Reproduction: Molecular, Subcellular, and Cellular

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Introduction

The purpose of this symposium is partly to clarify and define specific aspects of reproduction at different levels of biological organization, and partly, in common with other symposia held by the Society, to stimulate interaction between various intellectual approaches to a broad phenomenon of common interest. Whether or not a cell reproduces true to parental type, or whether a cell reproduces at all, are issues of fundamental interest in developmental biology. Reproduction of a cell is impossible without reproduction of molecules, but the kinds of molecules reproduced and the conditions effecting reproduction are dependent upon the intracellular pattern of molecular organization and the influence of the environment on the metabolic poise of the cell. The production of a chlorophyll molecule, for example, may be fully stated in terms of the enzymes in its biosynthetic chain. The reproduction of a chlorophyll molecule is, however, quite a different matter. A necessary condition for reproduction is the transmission of proplastids or plastids from parent cell to offspring. Even if such transmission occurs, the production of chlorophyll remains dependent upon the location of the cell within an appropriate region of the plant. Thus, excluding environmental and mutational factors, a clarification of the phenomenon of chlorophyll reproduction requires the elaboration of certain rules governing the behavior of subcellular organelles with respect to transmission, as well as rules governing the development of organelles with respect to cell differentiation. A counterpart of this example may be found in the reproduction of many other cell components. The involvement of a cytoplasmic particle is incidental to the general question of reproduction. Applicable to all situations, however, is the fact that just as the reproduction of cells does not assure the reproduction of all molecules, so the reproduction of molecules does not assure the reproduction of cells. We cannot avoid the

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challenge of defining rules for the formation of molecules within the biosynthetic framework of cells, for the assembly of molecules within the architectural framework of cells, and for the reproduction of molecules or their complexes within the genetic framework of cells as governed by the interaction between cells and their environment.

We may justifiably make the dogmatic assertion that a complete understanding of cell reproduction requires a complete understanding of the molecular events underlying such reproduction. We cannot, however, extrapolate from molecular behavior to cell behavior without guidance from the cells themselves. A molecular mechanism, to the extent that it is perfectly understood, imposes clear restrictions and suggests fruitful possibilities with respect to the elaboration of rules concerning reproduction at more complex levels of organization. But unless we make the extremely doubtful assumption that the present organization of living systems is a necessary and inevitable consequence of molecular properties, we cannot hope to achieve a knowledge of reproduction by a unidirectional acquisition of information from molecule to man. Even if we allowed for this theoretical possibility, we would have to attribute to biologists a flawless prescience never witnessed previously in the history of science. Were it not for the discovery of mitosis, meiosis, and Mendelian inheritance, the Watson-Crick helix would not have come into existence; and, if it were somehow envisaged, it would have remained a conversation piece. If the molecular biologist must be defined as one who chooses problems which can be solved, then the biologist must be defined as one who chooses problems which need to be solved. These appositions and oppositions are, however, trivial. The point of view that clarity of formulation is essential in approaching the phenomenon of reproduction at all levels of organization represents the spirit in which this symposium was fashioned.

We are generally concerned with two aspects of reproduction, the mechanisms themselves and the regulation of these mechanisms. Although a distinction between these two aspects is often difficult to make, we may nevertheless attempt to outline the structure of the symposium with this distinction in mind. We should be able to define reproductive mechanisms at the molecular, subcellular, and cellular levels with some degree of rigor. Similarly, we should be able to define molecular, subcellular, and cellular regulatory mechanisms.

**Molecular Mechanisms of Reproduction**

Our present concepts of molecular reproduction are based entirely on two fundamental principles, enzyme catalysis and complementary nucleo-
tide pairing. The first is an oldtimer in biology; the second is a newcomer. The enthusiasm which greeted the discovery and studies of enzymes in the late nineteenth century is now focused on investigations of the function of nucleic acids. Whether molecular biology begins with base pairing, or dates back to the studies of Claude Bernard, is a matter of pride and prejudice with no relevance to the symposium. Our interest lies in the fact that enzymes select from the thermodynamically possible world and construct the real world of living matter. Bond formation without enzyme mediation is an event which can at best be of only marginal interest in biological reproduction. All molecules are synthesized by virtue of specific enzymes. But the very specificity of an enzyme, the relatively small intramolecular distances which its active site can recognize, precludes its operation in specifying the order of extended intramolecular sequences. To this particular aspect of ordering nucleic acids furnish an essential contribution.

Three categories of molecular synthesis have been considered in this symposium—autosynthesis, heterosynthesis, and antibody synthesis. Whether the categories are variants in the operation of enzyme catalysis and nucleic acid pairing, or whether other mechanisms are yet to be disclosed, remains to be seen. At present, we feel confident that the two basic mechanisms described are sufficient to account for all forms of molecular biosynthesis. In autosynthesis, an enzyme transcribes a complementary base sequence out of subunits identical with those of the template. This type of synthesis is considered universal in DNA and operative in RNA only in the case of RNA viruses. In heterosynthesis, an enzyme also transcribes a nucleic acid template, but in the case of protein synthesis, the transcribed product must be translated into a sequence of amino acids by a different set of processes. Antibody synthesis may be formally described as the formation of proteins complementary to antigen molecules. The discussion of this topic by Nisonoff should make it amply clear, however, that formal identities between nucleic acid complementarity and antigen-antibody complementarity have no counterpart in molecular terms. Present evidence virtually rules out the possibility that an antibody molecule may be synthesized by a transcription of antigen. Doubt even exists as to whether the total sequence of molecular events, from antigen challenge to antibody production, is encompassed by a single cell. It would appear that rules must first be formulated at the cellular level before molecular mechanisms can be properly resolved.

The two basic molecular mechanisms, if rigorously examined, reveal certain limitations and some possibilities with respect to molecular reproduction. On the assumption that the mechanisms are properly under-
stood and that no other mechanisms exist, direct copies of molecules are ruled out. Since sequences are transcribed by base pairing, the primary product of transcription must always be complementary to the template. The molecular distinction between autosynthesis and heterosynthesis is blurred, inasmuch as the primary determinant of the subunits used in transcription (whether ribo- or deoxyribonucleotide) is the enzyme. Nothing in our present concept of nucleic acid reproduction excludes the possibility of RNA being transcribed into DNA. Our failure thus far to discover instances in which DNA is replicated from an RNA template, or in which native cellular RNA is transcribed from RNA templates, cannot be sanctioned by molecular rules of reproduction. Moreover, since the agent of transcription is the enzyme, we must also allow for the possibility that direct rather than complementary copying may occur if the appropriate enzyme is found. Either the rules must be tightened, or our knowledge of cell behavior must be broadened. We may, on the other hand, dogmatically assert that the amino acid sequences within a polypeptide chain can only be derived from nucleotide sequences and that the reciprocal derivation is impossible. We are thus compelled to account for the reproduction of protein molecules in terms of nucleic acid templates.

Subcellular Mechanisms of Reproduction

At the subcellular level, the existence of a supply of molecules is taken for granted and the question to which we must address ourselves is how the various structures in a cell are reproduced from the molecular pool. The presence of DNA templates in the cytoplasm mainly influences the phenomenon of intracellular regulation. The geography of transcribing systems is largely, though not entirely, incidental to the mechanisms involved in the generation of subcellular structures. Our knowledge of these mechanisms has none of the crispness characteristic of our present understanding of molecular events. Currently, we envisage three possible mechanisms in the formation of structures from molecular pools: self-assembly, accretion by intussusception or end addition, and assembly on preformed templates. The first of these has evoked considerable conceptual interest because of the implication that individual macromolecules contain sufficient information to arrange themselves in recognized biological patterns. Bacterial flagella and collagen fibers are among the structures thus studied for which the evidence pertaining to self-assembly is impressive. The biological consequences of such a process are clear. If the information contained in a coded amino acid sequence is sufficient to determine the tertiary structure of a macromolecule, and also to assemble
that molecule along with others similarly endowed into a discrete subcellular structure, then subcellular reproduction is an obligatory consequence of molecular reproduction. Even if allowance is made for the possibility that enzymes might catalyze the self-assembly process, a passive role would still have to be assigned to extragenic components in the generation of subcellular forms.

Whether the concept of self-assembly can be extended to all formed elements in a cell remains to be seen, but the prospect for such extension is, at least in some instances, not bright. "Membranes" have been generated by modifying the old technique of mixing lecithin with water to create myelin forms. Modification consists of the addition of protein to the initial mixture and observation of the product under an electron microscope. The artificial model is undoubtedly similar in appearance to a natural membrane. Unlike the self-assembled structures discussed above, however, membranes are transmitted as such from one cell generation to another; a membraneless cell is unknown. Where careful observations have been made of the formation of specific membranes, as in the chloroplast, evidence points to the growth of new membranes from preexisting ones. The functional and structural heterogeneity of membranes (e.g., inner membranes of mitochondria, lamellae of chloroplasts, limiting membranes of cytoplasm) apparently makes self-assembly from a pool of individual molecules a rather hazardous morphogenetic operation. The issue is whether the essential genetic complement of a cell is reducible to sequence coding, or whether it must also contain other preformed elements that serve as frameworks, however small and undistinguished, for the reproduction of recognized structures. Cortical inheritance in protozoa furnishes the best-studied example of such a requirement. Other examples of cytoplasmic inheritance (discussed by Srb) may be attributed either to a requirement for the transmission of preformed cytoplasmic structure or as elucidated by Schiff, to the presence of cytoplasmic DNA.

The reproduction of chromosomes is clearly not a process of self-assembly. The problem of chromosome reproduction is unique inasmuch as it is preceded by DNA replication, and the formation of the longitudinally differentiated structure as an apposition of materials to a preexisting DNA skeleton could be rationalized with some theoretical and empirical justification. Nevertheless, difficulties remain both in defining the actual molecular organization of the chromosome and in explaining how its characteristic cytochemical morphology is maintained. Swanson has pointed out that a single double helix of DNA is still a favorite model for the primary skeleton of the chromosome, although this repre-
sentation is not universally embraced. Modifications of the model have to be made in order to account for the many starting points of replication within a single chromosome. The acquisition of genetic data, inadequate as the data may be, would be helped rather than hindered by postulating the existence of specialized linkers between gene segments. Whatever the precise nature of the DNA skeleton, thought must be given to the mechanisms which confer upon that skeleton its full structure. Are histones deposited randomly, or are there localized sites in the DNA filament which interact with specific histones? If the latter is true, we must envisage a mechanism by which segments of a DNA chain recognize specific histones. Is the RNA in a chromosome a collection of unspent messages destined for the cytoplasm, or does it have some special function in making chromosomal proteins which are properly apposed to the DNA filament because they are locally synthesized? Curious though it may seem, the major problems in understanding chromosome reproduction do not center around the replication of DNA. Our preoccupation with nucleic acids and their potentialities in specifying macromolecules overshadows these other problems but does not diminish them. The fact remains that the reproduction of a chromosome is not implied in the replication of DNA. Only future studies will tell whether a total implication is even possible.

**Mechanisms of Cellular Reproduction**

In defining mechanisms of reproduction at the cellular level we must take for granted the operation of reproductive mechanisms at the molecular and subcellular levels. If this is done, we might wonder whether anything can be said about cell reproduction. Little in our experience leads to the view that an analogy can be drawn between the formation of subcellular organelles from molecules and the formation of cells from subcellular organelles. Indeed, the view that cells are aggregates of functionally integrated but structurally discrete systems is almost universal. As a consequence of this view, we are inclined to define the problems of cell reproduction entirely in regulatory terms. Nevertheless, however correct this view, one aspect of cell reproduction—the partitioning of subcellular components—cannot be considered except at the level of cell organization. If nothing else were said about this complex problem, we would have to concede that a mechanism hitherto unmentioned—polarity—is a necessary component of the reproductive process. This does not imply that polarity is characteristicly a supramolecular property which emerges only at the cellular level of organization. Indeed, a polarity
certainly exists in the structure of every nucleic acid and phospholipid molecule; polarity almost certainly exists in the myosin molecule and most probably is to be found in many types of proteins. The existence of polarized molecules is a necessary but hardly sufficient condition to account for the polarized movements of components which occur during cell reproduction.

Partitioning of a cell into daughter cells does not involve a polarized migration of all components. Obviously, some components do not move. Moreover, it is questionable whether subcellular populations occurring in relatively large numbers (chloroplasts, mitochondria) require equipartitioning to achieve equivalent numbers in the matured daughter cells. In *Euglena*, as pointed out by Schiff in the course of discussion, the imbalance created by a partial loss of proplastids or chloroplasts is eventually relieved by their reproduction at a compensatory rate. Nevertheless, although many components do not require polarized movement, it is apparent that chromosomes do. Indeed, one may extend this requirement even to prokaryotic organisms. Replication of DNA does not in itself account for equipartitioning, and without such partitioning genetic continuity could not operate. We may not have sufficient experimental and conceptual tools to cope with the problem of polarity, but one cannot avoid the impression that this phenomenon is commonly regarded as an eccentricity rather than as a profundity of natural organization. We must also recall that the role of polarity in reproduction is not a singular one. Meiosis and mitosis lead to completely different modes of cell reproduction, and one of the major differences between these two similar processes depends upon the packaging of polar movements. The separation of chromosome pairs in meiosis is probably the most outstanding example of differentiation effected by unequal partitioning of cell components. The once strongly held view that differentiation may arise from an unequal partitioning of components among daughter cells has lost its impact. Nevertheless, the fact remains that in at least some instances (stomatal formation, microspore maturation) unequal division of a cell leads to daughter cells with differentiated functions.

Regulation of Reproductive Mechanisms

Regulation is so diffuse a subject that changing fashions in its investigation are to be expected, the more so, since one good scheme can explain a lot without proving much. We might have focused exclusively on the regulation of gene action in relation to reproduction. The topic would
be timely, and carry the full prestige of profundity, but in doing so we would have chosen the less complex issue and avoided the more difficult challenge. On the other hand, it is doubtful whether we are in an effective position to take on the challenge. An attempt has been made in this symposium to classify regulatory mechanisms in terms of organizational level. Such sorting out should help to clarify our thinking and, hopefully, to emphasize the inherent limitations of any single control mechanism. In an integrated system such as a cell or organism, the profound mechanism may turn out to be the most invariant one, and the apparently trivial mechanism the one most sensitive to environmental fluctuations and hence the most important in the translation of subtle influences into profound reactions.

**Molecular Regulation**

At the molecular level, the regulation of reproduction can be defined in terms of the two much discussed mechanisms, complementary base pairing and enzyme catalysis. The first of these has lent itself to a variety of schemes all based upon the principle that linear nucleic acid templates must be transcribed directionally. Evidence that transcription of one nucleic acid molecule into another and translation of the latter into protein proceeds directionally, rather than by a simultaneous or random apposition of complement, is strong enough to be adopted, tentatively at least, as an axiom of the process. Direct evidence has been obtained from studies of protein synthesis and from tracing the reproduction of genetic markers in microbrial systems. The question mentioned by Atwood—whether transcription and translation occur in the same or in opposite directions—is important in detailing the mechanism, but does not affect the validity of linear reading as a regulatory device. The general evidence naturally invites the conclusion that the prime target of any regulatory mechanism is the starting point of transcription. Blockage or opening of the starting point encompasses all the schemes involving repressors, derepressors, operators, and regulators. Such schemes lend themselves to an infinitude of designs, and proof rather than explanation is the principal missing ingredient. The main problem, however, is not to prove the formal validity of a scheme too logical to be abandoned. It is on the more restricted issue of molecular recognition that the scheme requires investigation. For in a very real sense, the nucleic acid filament, although it is replete with information about sequence, appears to be rather static in its behavior. Unless we misunderstand its operation, its regulatory role must be confined to a choice between being read or not being read. Even so,
the choice must be effected by some substance which is not a part of the nucleic acid tape. Such a substance could be the direct product of a gene, but directly or indirectly it must certainly be the product of events in the cytoplasm. The case of the substrate inducer in microorganisms is now classical, but it takes little imagination to perceive that the possibilities of similar relationships are virtually unlimited in a multicellular system.

If we postulate that promotion of transcription for a defined portion of the nucleic acid filament is a function of the attachment between the transcribing enzyme and the starting region of the filament, a variety of conditions may be considered necessary for molecular biosynthesis. If the enzyme is lacking, no transcription will ensue. If the enzyme is present but the starting site is complexed with some other substance (e.g. a polynucleotide or protein), transcription will also be blocked. If another substance with a higher affinity for the blocking agent than the polynucleotide segment is produced, the block will be removed. This is essentially the repressor theory of regulation. Modifications of this scheme are clearly possible. The evidence that chemical alteration may occur in the intact chain (e.g. methylation) as a result of the action of specific enzymes, opens a new arena for speculation. Given the inherent linear orderliness of the nucleic acid molecule and the capacity of enzymes to recognize localized segments, we can visualize a variety of enzymes, activated under a variety of conditions, effecting specific changes in certain nucleotides which enhance the capacity of the chain to recognize and interact with specific substances in the environment. This possibility has been considered by Atwood and, in a somewhat different form, by Dulbecco who postulated the operation of a nuclease in effecting specific breaks in the chain.

Whatever our preferences for regulatory schemes, it is apparent that linearity has consequences for the cell which extend far beyond the coding of amino acid sequences. Granick has indicated in his comments that the amount of DNA per cell in higher organisms cannot be rationalized on the basis that such organisms effect more biosyntheses than do bacteria. The fact that regulation of molecular reproduction is far more extensive in higher organisms than in bacteria, however, may offer a possible explanation. But if we postulate more DNA lengths for regulatory purposes, we must also postulate more proteins and/or other substances which are the active components of any regulatory system.

All our information points overwhelmingly to protein molecules as the most sensitive receptors of intracellular or extracellular environmental stimuli. In many cases the protein molecule may be associated
with a chromophore or other prothetic group, but such an association may be regarded as an extension of its powers. If regulation were no more than synthesis or lack of synthesis of macromolecules, we could assign to proteins a purely supporting role. But the evidence is almost entirely against so narrow a definition. From the start, development is a process of eliciting the expression of genetic potential. Much, if not all, of that potential is linearly taped, but the tape states what can be done whereas the environment of that tape states what will be done. At the molecular level we have yet to learn how environment and tape interact.

Subcellular Regulation

If regulation of reproduction at the molecular level encompasses considerations well beyond the immediacies of the molecules involved, difficulties in drawing any clean line between regulation at the subcellular and cellular levels should not come as a surprise. The distinctions we make must be arbitrary and can be justified only by the extent to which they have some operational meaning. With this in mind we may say that the major impact of regulatory mechanisms at the subcellular level must be in the coordination of relative population numbers. In a clone of nondifferentiating cells this means equivalent rates of reproduction among all cellular constituents from one generation to the next; in the case of a differentiating clone this often means alterations in otherwise equal rates of reproduction. We must distinguish, however, between subcellular components having their own genetic continuity and those which do not.

For subcellular components lacking a genetic continuity of their own, regulation can be considered only in the same terms applied to molecular components. On the other hand, for subcellular components with their own genetic continuity we may at once define two requirements: Their transmission to daughter cells must be assured and their net rates of reproduction must equal that of the nucleus. In cases where unequal segregation occurs, an additional mechanism must be present to restore the original numbers. If these mechanisms do not operate then, the progeny undergo differentiation. The two mechanisms involve both cytoplasmic inheritance and cell differentiation. The various examples of cytoplasmic mutation discussed by Schiff and by Sröb invite us to consider not only molecular mechanisms but also subcellular ones. The chromosomal system is so organized that mutated genes are replicated and transmitted like their counterparts. So far as we know, this relationship does not hold for cytoplasmic systems. A mutation within a chloroplast,
for example, must be evaluated from the standpoint of two parameters: (1) the effect on development and on functions within the cell and (2) the effect on reproductive rate relative to other chloroplasts and to the cell as a whole. Only mutations which have no depressing effect on reproductive rate would survive, and those which elevated the reproductive rate would eventually displace the original population. We know too little about the relations between physiological function and reproductive rates to draw any general conclusions, but we may suspect an interaction between the two properties. Such an interaction would serve to stabilize the characteristics of cytoplasmic components with genetic continuity. The implications of this interaction are perhaps forgotten in pondering the significance of cytoplasmic DNA. If it can be assumed that such DNA does serve as a coding template and in this respect is identical with chromosomal DNA, an important distinction is to be found at the subcellular rather than at the molecular level. A single mutation within a chromosome is perpetuated for as long a period as the cell line itself; a single mutation within a cytoplasmic particle is perpetuated only if its reproductive rate remains unaffected by the mutation. From the standpoint of evolution, chromosome structure is the more effective vehicle.

The observations of Tulecke on the respective growth patterns of male and female haploid tissues raise an important question which invites exploration. The fact that female tissues contain not only chlorophyllous cells but also various other differentiated elements lacking in male tissues, suggests that their respective cytoplasms have different morphogenetic potentials. On first inspection, we cannot attribute these differences to the genome and are therefore compelled to consider the cytoplasm as a seat of heritable factors relevant to the differentiating process. If this is true, then totipotency cannot be considered entirely in terms of the genome. The hazards in simple interpretations of cytoplasmic inheritance have been stressed by Srb; this caution should be respected but not overemphasized in evaluating the behavior of haploid plant tissues.

The regulation of chromosome reproduction still remains a problem of major interest. The problem is somewhat simplified by our assumption that DNA provides a structural template to which other constituents must be apposed. The simplification permits us to probe some aspects of chromosome reproduction even though we are uncertain about the nature of the structure to be reproduced. Evidence that the mere presence of essential enzymes and substrates is insufficient to initiate chromosome reproduction is strong. We are therefore led to consideration of the remaining molecular mechanism—the exposure of starting points on the
template. Excluding, however, this particular mechanism which relates specifically to DNA, we are still uncertain, as Swanson has pointed out, about the regulation of other chromosomal components. We frequently make the tacit assumption that the macromolecules in a chromosome are reproduced in the same way as other cellular macromolecules, but this conclusion is something less than a fact, and we have yet to discriminate between nuclear metabolism which serves the cytoplasm and nuclear metabolism which serves the chromosome. We know from cytological data that DNA replication is not an assurance of chromosome reproduction. If it were, polyteny would be unknown. We must also elucidate the regulation of centromere reproduction, for in its control lies part of the answer to meiotic and mitotic reproduction. Moreover, to these difficult considerations another one which emerges from studies of viral infection must be added. However autonomous a replicating system appears to be, the introduction of another replicating system into the cell may lead to interactions which affect the rate of chromosome reproduction. If such effects occur between virus and genome, they may also occur between genome and normal cytoplasmic constituents.

The clarity which can be given even to partially proved schemes of molecular regulation stems from the fact that multiple interacting systems may be conveniently ignored. That multifactorial considerations enter, however, is only part of the problem. The other part relates to the obvious fact that we do not know enough about the mechanisms by which the subcellular structures are reproduced. Since our knowledge of the pure mechanisms of reproduction is uncertain, we can hardly expect to clarify the regulation of those mechanisms. We are well aware that in a developing organism we must deal not only with factors that assure reproduction, but also with factors that assure selective reproduction. At this moment, these factors require a much better understanding of how structures are assembled, rather than how frequently or under what circumstances message RNA is produced.

Cellular Regulation

If we turn from the subcellular to the cellular level of reproduction we are at once faced with a fact, too often ignored, that each cell has a history, and that whatever the modes and mechanisms of subcellular reproduction, cellular events occur in a characteristic pattern along the axis of time. Presumably, the information for temporal organization, just like that for spatial organization, is encoded in the hereditary apparatus. The challenge is not to prove the assumption, but to clarify the unfolding
of this temporal pattern. Studies of viral reproduction suggest that the reference axis for temporal behavior is in the axis of the DNA filament. Directional reading of a filament thus assures a sequential expression of events. Yet even if we accept as a certainty the occurrence of such a translation of temporal pattern, it is clear that the mechanism cannot be extended to the behavior of a whole cell, if for no other reason than the fact that cells may have large numbers of chromosomes, and in order to maintain total directional reading, gap messages would be required to trigger chromosomes sequentially. A sequence of chromosome readings is nevertheless open to question, since numerous translocations and inversions are known which do not have any marked effect on the developmental cycle of a cell. The possibilities for regulatory schemes in a system consisting of a linear template with a controllable starting point are unlimited, however, and a satisfactory temporal scheme could easily be designed out of DNA filament readings no matter how many discrete filaments we postulate for each cell.

Despite the fact that we can grossly ascribe to every cell an inherited temporal pattern of development, the pattern must be much more rigorously defined before it is subjected to meaningful study. The only operational value in ascribing the characteristic of temporal patterning to a cell, lies in being able to assert that events a,b,c,d, etc., must occur in sequence. Moreover, the order of such events must remain unaffected by environmental factors. We expect the environment to affect the rate of transformation, to interrupt it, or to initiate it, but not to change the order of occurrence. If we now ask ourselves which aspects of cell history have thus been adequately analyzed, the answer must be somewhat equivocal. The common target has been the “cell cycle” occurring in the sequence $G_1S G_2M$. There is no question that this cycle is common. Yet we know from some studies that the $S$ period may occur in the telophase of division, thus preceding the $G_1$. We also know that the common characteristics of the $G_1$ phase—protein and nucleic acid synthesis to the point of doubling the initial cell size of the daughter cell—may hardly occur in all even though a $G_1$ interval exists. In male gametogenesis in plants the microspores resulting from meiosis show little synthesis of nucleic acid or protein during an extended $G_1$ period, but form ribosomal RNA and protein only close to the time of DNA replication. And we already know from classical cytological studies that DNA synthesis need not be followed by mitosis.

Thus, if we examine the most thoroughly studied type of temporal pattern, the findings in support of a predetermined history are meager.
The only assertion we can make is that DNA synthesis must precede mitosis. Any of the other developments which apparently occur in a fixed sequence could be rationalized by assuming that one of the consequences of mitosis is an imbalance in the ratio of chromosomal to non-chromosomal components, and that such an imbalance is removed by the complex operation of feedback systems. The denial of a predetermined history to a system which repeatedly reproduces the same history may appear absurd, yet the absurdity may lie in our attempt to reveal a 1:1 correspondence between discrete physiological events and a linear tape. Perhaps the only linearly taped history is that of an operon as defined by Jacob and Monod.

We may relieve ourselves of the task of resolving the characteristics of a taped history, but we cannot relieve ourselves of the fact that each cell and each organism does have a finite history. If so, we must ask ourselves how such a history, whatever its motive source, is related to the problem of regulating cell reproduction. Clearly, no single cell goes through life by reading its chromosome filaments progressively. In a proliferating clone, a cell reads a fixed length of linear scripture and its daughters repeat the exercise. In a differentiated cell, a novel piece of text is read, but the end of the text does not coincide with the end of the cell. For a greater or lesser interval after the first reading, the cell eschews any new theology and retains the familiar text for re-reading. Furthermore, cells of all types have a common genetic text to provide for common requirements—energy transformation, carbon chain building, active transport, and mechanical work. How frequently a cell must consult its tape is a question which currently takes the form of a search for stable message. How readily a cell may be induced to transcribe a segment of its tape is a question which is now often framed in terms of the elicitation of RNA by hormones. Such studies may have overlooked the fact that the genetic tapes of cells at different developmental stages and in different developmental forms need not have identical responses to the same substance. The transcription of some segments may be open to stimulation; the transcription of others may not be. We do not know how the mechanisms of opening and closing operate, but we may hazard a prediction that such mechanisms involve the history of the cell. That history is embedded in an interaction between cell and environment, and the consequence of that history is to provide for a variety of cumulative changes within the cell. Such changes would be expected to filter environmental stimuli and hence affect the cellular response pattern.
The discussion of Königsberg points to the superfluity of certain profundities and reasserts the historic experience that the apparently trivial may be pregnant with profound implications. His principal target has been the differential reproduction of myoblasts, and he has sought to clarify the rules governing such reproduction. In seeking the pertinent rather than the profound, he has discovered, in common with others, that extracellular collagen may be a causative factor in differentiation. The line of communication between collagen and message may be long or short, but studies of this type point to the epistemological value of the clear fact as opposed to the hazy doctrine.

In a similar vein, one must take into account the provocative discussion of Kohn on the aging process. Some stress mutation of the template; others stress deterioration of the individual cell. Kohn stresses neither; his emphasis is on the organism which represents an integrated community of cells. Any process which interferes with the integrity of intercommunication is a potential factor in aging. Such interference may of course occur by virtue of the deterioration of certain cell types, brain cells, for example. Unequivocal evidence is not available, but Kohn does present some convincing evidence that the functional capacities of several cell types do not deteriorate with age and that the villain is a progressive alteration in collagenous material. A full reading of this text would lead to the conclusion that immortal organisms are stayed from immortality because their cells are mortal, and that immortal cells are stayed from immortality because their host organisms are mortal. The conclusion may offer little psychological comfort but it does afford intellectual stimulation.

We are still very much in need of some bold strokes which will sketch the capacities and limitations of the reproductive abilities of cells. We must be able to distinguish between flexibility of differentiated cells to adapt to varying environmental pressures and their flexibility to resume a novel life history. Ultimately, all the footnotes to the major phases of a cell's history will contain molecular explanations, but the process of learning is such that the history must be known before the footnotes are written. This symposium should tell us not only how well prepared we are for the footnotes, but also how well prepared the footnote writers are themselves.

These comments do little to reflect the alacrity of discussion at the symposium. The genesis of the alacrity must be attributed to a blend of circumstances: the personalities of speakers and chairmen, some per-
versely colorful, others poetically serious; the avid responses of the audience; and the ebullient hospitality of our host, Carleton College, which cemented social *gemütlichkeit* to intellectual venture. To all encompassed by these circumstances the Society expresses its deepest appreciation.