0023-6837/93/6902-0136$03.00/0
LABORATORY INVESTIGATION

Vol. 69, No. 2, p. 136, 1993
Printed in U.S.A.

Biology of Disease

Cellular Selection in the Genesis of Multicellular Organization

JAMES MICHAELSON

Department of Pathology, Harvard Medical School; Department of Pathology, Massachusetts General Hospital; Massachusetts General Hospital Cancer Center, Charlestown (Boston) Massachusetts

INDIVIDUALS AND POPULATIONS

Biologic populations display properties that are more complex and interesting than the individuals of which they are comprised. This ecological viewpoint lies at the heart of modern thinking on the nature of biologic order and evolution, a viewpoint that has been fruitfully applied to populations of individuals ranging in size from the smallest microbes, to the largest multicellular organisms. (1) The populations of cells within us may also be viewed in such ecological terms. A single human is a population of $\sim 10^{13}$ cells. In contrast, the species of human beings is a population of $\sim 10^{9}$ individuals. Thus, there is as much potential for ecological and evolutionary complexity within us, as among us. Each of us, then, has a double evolutionary history; we are each the descendant of a single primitive hominid ancestor, as well as the descendant of single fertilized ovum.

Here, I will review some of the properties of biologic populations, with emphasis on the populations of cells that make up metazoan organisms. In particular, this

review will examine the possibility that developmental order may arise in metazoan embryos through the action of the selection of cells, that is, by either differential cell growth or death (2–10). This cell selection is a familiar occurrence to those of us who utilize the methods of histology. Indeed, cell selection has a deceptive simplicity, which belies its power to mold the composition of the cellular populations of which we are comprised.

THE PROPERTIES OF DARWINIAN POPULATIONS

Selection

It was, of course, Darwin who was the first to discover that biologic populations have important properties not apparent in the biology of the individuals of which they are comprised. Thus, while Lamarck was able to discern the reality of evolution, he was misled by the illusion of the environment determining the heritable properties of individuals. Darwin showed that the environment, even without the capacity to affect the heritable properties of individuals, may change the heritable properties of populations, through the action of selection.

Diversification

Evolution may be molded by selection, but selection requires genetic innovation for the possibility of true progress. This innovation is created by a variety of processes of heritable diversification, such as mutation, duplication, deletion, meiotic recombination, and independent assortment. Each of these processes of heritable diversification possess the property of being stochastic, that is of being probabilistic processes occurring over time (11, 12). Mutation provides the prototype of such a stochastic process, but we will find this stochastic feature to appear again in a variety of unexpected and interesting places.

Biotic Potential

Reproduction gives all populations the capacity for exponential growth, and thus the inevitable potential to outstrip the resources that support the population. This enormous potential for exponential growth has been called the biotic potential of a population (13).

All living things, including the cells that make up metazoan organisms, possess a biotic potential that can never be achieved. For example, in amphibian embryos, such as the axolotl *Ambystoma mexicarum*, fertilization is followed by rapid exponential growth that continues through a dozen-or-so cell divisions (14). However, this growth rate can not continue unabated, so that by the gastrula stage the actual number of cells in the embryo is only 10% of its biotic potential, by the neurula stage 1%, by the tailbud stage .01%, while by the time that the embryo has reached the tadpole stage, it contains only .00000000001% of its biotic potential. In mammalian embryos, development likewise begins with an exponential growth of cells, doubling about every 8 hours (15), and a small number of cells continue to divide at this rate until quite late in development (16). If all of the cells of the embryo continued this rate of growth, by the time of birth (21 days after conception) a mouse embryo would comprise approximately 10^{18} cells, and would weigh about a million pounds. It is this difference between the embryo's enormous biotic potential, and its more modest actual potential, that allows the opportunity for cellular selection to occur.

The Organizing Power of Selection

The selection made possible by the biotic potential of cells has a powerful creative potential, as can be illustrated by a simple numerical example. Consider a population of 1,000 cells, containing just one cell with a unique, cell-heritable, property that makes it distinguishable from the other cells in the colony. If that distinctive cell divides (every 8 hours), while the other cells do not proliferate, then by 4.5 days, 50% of the colony will be made up of cells of the distinctive type, while by the end of the 7th day, 99.99% of the population will be of the special type.

CELL SELECTION

The Immune System

The production of a specific antibody by the immune system provides the prototype of a cellular system organized by cellular selection. In the presence of a foreign substance (an antigen) those few lymphocytes that happen, by chance, to express a complementary immunoglobulin are induced to divide. The key signal that induces the selective proliferation of these few lymphocytes is the binding of the antigen to the immunoglobulin, which, in immature lymphocytes, is located at the cell surface. This process of antigen-induced cellular selection creates a new population of cells, which can then secrete the specific antibody. Thus, it is by the power of cellular selection, that the immune system is capable of producing large amounts of specific antibody to any number of foreign substances.

The versatility and power of the selective mechanism of antibody production is amazing. It requires only one of the billion lymphocytes that comprise a mouse's immune system to produce specific antibody. If that single cell is made to divide, then by 21 cell divisions (approximately 8 days), the immune system will have selected a population of 10^7 antibody-producing cells. We know from the work of Conrad and Ingraham (17), and Perelson and Goldstein (18), that each of these terminally expanded cells is capable of producing immunoglobulin at the rate of ~15 ng/cell/hour. Thus, these 10^7 cells are capable of producing, in an hour, 15 mg of specific antibody, which constitutes 50% of the mouse's total plasma immunoglobulin.

The Liver

The liver has provided another system in which the role of cellular selection may be examined (19, 20). While the immune system produces the immunoglobulins of the blood, most of the rest of the plasma proteins, such as albumin, transferrin, fibrinogen, α_2-macroglobulin, complement-component-C3 and α-fetoprotein, are produced by the liver. It appears by immunofluorescence that each of these plasma proteins is present in, and presumably synthesized by, a separate population of

hepatocytes in the liver (Fig. 1). In the adult mouse liver, albumin-containing cells constitute somewhat less than 1% of hepatocytes. Transferrin-containing cells are even less abundant, being present in about 1/10th the number of albumin-containing cells, while 1/30th of this number of hepatocytes contains complement-component-C3 (Fig. 2). α-Fetoprotein-containing cells are quite rare in the adult liver, being present in only about 1/1000th of the abundance of albumin-containing cells, but are present in higher numbers early in life. These values roughly

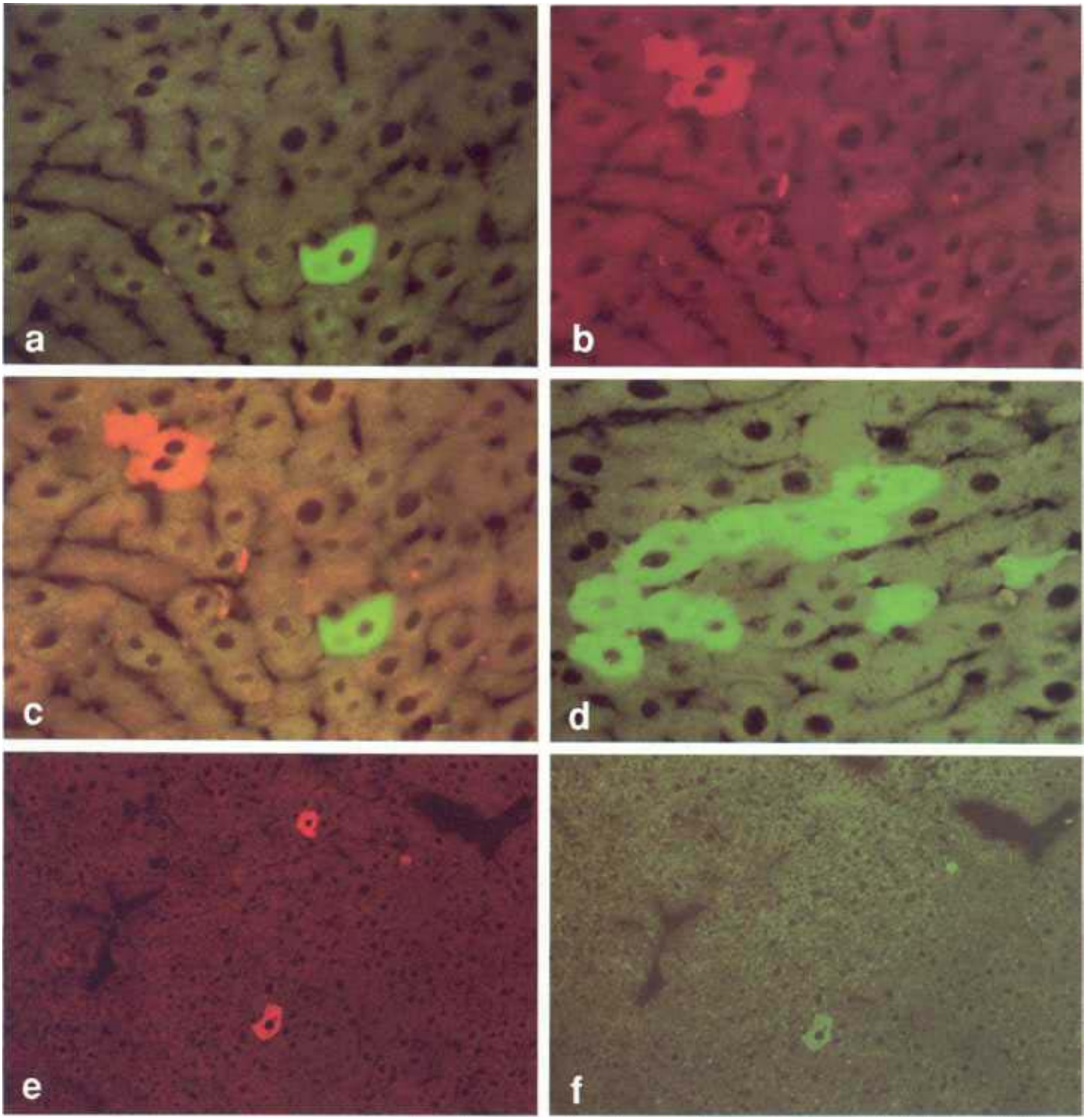

FIG. 1. Immunofluorescence of mouse liver. *a*, Albumin-containing cell (*green*) identified by immunofluorescence with rabbit anti-mouse albumin. *b*, C3-containing cell in same field (*red*) identified by goat anti-mouse C3. *c*, Double exposure of same fields as *a* and *b*, showing simultaneously, albumin (*green*) and C3 (*red*) containing cells. A large series of similar experiments established the separate identity of cells containing albumin, transferrin, C3, fibrinogen, α2 macroglobulin. *d*, Cluster of albumin-containing cells. *e* and *f*, Immunofluorescence of liver from an *Alb-1*^{a}/*Alb-1*^{c} heterozygous mouse. The reactivity of rabbit anti-mouse albumin is revealed in *red*, the reactivity of mouse Anti-Alb^c is seen in *green*. (Anti-Alb^c = (C57B1/6 × A)$_{F1}$ Anti-B6 *Alb-1*^{c} albumin).

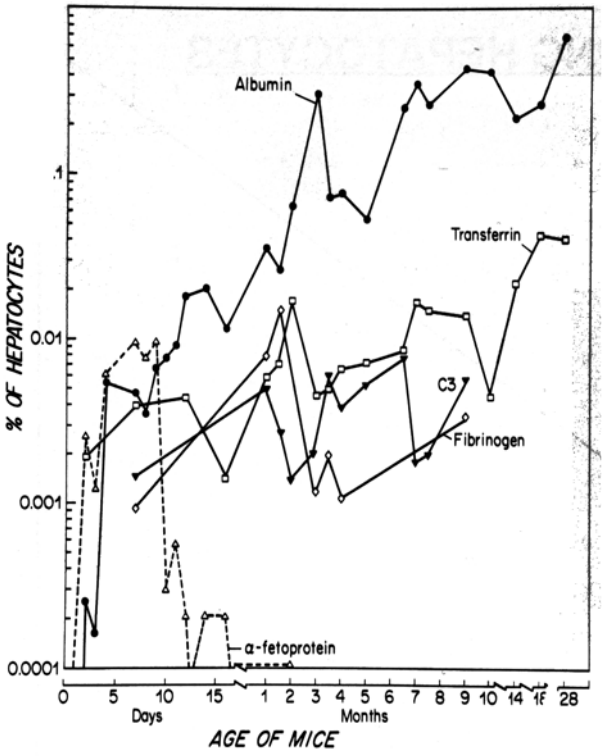

FIG. 2. Abundance of plasma protein-containing hepatocytes in the mouse.

reflect the relative rates of synthesis of each of these proteins (21).

Albumin is produced by the liver at a rate of approximately 0.4 milligrams/hour/gram of tissue (17, 18). If, as the immunofluorescence picture suggests, albumin synthesis is confined to just .1% of hepatocytes, then each albumin-containing hepatocyte would have to produce albumin at a rate of approximately 10 ng/cell/hour. This is not heavy labor for a cell; as we know from work cited above, single immunoglobulin-producing cells are capable of producing immunoglobulin at a rate of approximately 15 ng/hour. (17, 18)

The plasma protein-containing cells visualized by immunofluorescence are scattered throughout the liver, and are present both as individual cells and as clusters of multiple adjacent cells (Fig. 1*d*). As will be discussed later in this review, it appears that each of these clusters is a clonal group, that is to say, is the progeny of a single precursor cell. Because these clusters are clonal, the size of each cluster is a reflection of how much mitotic growth the clone has undergone. We will be able to utilize this information in order to make inferences about the nature of the cellular growth that occurs in the liver.

Cellular Selection in the Liver: Inflammation. For many years, it has been known that during an inflammatory response, there are many changes in the liver's pattern of plasma protein synthesis. For example, Schreiber *et al.* (23) has shown that in the rat, the inflammatory response induced by an injection of turpentine causes, over a 2-day period, a slight decrease in albumin synthesis, and a 5- to 10-fold increase in fibrinogen synthesis.

During an inflammatory response, the number of albumin-containing cells declines slightly, while the number of fibrinogen-containing cells increases six-fold, which parallels the liver's overall levels of plasma protein synthesis. (Fig. 4) (19, 20). It appears that this increase in the number of fibrinogen-containing cells can be ascribed to selective cellular proliferation, as has been determined from two types of experiments. First, there is a dramatic increase in the average number of hepatocytes in the clusters of fibrinogen-containing cells, indicating cell growth (Fig. 4). Apparently these clonal clusters become larger because they have undergone mitotic expansion. Second, the liver undergoes considerable hepatocyte proliferation during an inflammatory response, which has been detected by measuring uptake of a synthetic DNA precursor, bromodeoxuridine. The bromodeoxuridine can be visualized histologically, and by this method, it has been found that while there is a very low level of mitotic activity in livers from normal rats, livers from rats undergoing an inflammatory response contain many mitotically active hepatocytes.

It is striking that, unlike fibrinogen-containing hepatocytes, there is little change during inflammation in either the total number or the average clonal size of albumin-containing cells. In other words, the process of inflammation leads to the selection by the survival and mitotic expansion of a subpopulation of hepatocytes, which include the fibrinogen-containing cells.

Cellular Selection in the Liver: Neonatal Development. Early in life, α-fetoprotein is the principal plasma protein, whereas soon after birth, there is a gradual switch from α-fetoprotein synthesis to albumin synthesis (24, 25). These biochemical changes are paralleled by equivalent changes in the numbers of specialized hepatocytes containing each plasma protein (Figs. 2 and 3) (19, 20). Considerable numbers of α-fetoprotein-containing cells are present in the livers of neonatal rats and mice; the number of these cells declines as the animals mature, while the number of albumin-containing cells rises from being very rare to being the most prevalent plasma protein containing cell in the liver.

It appears that the abundance of α-fetoprotein-containing hepatocytes is a consequence of the rate of cell division of these cells, relative to the other cells in the liver. This was revealed from data on the number and size of the clonal clusters of plasma-protein containing hepatocytes (Fig. 5). For example, during the first few weeks after birth, α-fetoprotein-containing cells are distributed into clusters that are very large in comparison to albumin- or transferrin-containing clusters. However, at about 2 weeks of life, just before the time when the number of α-fetoprotein-containing cells drops precipitously, the average size of the clusters of α-fetoprotein-containing cells becomes much smaller. Apparently, early in life, α-fetoprotein-containing cells are plentiful because they divide more rapidly than the other cells in the liver, while they subsequent decline in number because of their failure to divide as rapidly as the other hepatocytes in the liver (19, 20).

These observations suggest that the developmental plasticity of the liver is mediated by differential cell growth. This is apparent both in the changes in the abundance of α-fetoprotein-containing cells that occur during the neonatal period, and in the changes in the

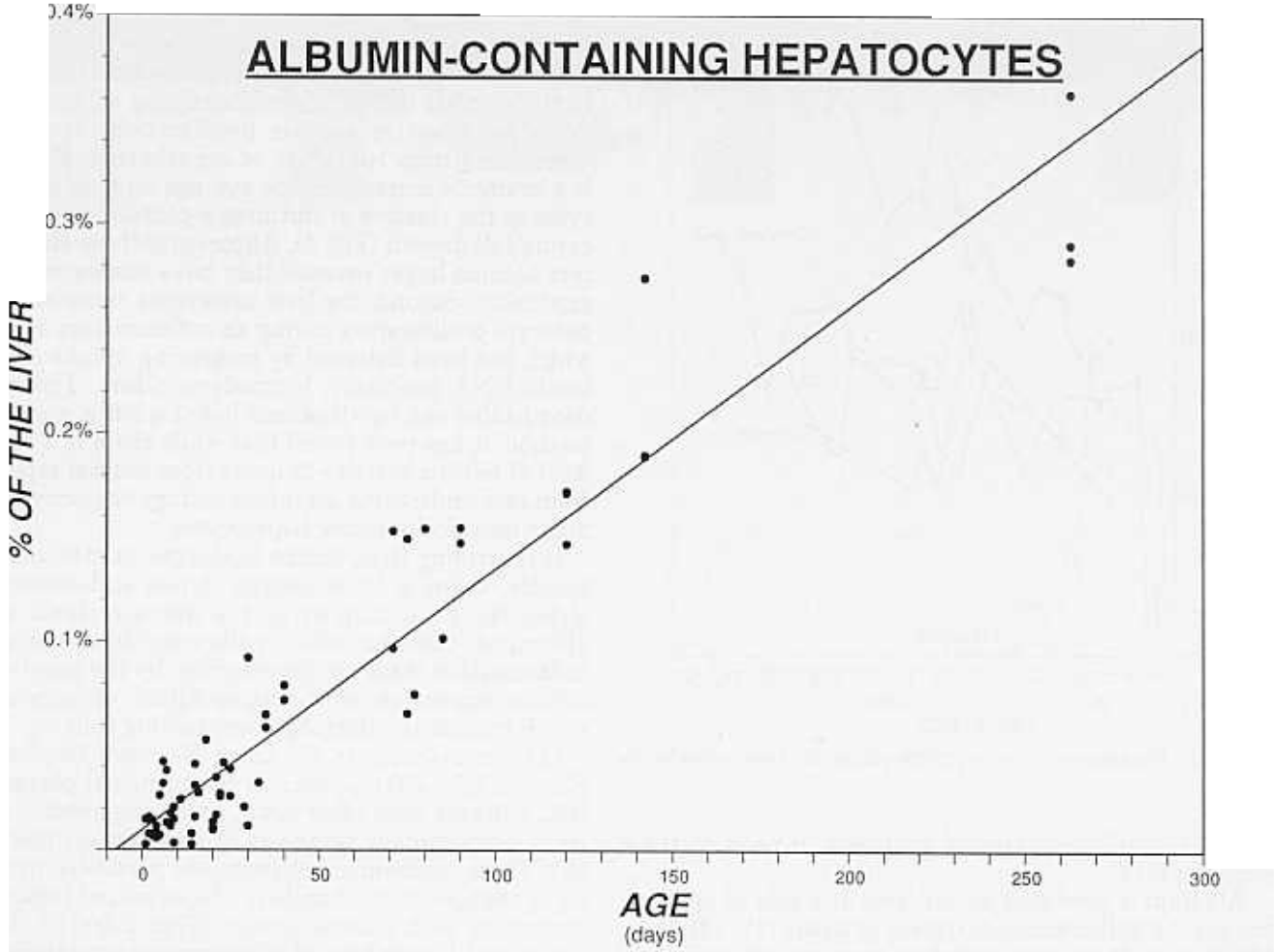

FIG. 3. Abundance of albumin-containing hepatocytes in the rat as a function of age. Each *point* represents the value from a single rat. Linear regression was carried out by utilizing a Delta Graph software program. $r^2 = 0.91$.

number of fibrinogen-containing cells that occur during inflammation. Thus, like the immune system, the liver appears to be a cellular population whose composition is molded by a Darwinian process of cellular selection.

DEVELOPMENT OF THE GENITOURINARY SYSTEM

Cellular selection also appears to be at work in the development of the genitourinary system (26, 27). The rudiments of the reproductive structures of both sexes are present in the early embryo. These rudiments may be seen in the Wolffian and Mullerian ducts, and their associated structures (Fig. 6). In female embryos, the final sexual structures arise by differential cell growth from a subset of these preexisting elements, particularly the Mullerian ducts. The uterus, Fallopian tubes, etc., arise by selective proliferation of their rudiments, whereas the rudiments of the male structures are not subject to differential cell growth, and thus do not appear. In male embryos, the reverse is true; small precursor elements of the Wolffian ducts and their related structures give rise to the male sexual structures such as the epididymus, the prostate, the seminal vesicle, etc., by differential cell proliferation, whereas the precursors of the female structures do not grow. Thus, the emergence of anatomical structure is determined not by the creation of rudiments, but in their realization by differential cell growth (26, 27).

THE NERVOUS SYSTEM

It is not just the rough mass of each embryonic structure that is created by differential proliferation, but specific anatomical detail as well. This is well illustrated by the development of the nervous system. As Balinsky (28) has described it, "when the neural tube is first formed, its walls consist of a single layer of pseudostratified epithelium" (28). The tube is initially uniform in thickness. The various subdivisions of the neural tube appear initially as local thickenings that subsequently grow into the forebrain, midbrain, spinal chord, and so on; these structures come into existence by a process of differential proliferation in the neural tube. "The uneven thickening of the neural tube... is a direct result of the differential rate of proliferation... It has been observed by Frank (29) that a local thickening of the neural tube is actually preceded by an increase in the number of dividing cells in the area where the thickening is to appear" (28).

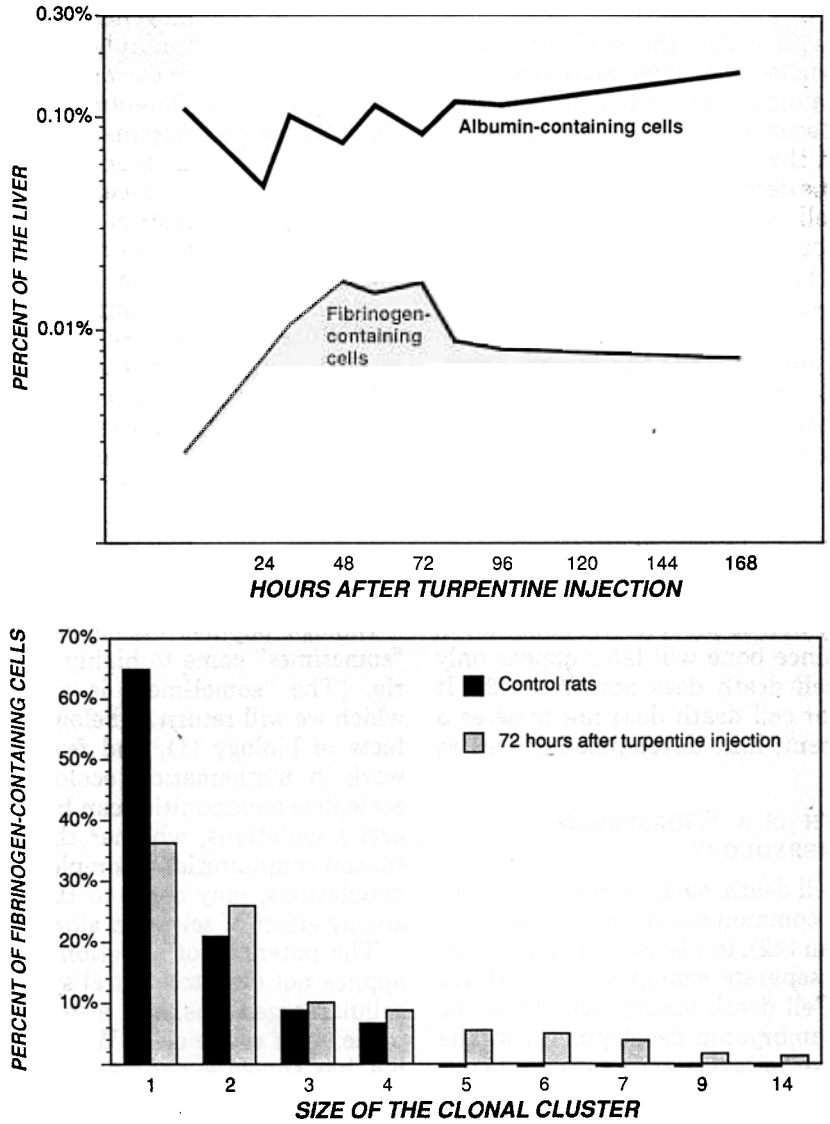

FIG. 4. Changes in the abundance and clonal constitution of plasma protein-containing hepatocytes during inflammation. *A*, Changes in the abundance of albumin- and fibrinogen-containing cells after the induction of an inflammatory response in rats by an injection of turpentine. *B*, Distribution of fibrinogen-containing hepatocytes into single cells and clonal clusters of various sizes during inflammation. Note that by 72 hours after the induction of the inflammatory response, there is a the general increase in size of the clusters of fibrinogen-containing cells, apparently by cell division.

SELECTIVE PROLIFERATION IS A WIDESPREAD OBSERVATION IN EMBRYOLOGY

Differential cellular proliferation is at work in many additional developmental processes. The heart, limbs, ear, liver, spinal cord, all begin as rudiments of small numbers of cells, which come to form their prominent structures by virtue of the simple process of growing faster than the rest of the embryo (30–35). Note in Figure 7, which shows a progressive series of external images of the chick embryo, how the emergence of the eye, the head, the limbs and the tail all appear to occur by the process of growing more rapidly than the embryo as a whole. This differential, or allometric, growth occurs throughout embryonic life, and provides a fundamental mechanism for the creation of anatomical structure during embryonic life (30–35).

THE VERTEBRATE LIMB

The creation of the vertebrate limb provides another illustration of the role that selective cell growth plays in the creation of anatomical structure. The limb traces its development to a small number of cells, called the limb bud. As noted above, the limb itself comes into existence from this bud by the simple mechanism of growing faster that the rest of the embryo (Fig. 7). This differential growth occurs at the growing edge of the limb, and Ede has found that the rate of cell replication, as measured by autoradiography, is greater in this growing portion than elsewhere in the limb. (36)

After the period of selective growth that creates the mass of the limb, condensations of bone-forming and muscle-forming cells appear roughly in the general pattern of bones and muscles which will constitute the

mature limb. It appears unlikely that limb morphogenesis occurs by a process in which the undifferentiated mesodermal cells are signaled to become muscle or bone, for the simple reason that undifferentiated mesenchymal cells with potential to become either myogenic or chondrogenic do not exist in the limb (2, 3, 37). In fact, the two cell types appear to be derived from entirely separate, noninterconvertable, cell lineages, which trace their origins to different regions of the embryo (2, 3, 38, 39). Thus, the mesoderm of the limb bud is a salt-and-pepper mixture of cells with distinct developmental potentialities.

The appearance of limb structure out of the macroscopically homogeneous, but cellularly diverse, mesoderm of the limb bud seems to arise by a process of cell selection, which takes the form of differential cell death. The signs of this selective process are apparent in the massive amounts of cell death throughout the developing limb (Fig. 8). Large areas of this cell death occur at the front and back of the limb bud (the anterior and posterior necrotic zones) and in the central area (the opaque patch) (37). This cell death may well be the selective elimination of chondrogenic cells, since bone will later appear only in those areas where cell death does not occur (2). It might be said, then, that cell death does not arise as a result of limb development; limb development arises as a result of cell death.

Selective Cell Death is a Widespread Observation in Embryology

Massive amounts of cell death, such as in the morphogenesis of the limb, is a common occurrence in development (42–59). Glucksman (42), in his early review of this subject, enumerated 74 separate examples of cell death during embryonic life. Cell death occurs right from the very earliest period of embryonic development, at the time when the primary germ layers arise (45), and continues throughout embryonic life. Examples of this cell death include destruction of larval structures during tissue reformation in insect and amphibian metamorphosis, closure of the palate, invagination of the optic cup, shaping of the nose, creation of the digestive tract and heart, and formation of the nervous system (2, 3, 42–59). Overall, then, cell death occurs throughout development, and it is hard to escape the impression that the embryo is one massive cellular battlefield, with the winners feeding off of the losers.

Selective Interactions

If there is any single theme in vertebrate embryology, it is induction, the principle of one embryonic tissue influencing the fate of another tissue. The embryologic data, however, do not indicate whether induction is mediated by the influence of the inducing tissue on the cellular phenotypes of the target population, caused by instructive alteration of gene expression, or on the cellular composition of the target population, caused by selective growth or death. In fact, much of embryogenesis can be interpreted in the light of the latter possibility.

Many observations made over the last few years have implicated the role of growth factors in inductive processes (60–66). For example, there have been a number of findings that suggest that retinoids play an important role in limb morphogenesis (40, 64). These findings are suggestive of limb morphogenesis as a selective process, as these compounds are well-known tissue-specific agents of both differential cell growth and death (64). Perhaps the most dramatic example from experimental embryology may be seen in the involvement of growth factors in the early development of the amphibian embryo, where a number of the earliest inductive events of development appear to be associated with, and indeed may be caused, by a variety of growth factors, a half a dozen-or-so specific examples of which have been identified (65, 66). The most straightforward interpretation of these observations is that the action of these proteins is mediated through their capacity as growth factors, that is, as agents of selective cell growth. As Gurdon has written "growth factors may act as survival factors, enabling cells with certain dispositions to proliferate" (66).

THE POTENTIAL OF SELECTION FOR CREATING ORDER

Biologic populations molded by natural selection may "sometimes" come to highly ordered and stable equilibria. (The "sometimes" is an important qualification, which we will return to below). We know from the basic facts of biology (1), and from the theoretical body of work in mathematical ecology (67–70), that balanced ecological communities can be created by selection. Natural populations, whether they are complicated multispecies communities or simple two-species predator/prey populations, may come to stable equilibria by the balancing effect of selection alone.

The potential of selection to generate biologic order applies not only to natural systems comprised of multicellular organisms, but also to ecological communities made up of single cells. Wimpenny *et al.* (71), for example, has shown experimentally that cultures of bacteria in a semisolid medium may grow to yield highly ordered and reproducible patterns visible in three dimensions. The communities of cells within multicellular organisms may also generate such highly ordered systems. This has been shown theoretically by Swindale (72) who demonstrated with an idealized computer model of cells interacting by selection alone, that a selective system of mammalian cells may give rise to a stripped pattern, such as in a zebra's stripes or in the stripped pattern of innervation in the visual cortex. Thus, as shown experimentally by Wimpenny, and theoretically by Swindale, the emergence of form may be a spontaneous consequence of selection, resulting directly from the internal dynamics of the selective process, without requiring any external guidance or information.

The properties of selective systems have been accurately simulated as one example of a large class of model systems, in which order arises arise under nonequilibrium conditions. These nonequilibrium structures have been called dissipative structures. The first example of a idealized system in which structure may arise under nonequilibrium conditions was proposed by Turing (73), in the reaction-diffusion model of morphogenesis, and in recent years, a variety of such non-equilibrium structures have yielded to mathematical modelling and computer simulation. Whirlpools, weather patterns, convection,

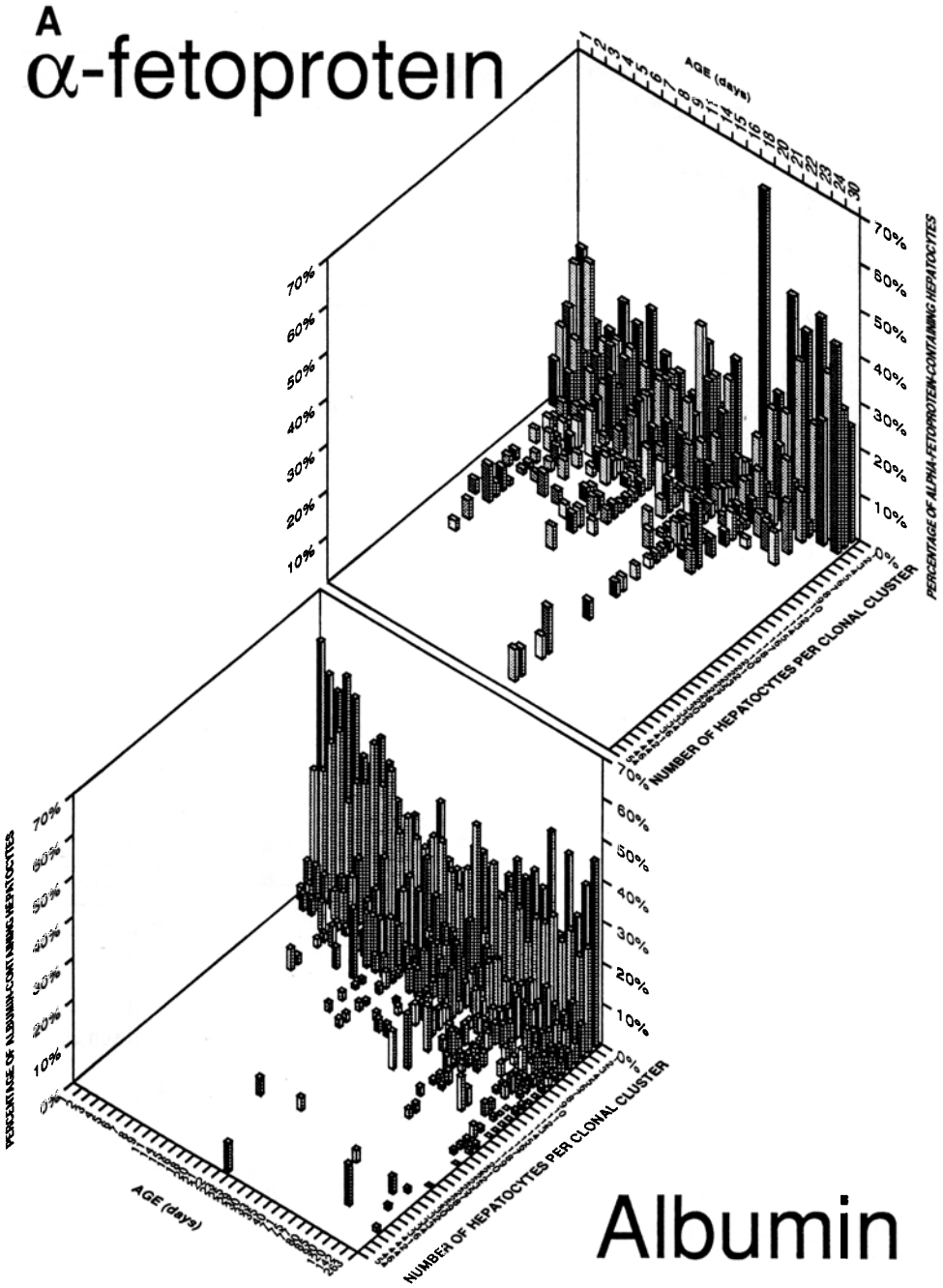

FIG. 5. *A*, Changes with age in the clonal constitution of plasma protein-containing hepatocytes. Shown is the distribution of α-fetoprotein- and albumin-containing hepatocytes into single isolated cells and clonal clusters of various sizes, as seen in livers from rats of various ages. *B*, Estimation of relative rates of cell growth of various types of plasma protein-containing hepatocytes throughout life. Displayed are the relative rates of cell division of α-fetoprotein- and albumin-containing cells in the rat, calculated from data on distribution of these hepatocytes into single isolated cells and clonal clusters of various sizes. Note that the growth rate of α-fetoprotein-containing cells exceeds that of and albumin-containing cells in 26 of 28 rats 16-days-old and less, while the growth rate of albumin-containing cells exceeds that of α-fetoprotein-containing cells in 7 of 8 rats older then 16 days of age. Apparently, the presence of large numbers of α-fetoprotein-containing cells early in life can be ascribed to their high mitotic rate at this time, whereas the decline with age in the abundance of these cells appears to be caused by their subsequent failure to divide as rapidly as the other hepatocytes in the liver.

heart-beats, the clustering of asteroids, have all proved susceptible to this type of analysis (74–78). These dissipative structures share a number of characteristic features; they contain the seemingly antagonistic tendencies toward both order and spontaneity (74–78). Dissipative structures arise without any pre-plan. That is to say, although the final structure that arises in a non-equilibrium system are determined by initial conditions, there is no obvious information in the initial conditions in which we can find an image of the final structure.

HEALTHY POPULATIONS, SICK POPULATIONS

As we noted above, selection is a mechanism that generates order, but only sometimes. Theoretical meth-

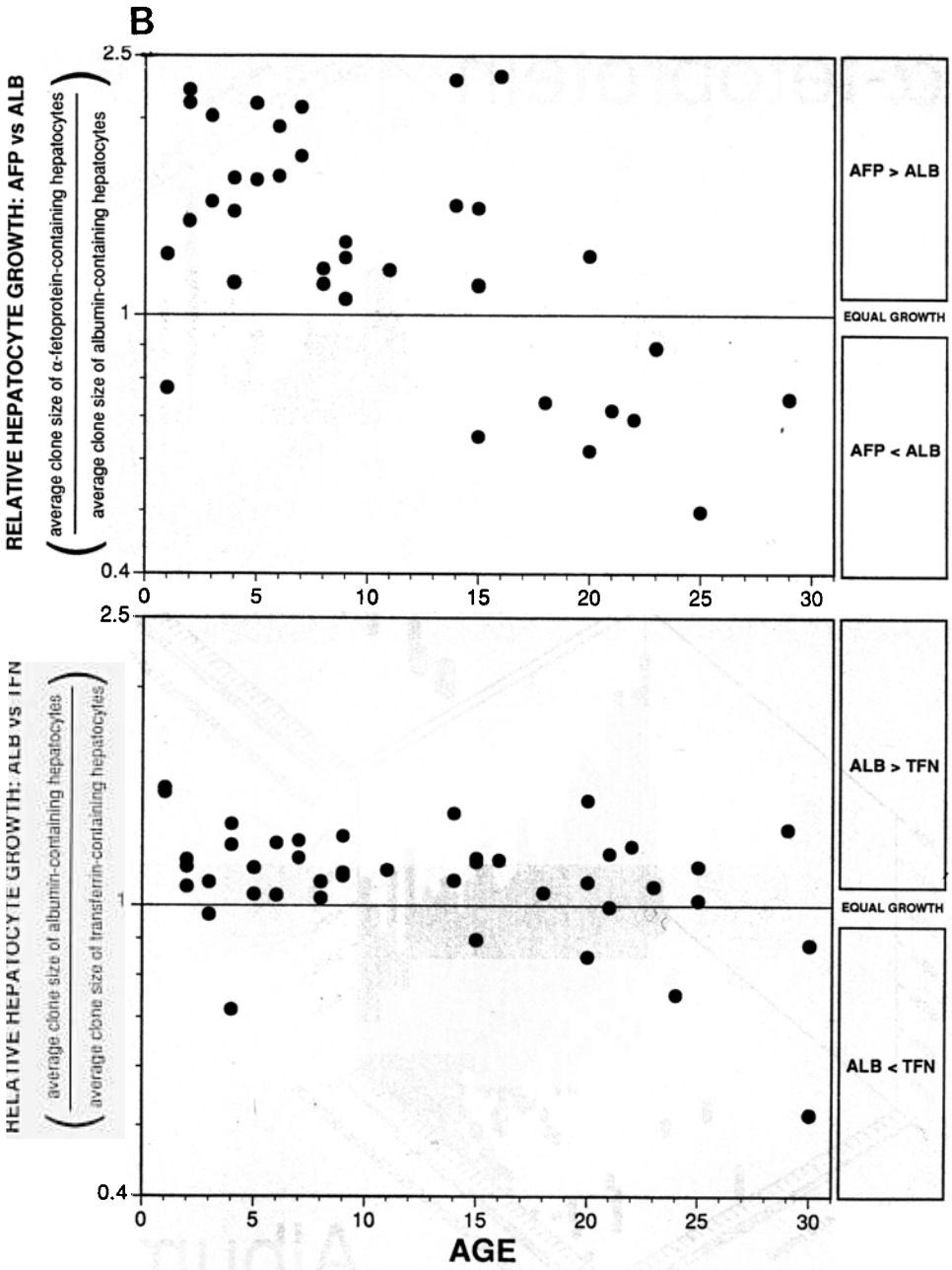

FIG. 5. (cont'd)

ods in ecology allow us to determine this sometimes quality of selective systems, that is to determine if the details of selection lead to an equilibrium, and if so, whether that equilibrium is stable or not. (To visualize the difference between a stable and an unstable equilibrium, an egg at the bottom of a bowl is at a stable equilibrium, while an egg balanced on the point of the Washington Monument is at an unstable equilibrium.)

If it is possible to think about a person as an ecological population of cells, might we not think about a healthy person as a stable population of cells at equilibrium? On the other hand, might we not be able to think of a patient with a growing tumor, or a malnourished child with a failure to thrive and grow, as unstable, nonequilibrium populations?

Consider the simplest model of population structure, the growth of a population until the resources of the population are totally utilized. It has been useful to think about such a simple ecosystem as an S-shaped curve. The simplest mathematical expression of this simple system is the logistic equation, where the rate of growth $= \mathbf{rn} - \mathbf{rn^2/k}$, where **r** is the intrinsic rate of growth of the population, **n** is the number of individuals in the population and **k** is the maximum number of individuals that can be sustained by the environment. All populations that grow according to this growth law have an equilibrium that will be reached when the population grows to a size of **k** individuals. However, as May (67–70) has shown, depending upon the specific growth values, particularly of **r**, the equilibrium can be stable or unstable. Generally, high values of **r** lead to unstable equilibria while low values of **r** yield stable equilibria.

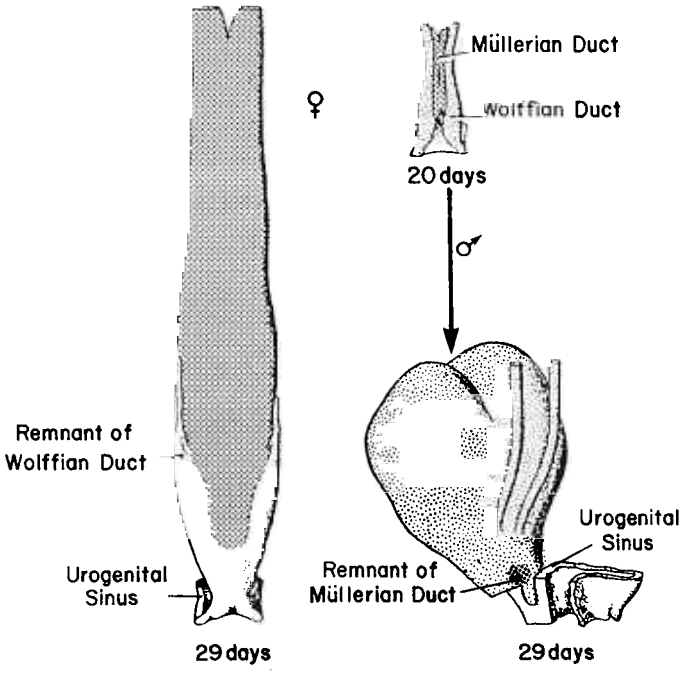

FIG. 6. Schemic illustration of the development of sexual structures in the rabbit. Only the posterior region shown. Mullerian duct (*dense stipling*), Wolffian Duct (*light stipling*), urogenital sinus (*white*). From Jost (26, 27).

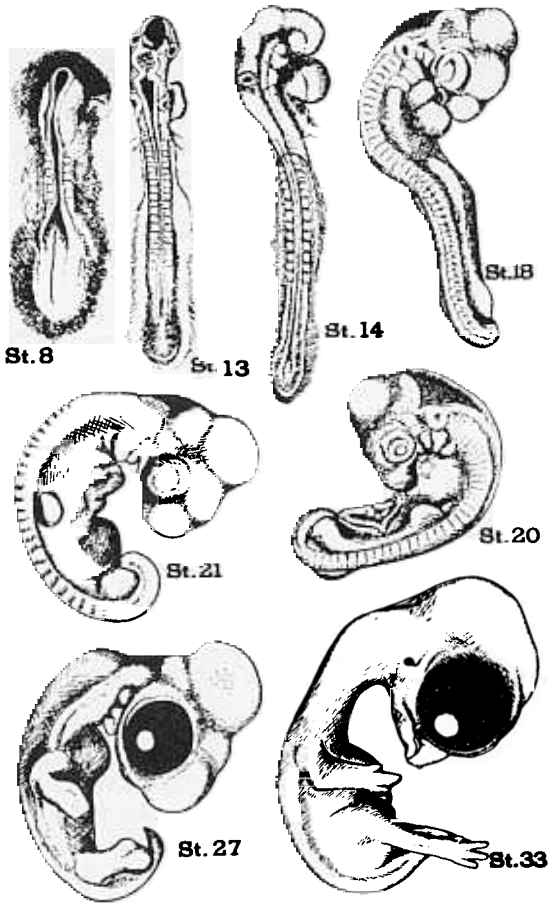

FIG. 7. Stages of development of a chick. From Balinsky (28).

Furthermore, when provided with additional information, such as the generation time, and the specific death and birth rates, it is possible to calculate the domains of stability and instability precisely.

May's analysis of the stability properties of populations has revolutionized ecology. The main limit to the application of this theory has been a scarcity of data. Ecological population data is enormously time-consuming and expensive to collect. Data on metazoan cellular populations may be far more easily assembled. Thus, not only does ecology have much to tell us about immunology, anatomy, and embryology, but immunology, anatomy, and embryology might also have much to tell us about ecology.

We have at our disposal a powerful theory that permits us to identify the stability properties of ecological populations. Perhaps this knowledge can be applied to the dissection of metazoan cellular biology. As in May's analysis of the stability properties of populations growing according to an S-shaped curve, there might be nothing obvious about the conditions that distinguished between a stable and an unstable outcome, but numerical methods might permit us to determine in advance the potential for stability (67–70). For example, if a patient with a tumor is a population of cells that is neither at equilibrium nor stable, is a patient with a pre-neoplastic condition a population of cells that is at an unstable equilibrium? Is the immune system of an individual with a genetic predisposition to autoimmune disease a population of immune cells at an unstable equilibrium? Might it be possible nudge these unstable populations into states of stability?

THE EVOLUTION OF SELECTION

GROUP SELECTION

Selection may seem, at first glance, to imply a desperate, selfish, struggle for survival, which does not match the commonplace biologic reality of cooperation and group behavior. How can selection and group behavior be reconciled? The answer to this problem lies in the fact that not only are self-centered genes favored by selection, but generous genes as well, if they rely upon the devious path of perpetuation through closely-related individuals. It may sometimes be better to help your children than to eat them. This concept of group selection was first proposed by Darwin, in order to explain the existence of sterile workers in colonies of social insects (79–85).

Of course, in order for a gene is perpetuated by group selection, the adaptive cost of the gene to the individual must be balanced by the likelihood that the gene will be perpetuated through a related individual. A familiar example might be the duplication and mutation of the lysozyme gene that occurred in the reptilian ancestors of the first mammals, which would become the α-lactoglobulin gene of milk (94). We may presume that the new, proto-lactoglobulin gene was favored by evolution, not because it helped the mother (in fact, it came with a definite cost) but because it favored the survival of the mother's offspring. Genes such as α-lactoglobulin will be

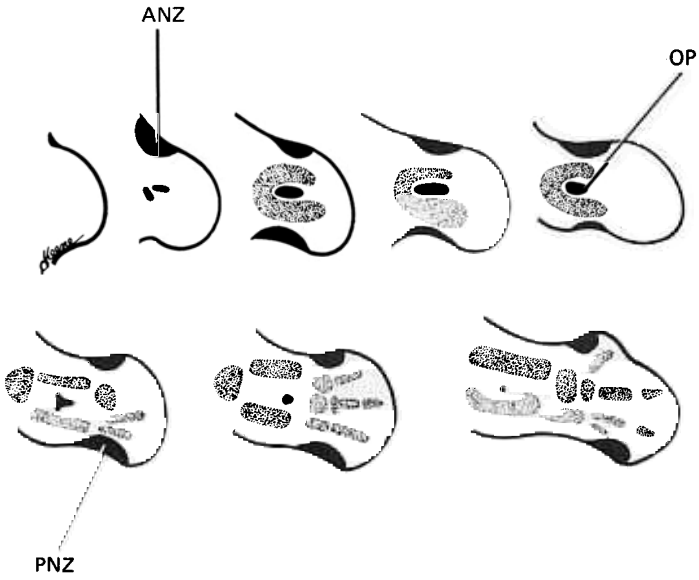

FIG. 8. Development of the chick wing, showing areas of highest cell death (*black*) and areas of chondrogenic condensation (*grey*). *ANZ*, anterior necrotic zone; *PNZ*, posterior necrotic zone; *OP*, opaque patch. From various sources, see Michaelson 1987 for references (2).

favored because they are perpetuated through the offspring, and similar genes will also be favored, although with less intensity, if they are perpetuated through more distantly related individuals. Thus behavioral genes, such as those that lead a mother to suckle both her own offspring and her sibling's offspring, will be favored, and, in fact, such behavior is known in mice.

GROUP SELECTION HAS LEAD TO THE CREATION OF HIGHER LEVELS OF BIOLOGIC ORGANIZATION

While the simplest, solitary, organisms may adapt by relying exclusively upon self-centered genes, extroverted organisms depend upon the force of group selection. Indeed, the very existence of group entities in biology can be traced to the evolutionary potential of group selection. Thus genes such as α-lactoglobulin make possible the entity of the family, while genes, such as those leading to foster-suckling, bring larger scale groups into being, in this case the deme.

Among unicellular organisms, group selection favors genes that limit individual reproduction, when this limitation favors the survival of the population as a whole. Yeast, for example, when the food supply becomes limited, do not engage in a desperate struggle for survival, but rather restrict their growth (86, 87). Presumably, this protects the local population, the shared gene pool, from the extinction that might result from the fratricidal struggle of individual cells.

THE CELL IS THE FUNDAMENTAL UNIT OF SELECTION

All group entities in biology are built up from cells, however diffuse these populations of cells might be: 10^6 cells in an ant, 10^9 cells in an ant colony, 10^{13} cells in a person, or 10^{21} cells in the species of humans. These group entities of cells—organisms, kin groups, demes, species, and so on—may well have come into being by the action of the group selection of cells, and are themselves subject to selection. Thus, an insect colony, such as a bumblebee nest, comprised of the offspring of a single queen, may be viewed as a single "diffuse organism", a thousand insects, or a billion cells (79, 83). A lichen plant may appear to be a single organism, but it is, in fact, an ecological community composed of two separate species, an algae and a fungus (88). It is only by a matter of convention that we consider each of ourselves to be a single individual rather than a colony of a trillion cellular organisms, or that we regard that peculiar clonal entity known as identical twins to be two separate organisms.

THE ORIGIN OF MULTICELLULARITY

Multicellular life is a relatively recent arrival in the history of life. Fossil evidence of unicellular organisms is at least 3 billion years old, yet the first metazoan fossils are only about 600 million years old (89–93). Earlier examples of multicellular life are hardly older than a billion years (114). Thus, for most of its history, the biologic world has been a world of unicellular organisms, a world exclusively ordered by the Darwinian struggle of cells.

It is easy to see why the first multicellular organisms might have had selective advantages over the unicellular organisms of the precambrian world; after all multicellular organisms are more flexible, versatile, resilient, and above all else, larger than unicellular organisms. How might such a desirable end have been achieved? We have seen how higher levels of cellular organization may appear among populations of unicellular organisms, such as yeast, by the mechanism of the group selection of cells. Group selection is strongest when acting upon closely related individuals, and this provides a powerful evolutionary force for the "walling of" these populations. Perhaps metazoans are one encapsulated version of such populations (95). If they are, then perhaps the essence of metazoan organization may be found in the internal domestication of the Darwinism, of the transformation of the natural selection of cellular populations into the cellular selection of metazoans.

DIVERSIFICATION

As we have seen, the population of cells that make up the metazoan body may be viewed much like any population of biologic organisms. Can the Darwinian lessons of natural populations tell us anything about the origin of cellular diversity in the cellular populations of embryos? In natural populations of organisms, heritable diversification arises by a variety of mechanisms that are stochastic. Here I am using the term stochastic according to its usage in probability and statistics, where it is defined as describing those processes that are probabilistic and occur over time (11, 12). In natural populations, these stochastic processes include, mutation, deletion, duplication, meiotic recombination, and independent assortment. Surprisingly, it has been found that, like these Darwinian events of heritable diversification, many of the events of cellular diversification that occur in embryonic populations are also stochastic in character (2, 3, 96–107).

The Stochastic Basis of Heritable Diversification in the Immune System

The classic example of a stochastic process of cell diversification occurs in the immune system. Indeed, cell selection is possible as a mechanism for the generation of antibody production because the immune system contains a preexisting cellular diversity of lymphocytes upon which selection may draw. This cellular diversity is created by a stochastic process of immunoglobulin gene assembly and scrambling. Thus, the immune system seems to have taken a leaf from the natural world, where genetic innovation created by heritable diversification arises by a variety of stochastic processes.

Of course, it might reasonably be viewed that the stochastic quality of cellular diversification that occurs in the immune system is a consequence of the immune system's unique process of immunoglobulin gene assembly. Nonetheless, although the process of gene recombination and scrambling is probably specific to the immune system, the more general stochastic kinetics that characterize the generation of lymphocyte diversity have been found in the generation of cellular diversity in a variety of developmental systems (2, 3, 96–107).

The Stochastic Basis of Heritable Diversification in the Liver

As noted earlier in this review, the hepatocytes of the liver possess a cellular heterogeneity, which may be visualized by immunofluorescence with antisera to plasma proteins (Fig. 1). It appears that this cellular heterogeneity of hepatocytes is generated stochastically. The simple fact that albumin, and other plasma proteins, are each present in small, separate, subpopulations of hepatocytes, is consistent with a stochastic model, in which activation of each plasma protein gene occurs with a probability **1/p**, the value of which is specific for each individual plasma protein gene. According to such a model, the probability of two copies of a gene being activated simultaneously in a single cell will be the much smaller value $(\mathbf{1/p})^2$. Of course, mice, being diploid organisms, possess two copies of each autosomal gene, and thus the stochastic model would predict that it would be much more likely for only one allelic form of a plasma protein gene to be expressed in a single cell than for both genes to be expressed. This is precisely what has been found with respect to the gene for albumin (19, 20).

Methodologically, this manifestation of the stochastic process of hepatocyte diversification was detected with an antiserum that reacts with just one of two allelic forms of albumin in the mouse. In the mouse, there are two allelic form of albumin, named *Alb-1*^{a} and *Alb-1*^{c} (4). *Alb-1*^{a} mice were immunized with albumin from *Alb-1*^{c} animals, and this resulted in an antiserum, Anti-Alb^c, which reacts with albumin of *Alb-1*^{c} genotype. The reactivity of this antiserum provided direct evidence that most cells expressing albumin are, in fact, expressing only one of the two allelic genes of albumin, as indicated by the observation that Anti-Alb^c reacts with only half of the albumin-containing cells in the livers from *Alb-1*^{a}/*Alb-1*^{c} heterozygous mice (Fig. 1*e* and *f*). Apparently, then, albumin gene activation occurs by a process that is stochastic, as made visible in the independent expression in single cells of each of the two allelic genes for albumin.

Stochastic processes display a second diagnostic feature, which can be detected if these processes are followed over time. As a general matter, stochastic models do not require **1/p** to be constant, but the stochastic processes familiar in natural populations, such as meiotic recombination, independent assortment and mutation, display this property. Stochastic models of this type are called by the somewhat incongruous name stationary stochastic processes, and may be detected by determining whether the value of **1/p** remains constant over time. That is, if activation of a gene occurs with a constant probability of **1/p**, occurring as a function of time **t**, then it follows that the total number of cells expressing that gene will be **(1/p × t)**, which, when graphed, will appear as a straight line intersecting the origin. In fact, the accumulation of cells expressing the albumin gene in the liver conforms quite closely to this expectation (Fig. 3).

By measuring the slope of the **(1/p × t)** line, it has been possible to derive an estimate of the value of the probability, $\mathbf{1/p_{alb}}$. As shown in Figure 3, in the rat, $\mathbf{1/p_{alb}}$ has a value of ~1/20,000, that is, each day, each uncommitted hepatocyte has a 1/20,000 chance becoming committed to the expression of albumin.

To summarize, then, the hypothesis that the creation of albumin-containing cells is a stochastic process is supported by two observations. First, it appears that each albumin gene activation event occurs independently, as revealed in livers from *Alb-1*^{a}/*Alb-1*^{c} heterozygous mice. Second, the rate at which albumin-containing hepatocytes are created is constant throughout life.

The Cell-Heritable Feature of the Liver's Process of Diversification

Plasma protein-containing hepatocytes are present in the liver both as individual cells and as clusters of multiple adjacent cells (Fig. 1*d*). Analysis of the clusters of albumin-containing cells in *Alb-1*^{a}/*Alb-1*^{c} heterozygous mice indicated that these clusters are clonal. Thus, although some clusters in these mice are Alb^{c+}, and some Alb^{c-}, within each cluster there is no variation. Presumably, each cluster is a clonal group. That is, each cluster is the mitotic product of a single progenitor hepatocyte. This progenitor may become committed either to express the *Alb-1*^{c} gene or not, but whatever option is taken, that property is inherited by the progeny of the progenitor cell. In other words, albumin gene expression, once achieved, becomes a cell-heritable property.

Stochastic Generation of Cellular Diversification in Other Systems

The stochastic quality that is characteristic of the cellular diversification in the immune system and the liver, has also been found in a variety of other events of cellular diversification. The first, and perhaps best known, identification of this stochastic quality was carried out by Till, McCollough, and Siminovitch (96), with respect to the creation of hematopoietic stem cells by the blood-forming tissues. Subsequently, the generation of cellular diversity has been found to occur stochastically

in a number of additional instances, including melanogenesis (97), globin gene expression (98, 99, 100), myogenesis, (101), and terminal differentiation (102).

The stochastic activation of individual genes, such as that which we observed for albumin genes, has also been observed in a variety of other systems. For example, the independent expression of allelic forms has been found to occur for a number of coat color genes, and is apparent as a patchy or variegated appearance in the pelt of heterozygous animals. This has been observed for coat color genes on half a dozen autosomes in the mouse, as well as for a variety of autosomal genes in guinea pigs, cattle, rabbits, dogs and pigs (see 2 and 103–105 for references). Independent expression of allelic genes has also been observed for globin genes, in the erythroid precursors from children heterozygous for a fetal globin genes. Apparently the two allelic forms of a fetal globin gene are not expressed coordinately in the progeny of single cells (106).

The stochastic quality has also been found in another aspect of gene expression, in DNA methylation. Silva and White have followed the creation of the cell-heritable acquisition of methylation at specific sequences, and have, in fact, found the acquisition of a methylated state to occur stochastically (107).

Stochastic Gene Activation and Gene Expression

The possibility that any aspect of gene expression may begin with an initial step that is stochastic may seem, at first glance, to be at odds with the striking order, which characterizes metazoan gene expression. Of course, part of this order may well be traced not to gene expression itself, but to the subsequent action of cellular selection. Furthermore, the stochastic quality that occurs during development may be only the first of many steps in the expression of genes. This first stochastic step appears to determine the cell-heritable component of gene expression (108), but there must certainly be other aspects of gene regulation that are reversible. It can hardly be doubted that once gene expression has begun, it is then subject to other levels of regulation, which are dependent upon environmental signals (109).

What is the Meaning of Stochastic Quality of Cellular Diversification?

The stochastic quality that is present in these processes of heritable diversification may seem strange and unlikely. There is, however, a fundamental physicochemical basis for suspecting that this might not, in fact, have been otherwise. The stochastic quality may be traced to a rather unusual realm of chemistry, the chemistry of single chemical events.

Most physicochemical processes are not stochastic in character, but are deterministic, that is to say, are ruled by cause and effect. There is, however, one area of physical behavior, to which the chemistry of single chemical events belongs, that is intrinsically stochastic; the domain of those physical process that involve change in the state of single atomic or subatomic elements. We recognize that the decay of an individual radioactive atom is random; we can not predict when the atom will decay, although we may model its behavior probabilistically and calculate the value of the probability of its decay for any given time. In the 1930's it was proposed that not only radioactive decay, but all of the elemental processes that involve the absorption or release of single quanta of energy, display this stochastic quality (110–113). While there were initially doubts as to the correctness of this concept (doubts about whether there really is a "God who plays dice" (78)), the empirical verification of this theory has been amply substantiated over the past 60 years. Accordingly, it is now generally held that all events that occur by the release or uptake of individual quanta of electromagnetic radiation, such as the change in the energy state of an electron, are fundamentally and irreducibly probabilistic (110–113).

If we could watch a single chemical event, such as the decay of a single molecule, we would find that the breakdown of this molecule would be well described by probability. The decay of a single molecule of H_2O_2, would provide a nice example of such a stochastic process. In large part, this stochastic quality may be ascribed to the random molecular motion of molecules, that is, the thermal quality of matter. However, even if we could isolate our molecule from this random noise, that is, if somehow we could "hold" the molecule in a molecular "tweezer", and observe its breakdown in isolation from thermal processes, we would still find that the breakdown of the molecule is utterly unpredictable. This is because the breaking of every chemical bond is a change in the energy state of a electron, and, as noted above, such quantal changes are inherently and irreducibly probabilistic (111–113). It is for this reason that single chemical events invariably display this characteristic stochastic quality.

Ordinarily, the stochastic feature of single chemical events has such a marginal effect on chemical processes that we may ignore their influence. In the chemical experiments that we usually carry out, we almost never see the effect of such single chemical events, because we observe bulk properties of enormous number of molecules. These bulk reactions of many molecules "smooth out" the stochastic unevenness of the individual molecules. However, the chemistry of nucleic acids is strikingly different in this regard, because all changes in the physicochemical state of nucleic acids are changes in or at single base pairs. Each nucleotide is like our molecule in a "tweezer", and the processes of life make visible the stochastic chemistry of each of these nucleotides. A child with the sickle-cell trait may have a disease that can be traced to a mutant globin protein, then to an altered amino acid, then to a change in a single nucleic acid residue, a change that is intrinsically probabilistic in character. Living things make the ultrafine stochastic graininess of the physical world visible on a macroscopic scale.

The fact that the chemistry of the genome is the chemistry of single chemical events insures that all changes in the state of the genome are stochastic. This is equally true whether the genomic change is mutation, methylation, or the noncovalent interaction of a particular DNA sequence with a regulatory molecule. Seen in this light, the many observations of stochastic gene activation no longer seem to be quite so strange, but appear

to be the biologic manifestations of stochastic chemistry of single chemical events.

The Usefulness of Chance

These stochastic changes in the genome, of which mutation is the most familiar example, are not simply accidents of nature, but provide the basic innovations upon which evolution depends. In fact, the resulting diversity is so useful to biologic adaptation that a variety of mechanisms have been developed that exploit this basic stochastic tendency of the genome. Sex, for example, with meiosis, independent assortment, and so on, is an elaborate mechanism that uses this inherent propensity for generating diversity. When the need is for variety, biologic systems have, time-and-time again, turned towards the natural stochastic tendency of nucleic acids. We have certainly seen this in the immune system. Perhaps the need for cellular variety in the early embryo has also been met by exploiting this essential stochastic quality of the genome, in order to provide the cellular diversity upon which selection depends.

Selection Makes Stochastic Gene Activation a Practicable Method of Cellular Diversification

At first glance, it may appear that a system of cellular differentiation based on stochastic gene activation would be enormously messy and inefficient. For example, as described above, albumin gene activation appears to occur with a probability $\mathbf{1/p_{alb}} = 1/20{,}000$. For any gene with such a probability value, only about .005% of cells will come to express the gene by the action of stochastic gene activation alone. However, if those few cells are selected (dividing every 8 hours), by 5 days the cells expressing the gene will constitute 50% of the population, by 6 days 85%, while by 7 days 98% of the population will express the stochastically activated gene. The enormous biotic potential of development makes the equally enormous inefficiency of stochastic gene activation a feasible mechanism of cellular diversification.

THE DARWINIAN WORLD WITHIN

As we have seen, two forces appear to be at work in the creation of cellular order: diversification and selection. The diversification that occurs during embryonic life appears to be cell-heritable and stochastic, and acts to create the many types of new cells that arise during developmental life. The cell selection of embryonic life appears to act in the creation of the order that arises during development. Immunologists have long found these forces to be at work in the development of the immune response, but these same Darwinian processes also appear to be at work in the liver, and in the many details of embryogenesis. Group selection provides an evolutionary mechanism by which the precambrian populations of unicellular organism may have evolved into the modern populations of metazoan cells. In short, each embryo appears to be a miniature version of the Darwinian world, in which the stochastic instability of DNA provides for the stochastic generation of cellular diversity, and in which the Darwinian selection of cells provides for the organizing mechanism of development.

Acknowledgments: I would like to thank Huber Warner of the National Institute on Aging for his advise and suggestions on the experiments concerning the effects of age on plasma protein synthesis, Tom Jessel and Jane Dodd for teaching us the immunofluorescence technique, my colleagues Phuong N. Huynh, Jing Xing Yu, Olga Falkowsky, Daniel Nicoli, Pei Ge, Takao Takahashi, Verne Caviness, Roger Anderson, Jonny Wee, Ken Winter, and Dan Michaelson for deciphering the selective basis of cellular order in the liver, and especially Igor Rosenvald for many stimulating discussions and for many excellent suggestions on this manuscript. This paper was written in the wonderful library of the Marine Biological Laboratory in Woods Hole, Massachusetts.

This work was supported by a Grant CA37374 from the National Institutes of Health.

REFERENCES

1. Begon M, Harper JL, Townsend CR. Ecology Sinauer Associates Sunderland 1985.
2. Michaelson J. Cell selection in development. Biol Rev 1987;62:115–139.
3. Michaelson J. The significance of cell death. In: Apoptosis (Eds Cope F.O., Tomlei, L.D.). Cold Springs Harbor Press, 1991.
4. Mintz B. Mosaic expression of a tyrosine fusion gene in albino mice yields a heritable striped coat color pattern in transgenic homozygotes. PNAS 1992;88:9643–9647.
5. Mintz B. Developmental Mechanism found in allophenic mice with sex chromosomal and pigmentary mosoaicism. Birth Defects Original Articles Series 1969;V:11–22.
6. Mintz B. Gene expression in allophenic mice. Symp Int Soc Cell Biol 1970;9:15–42.
7. Mintz B. Clonal basis of mammalian differentiation. Symp Soc Exp Biol 1971;25:345–342.
8. Till JE. Cellular Diversity in the Blood Forming System. American Scientist 1981;69:522–527.
9. Till JE, Mcculloch EA. Hemopoietic stem cell differentiation. Biochimica et Biophysica Acta 1980;605:431–459.
10. Lederberg J. Ontogeny of the clonal selection theory of antibody formation. Reflections on Darwin and Ehrlich. Ann NY Acad Aci 1988;546:175–182.
11. Thorp EO. Elementary probability. John Wiley and Sons Inc. New York, 1972.
12. Lindley DU. Probability and Statistics. Cambridge University Press. Cambridge, 1979.
13. Chapman RN. The Quantitative Analysis of Environmental Factors. Ecology 1928;9:111–122.
14. Hara K. The cleavage pattern of the Axolotl Egg Studied by Cinematography and Cell Counting. Wilham Roux's Archives 1977;181:73–87.
15. Theiler K. The House Mouse; Atlas of Embryonic Development. Springer-Verlag New York 1989.
16. Gamow EI, Prescott DM. The cell life cycle during early embryogenesis of the mouse. Exp Cell Res 1970;59:117–123.
17. Conrad E, Ingraham S. Rate of hemolytic antibody production by single cells in vivo in rabbits. The Journal of Immunology 1974;112:17–25.
18. Perelson A, Goldstein B. Antigen modulation of antibody forming cells: the relationship between direct plaque size, antibody secretion rate and antibody affinity. The Journal of Immunology 1977;115:1649–1651.
19. Michaelson J. The significance of cell death. In: Apoptosis (Eds Cope F.O., Tomlei, L.D.). Cold Springs Harbor Press, 1991.
20. Michaelson J. Heterogeneity of Liver Parenchymal Cells. Cell Diff Dev 1989;27:751.
21. Putnam W. The Plasma Proteins. Academic Press. Second Edition/Volume 1, 1975.
22. Morgan EH, Peters T, Jr. The biosynthesis of rat serum albumin. V. Effect of protein depletion and refeeding on albumin and transferrin synthesis. J Biol Chem 1971;246:3500–3507.

23. Schreiber G, Tsykin A, Aldred AR, Thomasm T, Fungm WP, Dickson PW, Cole T, Birch H, DeJong FA, Milland J. The acute phase response in the rodent. Ann NY Acad Sci 1989;557:61–85.
24. Abelev GI. Alpha-fetoprotein in oncogenesis and its association with malignant tumors. Adv Cancer Res 1971;14:2915–358.
25. Nahon JL. The regulation of albumin and α-fetoprotein gene expression in mammals. Biochimie 1987;69:445–459.
26. Jost A. Sur Le Control Hormonal de la Differentiation Sexualle du Lapin. Arch Anat Micro Morph Exp 1950;39:577–586.
27. Jost A. Problems of Fetal Endocrinology: The Gonadal and Hypophyseal Hormones. In: Recent Progress in Hormone Research 1952;8:379–418.
28. Balinsky BI. Embryology. Third edition, W.B. Saunders Co, Philadelphia, 1970. Emphasis has been added for this review.
29. Frank GM, Ueber Gesetzmassigkeiten in der Mitosenveteilung in den Gehirnblasen im Zusammenhang mit Formbildungsprozessen. Arch. miktrosk. Anat Entw Mech 1925;104:262–272.
30. Goedbloed JF. Embryonic and postnatal growth in the rat and mouse. Acta Anat 1980;106:108–128.
31. Huxley J. Problems of relative growth. MacVeagh, London 1932.
32. Siddiqui RA, McCutcheon SN, Blair HT, Mackenzie DDS, Morel PCH, Breir BH, Gluckman PD. Growth allometry of Organs, Muscles and Bones in Mice from Lines Divergently Selected on the Basis of Plasma Insulin-like Growth Factor I. Growth, Development and Aging 1992;56:53–60.
33. Gould SJ. Ontogeny and Phylogeny. Harvard University Press Cambridge 1977.
34. Gould SJ. Allometry and Size in Ontogeny and Phylogeny. Biol Rev 1966;41:587–640.
35. Gould SJ. Ontogeny and Phylogeny Revisited and Reunited. BioEssays 1992;14:275–279.
36. Ede DA. Control of Form and Pattern in the Vertebrate Limb, In: Control mechanisms of differentiation and growth. Society of Experimental Biology Symposium 25. Eds: Davies, D.D. and Balls, M. Cambridge University Press. Cambridge. 1971.
37. Hinchliffe JR, Johnson DR. The Development of the Vertebrate Limb. Clarendon Press, Oxford, 1980.
38. Abbott J, Schiltz J, Dienstman S, Holtzer H. The phenotypic complexity of myogenic clones. Proc Nat Acad Sci USA 1974;71:1506–1510.
39. Christ B, Jacob HJ, Jacob M. Experimental analysis of the origin of the wing musculature in avian embryos. Anatomical Embryology 1977;150:171–186.
40. Eichle G. Retinoids and vertebrate limb pattern formation. Trends in Genetics 1989;5:123–127.
41. Tabin CL. Retinoids, Homeopboxes and Growth Factors: Toward Molecular Models for Limb Development. Cell 1991;66:199–217.
42. Glucksmann A. Cell death in normal vertebrate ontogeny. Biological Review 1950;26:59–86.
43. Kerr JFR, Searle J, Harmon BV, Bishop CJ. Apoptosis. In Perspectives on mammalian cell death (ed. C.S. Potten), Oxford University Press, Oxford, p. 93, 1987.
44. Cope FO, Tomei LD. Apoptosis. Cold Springs Harbor Press 1991.
45. El-Shershaby AM, Hinchliffe JR. Cell redundancy in the zona-intact preimplantation mouser blastocyst: a light and electron microscope study of cell death and their fate. J Emb Exp Morph 1974;31:643–654.
46. Bellairs R. Cell Death in chick embryos as studied by electron microscopy. J Anat 1961;95:54–65.
47. Ashwell KW, Watson CRR. The development of facial motoneurons in the mouse-neuronal death and the innervation of the facial muscles. Journal of Embryology and Experimental Morphology 1983;77:117–141.
48. Beaulaton J, Lockshin RA. The relation of programmed cell death to development and reproduction: comparative studies and an attempt at classification. Int Rev Cytol 1982;79:215–35.
49. Bursch HS, Taper B, Lauer, Schulte Hermann R. Quantitative histological and histochemical studies on the occurrence and stages of controlled cell death (apoptosis) during regression of rat liver hyperplasia. Virchows Arch (Cell Pathol) 1985;50:153–166.
50. Buttyan R, Olsson CA, Pintar J, Chang C, Bandyk M, Ng PY, Sawczuk IS. Induction of the TRPM-2 gene in cells undergoing programmed death. Mol Cell Biol 1989;9:3473–81.
51. Columbano A, Ledda-Columbano GM, Coni PP, Faa G, Liguori C, Santa Cruz G, Pani P. Occurrence of cell death (apoptosis) during the involution of liver hyperplasia. Lab Invest 1985;52:670–5.
52. Duke RC. Self recognition by T Cells. I. Bystander killing of target cells bearing syngeneic MHC antigens. J Exp Med 1989;170(1):59–71.
53. Johnson EM, Jr, Chang JY, Koike T, Martin DP. Why do neurons die when deprived of trophic factor? Neurobiol Aging 1989;5:549–52.
54. Oppenheim RW, Nunez R. Electrical stimulation of hind limb increases neuronal cell death in chick embryo. Nature 1982;259:57–61.
55. Rotello RJ, Hockey MG, Gerschenson LE. Biochemical evidence for programmed cell death in rabbit uterine epithelium. Am J Pathol 1989;3:491–5.
56. Tomei LD, Kanter P, Wenner CD. Inhibition of radiation-induced apoptosis in vitro by tumor promoters. Biochem Biophys Res Commun 1988;30:155(1):324–31.
57. Trauth BC, Klas C, Peters AM, Matzku S, Mololer P, Falk W, Debatin KM, Krammer'PH. Monoclonal antibody-mediated tumor regression by induction of apoptosis. Science 1989;245:301–5.
58. Wadewitz AB, Lockshin RA. Programmed cell death: dying cells synthesize a co-ordinated, unique set of proteins in two different episodes of cell death. FEBS Lett 1988;241(1–2):19–23.
59. Raff MC. Social controls on cell survival and cell death. Nature 1992;356:397–400.
60. Witte ON, Steel locus defines new multipotent growth factor. Cell 1990;5:5–6.
61. Godin I, Deed R, Cooke J, Zsebo K, Wylie CC. Effects of the steel gene product on mouse primordial germ cells in culture. Nature 1991;352:807–8–9.
62. Matsui Y, Zsebo KM, Hogan BL. Embryonic expression of a hematopoietic growth factor encoded by the S1 locus and its ligand for kit. Nature 1990;347:667–669.
63. Berstein A, Forrester L, Reith AD, Dubreuil P, Rottapel R. The murine W/c-kit and Steel loci and the control of hematopoiesis. Semin Hematol 1991;28:138–142.
64. Favennec L, Cals MJ. The Biological Effects of Retinoids on Cell Differentiation and Proliferation. J Clin Chem Biochem 1988;26:479–489.
65. Jessell TM, Melton DA. Diffusible Factors in Vertebrate Embryonic Induction. Cell 1992;68:257–270.
66. Gurdon JB. The Generation of Diversity and Pattern in Animal Development Cell 1992;68:185–199.
67. May RM, Seger J. Ideas in Ecology. American Scientist 1988;74:256–267.
68. May RM. Chaos and the dynamics of biological populations. Proc Roy Soc Lond A 1987;413:27–44.
69. May RM. Stability and Complexity in Model Ecosystems 1973.
70. May RM, Oster GF. Bifurcations and Dynamic Complexity in Simple Ecological Models. The American Naturalist 1976;110:573–599.
71. Wimpenny JWT, Coombs JP, Lovit RW, Whittaker SG. A gel stabilized model ecosystem for investigating microbial growth in spacially ordered gradients. J Gen Micro 1981;127:277–287.
72. Swindale NU. A model for the formation of occular dominance stripes. Proceedings of the Royal Society of London 1980;208:243–264.
73. Turing A. The Chemical Basis of Morphogenesis. Phil Trans Roy Soc London Ser B 1952;237:37.
74. Nicolis G. Prigogine. Self-Organization in Nonequilibrium systems. John Wiley and Sons, New York. 1977.
75. Prigogine I, Stengers I. Order Out of Chaos., Bantam Books, Toronto, 1984.
76. Prigogine I. From Being to Becoming. W.H. Freeman and Co. San Francisco, 1980.
77. Ekland I. Mathematics and the Unexpected., The University of Chicago Press. Chicago, 1988.
78. Stewart I. Does God Play Dice? The Mathematics of Chaos. Basil Blackwell Inc. Cambridge, 1989.
79. Wilson EO. Insect Societies, Harvard University Press, Cambridge, 1971.
80. Hamilton WD. The Genetical Theory of Social Behavior. J Theoret Biol 1962;7:1–52.
81. Brown JL. Types of Group Selection. Nature 1966;211:870.

82. Lewantin RC. The Units of Selection. Ann Rev Ecol Evol p1–18 1970.
83. Holldober B, Wilsom EO. The Ants Harvard University Press Cambridge 1070.
84. Wilson EO. Insect Societies, Harvard University Press, Cambridge, 1971.
85. Haldane JBS. On Being the Right Size, and Other Essays. Oxford University Press, Oxford, 1985.
86. Forsburg SL, Nurse P. Cell Cycle Regulation in the yeast Sachromyces cerervisiae and Schizossaccharomyces pombe. Ann Rev Cell Biol 1991;7:227–256.
87. Pringle JR, Hartwell LH. The Sachromyces cerervisiae Cell Cycle. In: The molecular biology of the yeast sachromyces. Eds. Strathern, J.N. Jones, E.W., and Broach, J.R. Cold Springs Harbor Press Cold Spring Harbor 1981.
88. Mason EH. The biology of Lichens. Third edition, Edward Arnold Co., New York, 1982.
89. Schopf JW. The oldest fossils and what they mean. In: Major Events in the History of Life. Ed. Schopf, J.W. Jones and Barletts Publishers, Boston 1992.
90. Runnegar B. Evolution of the earliest animals. In: Major Events in the History of Life, Ed. Schopf, J.W. Jones and Barletts Publishers, Boston 1992.
91. Gould SJ. Wonderful Life. W.W. Norton Co, New York 1989.
92. Wittington HB. The Burgess Shale Yale University Press. New Haven 1985.
93. Jun-Yuan C, Bergstrom J, Xianhuang H. Fossilized Soft-bodied Fauna. Research and Exploration 1991;7:8–19.
94. Dickerson RE, Geis I. The Structure and Action of Proteins. W.A. Benjamin Inc. Menlo Park 1969.
95. Buss L. The Evolution of Individuality. Princeton University Press. Princeton. 1987.
96. Till JE, McCulloch EA, Siminovitch L. A stochastic model of cell proliferation based upon the growth of spleen colony-forming cells. PNAS 1964;51:29–36.
97. Bennett DC. Differentiation in mouse melanoma cells: initial reversibility and an on-off stochastic model. Cell 1983;34:445–453.
98. Gusella J, Geller R, Clarke B, Weeks V, Housman D. Commitment to erythroid differentiation by Friend erythroleukemia cells: a stochastic analysis. Cell 1976;9:221–229.
99. Levenson R, Housman D. Commitment: How do cells make the decision to differentiate? Cell 1981;25:5–6.
100. Orkin SH, Harosi FI, Leder P. Differentiation in erythroleukemic cells and their somatic hybrids. Proc Natl Acad Sci 1975;72:98–102.
101. Nadal G. Myoblast differentiation in the absence of DNA synthesis. Cell 1978;11:855–864.
102. Smith JR, Whitney RG. Intraclonal variation in proliferative potential of human diploid fibroblasts: stochastic mechanism for cellular aging. Science 1980;208:82–84.
103. Searle AG. Comparative Genetics of Coat Color in Mammals. Logos Press, London 1965.
104. Silvers WK. The Coat Colors of Mice. Springer-Verlag New York, 1979.
105. Mintz B. Mosaic expression of a tyrosine fusion gene in albino mice yields a heritable striped coat color pattern in transgenic homozygotes. PNAS 1992;88:9643–9647.
106. Kidoguchi K, Ogawa J, Karam D, Schneider G, Carpentieri U. Synthesis of Hemoglobin F. malta-I in culture by adult circulating erythropoietic precursors. Blood 1980;555:334–337.
107. Silva AJ, White R. The Genetics of Mosaic Methylation Patterns in Humans. In: Nucleic Acid Methylation. p395–408 1990.
108. Riggs Arthur D. DNA Methylation and Cell Memory. Cell Biophysics 1989;15:1–13.
109. Mitchel PJ, Tijan R. Transcriptional Regulation in Mammalian Cells by Sequence-Specific DNA Binding Proteins. Science 1989;245:371–378.
110. Feynman RP, Leighton RB, Sands M. The Feynmann Lectures on Physics. Vol III. Addison-Wesley Publishing House. Reading. 1965.
111. Steinfeld JI, Francisco JS, Hase WL. Chemical Kinetics and Dynamics. Prentice Hall, Englewood Cliffs, 1989.
112. Bartholomay AF. Stochastic Models for Chemical Reactions. Bull Math Biophys 1958;20:175–190.
113. McQuarrie DA. Stochastic Approaches to Chemical Kinetics. J Appl Prob 1967;4:413–478.
114. Knoll AH. The early evolution of eukaryotes: A geological perspective. Science 1992;256:622–627.