THE NINTH FREDERICK H. VERHOEFF LECTURE

THE LIFE HISTORY OF RETINAL CELLS*

BY *Richard W. Young*, PHD (BY INVITATION)

IT IS A GREAT HONOR TO HAVE BEEN GIVEN THE PRIVILEGE OF DELIVERING THE Frederick H. Verhoeff Lecture to the American Ophthalmological Society—the first national society to be devoted to a special branch of medicine in this country.[1] Doctor Verhoeff was a prominent member of this society for 63 years.[2] In this lectureship, initiated in 1961, it was envisioned that the speakers would be "animated by his ideals to the end that the name of Verhoeff will always be revered."[3]

The opportunity to present the Ninth Verhoeff Lecture represents a peak experience in my life in science, which now spans a quarter of a century, and thereby overlaps the period of Verhoeff's career. My theme—*the life history of retinal cells*—is a topic with a long tradition, and one that Verhoeff would certainly have found familiar. Nevertheless, I suspect that much of my presentation would have sounded strange to Verhoeff and his contemporaries. In the last 25 years we have lived through a revolution in biological science that has irrevocably changed the perspective with which we view and interpret the traditional problems. Important new theories, which did not exist in Verhoeff's day, have altered our concept of the nature of life itself.

I propose to describe the life history of retinal cells as consisting of three stages: a stage of *multiplication*, in which all of the cells repeatedly divide; a stage of *differentiation*, in which the homogeneous population is transformed into a complex network of heterogeneous cells; and a stage of *renewal*, in which nothing seems to change at all. The first stage is characterized by the primitive, repetitive mitotic cycle, which in the

*From the Department of Anatomy and Jules Stein Eye Institute, University of California, Los Angeles.

TR. AM. OPHTH. SOC. vol. LXXXI, 1983

retina takes place with a curious, back-and-forth "elevator movement." Next, the cells stop dividing forever, and embark upon a rebuilding process which creates an impressive variety of cells of strikingly different sizes and shapes. Finally, in the last and longest stage, the apparently stable structure of the mature retina proves to be in continual flux, with each cell engaged in an endless process of self-reconstruction.

STAGE OF MULTIPLICATION

The mature ovum accumulates beyond its own needs fundamental cell constituents (such as mitochondria and ribonucleic acid [RNA]) which can be used for the construction of other cells. Deoxyribonucleic acid (DNA) is not stored, however, so that when mitosis is activated by fertilization, the egg undergoes a series of rapid divisions, separated by intervals only long enough to permit the replication of the genetic material.[4-7] During this process, the cells progressively diminish in size, although each retains a full complement of chromosomes (Fig 1). The rate of cell division soon decelerates, and within a few days the size of new cells becomes stabilized. The expanding population then gradually arranges itself in various sheets and masses through a series of "morphogenetic movements," but the metabolism of each cell continues to be devoted primarily to self-reproduction.

In the anterior part of the primitive embryo, the cells form a tube, the future forebrain, from which two stalks emerge.[8] These invaginate to form cup-shaped structures (Figs 2 and 3) in which the inner layer represents the future neural retina (Figs 3 and 4). It consists entirely of elongated ventricular cells[9] aligned in parallel, and capped by a single row of spherical cells undergoing mitosis. This is not a layer of specialized "stem cells." On the contrary, all of the cells are homogeneous and proliferating.[10-13] In a peculiar process typical of the developing central nervous system, the cells shift position as they traverse the cell-division cycle. A cell newly formed by mitosis in the outer layer elongates until its inner cytoplasmic process reaches the vitreal surface and its nucleus is situated nearby. The opposite cytoplasmic strand remains permanently attached to adjacent cells at the ventricular surface. Next, the chromosomal DNA is duplicated, the cell detaches its inner cytoplasmic process, and gradually contracts itself into a sphere, progressively pulling its nucleus to the ventricular edge, where the cell undergoes mitosis. The two daughter cells then elongate, and the process begins anew (Figs 5 to 7).[14-18]

All of the ventricular cells are engaged in the same repetitive, cell-division cycle, but they are not synchronized. All phases of the cycle are in

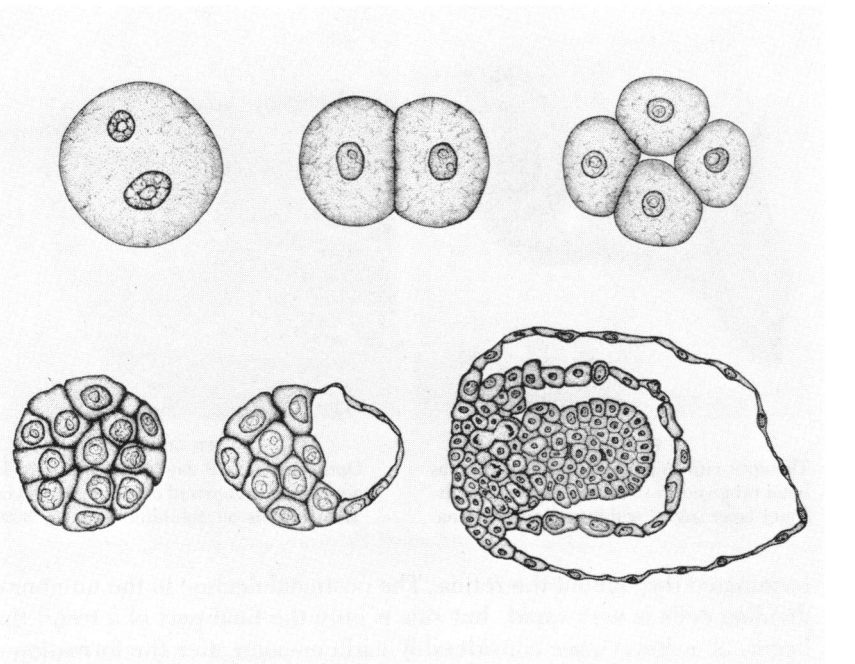

FIGURE 1
Early stages in the development of mouse embryo from fertilized ovum (upper left) to gastrula (lower right). Note progressive reduction of cell size. All cells are mitotically active. (Drawing modified from Rugh, 1968.[5])

progress simultaneously in different cells and in different layers: the population of homogeneous cells is metabolically stratified (Fig 7).

As the number of cells increases (Fig 8), the back-and-forth movement gradually slows. In the embryonic mouse at 7 days, the duration of the total mitotic cycle in ectoderm is 6 hours.[19] By 10 to 11 days, a new generation of cells is produced in the retina every 9 to 10 hours.[20,21] In the 15-day embryo, 20 hours elapse between mitotic divisions of retinal ventricular cells.[22] Five days later (1 day after birth), the cell generation time is 30 hours.[23] The progressive increase in duration of all phases of the cell-division cycle (Fig 9) appears to be a general rule in embryonic tissues.[19,24-26]

Ever more slowly the cells traverse the increasingly thicker layers of nuclei until a point is reached, determined separately in each cell, when mitosis ceases. In the mouse, cell division stops entirely in the center of the retina on about the sixth postnatal day; within 4 more days it has

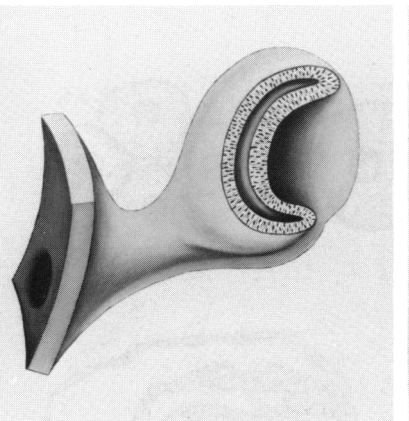

FIGURE 2
The optic cup. A portion of side of cup has been removed to show two layers of cells. Inner layer (right) will form neural retina.

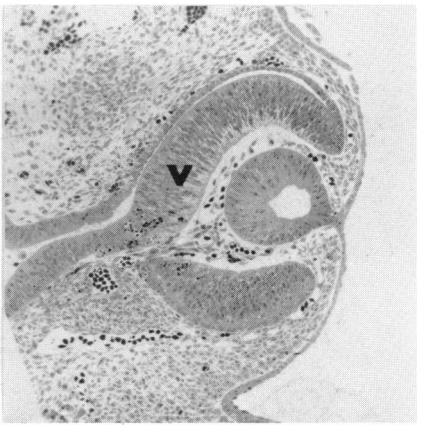

FIGURE 3
Optic cup in a 12-day mouse embryo. Inner layer is comprised of proliferating ventricular cells (v) (toluidine blue, × 850).

terminated throughout the retina. The postnatal decline in the number of dividing cells is very rapid, but this is only the final part of a trend that begins at a slower pace considerably earlier—soon after the formation of the optic cup.[27,28] Ultimately, not a single mitotically-active ventricular cell remains. The process of mitosis which completely dominated the metabolism of the cell lineage from the very onset of embryological development ceases forever. This marks the end of the stage of multiplication.

STAGE OF DIFFERENTIATION

The stage of differentiation overlaps the stage of multiplication, but in the life history of each cell, mitosis and differentiation are mutually exclusive. The first readily visible sign of the onset of differentiation is the appearance along the inner surface of the presumptive retina of nuclei that are larger and paler than those of the ventricular cells (Fig 10). In the mouse, these cells—future ganglion cells—begin overtly to differentiate in the center of the retina at about the 13th day of embryonic development. The process continues for several days, gradually progressing toward the periphery. While this is taking place, other types of retinal cells also begin to differentiate.

Soon after ganglion cells are first discernible, nuclei situated on the inner edge of the ventricular layer begin to take on pale-staining charac-

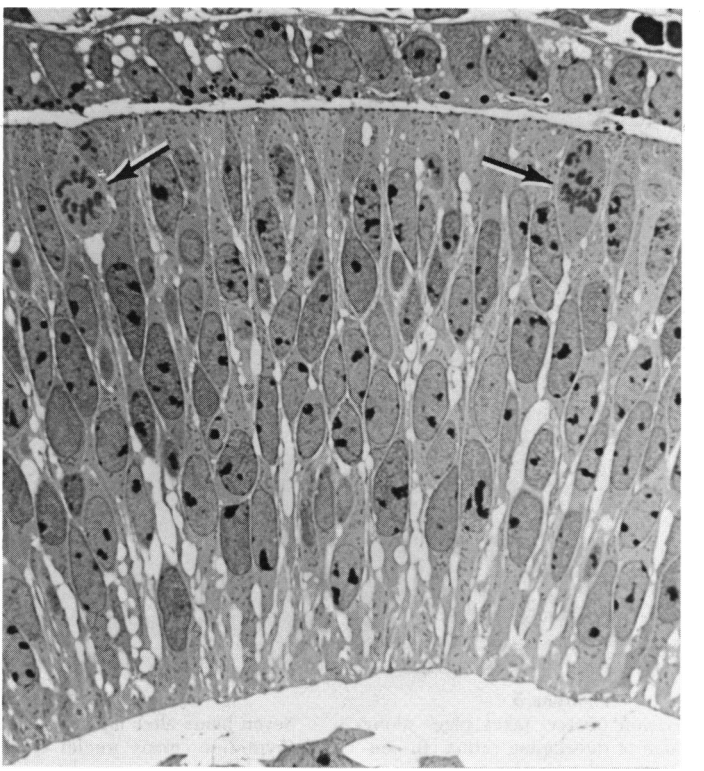

FIGURE 4
Ventricular cells in 12-day mouse embryo optic cup. Cells undergoing mitosis (arrows) are situated in outer layer. Uppermost is single layer of pigment epithelial cells (toluidine blue, × 850).

teristics which signal the onset of amacrine cell differentiation (Fig 11). The recruitment of amacrine cells from the ventricular cell population also continues for an extended period, overlapping the onset of visible differentiation not only in ganglion cells, but also of horizontal cells, which in the mouse are clearly evident at the time of birth (Fig 11). At first in the center of the retina (4 to 8 days after birth), then spreading to the periphery, the remaining specialized cells of the neural retina begin to appear. Practically simultaneously, rods, cones, bipolars and Müller cells can suddenly be distinguished (Figs 12 and 13). Differentiation, like the cessation of mitosis, begins gradually and finishes rapidly.

Although in each species of vertebrate animal there is a consistent sequence in which different kinds of cells appear, there are some differ-

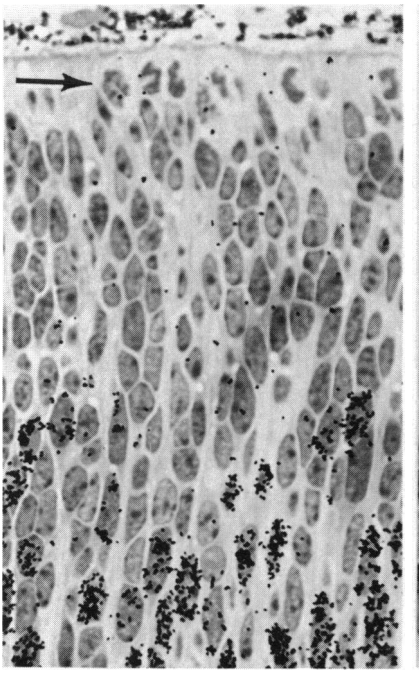

FIGURE 5
Cell division (arrow) takes place along outer edge of developing retina. In contrast, DNA synthesis prior to mitosis occurs in nuclei situated deep within population of ventricular cells, as shown by presence of labeled cells in 1-day-old mouse 45 minutes after injection with radioactive thymidine (a specific DNA precursor) (autoradiogram, toluidine blue, × 850).

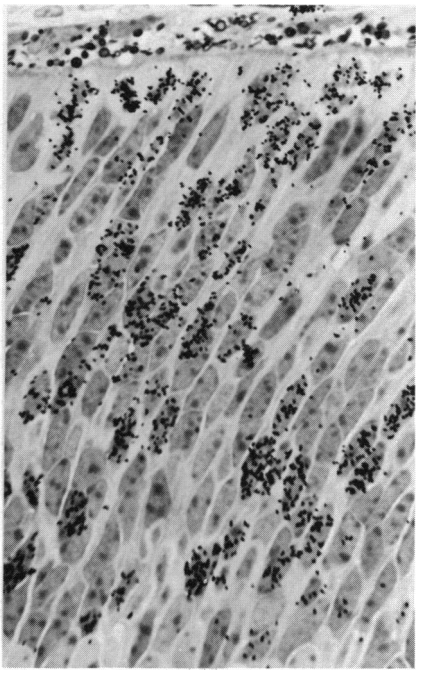

FIGURE 6
Seven hours after injection of radioactive thymidine, many nuclei containing labeled DNA have moved to outer edge of retina and are undergoing mitosis (autoradiogram, toluidine blue, × 850).

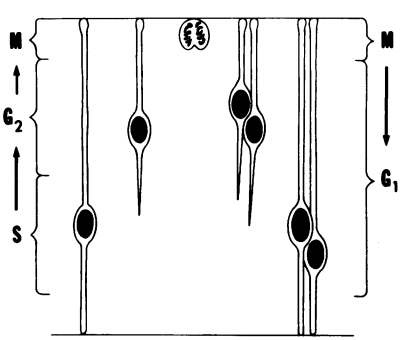

FIGURE 7
Diagram showing back-and-forth movement of ventricular cells during mitotic cycle. After completing DNA synthesis (S), cells migrate outward (G_2) and contract into a spherical shape for mitosis (M). Next, in G_1 phase, cells elongate, and their nuclei return to inner layers of developing retina.

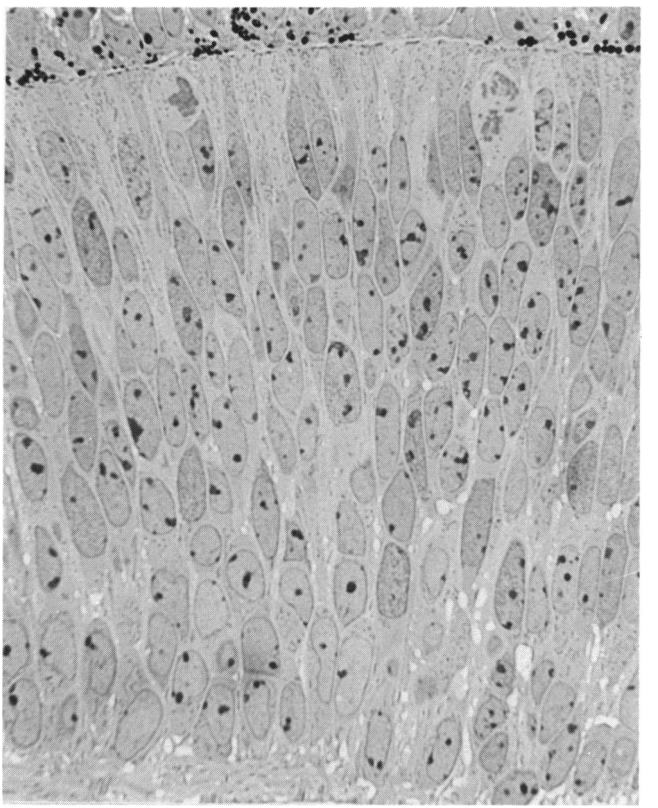

FIGURE 8
Thirteen-day mouse embryo, presumptive neural retina. Note increased number of ventricular cells (compare with Fig 4). There is as yet no visible cell differentiation (toluidine blue, × 850).

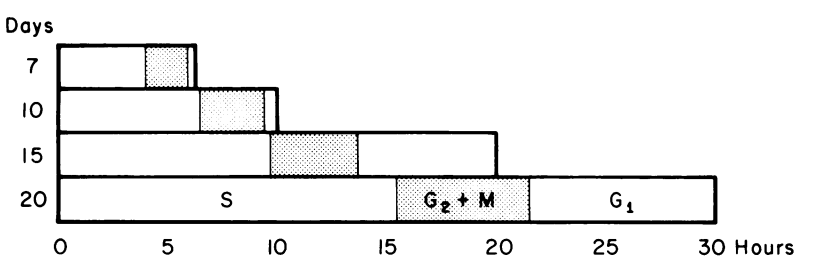

FIGURE 9
Pace of cell-division cycle grows progressively slower during embryological development of mouse retina.

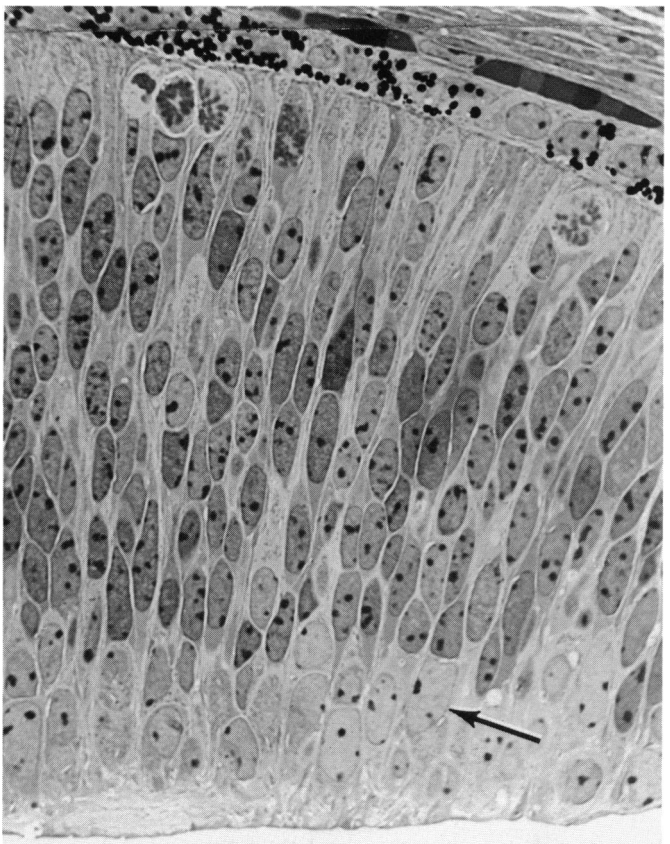

FIGURE 10
Appearance of a layer of larger, paler-staining nuclei (presumptive ganglion cells) on inner surface of ventricular cell population is first visible sign of cell differentiation (14-day mouse embryo, toluidine blue, × 850).

ences in sequence between species. In all cases, differentiation of several cell-types occurs concurrently among groups of ventricular cells which synthesized DNA and completed their final mitotic division at essentially the same time (Figs 14 and 15).[13,29,30] Even the two daughter cells produced at the final mitosis may differentiate in diverse ways—for example, one becoming a ganglion cell, the other a horizontal cell.[29] This indicates that the crucial event determining the fate of the ventricular cell occurs after the final mitosis; that is, in the G_1 period of the mitotic cycle (Figs 7 and 9). It is in this phase that terminal differentiation occurs in the neural retina, as it does in other kinds of cells.[31-33]

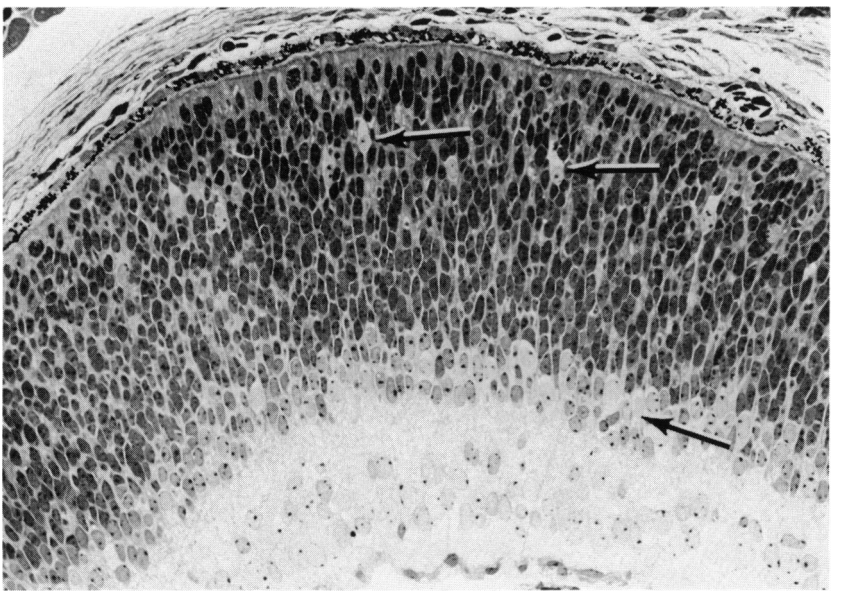

FIGURE 11
Newborn mouse. Although mitosis of ventricular cells continues, horizontal cells (upper arrows) and amacrine cells (lower arrow) can now be distinguished. Ganglion cells form a separate layer (below) (toluidine blue, × 330).

The retinal ventricular cell evidently is capable of several, mutually exclusive pathways of differentiation. When one course is initiated, all others are suppressed. No intermediate mixtures or cell chimaeras are produced. Furthermore, the differentiation is perfectly stable. For the retinal cell, it is an irreversible, once-in-a-lifetime event.

Cell differentiation involves a complete restructuring of nucleus and cytoplasm alike. Visual cell outer segments, ganglion cell axons, a variety of synaptic interconnections, all must be constructed where none existed previously. In rats and mice this takes about 1 month to complete (Fig 16). In the human retina 1 year is required (although visual function is established before then, and further expansion of the eye may stretch the retina, thinning it slightly).[34-36] Thereafter, no further alterations of any kind are apparent.

Two brief episodes, involving profound and rapid changes of a different nature, are followed by a much longer period (Fig 17) in which nothing seems to change at all. This is the stage of renewal.

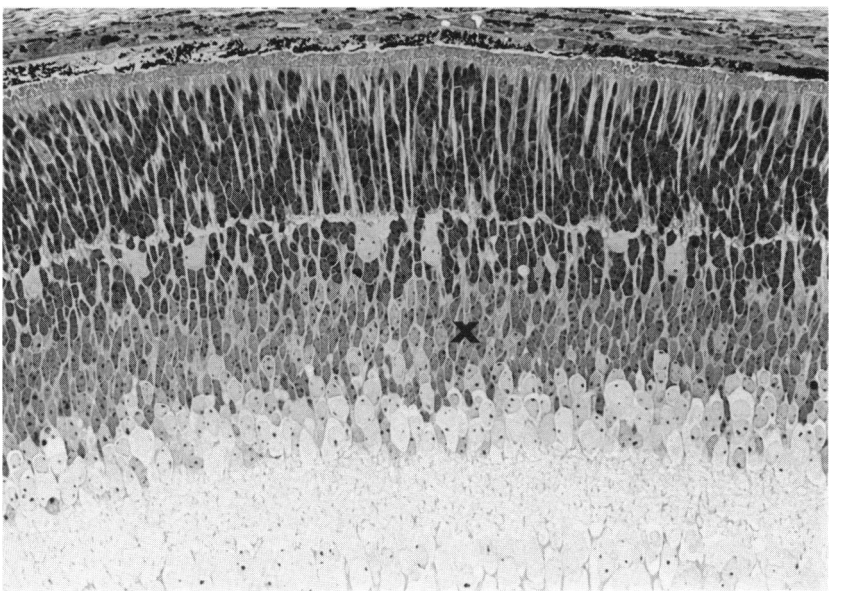

FIGURE 12
Six days after birth in mouse, stage of multiplication has terminated in center of retina and cell differentiation is proceeding rapidly. Smaller, dark-stained rod nuclei (outer layers) can be distinguished from paler nuclei of bipolar and Müller cells (x) (toluidine blue, × 330).

STAGE OF RENEWAL

For many years it was thought that the major metabolic activity of retinal cells was concerned with *vision*, and consisted in the intermittent, transient rearrangements of a restricted set of molecules and ions, associated with the bleaching of rhodopsin and the transmission of nerve impulses, consequent to the absorption of radiant energy in rod or cone outer

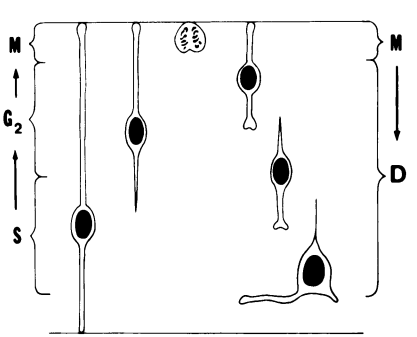

FIGURE 13
Following their final mitotic division, offspring of ventricular cells undergo differentiation (D).

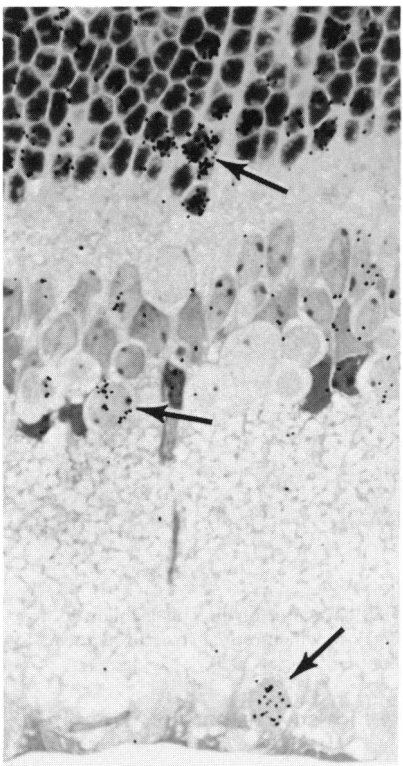

FIGURE 14
Differentiation of different kinds of cells takes place concurrently. Ventricular cells whose DNA was being replicated at time of injection of radioactive thymidine in 1-day-old mouse subsequently differentiated as rods (upper arrow), amacrine cells (middle arrow), and ganglion cells (lower arrow) (autoradiogram, toluidine blue, × 850).

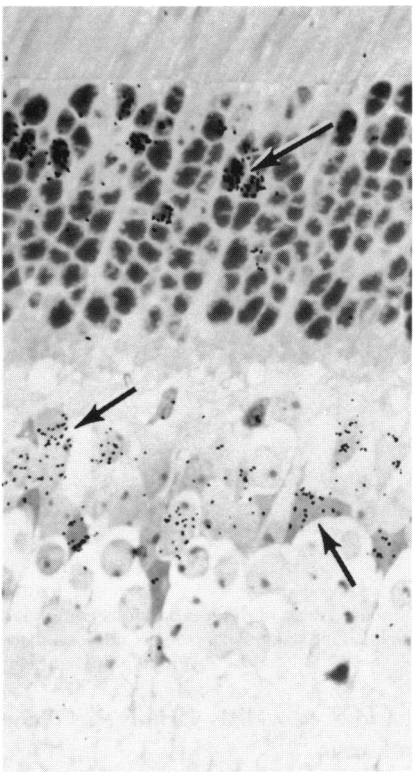

FIGURE 15
Ventricular cells exposed to radioactive thymidine during DNA-synthesis phase of mitotic cycle in 5-day-old mouse were subsequently transformed into rods (upper arrow), bipolar cells (middle arrow), and Müller cells (lower arrow) (autoradiogram, toluidine blue, × 850).

segments. It now seems that these transitory chemical changes are a "sideline" to the main metabolic activity of retinal cells, which is *incessant self-reconstruction*.[37,38] Dating from early studies which demonstrated "axonal flow" in ganglion cells[39] and the renewal of outer segments in rod visual cells,[40] the concept that retinal cells of all kinds are continuously engaged in the rebuilding of their own molecular structure was gradually developed from studies which documented the renewal of RNA, protein, phospholipid, glycolipid, gangliosides, glycoprotein, and mucopolysac-

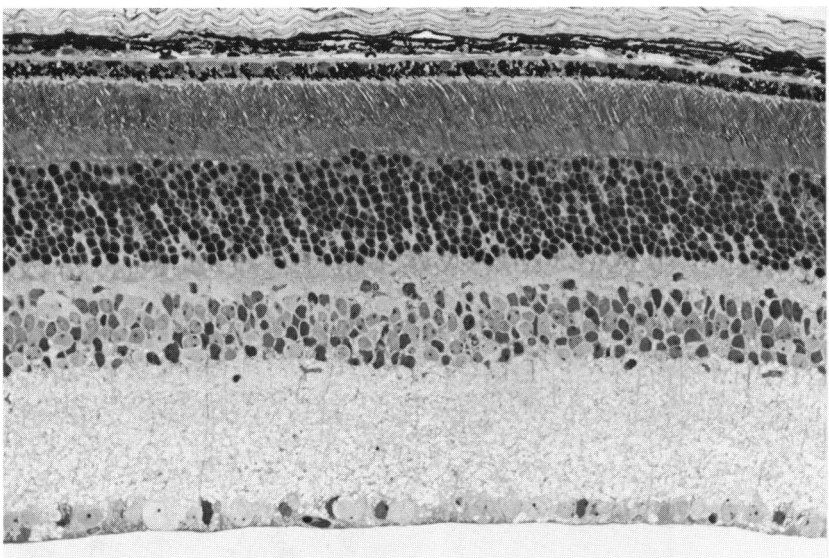

FIGURE 16
In mouse, stage of differentiation is completed by 30 days after birth. This is followed by a much longer stage of continuous intracellular renewal, in which retinal structure appears to be essentially unchanging (toluidine blue, × 330).

charides, as well as various specific molecules such as taurine, vitamin A, and opsin.[38]

The lifetimes of the molecular constituents are impressively brief. For retinal proteins, the average half-life (the period during which half the molecules are replaced) is about 5 days,[41,42] but there is a considerable range, from a few hours to several weeks (Table I). New cell constituents are continuously produced in synthetic centers near the nucleus (except for RNA, which arises in the nucleus itself), and are then distributed to sites of utilization throughout the cell. For ganglion cells, with a complex

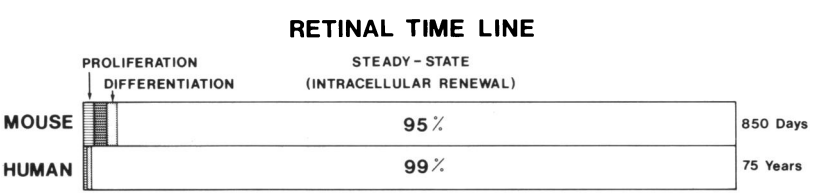

FIGURE 17
Retinal time line illustrates that most of the life history of retinal cells is spent in stage of intracellular renewal, a steady-state in which molecular synthesis and degradation are in balance.

TABLE I: RENEWAL IN THE RETINA*

COMPONENT	SPECIES	HALF-LIFE
		(days)
Retinal protein	Rabbit	5-6
Ganglion cell axon-terminal components	Various	1
		RENEWAL TIME
		(days)
Rod outer segment integral proteins	Pigeon	3-4
	Mouse	10
	Rat	10-14
	Squirrel	5
	Dog	6
	Monkey	9-13
Ganglion cell axoplasmic framework	Mouse	3-4
	Monkey	30

*Adapted from Young (1982).[38]

arborization of dendrites, and an axon that may extend for 90,000 μ, replacement of cell constituents presents formidable logistical problems. These are met by complex systems of intracellular transport. The major component of the axon, a framework of microtubules, neurofilaments, and microtrabeculae, forms a cohesive column, extending from cell body to axon terminal, which moves outward at the rate of 1 mm per day. This framework is relentlessly assembled at the entrance to the axon and disassembled at the entrance to the axon terminal. Other axonal constituents travel rapidly from the cell body, moving along the surface of the axonal framework, and arrive in the axon terminal within a few hours after their synthesis. Molecular lifetimes in the terminals seldom exceed 1 or 2 days. Some molecules are destroyed in the terminals; others are returned to the cell body for destruction.[38]

Visual cells similarly produce the molecules of which they are composed at a prodigious rate in the mature retina. In rods, the entire outer segment, consisting of a stack of up to 1000 double-membrane discs, is completely replaced within 2 weeks or less (Table I). Neither rods nor cones are capable of destroying the membranes which they unrelentingly assemble. Instead, groups of discs are periodically detached from the tips of the cell. The shed discs are then phagocytosed and destroyed by the pigment epithelium (Fig 18).[43,44] The net result, however, is that the membranes are destroyed just as rapidly as they are assembled.

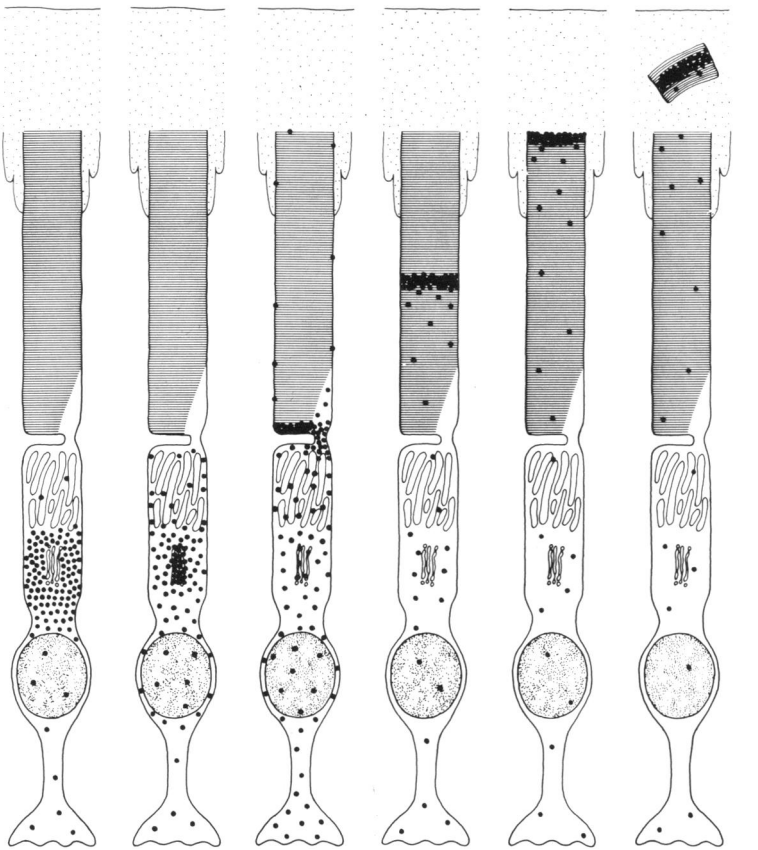

FIGURE 18
Diagram illustrating mechanism of renewal of rod outer segment membranes. Pigment epithelium phagocytizes and destroys membranes detached from ends of cell. Although rods and cones do not degrade their own photosensitive membranes, net result is that membranes are destroyed as rapidly as they are produced (from Young, 1976[37]).

Of all the constituents of differentiated retinal cells, only one has so far been shown to be spared from incessant replacement: DNA. The absence of renewal of DNA[45-48] is readily demonstrated in retinal cells. If the DNA is labeled in ventricular cells by providing them with radioactive thymidine as they replicate their genetic molecules just prior to their final mitosis, the radioactive constituents are retained in the DNA for the remainder of the cell's lifetime; the atomic bonding pattern is stable (Figs 15 and 16). We now have evidence of another class of stable molecule in retinal cells. If the same experiment is carried out with a radioactive

amino acid, all of the proteins undergoing synthesis become radioactive, in nucleus and cytoplasm alike. With the passage of time, however, the labeled proteins gradually disappear, due to incessant replacement, *except* for some of the nuclear proteins in certain retinal cells (Fig 19). We have tentatively identified the stable nuclear proteins as histones.

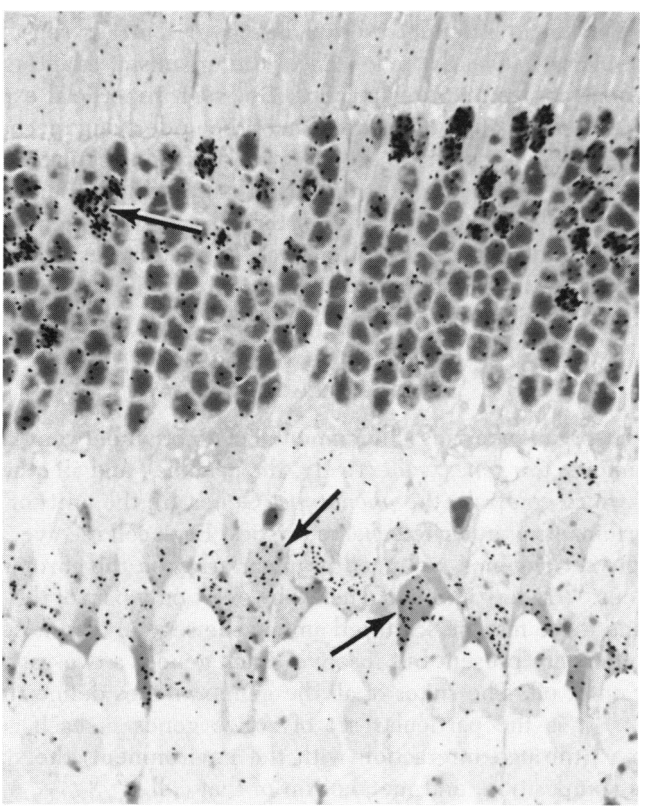

FIGURE 19

Retinal cells contain nuclear proteins that apparently are not renewed. This experiment is comparable to that depicted in Fig 15, except that mouse was injected with radioactive arginine (a protein-precursor), instead of DNA-precursor, thymidine. Proteins which have not been replaced during a 45-day interval are situated in nuclei of certain rods (upper arrow), bipolar cells (middle arrow), and Müller cells (lower arrow). Stable proteins are believed to be histones (autoradiogram, toluidine blue, × 850).

THEORETICAL EXPLANATIONS

The three stages of the life history of retinal cells are in the category of "empirical laws"—regularities of natural processes that can be observed, measured and recorded. An ultimate goal of science is to be able to *explain* such regularities. It is the role of scientific theories to account for and predict groups of related empirical laws, and to provide a deeper understanding of them, by demonstrating that they are manifestations of more fundamental and general principles.

Generating rapidly a large population of retinal cells, and then converting it into a heterogeneous network capable of serving as a functioning sense organ for an extended period intuitively "makes sense." Such a course of events is clearly beneficial to the organism, adapting it to the environment, and promoting survival. But such superficial explanations do little to enhance our comprehension of the underlying mechanisms by which these cellular activities are accomplished and regulated.

It is now my purpose to illustrate that a more profound understanding may be derived from three biological theories: developmental genetics, molecular biology, and biological renewal. These theories, which are fully consistent with one another, focus attention on different aspects of the complex biological process.

DEVELOPMENTAL GENETICS

Genetic theory emphasizes a fundamental distinction between the hereditary elements, the *genes* (collectively, the *genome*), and all other parts of the cell, which comprise the *phenotype*. Genes are the units of heredity, the determinants of inherited characteristics. Each cell receives at mitosis a complete set of genes, arranged in linear array on the chromosomes in the nucleus.[5-7] In any individual cell, however, only part of the genes are active, and these may be scattered among the several chromosomes.[49,50] All the others are present but inactive. Each gene is a two-way switch; it is either on or off. The states of all the gene-switches define the state of the cell.[51] It is the particular set of active genes in each cell which determines (through interaction with the environment) the size, shape, chemical composition, and metabolism of that cell.[5-7,50-53]

Cell differentiation is discrete, without overlapping, intermediate types, because there are a limited number of integrated gene-sets which may be activated, and these produce distinctly different phenotypes. However, some cell constituents and activities are common to different kinds of cells. Thus, certain genes may be active in more than one type of cell.[50]

When a cell changes from one differentiated state to another, it is the result of a new set of genes becoming active, while some (or all) of the precedingly active set has been repressed. Genetic regulation is therefore the source of the distinctly different cellular activities which characterize the three stages in the life of retinal cells. What produces the changes in the gene-sets that are active?

Gene regulation is responsive to factors in the environment, including the cell's own cytoplasm, as shown by nuclear transplantation and cell fusion experiments.[6,7,54-56] In the fertilized egg, there is an asymmetric distribution of various cytoplasmic factors. These are unequally dispersed among the offspring of the first few cell divisions, leading to a differential activation of genes, and the first signs of cell differentiation.[5-7,52] The subsequent course of embryogenesis involves a progressively unfolding series of differentiations, which are attributed to *induction*, in which the differentiation of a particular cell-type requires the action of a factor produced by another kind of cell.[5,6,52,57] Inducers may be transferred from cell to cell by direct contact, or by transport from a distant site. Interaction with an appropriate inducer results in a change in the set of genes that are active in the induced cell, thereby changing its state of differentiation.[58,59] There is no correlation between the nature of the inducer and the kind of cell differentiation produced. Induction acts as a non-specific cue, triggering a sequence of events specific to the reacting cell.[60]

Cells respond to signals only during some particular time interval, called the period of *competence*.[6,61] A cell is (or is not) competent to respond to an inducer by virtue of its phenotypic composition, which is the result of an earlier inductive event.

How can a non-specific inducer bring about the coordinated activation and repression of numerous genes? To account for this, the theory postulates the existence of different *categories* of genes. Some genes are the determinants for special cell characteristics, whereas other genes are of a regulatory nature. "Integrator genes," relatively few in number, would respond to appropriate inducers by producing factors that would activate the gene-set controlled by that particular regulatory gene.[49,51,62] A major genetic event, termed "commitment," occurs when the inducer initiates the coordinated activation of a set of genes.[49,63,64] Continued presence of the inducer is not required after the cell has become committed to a particular course of differentiation, because the new phenotype includes a factor that replaces the inducer, thereby sustaining the differentiated state. Once activated, the genes could remain "turned on" indefinitely. The stability of the differentiated state is variable, being dependent upon

whether the cell is competent to respond to inducers it may encounter.

These principles help to explain the stages in the life history of retinal cells: In the stage of multiplication, the genes that are active determine the operation of the mitotic cycle. The phenotype of the ventricular cells also includes elements which render them competent to respond to a particular group of inducers. Although for simplicity and emphasis I have suggested that there is a single event of cell differentiation in the life history of retinal cells, there must be some rearrangements of the pattern of active genes prior to the appearance of the ventricular cells to account for their elevator movements as well as their limited range of competence. (Some differentiation may be detected very early in embryologic development, even prior to implantation in the uterus.)[65-68] Perhaps during their encounter with the presumptive lens or the outer layer of the optic cup they become competent to respond to inducers which activate the gene-sets responsible for the several types of cells in the neural retina.

In the developing retina, there is no ingrowth of axons or neurons arising outside the organ, and the invasion of vascular elements (in species where it occurs) takes place too late to serve as the source of inductive stimuli. Consequently, the mechanism of differentiation appears to be intrinsic to the retina. After formation of the optic cup, the ventricular cells are bounded on the ventricular surface by the pigment epithelium, and on the opposite surface by the vascularized, embryonic vitreous body. There must be chemical differences between these two regions. This might account for the inductions of the ganglion and amacrine cells from ventricular cells whose nuclei have come to be situated adjacent to the vitreal border. Due to the back-and-forth motion of the ventricular cells, each nucleus passes through a continuously changing microenvironment, stratified both structurally and metabolically. There appear to be abundant opportunities for inductive interactions which increase in complexity as cell differentiation progresses.

The cells move asynchronously, traversing the cycle at slightly different rates. Perhaps individual cells pause momentarily at one level or another, thereby becoming sufficiently exposed to regionally concentrated inducers which trigger one or the other of the differentiated states for which the cell is competent. The progressive slowing of the elevator movement which accompanies retinal differentiation may enhance this effect, accelerating the pace of differentiation and the cessation of mitosis as this stage draws to a close.

The incessant self-rebuilding (without change in the state of differentiation) that characterizes the stage of renewal can be accounted for by

assuming that the set of genes underlying the differentiation of each kind of cell in the neural retina remains "turned on" permanently. Cell differentiation in the mature retina is presumed to be stable because the cells are incompetent to respond to any inducing agents.

MOLECULAR BIOLOGY

The core principle of this theory is simple and grand: *All of life is but the interaction of molecules.* The theory is supported by the entire theoretical structure of physics and chemistry, since it explicitly states that the chemical reactions within living systems occur in exactly the same manner as they do in systems that are not living, determined by the principle of entropy and the quantum rules of atomic structure and bonding.[69,70] Nevertheless, chemical reactions in living systems appear to be uniquely goal-directed. This is attributed to the principle of *molecular recognition.*

Within cells, molecules in thousands of different forms incessantly collide with each other in random orientations. Most collisions are nonproductive, but some elicit changes that are exceedingly specific. The specificity results from molecular recognition, which is based upon the surface properties of one molecule matching in a reciprocal manner the surface properties of another.[69,70] The relatively enormous size of proteins (several thousand atoms) provides the opportunity for endless variations in their surface characteristics. Certain "active sites" have a specific size, shape, and distribution of electrical charge to which only a molecule with reciprocally matching properties will adhere. By this means, proteins can "select" a particular molecule from a large and heterogeneous mixture. Molecular recognition determines a wide range of metabolic activities, including self-assembly of molecular aggregates, the action of enzymes, specificity of receptors, and the recognition of specific loci in the DNA.

A protein changes shape when another molecule becomes bound to it. In the case of enzymes, the change in conformation may deform an atomic bond in the bound molecule until it ruptures, or it may displace two bound molecules into a position favoring bond formation. When there are two (or more) binding sites on a protein, adhesion of a molecule at one site may change the protein conformation so that another site is rendered either inactive or active, depending on whether or not it will now bind another molecule.[71-73] DNA and RNA may also interact by a form of molecular recognition called *base-pairing.*

Genes are considered to be segments of DNA molecules that are repositories of "information" which specifies the structure for RNA, which

in turn may specify the structure of proteins. The information in DNA is determined by the sequence of its purine and pyrimidine bases, which may comprise a code for the amino acid sequence of a particular protein. This information can be released a limitless number of times by a base-pairing mechanism which transfers the coded instructions from DNA to RNA. Sets of instructions transferred from nucleus to cytoplasm through the intermediary of messenger RNA (mRNA) molecules lead to the formation of sets of specific proteins. These proteins, in turn, control the entire metabolism of the cell. DNA and RNA are passive and inert. They are manipulated by proteins, guided by the principle of molecular recognition.

Evidently, if we are to understand the three stages in the life history of retinal cells, we must elucidate the molecular processes which regulate the release of coordinated sets of information stored in the structure of DNA. We must concentrate our attention on the metabolism of the nucleus, where the genes are sequestered.

In the special chemical environment enveloped by the nuclear membrane, lipids and carbohydrates are excluded, and essentially all of the metabolism is concerned with the regulated production of RNA molecules (interrupted in mitotically active cells by intermittent intervals in which the genetic material is replicated). More than 450 different kinds of proteins have been detected in the nucleus.[74] These comprise a small group present in large amounts, the *histones*, and the *non-histone proteins* which include all the nuclear enzymes.

The long threads of the DNA double helix are associated with histones in the manner depicted in Fig 20. Two each of four different histone species are assembled into tiny octameric structures to form the *nucleosome core particle*. The DNA is wrapped twice around the outside of the particle and is sealed at its entrance and exit by a single molecule of the fifth histone variety, the *linker* histone.[75-77] The particles are spaced with a periodicity of about 200 pairs of DNA bases. This repeating structure comprises the *nucleosome*.[75,78-80] All of the DNA is ordered into nucleosomes.[81-83] Linker and core histones isolated from retinal cells are shown in Fig 21.

In the nucleus of each human diploid cell, the total length of DNA, expressed as the number of constituent bases, is 5.6 billion,[84] ordered into 28 million nucleosomes. Parts of this are greatly compacted, largely due to the interactions of the linker histones, forming dense masses of "heterochromatin," which are inaccessible to the enzymes required to release its genetic information.[85,86] Formation of heterochromatin is pri-

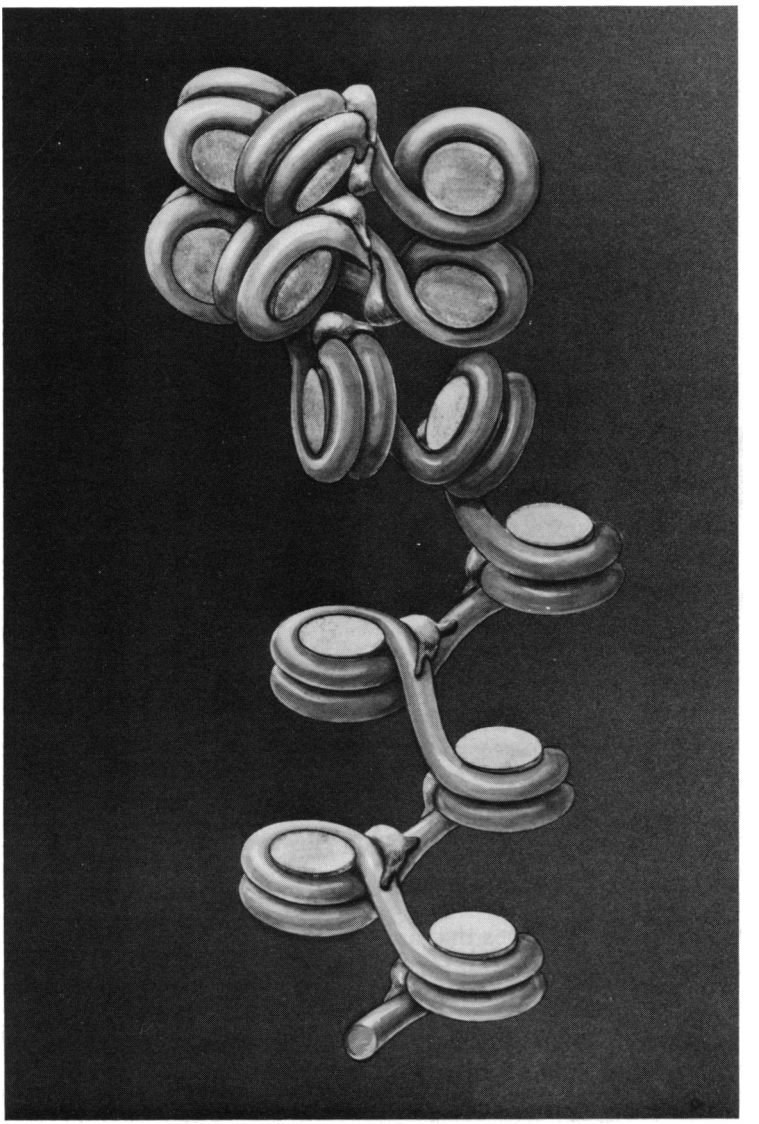

FIGURE 20
Model of nucleosome structure of DNA. Two each of four different histone proteins form a disc-shaped core, around which DNA makes two complete turns. DNA is sealed around core by a fifth type of protein, linker histone. This combined structure of DNA and histone, termed "nucleosome," occurs throughout all of DNA, repeated at regularly spaced intervals. Inactive regions of genome may be compacted into dense masses due to interactions of linker histones (top of drawing).

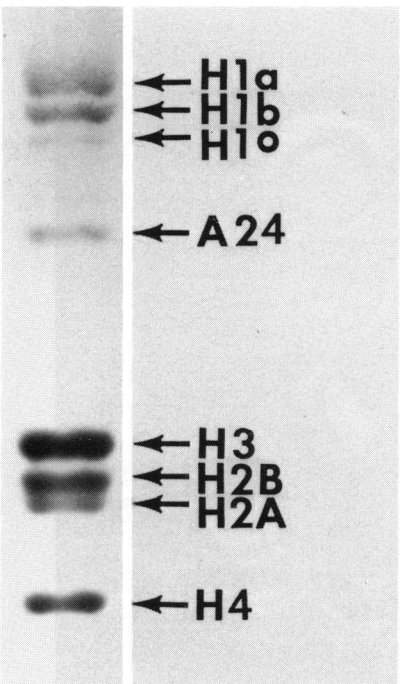

FIGURE 21
Histone proteins isolated from retinal nuclei in adult mouse. H1a, H1b, and H1° are linker histones. A24 is a modified core histone (H2A covalently bound to ubiquitin). H3, H2B, H2A, and H4 are nucleosome core histones. (Preparation by Dr TR LeBon.)

marily concerned with the packaging of unused parts of the genome after a particular set of genes has been activated.

The number of protein-coding genes in the human genome is estimated to be between 30,000 and 100,000.[87-91] However, there is enough DNA to code for over 2 million genes—20 times the maximum estimate! Evidently, much of the DNA does not code for protein. Nearly half of the DNA consists of sequences that are repeated between a thousand and a million times,[91] yet the vast majority of protein-coding genes exist in only one copy.[84,92] Some of the repeated sequences are believed to have a structural role,[93-95] and others may determine the boundaries of replication units,[31,96] or mediate circadian rhythms. Of primary interest here are the reiterated sequences, commonly a few hundred bases in length, interspersed with longer, single-copy sequences. It seems likely that these constitute gene regulatory sites.[97]

There are a few thousand protein-coding genes active in each cell, and these exist as unique copies, dispersed among the chromosomes. Some way of coordinating this dispersed cluster must exist. Some marker sequence associated with each protein-coding gene must identify it as be-

longing to the set "cone" or "Müller cell." This sequence must be repeated a few thousand times, once for each gene belonging to the set. This is also the case for each of the other distinct cell types of which our bodies are comprised. Since many genes may be activated in more than one kind of cell, each protein-coding gene may be associated with many repetitive (cell-type marker) coding regions. In fact, analysis of mRNA populations in different kinds of cells reveals the presence of overlapping sets, with as much as 80% of the mRNA being shared by some tissues.[98-101]

Even the simplest protein-coding gene is a very complex unit, interrupted by several stretches of DNA that are not part of the genetic message—a mosaic of expressed sequences in a matrix of silent DNA.[102] Most of the untranslated sequences—repetitive and non-repetitive alike—are extirpated from the gene transcript before it is released to the cytoplasm.[101,103-105] The heterogeneous nuclear RNA (hnRNA) consists of the precursors of mRNAs at different stages of post-transcriptional processing. This is summarized in Fig 22. The entire gene is transcribed into a single-stranded ribbon of hnRNA. This primary transcript may be as much as six or seven times longer than the contained segments that are ultimately translated into amino acid sequences.[106,107] Cutting and splic-

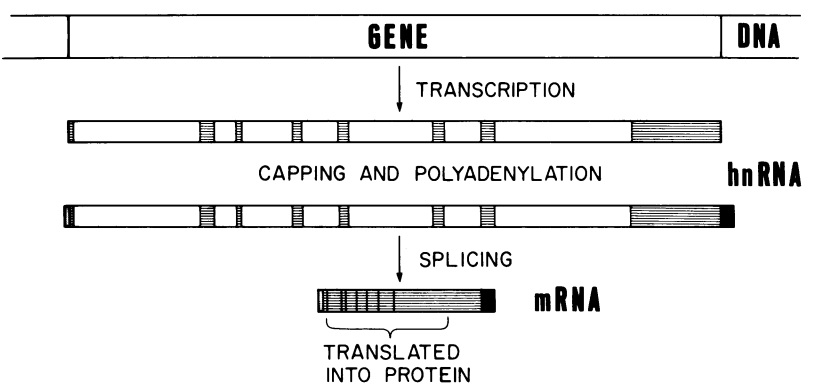

FIGURE 22

Protein-coding genes are complex structures which contain large segments of DNA that are not translated into protein structure. Heterogeneous nuclear RNA (hnRNA) consists of primary gene transcripts in various stages of post-transcriptional modification, during which nuclear enzymes extirpate many of "silent" regions before RNA is transported to cytoplasm as messenger RNA (mRNA). Note that not all of mRNA is translated. (Figure modified from Chambon, 1981[106]).

ing enzymes then excise the silent sequences and join the coding sequences.[102,106,107] Even then, as much as 40% of the mRNA is not translated into protein structure.

The production of primary gene transcripts from active genes takes place in regions of the genome that are in the diffuse, uncompacted state called "euchromatin." It is here that the non-histone proteins are concentrated.[108-110] The mechanism by which the RNA polymerase molecule may gain access to the coded sequence of bases in the DNA is indicated in Fig 23. Electrostatic bonds between the negatively charged phosphate groups of the DNA and the positively charged core histones are temporarily broken by an enzyme that adds acetate groups to the histones.[111-115] This permits uncoupling of the double helix from the nucleosome core. The linker histone is detached,[91,116-118] permitting the further loosening and unwinding of the DNA. The core splits into two parts, and each half remains attached to one strand of the double helix.[119-122] Other enzymes conspire to uncoil the double helix and separate the two strands. Meanwhile, an RNA polymerase molecule initiates transcription at a site called the "promoter," then moves along one strand of the DNA, gradually traversing the gene, polymerizing nucleotides into an increasingly long ribbon of hnRNA, which reproduces the coded sequence in the DNA. Immediately after passage of the polymerase molecule, the double helix is reformed, the half-nucleosomes rejoin to form a complete nucleosome core, the acetate groups are enzymatically removed, the DNA winds itself around the core, and the two turns are resealed by the histone linker protein. The distorted area moves along the DNA molecule slightly in advance of the enzyme until the polymerase recognizes a base sequence comprising a termination signal, which causes the entire complex to be detached and the nucleosome structure to be fully restored.

The molecular mechanisms by which a cell may be induced to change its state of differentiation may now be summarized. Competence to be induced depends upon the presence of protein receptors that can bind the inducer.[123,124] Ventricular cells apparently have several alternative receptors. Proteins can bind any type of molecule, so there are no restrictions regarding the chemical nature of inducers. The receptor protein, its conformation changed upon binding the inducer, would be displaced to the nucleus, where its now-exposed active site would bind to the specific sequence of DNA bases identifying an integrator gene, which would be in the extended (euchromatic) state. Binding of the receptor would expose the promoter site, permitting the attachment of RNA polymerase, and the synthesis of an hnRNA molecule. After processing and transfer of the cytoplasm, the resultant mRNA would yield protein molecules which

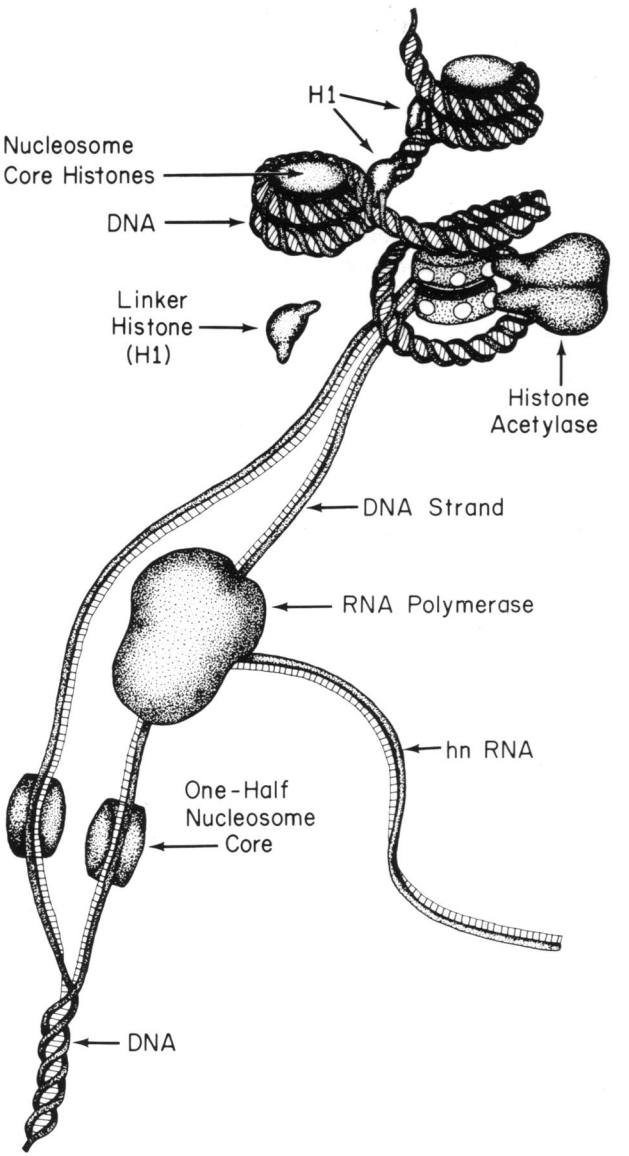

FIGURE 23
Model showing some of molecular events which take place when an active gene is transcribed. Explanation in text.

enter the nucleus, and bind to repeated sequences on DNA distributed throughout the genome, activating the promoter sites of a new gene-set. This would result in the production of a few thousand different mRNA molecules—those required to produce the spectrum of proteins which direct the metabolism of the new differentiated state. Among the new proteins would be species that maintain the integrator gene in the active state. Some genes would be newly active; some would be newly repressed, and some, perhaps a majority, would remain active.

As the new cell type is reconstructed by the new assemblage of proteins, rearrangements of the chromatin pattern would occur, mediated by the linker histones, involving compaction of much of the inactive portions of the genome. Whether the differentiated state would be terminal, or would contain the potential for subsequent changes, would depend upon what kinds of protein receptors were produced, and what parts of the genome remained extended, and thereby accessible to induction.

BIOLOGICAL RENEWAL

The theory of biological renewal was developed to account for numerous empirical laws which reveal that the substance of living systems is profoundly unstable, consisting of cells and molecules that seldom endure for more than a few weeks at most.[38] In the tissues of mature, multicellular creatures, biological renewal takes one or the other of two forms: *cellular renewal,* in which the constituent cells are incessantly replaced, or *intracellular renewal,* in which individual cells survive indefinitely, while their molecular constituents are relentlessly reconstructed.

The continuous reformation of cells and molecules is a general property of life. The theory of renewal asserts that this fundamental and ubiquitous process is a necessary outcome of the central principle of physical science—the principle of *entropy.* According to the entropy principle, the predominant process in all natural events—including every chemical reaction—is a dissipation of energy (scattering in space and fragmenting into smaller packets), and a degradation of structure. Life persists despite the universal trend towards decay and disorder by the expedient of continually rebuilding itself.[38] *Self-synthesis, repeated incessantly,* is the key process which enables living systems to endure.

Relentless renewal is therefore a biological imperative, in the absence of which life would long ago have disappeared into the crust of the earth from which it arose. The principle of renewal exists because the principle of entropy exists. If it were not for the ineluctable tendency of *all* systems to decay, there would be no requirement for the perpetual reconstruction

of the life substance.[38] This means of persistence is unique to living systems. Nothing in the world of the non-living endures by repeatedly synthesizing its own molecules. This basic feature may be incorporated into a definition of life: *Life is an organized system of molecules that persists by means of repeated self-synthesis.*[38]

Although the fundamental process is self-synthesis, controlled self-degradation is also significant. All forms of life are able to destroy their own molecules. There are no physical laws that set a requirement for regulated self-destruction. Controlled degradation of self is a uniquely biological phenomenon. When the rate of production of new cells or molecules exceeds the rate at which they are destroyed, the system grows. When degradation exceeds formation, the system diminishes in size. When rates of formation and degradation are in balance, the resulting condition is called a *steady-state.*

The theory of biological renewal reveals a common denominator in the three stages of the life history of retinal cells: Incessant self-synthesis. In the stage of multiplication, self-synthesis is manifest in the repeated formation of new cells. But even during the period of relentless cell division, there is evidence of molecular self-degradation. Intracellular renewal is observed at all times, beginning even in the unfertilized ovum,[125-128] and continuing during the period of rapid mitosis that characterizes the onset of embryogenesis.[4,128-133]

Nevertheless, self-synthesis far exceeds self-degradation during the stage of multiplication, resulting in rapid net growth. During the initial rapid cleavage divisions, production of many cell constituents lags behind the production of new DNA and histone, so that the cells progressively diminish in size. When formation of new molecules comes into balance with their dispersion by mitosis and their destruction by self-degradation, then the size of cells is stabilized (Fig 1). (Cells are of similar size in mice and humans. The prolonged period of multiplication in our species is not due to differences in the size of cells or the rate at which they are formed, but is the result of a genetic program that produces a much larger population of cells before terminal differentiation begins.)

In the stage of differentiation, the synthesis of self-molecules continues without respite. However, due to the activation of a different set of genes, there is a change in the spectrum of molecules that is produced. Molecular degradation continues, but when molecules of the previous state of differentiation (the ventricular cell) are degraded, they are not replaced—unless they also participate in the newly activated state of differentiation. In this manner, the ventricular cell gradually transforms itself into a cell of a different kind. During differentiation, cells synthesize and destroy a

changing constellation of molecules. After differentiation, they synthesize and destroy the same molecules, over and over again.[38]

There is not the slightest abatement of synthesis and degradation during the stage of renewal. Instead, the cells maintain themselves, apparently unchanging, by a perpetual process of balanced formation and degradation of molecular structure. This is the condition of the *steady-state* of intracellular renewal (Fig 17). The retina maintains the appearance of inert stability despite the underlying turmoil of unrelenting formation and degradation because the synthesis of each new cell constituent is precisely matched by the coordinated destruction of older constituents of the same kind. The cell is at all times comprised of new molecules, recently assembled according to the genetic instructions. (If renewal worked perfectly, we would grow old without aging, but due to the inevitable imperfections of biological systems in an imperfect environment, entropic decay gradually pervades the system, producing senescence.)[38,134]

When differentiation is evoked by the induction of a ventricular cell, the newly activated set of genes remains thereafter continuously in the "on" position. Although it has been suggested that the stability of the differentiated state might be accounted for by stable mRNA molecules,[135-137] these prove to be even more ephemeral than proteins, and seldom persist for more than a few days.[132,133,138,139] Nor is the stability of differentiation due to the permanence of the molecules which control genetic regulation—the non-histone nuclear proteins. These too are rapidly replaced.[140-143] The unchanging appearance of mature retinal cells is due to the unceasing activities of impermanent molecules.

After several decades of analysis using isotope-labeled molecules, only a single exception has emerged to the rule that all parts of cells are continually renewed. But the exception is not trivial. It resides at the center of our current concept of the organization of living cells: DNA is not renewed. In addition, there is increasingly substantial evidence that the nucleosome core histones are also stable against incessant replacement.[38,144-149] (The linker histone is renewed.)[145,150-153] The genetic substance, DNA, and the core histones to which it is permanently bound, turn out to be the only parts of the living system that are not repeatedly reconstructed.

The theory of biological renewal accounts for their temporal stability as follows: The genes underlying the replication of DNA and the synthesis of the core histone mRNAs are only activated during the DNA-synthesis period of the mitotic cycle.[154-156] When cells are induced to activate a state of differentiation in which they cease to divide, they become ar-

rested in the stage of the cycle that follows mitosis (the G_1 phase), in which the synthesis of DNA and core histone proteins is not possible.

If the incessant synthesis of molecules is made imperative by the entropic tendency to decay, why are the most important molecules of all exempt from replacement? Renewal of the genetic material evidently was sacrificed in order to achieve a result that was even more advantageous—liberation from the stereotyped, repetitive chemistry of the cell-division cycle, so that new chemical pathways could be elaborated. From such evolutionary experimentation our most highly differentiated tissues have emerged, including the cells of the neural retina, whose remarkably complicated forms are incompatible with mitosis.

CONCLUSION

In 1951, Verhoeff's contemporary, Müller, the geneticist and Nobel Laureate, summarized his views on heredity and the nature of the gene. He concluded with this poignant phrase: ". . . whatever the secret of the gene's ability to reproduce itself and its mutations may consist in, it seems today clearer than ever . . . that this is also the most fundamental secret of life itself."[157] The secret of life! For centuries philosophers and scientists alike sought in vain the elusive secret of life. Why do we no longer hear this phrase? What happened to the glorious quest?

Science revealed the nature of the gene: the hereditary material is a *molecule*.[158,159]

The secret of life turned out to be just this: We and all other living creatures are organized systems of *molecules*—no more and no less—systems of molecules that persist because they *continually rebuild themselves*. You and I are constructed from transitory clusters of atoms that aggregate to form the molecular structure of our bodies for only a few brief moments before they are destroyed and then reformed again by other molecules. What is there about us that persists, if our atomic structure is impermanent? What endures is an organized pattern of energy and matter, determined by information stored in the inert molecules of DNA. Our new conception of the nature of life must influence our explanations of everything biological, including the life history of retinal cells.

ACKNOWLEDGMENTS

Drs William R. Green and Toichiro Kuwabara kindly supplied photographs of Dr Verhoeff that were displayed during the lecture. The assis-

tance of Dr Thomas R. LeBon, Penny Perkins, Mirdza Lasmanis, and Zoja Trirogoff is gratefully acknowledged. The author's research is supported by grant EY 00095 from the United States Public Health Service and by an award from the Retina Research Foundation, Houston, Texas.

REFERENCES

1. Duke-Elder S: The saga of a century: Second Frederick H. Verhoeff Lecture. *Trans Am Ophthalmol Soc* 1964; 62:193-202.
2. Cogan DG: Frederick Herman Verhoeff—Personal Recollections: Third Frederick H. Verhoeff Lecture. *Trans Am Ophthalmol Soc* 1969; 67:96-109.
3. Bedell AJ: Angioid streaks: A kodachrome exposition: First Frederick H. Verhoeff Lecture. *Trans Am Ophthalmol Soc* 1961; 59:110-140.
4. Chase JW, Dawid IB: Biogenesis of mitochondria during *Xenopus laevis* development. *Dev Biol* 1972; 27:504-518.
5. Davidson EH: *Gene Activity in Early Development*, ed 2. New York, Academic Press, 1976.
6. Gurdon JB: *The Control of Gene Expression in Animal Development*. Oxford, Clarendon Press, 1974.
7. ———: *Gene Expression During Cell Differentiation*. Burlington, NC, Carolina Biological Supply Co, 1978.
8. Rugh R: *The Mouse: Its Reproduction and Development*. Minneapolis, Burgess Publishing Co, 1968, pp 51, 62, 63, 84.
9. Angevine JB Jr, Bodian D, Coulombre AJ, et al: Embryonic vertebrate central nervous system: Revised terminology. *Anat Rec* 1969; 166:257-262.
10. Fujita S: Kinetics of cellular proliferation. *Exp Cell Res* 1962; 28:52-60.
11. ———: The matrix cell and cytogenesis in the developing central nervous system. *J Comp Neurol* 1963; 120:37-42.
12. Fujita H, Fujita S: Electron microscopic studies on neuroblast differentiation in the central nervous system of domestic fowl. *Zeit Zellforsch* 1963; 60:463-478.
13. Hinds JW, Hinds PL: Early development of amacrine cells in the mouse retina: An electron microscopic, serial section analysis. *J Comp Neurol* 1978; 179:277-300.
14. Sauer FC: Mitosis in the neural tube. *J Comp Neurol* 1935; 62:377-397.
15. Sidman RL, Miale IL, Feder N: Cell proliferation and migration in the primitive ependymal zone: An autoradiographic study of histogenesis in the nervous system. *Exp Neurol* 1959; 1:322-333.
16. Fujita S: An autoradiographic study on the origin and fate of the sub-pial glioblast in the embryonic chick spinal cord. *J Comp Neurol* 1965; 124:51-60.
17. ———: Application of light and electron microscopic autoradiography to the study of cytogenesis of the forebrain, in Hassler R, Stephan H (eds): *Evolution of the Forebrain: Phylogenesis and Ontogenesis of the Forebrain*. Stuttgart, Georg Thieme Verlag, 1966, pp 180-196.
18. Hinds JW, Hinds PL: Early ganglion cell differentiation in the mouse retina: An electron microscopic analysis utilizing serial sections. *Dev Biol* 1974; 37:381-416.
19. Solter D, Skreb N, Damjanov I: Cell cycle analysis in the mouse egg-cylinder. *Exp Cell Res* 1971; 64:331-334.
20. Sinitsina VF: DNA synthesis and kinetics of cellular populations in embryo histogenesis of mice (in Russian). *Arkh Anat Gistol Embriol* 1971; 61:58-67.
21. Konyukhov BV, Sazhina MV: Genetic control over the duration of G_1 phase. *Experientia* 1971; 27:970-971.
22. ———: The cell cycle and retinal histogenesis in fidget mutant mice. *Dev Biol* 1976; 54:13-22.

23. Young RW: Unpublished material.
24. Graham CF, Morgan RW: Changes in the cell cycle during early amphibian development. *Dev Biol* 1966; 14:439-460.
25. Kauffman SL: Lengthening of the generation cycle during embryonic differentiation of the mouse neural tube. *Exp Cell Res* 1968; 49:420-424.
26. Malamud D: Differentiation and the cell cycle, in Baserga R (ed): *The Cell Cycle and Cancer*. New York, Marcel Dekker, 1971, pp 132-141.
27. Sidman RL: Histogenesis of mouse retina studied with thymidine-H^3, in Smelser GK (ed): *The Structure of the Eye*. New York, Academic Press, 1961, pp 487-506.
28. Carter-Dawson LD, LaVail MM: Rods and cones in the mouse retina. II. Autoradiographic analysis of cell generation using tritiated thymidine. *J Comp Neurol* 1979; 188:263-272.
29. Hinds JW, Hinds PL: Differentiation of photoreceptors and horizontal cells in the embryonic mouse retina: An electron microscopic, serial section analysis. *J Comp Neurol* 1979; 187:495-512.
30. Spira AW, Hollenberg MJ: Human retinal development: Ultrastructure of the inner retinal layers. *Dev Biol* 1973; 31:1-21.
31. Prescott DM: *Reproduction of Eukaryotic Cells*. New York, Academic Press, 1976.
32. Brooks RF: Variability in the cell cycle and the control of proliferation, in John PCL (ed): *The Cell Cycle*. London, Cambridge University Press, 1981, pp 35-61.
33. Fantes PA, Nurse P: Division timing: Controls, models and mechanisms, in John PCL (ed): *The Cell Cycle*. London, Cambridge University Press, 1981, pp 11-33.
34. Braekevelt CR, Hollenberg MJ: The development of the retina of the albino rat. *Am J Anat* 1970; 127:281-302.
35. Beach DH, Jacobson M: Patterns of cell proliferation in the retina of the clawed frog during development. *J Comp Neurol* 1979; 183:603-614.
36. Robb RM: Increase in retinal surface area during infancy and childhood. *J Pediatr Ophthalmol Strabismus* 1982; 19:16-20.
37. Young RW: Visual cells and the concept of renewal. *Invest Ophthalmol* 1976; 15: 700-725.
38. ———: Biological renewal: Applications to the eye. *Trans Ophthalmol Soc UK* 1982; 102:42-75.
39. Taylor CA, Weiss P: Demonstration of axonal flow by the movement of tritium-labeled protein in mature optic nerve fibers. *Proc Natl Acad Sci USA* 1965; 54:1521-1527.
40. Young RW: The renewal of photoreceptor cell outer segments. *J Cell Biol* 1967; 33:61-72.
41. Ames A III, Parks JM, Newbett FB: Protein turnover in retina. *J Neurochem* 1980; 35:131-142.
42. ———: Synthesis and breakdown of different sized retinal proteins in darkness and during photic stimulation. *Neurochem* 1980; 1:393-403.
43. Young RW, Bok D: Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J Cell Biol* 1969; 42:392-403.
44. Bok D, Young RW: Phagocytic properties of the pigment epithelium, in Zinn KM, Marmor MR (eds): *The Retinal Pigment Epithelium*. Cambridge, Mass, Harvard University Press, 1979, pp 148-174.
45. Watts JW, Harris H: Turnover of nucleic acids in a non-multiplying animal cell. *Biochem J* 1959; 72:147-153.
46. Hughes WL: The metabolic stability of DNA, in Stohlman F (eds): *The Kinetics of Cellular Proliferation*. New York, Grune & Stratton, 1959, pp 83-94.
47. Fresco JR, Bendich A: The metabolic stability of rat liver deoxyribonucleic acid: a turnover study. *J Biol Chem* 1960; 235:1124-1128.
48. Bennett LL Jr, Simpson L, Skipper HE: On the metabolic stability of nucleic acids in mitotically inactive adult tissues labeled during embryonic development. *Biochim Biophys Acta* 1960; 42:237-243.

49. Davidson EH: Note on the control of gene expression during development. *J Theor Biol* 1971; 32:123-130.
50. Grobstein C: What we do not know about differentiation. *Am Zool* 1966; 6:89-95.
51. Wolpert L, Lewis JW: Towards a theory of development. *Fed Proc* 1975; 34:14-20.
52. Seglen PO: Differones: Control of gene expression and cellular differentiation by hormones and other agents, with particular emphasis on liver tissue. *Norwegian J Zool* 1974; 22(Suppl 1):1-132.
53. Sonneborn TM: The differentiation of cells. *Proc Natl Acad Sci USA* 1964; 53:915-929.
54. Johnson RT, Rao PN: Nucleo-cytoplasmic interactions in the achievement of nuclear synchrony in DNA synthesis and mitosis in multinucleate cells. *Biol Rev* 1971; 46: 97-155.
55. Gurdon JB: Nuclear transplantation and the cyclic reprogramming of gene expression, in Reinert J, Holtzer H (eds): *Cell Cycle and Cell Differentiation*. New York, Springer-Verlag, 1975, pp 123-13.
56. Harris H: *Cell Fusion*. Oxford, Clarendon Press, 1970.
57. Grobstein C: Cytodifferentiation and its controls. *Science* 1964; 143:643-650.
58. Needham J: Organizer phenomena after four decades: A retrospect and prospect, in Dronamraju KR (ed): *Haldane and Modern Biology*. Baltimore, The Johns Hopkins Press, 1968, pp 277-298.
Locke M (ed): *Major Problems in Developmental Biology*. New York, Academic Press, 1966, pp 29-84.
60. Holtzer H: Comments on induction during cell differentiation, in *Induktion und Morphogenese*. Colloquium d Gesellschaft f Physiol Chemie. Berlin, Springer-Verlag, 1963, pp 127-143.
61. Saxen L, Toivonen S: *Primary Embryonic Induction*. London, Academic Press, 1962.
62. Georgiev GP: On the structural organization of the operon and the regulation of RNA synthesis in animal cells. *J Theor Biol* 1969; 25:473-490.
63. Wessells NK, Rutter WJ: Phases in cell differentiation. *Sci Am* 1969; 220:36-44.
64. Levenson R, Housman D: Commitment: How do cells make the decision to differentiate? *Cell* 1981; 25:5-6.
65. Barton SC, Johnson MH: Molecular differentiation in the preimplantation mouse embryo. *Nature* 1976; 259:319-321.
66. Berkom JV, Brockway GO: Qualitative patterns of protein synthesis in the preimplantation mouse embryo. 1. Normal pregnancy. *Dev Biol* 1975; 44:148-157.
67. Brandhorst BP: Two-dimensional gel patterns of protein synthesis before and after fertilization of sea urchin eggs. *Dev Biol* 1976; 52:310-317.
68. Levinson J, Goodfellow P, Vadeboncoeur M, et al: Identification of stage-specific polypeptides synthesized during murine preimplantation development. *Proc Natl Acad Sci USA* 1978; 75:3332-3336.
69. Watson JD: *Molecular Biology of the Gene*, ed 3. Menlo Park, Calif, Benjamin, 1976, pp 85-111.
70. Monod J: *Chance and Necessity*. Glasgow, William Collins Sons & Co, 1972.
71. Koshland DE: The role of flexibility in enzyme action. *Cold Spring Harbor Symp Quant Biol* 1963; 28:473-482.
72. ———: Protein shape and biological control. *Sci Am* 1973; 229:52-64.
73. Monod J, Changeux JP, Jacob F: Allosteric proteins and cellular control systems. *J Mol Biol* 1963; 6:306-329.
74. Peterson JL, McConkey EH: Non-histone chromosomal proteins from HeLa cells: A survey by high resolution, two-dimensional electrophoresis. *J Biol Chem* 1976; 251:548-554.
75. Thoma F, Koller T, Klug A: Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J Cell Biol* 1979; 83:403-427.

76. Boulikas T, Wiseman JM, Garrard WT: Points of contact between histone H1 and the histone octamer. *Proc Natl Acad Sci USA* 1980; 77:127-131.
77. Allan J, Hartman PG, Crane-Robinson C, et al: The structure of histone H1 and its location in chromatin. *Nature* 1980; 288:675-679.
78. McGhee JD, Felsenfeld G: Nucleosome structure. *Annu Rev Biochem* 1980; 49: 1115-1156.
79. Oudet P, Germond JE, Bellard M, et al: Nucleosome structure. *Philos Trans R Soc Lond [Biol]* 1978; 283:241-258.
80. Kornberg RD, Klug A: The nucleosome. *Sci Am* 1981; 244:52-64.
81. Garel A, Axel R: Selective digestion of transcriptionally active ovalbumin genes from oviduct nuclei. *Proc Natl Acad Sci USA* 1976; 73:3966-3970.
82. Garel A, Zolan M, Axel R: Genes transcribed at diverse rates have a similar conformation in chromatin. *Proc Natl Acad Sci USA* 1977; 74:4867-4871.
83. Gottesfeld JM, Butler PJG: Structure of transcriptionally-active chromatin subunits. *Nucleic Acids Res* 1977; 4:3155-3172.
84. Szekely M: *From DNA to Protein—The Transfer of Genetic Information*. New York, John Wiley & Sons, 1980.
85. Brown SW: Heterochromatin: Heterochromatin provides a visible guide to suppression of gene action during development and evolution. *Science* 1966; 151:417-425.
86. Sieger M, Pera F, Schwarzacher HG: Genetic inactivity of heterochromatin and heteropycnosis in *Microtus agrestis*. *Chromosoma* 1970; 29:349-364.
87. Bishop JO: The gene numbers game. *Cell* 1974; 2:81-86.
88. McKusick VA, Ruddle FH: The status of the gene map of the human chromosomes. *Science* 1977; 196:390-405.
89. Neel JV, Schull WJ: On some trends in understanding the genetics of man. *Perspect Biol Med* 1968; 11:565-602.
90. Ohta T, Kimura M: Functional organization of genetic material as a product of molecular evolution. *Nature* 1971; 233:118-119.
91. Bradbury EM, Maclean N, Matthews HR: *DNA, Chromatin and Chromosomes*. New York, John Wiley & Sons, 1981.
92. Lewin B: Units of transcription and translation: Sequence components of heterogeneous nuclear RNA and messenger RNA. *Cell* 1975; 4:77-93.
93. Britten RJ, Kohne DE: Repeated sequences in DNA. *Science* 1968; 161:529-540.
94. Yunis JJ, Yasmineh WG: Heterochromatin, satellite DNA, and cell function. *Science* 1971; 174:1200-1209.
95. Paul J: The transcriptional unit in eukaryotes. *Genetics* 1975; 79:151-157.
96. Hand R: Eucaryotic DNA: Organization of the genome for replication. *Cell* 1978; 15:317-325.
97. Britten RJ, Davidson EH: Gene regulation for higher cells: A theory. *Science* 1969; 165:349-357.
98. Grouse L, Chilton M, McCarthy BJ: Hybridization of ribonucleic acid with unique sequences of mouse deoxyribonucleic acid. *Biochem* 1972; 11:798-805.
99. Galau GA, Klein WH, Davis MM, et al: Structural gene sets active in embryos and adult tissues of the sea urchin. *Cell* 1976; 7:487-505.
100. Chikaraishi DM, Deeb SS, Sueoka N: Sequence complexity of nuclear RNAs in adult rat tissues. *Cell* 1978; 13:111-120.
101. Hastie ND, Bishop JO: The expression of three abundance classes of messenger RNA in mouse tissues. *Cell* 1976; 9:761-774.
102. Gilbert W: Why genes in pieces? *Nature* 1978; 271:501.
103. Schmid CW, Deininger PL: Sequence organization of the human genome. *Cell* 1975; 6:345-358.
104. Savage MJ, Sala-Trepat JM, Bonner J: Measurement of the complexity and diversity of poly(adenylic acid) containing messenger RNA from rat liver. *Biochem* 1978; 17: 462-467.

105. Bantle JA, Hahn WE: Complexity and characterization of polyadenylated RNA in the mouse brain. *Cell* 1976; 8:139-150.
106. Chambon P: Split genes. *Sci Am* 1981; 244:60-71.
107. Trapnell BC, Tolstoshev P, Crystal RG: Secondary structures for splice junctions in eukaryotic and viral messenger RNA precursors. *Nucleic Acids Res* 1980; 8:3659-3672.
108. Bonner J: Chromatin structure and gene activity, in Nover L, Mothes K (eds): *Cell Differentiation in Microorganisms, Plants and Animals*. Amsterdam, North Holland Publ Co, 1977, pp 76-93.
109. Franke WW, Scheer U, Trendelenburg M, et al: Morphology of transcriptionally active chromatin. *Cold Spring Harbor Symp Quant Biol* 1977; 42:755-772.
110. Gottesfeld JM, Murphy RF, Bonner J: Structure of transcriptionally active chromatin. *Proc Natl Acad Sci USA* 1975; 72:4404-4408.
111. Allfrey VG: Post-synthetic modifications of histone structure: A mechanism for the control of chromosome structure by the modulation of histone-DNA interactions, in Li HJ, Eckhardt RA (eds): *Chromatin and Chromosome Structure*. New York, Academic Press, 1977, pp 167-19.
112. ———: Molecular aspects of the regulation of eukaryotic transcription: Nucleosomal proteins and their postsynthetic modifications in the control of DNA conformation and template function, in Goldstein L, Prescott DM (eds): *Cell Biology*. New York, Academic Press, 1980, vol 3, pp 347-437.
113. Nelson DA, Perry WM, Chalkley R: Sensitivity of regions of chromatin containing hyperacetylated histones to DNase I. *Biochem Biophys Res Commun* 1978; 82:356-363.
114. Sealy L, Chalkley R: DNA associated with hyperacetylated histone is preferentially digested by DNase I. *Nucleic Acids Res* 1978; 5:1863-1876.
115. Davie JR, Candido EPM: Acetylated histone H4 is preferentially associated with template-active chromatin. *Proc Natl Acad Sci USA* 1978; 75:3574-3577.
116. Shirley MA, Anderson KM: Electron-microscopic visualization of transcriptionally active and less active chromatin fractions from the rat ventral prostate and their content of histones. *Can J Biochem* 1977; 55:9-18.
117. Levy BW, Connor W, Dixon GH: A subset of trout testis nucleosomes enriched in transcribed DNA sequences contains high mobility group proteins as major structural components. *J Biol Chem* 1979; 254:609-620.
118. Weisbrod S: Active chromatin. *Nature* 1982; 297:289-295.
119. Bradbury EM, Javaherian K: *The Organization and Expression of the Eukaryotic Genome*. New York, Academic Press, 1977.
120. Weintraub H, Worcel A, Alberts B: A model for chromatin based upon two symmetrically paired half-nucleosomes. *Cell* 1976; 9:409-417.
121. Richards BM, Pardon JF, Lilley DMJ, et al: Nucleosome sub-structure during transcription and replication. *Philos Trans R Soc Lond [Biol]* 1978; 283:287-289.
122. Oudet P, Spadafora C, Chambon P: Nucleosome structure II: Structure of the SV40 minichromosome and electron microscopic evidence for reversible transitions of the nucleosome structure. *Cold Spring Harbor Symp Quant Biol* 1977; 42:301-312.
123. McMahon D: Chemical messengers in development: A hypothesis. *Science* 1974; 185:1012-1021.
124. Brunner G: Membrane impression and gene expression: Towards a theory of cytodifferentiation. *Differentiation* 1977; 8:123-132.
125. Levner MH: RNA transcription in mature sea urchin eggs. *Exp Cell Res* 1974; 85: 296-302.
126. Thomas C: RNA metabolism in previtellogenic oocytes of *Xenopus laevis*. *Dev Biol* 1974; 39:191-197.
127. Leonard DA, LaMarca MJ: *In vivo* synthesis and turnover of cytoplasmic ribosomal RNA by stage 6 oocytes of *Xenopus laevis*. *Dev Biol* 1975; 45:199-202.

128. Schultz RM, Letourneau GE, Wassarman PM: Program of early development in the mammal: Changes in patterns and absolute rates of tubulin and total protein synthesis during oogenesis and early embryogenesis in the mouse. *Dev Biol* 1979; 68:341-359.
129. Berg WE, Mertes DH: Rates of synthesis and degradation of protein in the sea urchin embryo. *Exp Cell Res* 1970; 60:218-224.
130. Brinster RL, Wiebod JL, Brunner S: Protein metabolism in preimplanted mouse ova. *Dev Biol* 1976; 51:215-224.
131. Epstein CJ, Smith SA: Electrophoretic analysis of proteins synthesized by preimplantation mouse embryos. *Dev Biol* 1974; 40:233-244.
132. Galau GA, Lipson ED, Britten RJ, et al: Synthesis and turnover of polysomal mRNAs in sea urchin embryos. *Cell* 1977; 10:415-432.
133. Nemer M, Dubroff LM, Graham M: Properties of sea urchin embryo messenger RNA containing and lacking poly(A). *Cell* 1975; 6:171-178.
134. Young RW: A theory of central retinal disease, in Sears ML (ed): *New Directions in Ophthalmic Research*. New Haven, Conn, Yale University Press, 1981, pp 237-270.
135. Jacob F, Monod J: Genetic repression, allosteric inhibition, and cellular differentiation, in Locke M (ed): *Cytodifferentiation and Macromolecular Synthesis*. New York, Academic Press, 1963, pp 30-64.
136. Kafatos F, Reich J: Stability of differentiation-specific and nonspecific messenger RNA in insect cells. *Proc Natl Acad Sci USA* 1968; 60:1458-1465.
137. Kafatos FC: mRNA stability and cellular differentiation, in Diczfalusy E (ed): *Gene Transcription in Reproductive Tissue*. Stockholm, Karolinska Institutet, 1972, pp 319-345.
138. Scott RB, Bell E: Protein synthesis during development: Control through messenger RNA. *Science* 1964; 145:711-714.
139. Darnell JE: mRNA structure and function. *Prog Nucleic Acid Res Mol Biol* 1977; 19:493-511.
140. Laurence DJR, Butler JAV: Metabolism of histones in malignant tissues and liver of the rat and mouse. *Biochem J* 1965; 96:53-62.
141. Stein G, Baserga R: Continued synthesis of non-histone chromosomal proteins during mitosis. *Biochem Biophys Res Commun* 1970; 41:715-722.
142. Appels R, Bolund L, Ringertz NR: Biochemical analysis of reactivated chick erythrocyte nuclei isolated from chick/HeLa heterokaryons. *J Mol Biol* 1974; 87:339-355.
143. Burdman JA, Haglid K, Dravid AR: Protein synthesis in fractions from isolated brain cell nuclei. *J Neurochem* 1970; 17:669-676.
144. Young RW: The chemistry of the retina: Function, renewal, rhythms, and the nucleus. *Neurochem* 1980; 1:123-142.
145. Sakagami H, Mitsui Y, Murota SI, et al: Effect of growth stage on histone H1 metabolism in human diploid fibroblasts. *J Cell Physiol* 1982; 110:213-218.
146. Balhorn R, Oliver D, Hohmann P, et al: Turnover of deoxyribonucleic acid, histones, and lysine-rich histone phosphate in hepatoma tissue culture cells. *Biochem* 1972; 11:3915-3920.
147. Hancock R: Conservation of histones in chromatin during growth and mitosis *in vitro*. *J Mol Biol* 1969; 40:457-466.
148. Piha RS, Cuenod M, Waelsch H: Metabolism of histones of brain and liver. *J Biol Chem* 1966; 241:2397-2404.
149. Byvoet P: Metabolic integrity of deoxyribonucleohistones. *J Mol Biol* 1966; 17: 311-318.
150. Tarnowka MA, Baglioni C: Synthesis of H1 histones by BHK cells in G1. *Cell* 1978; 15:163-171.
151. Gurley LR, Hardin JM: The metabolism of histone fractions III. Synthesis and turnover of histone f1. *Arch Biochem Biophys* 1970; 136:392-401.
152. Herve B, Jacquemin E, Courtois Y: Histones biosynthesis and turnover in epithelial lens cells cultured *in vitro*. *Cell Biol Int Rep* 1979; 3:271-281.

153. Ohba Y, Hayashi K, Nakagawa Y, et al: Metabolic activities of histones in rat liver and spleen. *Eur J Biochem* 1975; 56:343-352.
154. Robbins E, Borun TW: The cytoplasmic synthesis of histones in HeLa cells and its temporal relationship to DNA replication. *Proc Natl Acad Sci USA* 1967; 57:409-416.
155. Rickles R, Marashi F, Sierra R, et al: Analysis of histone gene expression during the cell cycle in HeLa cells by using cloned human histone genes. *Proc Natl Acad Sci USA* 1982; 79:749-753.
156. Elgin SCR, Weintraub H: Chromosomal proteins and chromatin structure. *Annu Rev Biochem* 1975; 44:725-744.
157. Müller HG: The development of the gene theory, in Dunn LC (ed): *Genetics in the 20th Century*. New York, Macmillan Co, 1951, pp 77-99.
158. Watson JD, Crick FHC: Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. *Nature* 1953; 171:737-738.
159. ———: Genetical implications of the structure of deoxyribonucleic acid. *Nature* 1953; 171:964-967.