 5–10 the viscosity is rather constant (Fig. 3), dropping at higher and lower values. Below *p*H 5 a decline in viscosity is believed to be due to repression of ionization of the phosphoric acid groups, with the result that the molecule collapses. Thymus nucleoprotein in 1 *M* sodium chloride exhibits a similar uniform viscosity over a range of about *p*H 5–9.5, where a sharp rise and fall are observed (Fig. 4). At alkaline *p*H's the DNA is thought to extend and then hydrolyze spontaneously. Isolated nuclei exhibit remarkably similar behavior, having essentially constant volume over the *p*H range 5–9 in the presence of equimolar phosphate buffers (Fig. 5). At higher *p*H values the nuclei swell and go into solution; below *p*H 5 they shrink. A comparison of the physical properties of the nucleus or of chromatin and of DNA in solution indicates that considerable segments of the DNA chains in the nucleus are free to coil and uncoil, exchange ions, and to behave very much as DNA does in solution. It is difficult to escape the conclusion that the colloidal properties of the somatic cell nucleus are very largely

reflections of the physical properties of the DNA molecule, subject to the following qualifications.

Firstly, it is evident that the DNA in a nucleus suspended in a salt solution approximating the intracellular environment is not in a soluble form. Even if the nuclear envelope is ruptured, it does not pass into solution. This has been demonstrated in the many experiments where nucleoprotein threads or isolated chromosomes have been prepared from nuclei by the use of high shearing forces (Mirsky and Ris, 1947a, b). Schneider and Hogeboom (1951) have questioned whether DNA in the nucleus is in a different state than has been supposed, since a large portion of the DNA from nuclei disrupted in sucrose was not easily sedimentable. The suggestion was made that DNA is not associated with structures comparable to chromosomes but is colloidally dispersed within the resting nucleus. Since nuclei and chromosomes swell markedly in ion-free sucrose media, and since in the absence of salts nucleoproteins can be dissolved out of nuclei (Bernstein and Mazia, 1953) and chromosomes, this argument does not appear to hold. Certainly the interphase nucleus does not normally exist in a salt-free environment.

Secondly, although the volume changes exhibited by isolated nuclei (Anderson and Wilbur, 1952), chromosomes, and nucleoproteins (Jeener, 1946) in response to salts indicate that a considerable number of segments of the DNA chains are free to bind various ions and to coil and uncoil, parts of these chains must be concerned with cross-linking or interlinking. The available data suggest that both in the nucleus and in isolated nucleoproteins the cross-linking and restraining is done by basic proteins such as histone or protamine.

The following evidence suggests that the bonds are predominantly of the salt or ionic type. Strong salts, which may be expected to dissociate salts of polycations with polyanions, extract nucleoproteins from a variety of nuclei (Pollister and Mirsky, 1946; Mirsky and Pollister, 1946). The splitting of the basic proteins from DNA under these conditions has been demonstrated by dialyzing them out (Mirsky and Pollister, 1946), and by ultracentrifugation and electrophoresis (Cohen, 1945; Stern and Davis, 1946; Petermann and Lamb, 1948; Stern, 1949). Later studies indicate that this splitting may not occur at once in fresh preparations (Shooter, 1954). Either the nucleic acid or the histone moieties may be displaced from isolated nuclei by substances possessing a higher charge density. Thus heparin may displace DNA (Anderson and Wilbur, 1950, 1952; Roberts and Anderson, 1951), and protamine may displace histone (Mirsky and Ris, 1950). X-ray-scattering evidence also points to the view that nucleoproteins exist as simple addition products (Riley and Arndt, 1953). It should be emphasized, however, that certain very obvious differences exist between nucleoproteins as they appear in the nucleus and nucleohistones formed by mixing the two purified substances. In 0.14 *M* sodium chloride, nucleohistone is insoluble (Mirsky and Pollister, 1946) and nuclei are somewhat shrunken, with evidence of internal structure. The nuclei, however, respond readily to changes in salt concentration, whereas the dense nucleohistone fibers respond very slowly. The difference may very well be that the histone in the nucleus occupies a characteristic position along the DNA chain, but in the artificial nucleohistone fiber the histone is randomly associated with the DNA. This view receives some

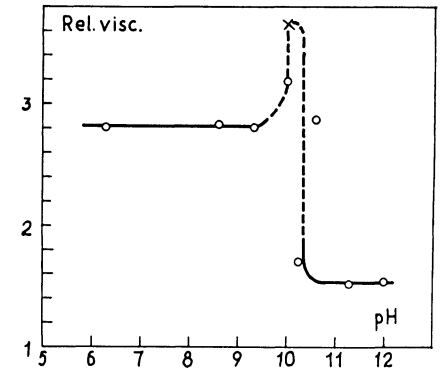

FIG. 4. VISCOSITY OF NUCLEOPROTEIN SOLUTIONS IN 1 *M* NaCl AS A FUNCTION OF *p*H
(From Frick, 1949).

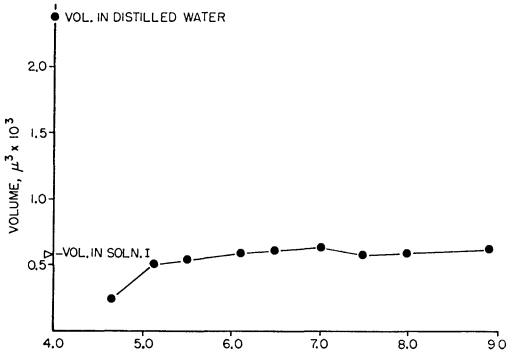

FIG. 5. EFFECT OF *p*H ON THE VOLUME OF ISOLATED RAT LIVER NUCLEI IN SOLUTIONS CONTAINING 0.023 *M* PHOSPHATE AND 0.145 *M* SUCROSE
(From Anderson and Wilbur, 1952).

confirmation from the work of Alexander (1953), who was able to prepare soluble DNA-protamine gels by allowing the two components to interact at extreme dilution. The suggestion was made that the protamine under these conditions attaches the DNA chains together end to end. A similar situation may well exist in the intact nucleus. Stern (1952) has considered that the spatial distribution of histone along the DNA chain is also of importance from a functional point of view. He suggests that histone contributes to the maintenance of a genetically important configuration of the DNA molecule, and that artificially recombined nucleohistone is an "artifact."

The nature of the linkages which hold the remaining protein (nonhistone) in the nucleus is not clear. The view that these are not held in the nucleus by virtue of their inability to pass out through the nuclear envelope has been previously presented (Anderson, 1953a; see comments by Hogeboom and Schneider, 1953; and by Stern and Mirsky, 1953). Rather, it appears that nonhistone protein may be linked to the DNA itself or to the histone. The complexing of a number of proteins by DNA has been reported (Greenstein, 1943; Greenstein and Hoyer, 1950; Goldwasser and Putnam, 1950; Geiduschek and Doty, 1952); the interaction of proteins such as serum albumin (Pederson, 1938) and insulin with basic proteins has long been known. In support of this concept, it should be noted that a variety of proteins can be adsorbed on isolated nuclei and displaced by other more basic proteins (Ohlmeyer et al., 1949; Ohlmeyer, 1950). A considerable amount of protein can also be removed from isolated nuclei by washing with saline (Dounce, 1950, 1952b; Stern and Mirsky, 1953; Kirkham and Thomas, 1953).

Assuming, therefore, that the "statistical chromosome" reflects the properties of a DNA-protein gelwork, the problem of chromosomal condensation then becomes essentially that of coiling up DNA molecules. Since changes in *p*H and simple salts sufficient to affect DNA coiling are not thought to occur in the dividing cell, the most probable mechanism remaining is one in which condensation is produced by polyvalent cations such as constrain the DNA in the nucleus —namely, histones, protamines, and similar basic substances. It is proposed that substances of this general class are usually related to the condensation of the chromosomes during prophase.

The essential characteristic of the general model presented here is that DNA chains are linked together at intervals by basic proteins, and that fairly long interhistone lengths of DNA exist. On both the histone and the DNA, nonhistone proteins are loosely held. In the "extended" state most of the DNA phosphate groups are not close to the positively charged groups of the histones. A number of slight changes may cause such a system to condense. Thus, if the loosely held protein is removed, the interhistone DNA segments will tend, at ionic strengths believed to obtain in the cell, to coil and cross-link with adjacent histone molecules so as to result in condensation or shrinkage. Similar results may be obtained by changes in the ionic character of the environment, or by the addition of *polycations*. In the light of the theory presented here, the key to the condensation mechanism is believed to be an increase in the latter.

Experimentally, the addition of small amounts of basic protein to isolated nuclei has a most profound condensing effect (Anderson, 1951), and only by the use of special techniques can chromosome-like bodies be condensed in isolated rat liver nuclei by polycations. The chromosomes seen in Fig. 6 were condensed by the addition of the dibasic amino acid, arginine.

It should be noted that the changes required to condense chromosomes may be somewhat less than might be supposed. If the system described is permeated by a solution of slightly acidic proteins (at cellular *p*H's most tissue proteins are on the alkaline side of their isoelectric points), it is evident that part of the reason for the noncontraction is the very presence of this acid protein which may reversibly associate with the nucleohistone. The acid protein serves to alter the polyelectrolyte balance in favor of the polyanions. Now if, through a small increment in polycation concentration, the chromosomal volume is decreased slightly, less of the permeating acidic protein will be contained in the gel, and a still further decrease in volume will result. Thus, it appears that swelling in a solution of cytoplasmic proteins may, since more of the protein is now in the gel, promote further swelling. Condensation, on the other hand, with consequent loss of such protein, may promote further contraction. If properly balanced, such a system could show an almost isoenergetic volume change. It makes little difference whether the added polycation attaches itself to the acidic protein or to the DNA chains, since it appears that sufficient histone is usually present in the nucleus to condense the DNA.

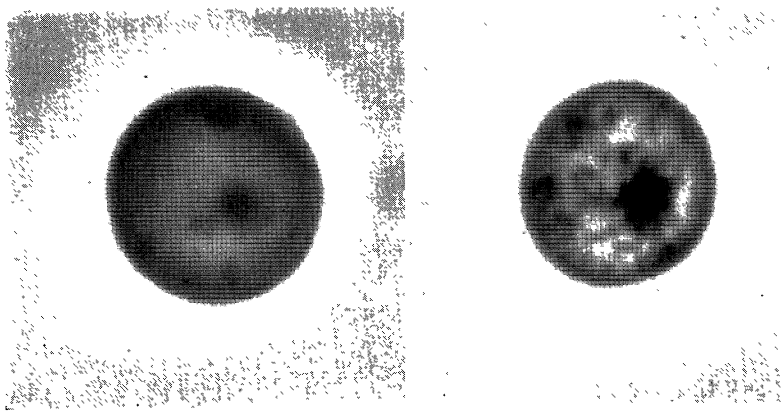

FIG. 6. LEFT, ISOLATED RAT LIVER NUCLEUS IN SALT-SUCROSE SOLUTION USED FOR NUCLEAR ISOLATION RIGHT, AFTER TREATMENT WITH 0.2 *M* ARGININE
(From Wilbur and Anderson, 1951).

Vendrely and Vendrely (1953) suggest that one arginine per DNA phosphate group is present in many nuclei. The amount of basic protein per nucleus in a given tissue is rather uniform (Alfert and Geschwind, 1953), but need vary only slightly to produce condensation or extension.

Stated somewhat differently, the volume occupied by chromatin material is thought to be controlled by competition between acidic and basic proteins or other polycations for sites on the nucleohistone. When the balance between these competing substances is shifted slightly in favor of the polycations, the DNA chains may now coil, with more of the surface of the DNA and histone molecules becoming available for mutual association. The net result would be (1) a loss from the chromatin of a large part of the proetin associated with it in the interphase nucleus, and (2) a decrease in volume.

As shown by Caspersson (1950), *the most marked change which occurs during prophase is a decrease in the amount of protein in the nucleus. The protein which remains is rich in arginine* (Serra, 1947) *and represents most of the basic protein of the cell* (Alfert and Geschwind, 1953).

Dounce (1952) has suggested that the DNA and histone are not present as a simple salt in the nucleus, since not all of the DNA phosphate is complexed with histone in the interphase nucleus. The structure proposed here would account for this.

Inasmuch as isolated nuclei can be made to show large volume changes, depending on the ionic character of the environment, it was suggested that variations in nuclear volume seen in various tissues and organs of the same animal could possibly be due to differences in the ionic composition of the intracellular fluid of the several types of cells (Anderson and Wilbur, 1952). However, further studies on isolated nuclei from several different organs (brain, kidney, and liver) of the rat showed that, when these were suspended in identical solutions, they maintained approximately the same relative size ratios found in fixed tissues. These differences persisted even when the nuclei were made to swell and shrink by appropriate changes in the suspending medium. The volume of the intracellular nucleus appears, therefore, to reflect both the ionic composition of the cell (which is probably rather similar in most tissues of the same animal) and the molecular organization of the nucleus itself. It has been shown that the protein content of many nuclei is proportional to the nuclear volume (Leuchtenberger and Schrader, 1951). If the nucleus is considered as largely made up of strands of DNA interlinked and probably cross-linked by histone, it is evident that the amount, charge density, and disposition of the histone would set some upper limit for the degree of swelling of such a system, short of complete dissolution. The nonhistone protein would be adsorbed on the unoccupied surfaces of the DNA and histone or would remain in solution in the interstices. Since the nuclear envelope is permeable to soluble cell proteins (Anderson, 1953a), it would be expected that the quantity of the protein inside the nucleus would bear a rather constant relation to the volume of the nucleus. Also, if cytoplasmic proteins tended to be very acid, the nuclei might tend to be large, and if more basic, would tend to be smaller. There is work on soluble tissue proteins which shows that the more basic soluble proteins are

present in lower concentrations in certain tumors (Sorof and Cohen, 1951). Although exceptions exist, it should be noted that tumor nuclei are generally larger than normal tissue nuclei (Miyaji, 1952). This increase in volume is not always due to an increase in ploidy. These considerations support the view that the volume of the somatic cell nucleus reflects a differentiation in the properties, amount, and distribution of basic proteins, and in the charge and concentration of nonhistone nuclear proteins and soluble tissue proteins.

In regard to the functions of histones other than their purely structural ones, it has been proposed that certain histones are specific gene inhibitors and differ in different tissues (Stedman and Stedman, 1950, 1951), a view supported by differences in the electrophoretic mobility of histones. Differentiation, from this point of view, would consist in the inhibition of a certain pattern of genes. Danielli (1953) has proposed that histones may act as inhibitors of protein synthesis generally, by virtue of their affinity for nucleic acids. This is a somewhat more general statement of the view proposed by the Stedmans. If the amount of histone is greater in an inactive gene than in an active one, it might be expected that the inactive gene would occupy a smaller volume. This problem may well be approached by the study of variations in the volume occupied by specific chromosome bands in cells of different organs. These are known to vary (Kosswig, 1948; Beermann, 1952). The concept that an inactive gene occupies a smaller volume than an active one supplies a clue to the mechanism behind the generalization that nuclear volumes vary with cell size. If the cytoplasm is considered as being in equilibrium with the nucleus (Anderson, 1953a), then a nucleus containing more active genes could maintain a larger volume of cytoplasm.

Additional support for the idea that the degree of condensation of DNA controls its activity comes from studies on heterochromatin, which may be considered as material remaining in the condensed state during interphase. When genes of euchromatic regions of the chromosome come into the neighborhood of heterochromatin by crossing over, their manifestation is lost (Prokofyeva-Belgovskaya, 1948; E. B. Lewis, 1950) or changed from dominant to recessive (McClintock, 1950). The same genes which in one species, sex, or tissue behave as heterochromatin in another may behave as euchromatin (Darlington, 1947), so as to suggest a relation between condensation and differentiation. It is possible that much of Darlington's work on so-called "nucleic acid starvation" and "overcharging" of chromosomes may be reinterpreted in terms of "undercondensation" and "overcondensation" of a relatively constant amount of DNA. If, as proposed here, the condensing basic proteins also cross-link adjacent DNA strands, then an excess of basic protein might tend to cross-link DNA strands in adjacent chromosomes. This would explain the stickiness observed between heterochromatic segments, and between so-called "overcharged" chromosomes.

The coiling and uncoiling of DNA in the chromosome is based on the assumption that segments of the chain exist which are not firmly or permanently bound to specific proteins or other substances. If DNA serves as a template for protein synthesis (Dalgliesh, 1953; Gamow, 1954; Dounce, 1952a; Campbell and Work, 1953), it would be expected that the length of the template would bear some relation to the unrolled length of the protein chain. In an average protein having a molecular weight of about 70,000, the combined chain length of the polypeptide chains would be approximately 2100 Å, assuming 3.5 Å per amino acid residue and an average amino acid molecular weight of 117. A DNA molecule of similar length would have a molecular weight of approximately 195,000 based on an assumed average nucleotide weight of 325 and an internucleotide distance of 3.5 Å. If DNA exists as a two-stranded coil (Watson and Crick, 1953), then a template of similar length would have slightly more than twice this molecular weight. A DNA molecule having a molecular weight of six million would then have the same length as a polypeptide chain of approximately one million molecular weight. It is therefore evident that the average DNA molecule could consist of more than one uncross-linked template segment and still be cross-linked with histone.

The length of the DNA molecule is of considerable importance in any consideration of the mechanism of chromosomal condensation from quite another point of view, since, in general, the longer a molecule is, the more likely it is to condense on itself.

The mechanism for chromosomal condensation proposed involves the coiling or kinking of DNA molecules. This coiling or condensation may be effective in itself in separating the daughter molecules formed by the autoduplication of DNA. The single DNA chain would be much less stiff

than the double coiled strand proposed by Watson and Crick (1953), and more easily compacted. Thus, the double-strand state may be more characteristic of interphase, whereas the single-strand condition may prevail during mitosis.

In connection with the effects of basic proteins on the volume of DNA-protein gels, it is of interest to note that the smallest and most basic proteins, the protamines, are found only in sperm nuclei, where space is most certainly at a premium, and where any mechanism for compacting genetic material would be expected to find its most highly developed expression.

To this point chromosome condensation has been treated in terms of the extension and condensation of a nucleoprotein gelwork without considering the fine structure of the chromosome. Unfortunately there has been little agreement as to what the ultrastructure of the chromosome really is, although it has been often concluded that the chromosome is held together by some type of continuous thread-like structure or chromonema. This "continuous skeleton" was thought by Serra (1947) to be of nonbasic protein with the nucleoprotein attached at intervals. The residual tryptophane-containing protein left after removal of histone and DNA was somewhat similarly considered by Mirsky and Ris (1947a and b). If histone is removed by acid treatment, or if DNA is digested away with DNAase (Mazia and Jaeger, 1939; Mazia, 1941 Catcheside and Holmes, 1947; McDonough, Rowan, and Mohn, 1952) the structural integrity of the chromosome is not destroyed. If both DNA and histone are removed, only very small coiled fragments composed of residual protein remain (Mirsky, 1951). But the "residual chromosomes" of Mirsky and Ris (1947a), obtained after extraction of thymus "chromosomes" in bulk with 1 *M* sodium chloride, are not seen when the same structures are similarly treated while being observed under the phase contrast microscope (Pollister, 1952). It is possible that the "residual chromosomes" are actually nuclear envelope material which has been stretched out during the course of nuclear rupture by high shearing forces. The existence and composition of a chromosomal backbone is therefore not a settled matter.

Both DNA and histone appear to have a non-uniform distribution on the chromosome. It cannot be assumed that DNA is absent from any region of the chromosome, however, since cytological methods can detect this substance only in comparatively high concentrations. Since DNA in crude preparations in a concentration hundreds of times lower than that seen in rat liver nuclei (50 mg./liter, for example) can produce a very high anomalous viscosity (Anderson, 1953c), concentrations of DNA below the limits of cytological detection may still have a structurally important effect. In these preparations cross links between parallel nucleoprotein strands are thought to be disrupted, while stronger bonds linking the molecules end-to-end remain.

Concepts of the association of DNA with a chromosomal backbone have been based, in many instances, on the following dubious generalizations: (1) that DNA varies in quantity during division in such a manner as to bear no relation to the number of chromosomes, chromomeres, or genes present; (2) that the amount of DNA on the chromosome could be varied experimentally, producing so-called "nucleic acid starvation" (Darlington, 1947); (3) that DNA moves off and onto the chromosomes during telophase and prophase with a possible accompanying change in polymerization (Darlington and Mather, 1950); (4) that DNA is a non-specific substance incapable of sufficient specificities to account for genetic activity; and (5) that enzyme-digestion and salt-extraction experiments indicate the existence of a ground structure which does not contain DNA.

The concepts of the role of DNA have undergone a revolution with the application of quantitative microspectrophotometric techniques (reviewed by Swift, 1953). With few exceptions, the amount of DNA *in the nucleus* appears to be directly related to the number of chromosomes present (Vendrely and Vendrely, 1948, 1949; Ris and Mirsky, 1949a; Pollister, Swift, and Alfert, 1951). Biochemically, DNA appears to be one of the most stable substances present in the cell. No change in the amount of DNA per diploid nucleus is seen with a wide variety of experimental treatments. As a result, the quantitative determination of DNA in a tissue has been considered as a direct indication of the number of cells present. No evidence that DNA depolymerizes in tissues except as a result of irradiation (Limperos, 1951) or cell death (Leuchtenberger, 1950) has been presented. The suggestion of Darlington and Mather (1950) that changes in DNA polymerization occur during mitosis has no experimental basis. The results of two lines of evidence, one chemical and physical (Watson and Crick, 1953; Gamow, 1954) and the other biological, based on the transforming effects seen in bacteria (Avery, McLeod, and McCarty,

FIG. 7. A HELICAL POLYMER WITH VARIATION IN PITCH OF HELIX

Two stable configurations with different pitches are illustrated at the two ends of the molecule. (From Pauling, 1953).

1944; Zamenhof, Alexander, and Leidy, 1953), indicate that the DNA molecule is capable of the internal complexity required of a genetic unit. These considerations support the view that DNA is an essential constituent of the gene.

An approach to the problem of chromosome structure may be made through the use of displacement effects involving large molecules. These may be termed macromolecular metathetical reactions. An example is found in the effect of heparin on isolated nuclei. The highly charged heparin molecule competes with DNA for basic sites on the histone molecule. Since heparin is more strongly charged, DNA is displaced from some of the sites. The result is the rapid swelling of the nuclear constituents into a large gel-like mass. No microscopically visible proteins strands or chromonemata may be discovered inside the intact nuclear membrane. These and other lines of evidence, including the breakage of chromosomes by ionizing radiations and ultraviolet light, lead to the conclusion that the DNA and protein of the nucleus are bound together to form a structure which is not dependent for its integrity on any central structure or backbone. Rather, as concluded by Kaufmann, Gay, and McDonald (1950), *the whole chromosome must be considered as an integrated structure with no single structural element.*

Numerous views of chromosome structure have been presented which range generally between two types of structure on the molecular level. The first suggests that discrete, particulate gene loci exist which find expression in discrete particles (Muller, 1947) bonded together. These have been thought by Mazia (1954) to be approximately 4000 Å long and 200 Å wide. The second view is that the chromosome is a genetic continuum in which discrete functional particles cannot be defined (Goldschmidt, 1951). A corresponding structural representation would be a continuous DNA-protein gelwork or a system composed of very long strands in parallel array. Fundamentally, the particulate and non-particulate concepts involve the question of the existence of bonds of considerably different energies along the chromosome.

A structure composed of individual particles may be compacted by condensation within the particles or micelles, or by packing or rearranging the particles themselves. As pointed out by Crane (1950), particles which are similar often condense most easily as spiral structures. This follows from the principle that a helical structure results from the continued application of a general identity operation to an asymmetric element, and accordingly that the helix is to be considered as the simplest infinite aggregate of units. Pauling (1953) has applied this general principle to the aggregation of globular proteins (Fig. 7) and has suggested that such helices could exist in two stable states having different pitch. While condensation can be accounted for in such a system, other problems arise. The chief of these, in the case of a chromosome composed of a series of discrete particles loosely bound together (as contrasted to a long bundle of parallel strands), concerns the reduplication of the particles. The most plausible mechanisms for exact molecular reduplication involve reduplication of the individual linear molecules themselves without recourse to long-range forces or multistep syntheses. In dealing with the reduplication of a multimolecular particle the question arises whether all the molecules or simple histone-DNA chains are identical or not. If they are different, then some mechanism for sorting them out and dividing the particle into two identical ones must be considered.

A number of authors have reported evidence for the existence of fibers running parallel to the length of salivary gland chromosomes (Palay and Claude, 1949; Yasuzumi, Odate, and Ota, 1951). Beermann (1952) considers these strands to be wound into a loosely cabled spiral, with each strand extending the length of the chromosome. Ambrose, Cuckow, and Gopal-Ayengar (1952) reported the threads in *Chironomus* salivary glands to have a diameter of 100–200 Å. Observations with the polarization microscope (Schmidt, 1941; Ambrose and Gopal-Ayengar, 1952) indicate that the long nucleoprotein molecules are arranged approximately parallel to the axis of the chromosome.

On the basis of these and other findings, Ambrose (1956) has proposed the following model of the

structure of the salivary gland chromosome. All structures are parallel to the long axis:

(1) Nucleic acid and protein chains with a diameter of 15–20 Å. These are inferred from x-ray and optical effects.

(2) Bundles of these chains arranged to form microfibrils 100–200 Å in diameter. Observable in the electron microscope.

(3) The microfibrils are arranged in bundles to form the chromonemata with a diameter of 5000–10,000 Å. These may be observed with the light microscope.

(4) Bundles of chromonemata form the salivary gland chromosome, which may have a diameter up to 20 μ.

The microfibrils may actually be formed from sections 3000–4000 Å in length which are held together by secondary forces (Mazia, 1954; Ambrose, 1956). The nucleoprotein-protein chains could therefore be continuous the entire length of the chromosome, but could have very weak links at points along the chain. If these weak links were identically spaced along the chains making up the microfibril, the microfibril would behave as if it were composed of long particles with a diameter of 100–200 Å. These particles are therefore in a sense artifacts of the isolation procedure. Such a model reconciles some of the contradictory views of chromosome structure which have been put forward previously, and is not at variance either with the concept that the chromosome contains discrete particulate gene loci (Muller, 1947), or that the chromosome is a genetic continuum in which discrete functional particles cannot be defined (Goldschmidt, 1951).

It is of interest that the available data can be used to construct a model which accounts for the structure of the extended and the condensed chromosome if the following assumptions are allowed:

(1) The ultimate genetic structure is a linear system, able to duplicate itself, and to engage in some other specific activity such as RNA or protein synthesis.

(2) The ultimate linear structure is based on the DNA molecule, probably interlinked end to end by protein. This structural unit may contain information for several genetic effects and is not necessarily synonymous with the gene.

(3) The linear structure is not single, but is composed of a number of similar, generally parallel, strands which form a loose skein in the extended state.

(4) The molecular strands or chains are identical in *all* respects, including the structure and orientation of protein attached to the strands.

(5) Condensation involves a change in affinity between adjacent strands such as would occur on the unmasking of basic charges on the histone molecule, the addition of small polycations, etc.

(6) In the resting or extended condition the chains forming the skein are *very* loosely cross-linked, and such linkages as occur are rather widely separated.

(7) Identical molecular chains or strands condense against each other best by moving parallel to one another a sufficient distance to allow non-identical segments to condense.

The first assumption might be justified on the basis of economy of ideas and simplicity. The second is based partly on data previously listed. The concept that one DNA molecule may be part of more than one gene deserves comment, however. Kurnick and Herskowitz (1952) calculated that a haploid set of Drosophila salivary chromosomes contains 44,000 DNA molecules if a molecular weight of 10^6 is assumed. If the number of genes is taken as approximately 5,000, then about nine DNA molecules per gene would be present. But if the molecular weight is taken as being around 8×10^6, then the ratio would be nearly that of one gene per DNA molecule. However, the finding that two transforming activities may exist in the same DNA molecule in experiments with pneumococcus (Hotchkiss and Marmur, 1954) argues against the "one gene, one DNA molecule" concept. It does not appear that any data are at present available from which the number of duplications (the number of identical molecular strands) can be calculated. The stability of the genetic mechanism argues in favor of a multiple-strand system, with some process for intercomparison and elimination of strands or sections of strands which do not match the rest. The necessity for such "inspection systems" has been discussed by Crane (1950). If the chromosome was built up from only one long strand in which DNA played a major role, it would be difficult to see how chromosome stickiness, which is thought to be due to loose DNA segments, could occur without chromosome breakage.

If one takes the available data at face value, the overall statistical model arrived at is that of a skein of DNA chains, each chain being approximately 20 Å in diameter, and between 5 to 40 $\times$ 10^6 Å long. The associated histone molecules

(data from Davison, James, Shooter and Butler, 1954, for thymus histone) appear to be of two types: one of low molecular weight (18,000) and a high axial ratio (1:28); the other much larger, with a molecular weight of 35,000. If the dimensions of the smaller molecule are calculated as described by Neurath (1939) they are found to be 11.6 and 319 Å. This can only be true if the molecule exists as an essentially linear polypeptide chain. As pointed out by Watson and Crick (1953), ". . . we know almost nothing about the structural features of protamines and histones. Our only clue is the finding of Astbury (1947) and of Wilkins and Randall (1953) that the x-ray pattern of nucleoprotamine is very similar to that of DNA alone. This suggests that the protein components or at least some of it, also assumes a helical form and in view of the very open nature of our model we suspect that proteins form a third helical chain between the pair of polynucleotide chains. As yet nothing is known about the function of the protein; perhaps it controls the coiling and uncoiling and perhaps it assists in holding the single polynucleotide chains in a helical configuration." The low molecular-weight histones would be admirably suited for this function in view of their threadlike configuration. From calculations based on the amount of this smaller histone in thymus nuclei and the amount of DNA present, it can be shown that the sum of the lengths of these small histone molecules is almost twice the sum of the lengths of the DNA chains. The larger histone molecules appear to be spaced along the DNA chains at average distances of about 700 Å.

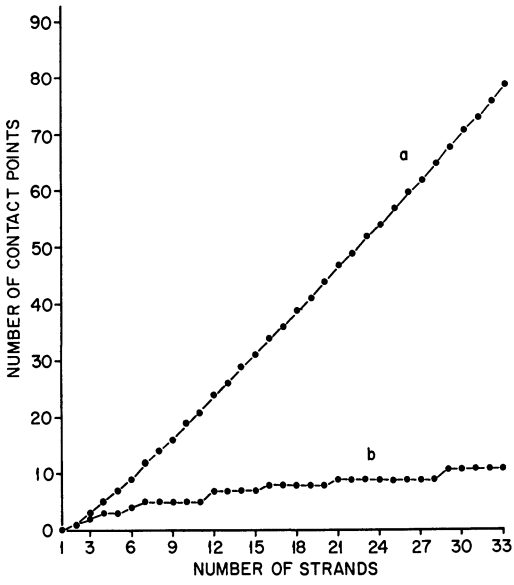

FIG. 8. TOTAL NUMBER OF CONTACT POINTS BETWEEN STRANDS IN A HELIX OBSERVED IN A THIN SECTION PERPENDICULAR TO THE AXIS AS A FUNCTION OF THE NUMBER OF STRANDS (CURVE *a*).

The number of such contact points which must be broken to separate the strands into two groups, as evenly matched as possible (Curve *b*).

It follows from the identical configuration of adjacent chains that they would not tend to condense in a parallel fashion (DNA against DNA, large histone molecule against large histone molecule), although conceivably some interaction between the smaller histone molecules in one strand and the DNA in another might occur. The simplest scheme for condensing identical chains is for each to be displaced parallel to its axis a sufficient distance to allow non-identical segments to condense, i.e., large histone molecule with DNA. The only way in which this can occur efficiently in a system where longitudinal translation of alternate molecules is prevented is by the formation of a helix. The distance which each strand moves relative to its neighbors will determine the pitch of the helix.

If one considers a very long skein of strands, it is evident that if a helix forms in one part of the bundle, twisting in the opposite direction will occur on either side of the segment where the original helix formed, giving three segments which alternate in direction of twist. (Coiling of the molecular strands described here should not be confused with the helical structure of DNA itself.) It can be easily shown that stresses will be set up between loosely cross-linked chains at the site where the direction of twist changes. The magnitude of the forces tending to split the chains away from each other at this point will be proportional to some function of the number of junction points between neighboring chains. This number increases rapidly as the number of chains increases, while the number of such contact points which must be broken to separate the skein into two smaller skeins increases at a much slower rate as the number of strands in the skein is increased. This is shown in Fig. 8, where the number of contact points between strands observed in a thin section perpendicular to the axis of a twisted cable, and the number of such contact points which will be broken when the strands are separated into two nearly equal groups, are plotted against the total number of strands. With seven strands, twelve contact points will exist, five of which are broken when the cable is divided into two groups with

four in one and three in the other. If the cable has fourteen strands, however, twenty-nine junctions points will exist, while only seven need be broken. It is evident that a number of strands N can be chosen such that it can be twisted in alternate directions at intervals (assuming a certain bonding energy between adjacent strands), while an attempt to do the same thing with twice this number ($2N$) will produce shearing forces sufficient to cause the skein to form two parallel helices. This mechanism, shown schematically in Fig. 9, is proposed as the origin of the division of the chromosome. As the coiling of the skein proceeds, the original $2N$ helix segment is untwisted and recoiled into two helices.

Further condensation produces more segments, each twisted in alternate direction. Unless the number of strands in the $1N$ skein is less than five, or unless a hollow tube is produced, a number of unresolved forces exist. For example, a seven-strand system with one strand in the center surrounded by six others has no provision for shortening the center strand as the others coil about it. The probability therefore exists that the original molecular coil will form a second tight coil in an attempt to resolve these forces, and may also form larger coils. While it is not possible to make any very accurate approximation of the size of the various coils, the second coil is of the same magnitude as the subminor coil of a small chromosome.

It is apparent that this model allows for the reduplication of single molecular strands in an uncoiled condition, separation of these into two skeins of strands coiling at intervals in opposite directions and the further coiling of the separate skeins into more complex higher order coils. It does not require reduplication of large beaded structures by long range forces, the separation of coiled systems by extensive breaking and rejoining, or the spiralization of long structures where the free ends would be required to rotate at a high rate of speed, as might be the case if the original skein were twisted in one direction along its entire length. The alternation of direction occurring in the first or molecular coil might suggest that the direction of coiling of the larger, microscopically visible coils would be indeterminate. Actually there is no reason (or space for that matter) for the interphase chromosome to uncoil completely. Rather, it will retain part of the same spiral structure of the condensed chromosome. When condensation with twist reversal at the molecular level occurs,

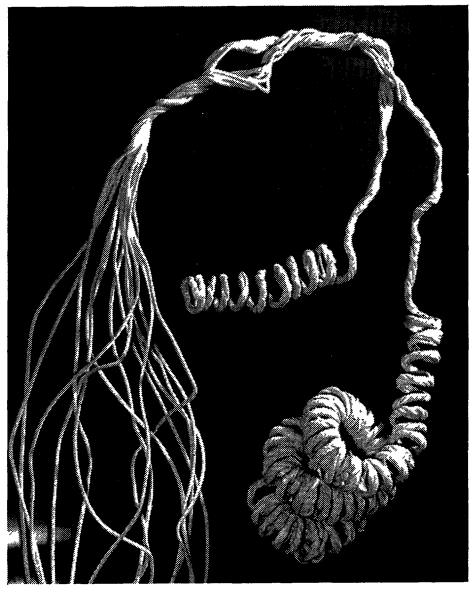

FIG. 9. A MODEL OF A CHROMOSOME CONSISTING OF LONG STRANDS OF NUCLEOPROTEIN

In the extended state (lower left) very little cross-linking exists. As an attempt is made to condense this system, with the restriction that identical segments of the chains cannot condense immediately next to each other, a helix altering in direction of twist at intervals is produced (upper left). Stresses set up at the point of twist reversal separate the skein into two bundles (upper center and right center). The two bundles form separate helices which coil further to produce the second and third coils shown (center and lower right). While the original bundles exhibit twist reversal along their entire length, the larger coils rarely do so because of their stiffness.

segments of the skein may attempt to form larger (subminor) coils going in opposite directions. For reasons of stiffness and efficiency of packing these will tend to revert to the one direction which is predominant. If 50 per cent of the length of the skein attempts to produce a second coil in one direction, and 50 per cent coils the other way, the interplay of forces will be resolved in terms of that small amount of coiling remaining from the previous condensation cycle. It would be expected, therefore, that the direction of coiling would be rather constant, but reversal of direction along part or all of the chromosome should be occasionally observed, as is indeed the case (Manton, 1950).

It is not unlikely that the points where twist reversal occurs are the points of rupture when the chromosome is broken down, as in the distilled water extractions of Mazia (1954). A coil of the dimensions of the second coil shown in Fig. 9 has

been shown in an electron micrograph by Frajola, Greider, and Rabatin (1954).

This condensation mechanism, by condensing only identical strands, may serve to eliminate those which are different, damaged, or defective. The stability of genetic patterns through tens of thousands of reduplications may well be due to such an intercomparison and defect-elimination system. The rujevenating effect of cell division (or conversely the lack of aging effects in cells which continue to divide) may well be due to this mechanism.

In considering the relation of microscopic observation to the problem of chromosomal condensation it should be noted, first, that most concepts and models of chromosome structure are largely derived from the study of giant non-condensing chromosomes, and second, that the problem of fixation alterations in chromosomal material is still far from being solved.

It is the purpose of the remainder of the papers in this series to show that the simple mechanism involving cyclical variations in the relative amounts of polymerized acidic and basic groups (shifts in polyelectrolyte balance) occurring in a loosely organized nucleic-acid–protein gelwork, and which are thought to be causally related to the division of primordial cells, may be extended to explain a number of other aspects of mitosis seen in contemporary life.

The pessimistic views concerning the complexity of mitosis and the difficulties attendant on any studies seeking to supply solutions to the problems involved, which have been expressed by Schrader (1953), undoubtedly represent a mature and realistic evaluation of the present status of the field. The large cytological literature appears to contain instances which may be cited as evidence against almost any conceivable detailed theory of cell division. It may be well to inquire, therefore, into the justification of an approach such as is presented in this series.

If a science is made up of those hypotheses which have not yet been discarded, then the history of a science is the history of hypotheses. Each new hypothesis or generalization at the outset lacks the pertinent experimental data for its proper evaluation and delimitation. Only by exploring an idea thoroughly, by pushing it as far as it will go, can such an evaluation be obtained. A case in point is the history of the study of *p*H in biological materials. Only after a long series of heated debates and claims that *p*H controlled a variety of processes ranging from aging to the determination of the sex of human offspring, has the whole matter fallen into proper perspective. The present evaluation rests on a mass of experimental data, which in turn owes its existence to a number of theories, some of which are now merely amusing.

The object here is to present a point of view which will stimulate a line of approach to the problem of cell division. The justification for such an endeavor lies not in that a theory may prove to be correct, partially correct, or even slightly correct. Rather, a theory is justified by the experimental data which it serves to generate.

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