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# Cells and Senescence

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## I. General Introduction

Senescence is customarily defined, at least roughly, as an increase in the force of mortality with time, that is, the probability that an organism will die within a unit time interval successively increases with the age of the organism. As we shall see, this crude definition contains a multitude of hidden pitfalls and obscurities which must be resolved before we can hope to have any comprehensive understanding of the mechanisms by which senescence occurs. The main purpose of this article is to come to grips with these basic problems within the context of recent experimental and theoretical work.

As defined above, senescence is a concept which can be meaningfully applied at any biological level. In its original usage, it pertained most directly to the observed progressive loss of fitness and vitality which occurs in the aging multicellular organism. However, it is also reasonable to inquire whether cells, or cell populations, also senesce, and if so, by what mechanisms. As we shall see later, senescence is meaningful in nonbiological contexts as well; mechanical and technological artifacts can become progressively less reliable in time, and historians such as Toynbee have called attention to similar phenomena at the level of human culture.

The original focus of research on senescence was on multicellular organisms. The cell theory suggests that senescence, like every other physiological property of multicellular organisms, finds its causes and explanation in events which occur at the cellular level. Thus cell biology becomes intimately intertwined with an understanding of whole-organism senescence. This article is devoted to a consideration of three related questions, all of which ultimately relate back to the basic problem of the interrelation between the cell and the organism:



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1. Do individual cells undergo senescence?
2. If so, does cellular senescence in a multicellular organism suffice to explain the senescence of the organism?
3. If not, what cellular properties, occurring in which cells, do suffice to explain organism senescence?

The first question is primarily of an experimental character; the other two must involve a substantial amount of theory.

Just as physiological events in the organism are reflected back to properties of the constituent cells, so cellular events are referred back to properties of the constituent molecules. Thus the three basic questions we have posed can be, at least to some extent, referred to properties of systems of interacting molecules. To this extent, a cell theory of senescence must also be a molecular theory of senescence as well.

It should also be observed that the tacit homology between cells as elements of organisms, and molecules as elements of cells (which may be crudely summed up by the relation cell:organism = molecule:cell) is, as we shall see, responsible for the striking similarities between molecular theories of cellular senescence and cell theories of organism senescence. Such homologies must be carefully distinguished from reductionistic *implications* of the form: (molecular properties) imply (cellular properties) imply (organism properties). Both the homology aspects and the implication aspects play an important part in the following discussion.

This article is organized as follows. In Section II, we consider the experimental results which bear on the three questions posed above. First, we consider the evidence bearing on the existence of cellular senescence as an independent phenomenon. We then consider some of the experimental work bearing on whole-organism senescence and its relation to senescence in cells. In Section III, we present a review of the various theories which have been proposed (1) as mechanisms for cellular senescence itself and (2) for establishing a relationship between cellular and organism senescence. In Section IV, we provide a critical evaluation of the work we have reviewed and propose a variety of alternative approaches based on the concept of senescence as a general property of complex systems. Our conclusions are summarized in Section V.

## II. The Experimental Background

### A. Cellular Senescence

Until perhaps 20 years ago, it was widely believed that free-living cells do not generally exhibit senescence, hence that senescent behavior arises as a conse-

quence of supracellular organization. There were two main supports for this belief: (1) the cell culture experiments of Alexis Carrel (1912) and others, which indicated that differentiated cells apparently could be kept in a state of continuous multiplication indefinitely; (2) the apparent "immortality" of protozoan cultures, such as *Paramecium* and *Amoeba*.

Closer observation of the situation in protozoa revealed that the actual behavior of protozoan cultures was far more complex than had been originally supposed. In ciliates such as *Paramecium* and *Tetrahymena*, complex, genetically controlled life cycles were revealed, with well-defined states of immaturity, maturity, and senescence, terminating in death or rejuvenation through a sexual mechanism (cf. Nanney, 1974, for a review). In *Amoeba*, Danielli and Muggleton (1959) showed that normally immortal clones could be "spanned" by appropriate treatment, such as starvation; such a spanned clone proceeded normally for a fixed number of divisions and then all the cells died. Senescence in ciliates and spanning in *Amoeba* can both be argued to provide a selective advantage in terms of the general biology of the species.

Doubt was thrown on the immortality of cell cultures by the experiments of Hayflick and Moorhead (1961). These experiments showed that normal human diploid fibroblasts possess a finite replicative capacity *in vitro*. It was shown that this limited replicative capacity, which had in fact been observed by many others, is not due (as had been supposed) to conditions of cell culture, nutritional factors, viruses, or the depletion of some essential metabolic pool. For human fibroblasts, this "Hayflick limit" was of the order of 50 ± 10 cell doublings. The cells in such a system seem to pass through a definite "life cycle," which can be roughly analogized to that of ciliated protozoa; in the final phase (phase III) loss of replicative capability is accompanied by gross morphological changes and accumulation of debris.

Such observations have been repeated on a variety of other normal cell types (cf. le Guilly *et al.*, 1973). On the basis of such experiments, Hayflick has postulated that *all normal cell types have a limited intrinsic replicative potential* (now called the Hayflick limit). Conversely, the capability to show unlimited proliferation in culture is diagnostic of cell abnormality, which can generally be independently recognized (e.g., aneuploidy, viral infection or transformation, and malignancy).

Several other suggestive observations have been made by Hayflick and others. It has been shown that there exists at least a rough inverse correlation between the replicative capabilities of normal fibroblasts in culture and the age of the organism from which the cells have been taken (Hayflick, 1965). This correlation is not perfect, but it can be said that the average number of population doublings of cells taken from embryonic donors is significantly larger than the corresponding number of doublings of cells taken from adults. [It cannot be excluded, however, that this observation reflects further endocrine-induced differentiation

of fibroblasts *in vivo* in the adult (Finch and Hayflick, 1976).] Further, there is a direct correlation between replicating capability and the life span of species; the longer-lived the species, the greater the replicative capability (Hayflick, 1973). In human pathological conditions characterized by apparently precocious senescence, such as progeria and Werner's syndrome, fibroblasts are capable only of a very small number of doublings as compared with cells taken from normal individuals of the same age (Segal and McCoy, 1974; Danes, 1971). However, such conditions may not truly represent accelerated senescence (Spence and Herman, 1973).

However, it is observed that the Hayflick limit is most accurately expressed in terms of the number of *doublings* rather than in terms of clock time. For instance, human fibroblasts known to be capable of 50 doublings may be arrested at an intermediate doubling level (say 20 doublings) by immersion in liquid nitrogen for an arbitrary period of time. When restored to physiological conditions, these cells proceed through another 30 doublings; that is, they "remember" how far they have proceeded in the life cycle (Hayflick, 1965). The same is true for other kinds of arrests of proliferation in culture (cf. Dell'Orco *et al.*, 1973), and for serial propagation (Daniel and Young, 1971) in whole organisms.

One of the characteristics of aging fibroblast cultures is a gradual increase in intermitotic time. For instance, Merz and Ross (1969) observed that the proportion of nondividing cells increased from 1% to nearly 50% as the passage level increased. Macieira-Coelho and his colleagues (cf. Macieira-Coelho *et al.*, 1966) showed that the preponderance of nondividing cells (up to 85%) in old cultures were blocked in $G_1$, and the remainder in $G_2$. These observations have been confirmed by Yanishevsky and Carrano (1975). Gelfant and Graham Smith (1972) argued that senescence in fibroblast cultures, and also in the intact organism, could be understood as the trapping of cells in noncycling states.

It is interesting to compare nondividing phase III cells with phase II (actively growing) cells in *confluent* culture, which are also nondividing as a result of contact inhibition. These cells are also predominantly blocked in $G_1$ (Gelfant and Graham Smith, 1972), but clearly the mechanism of blockage is different than in the older cells; contact-inhibited cells can be released from the block simply by restoring them to less crowded conditions.

It should be borne in mind in evaluating all these observations that the *fibroblast cultures represent heterogeneous populations*. This is best seen in the detailed genealogical studies of Absher, *et al.* (1974), in which wide variations in interdivision time and proliferative capability of individual cells were noted, whether they were taken from young or old cultures. Indeed, these investigators found cells which morphologically and behaviorally resembled phase III cells in cultures of all ages (cf. also Brandes *et al.*, 1972).

In connection with Hayflick's postulate of limited doubling potential of normal cells, it should be mentioned that Moore and his co-workers (1967; Moore and

McLimans, 1968) reported that suspension cultures of normal human lymphoblasts apparently can be maintained indefinitely. See also Hsu and Cooper (1974).

Complementary to these *in vitro* studies are various *in vivo* investigations utilizing serial transplantation of cells and tissues from older organisms into younger recipients. For instance, Daniel and his collaborators (1968; Daniel and Young, 1971) serially transplanted epithelial cells from mouse mammary gland. It was found that the line was lost after at most seven serial transplantations over a total duration of about 2 years; this is of the order of the life span of an intact mouse. In another series of experiments (Siminovitch *et al.*, 1964; Cudkowicz *et al.*, 1964), bone marrow cells were innoculated into recipient mice which had received massive doses of radiation. Such cells form colonies in the spleen of the recipient, and cells from these colonies can be serially transferred to new recipients. It was found that the ability to repopulate the spleens of heavily irradiated recipients was lost after about four transplantations.

However, Krohn (1962, 1966) maintained grafts of mouse skin through serial transplantation for at least 6 years. Similarly, Franks (1970) has reported that mouse prostate tissue grafted onto the flank of a recipient survived serial transplantation, with no visible deterioration, for at least 6 years. Thus, the cells in these serially transplanted tissues have apparently survived for several times the normal life span of the mouse without overt signs of senescence. Harrison (1972) has reported that mouse bone marrow serial transplants can persist long beyond the life of a single mouse if the interval between transplants is lengthened.

A study closely related to cellular senescence is that of "programed cell death." However, as we shall see, the two concepts are quite distinct; whereas senescence is defined as involving an increasing probability of death, and a corresponding loss of fitness, with increasing age, cells programed to die may typically remain fully viable up to the catastrophic event which terminates their life. Cell death plays an important role in morphogenesis, in the maintenance of normal physiological structures such as skin, and in reorganizations such as are found in insect and amphibian morphogenesis (for reviews, see, for instance, Saunders and Fallon, 1966; Lockshin and Beaulaton, 1975, and the bibliographies contained therein). As an example, the phenomenon of spanning in *Amoeba*, mentioned above, may be more closely related to programed cell death than to cellular senescence.

Several different lines of research have been directed toward elucidating the mechanism of cellular senescence and cell death. In an investigation of the spanning phenomenon in *Amoeba*, Danielli and his co-workers (Muggleton and Danielli, 1968) found that unspanned or immortal amebas could be switched to the spanned state by either the injection of a small amount of cytoplasm taken from a spanned cell, or the transplantation of a nucleus taken from such a cell into an enucleated unspanned cell. Other kinds of hybridization experiments have been performed with cells from fibroblast cultures using standard techniques of

somatic cell hybridization. Davidson and Ephrussi (1970) and Goldstein and Lin (1972) showed that senescent fibroblasts could be hybridized with a permanently established (heteroploid) cell line; the hybrids were capable of unlimited proliferation; this, it could be argued, shows that senescence is "recessive" in hybridization with a proliferating cell. A similar result was obtained by Norwood *et al.* (1975) on hybridizing senescent fibroblasts to HeLa cells or SV40-transformed fibroblasts. These workers showed that DNA synthesis could be reinitiated in the "old" nuclei in such hybrids. However, Norwood *et al.* (1974) reported that similar hybrids between early- and late-passage diploid "fibroblast-like" cells showed an opposite behavior; DNA synthesis in the "young" nuclei in such hybrids was extinguished, indicating a dominance of the senescent character in this case. In more recent work along these lines, Muggleton-Harris and Hayflick (1976) fused enucleated cytoplasts from young fibroblasts with nuclei from older cells, and conversely, using cytochalasin B and Sendai virus. They observed that karyoplast-cytoplast fusion was accomplished most successfully when old karyoplasts were involved, and that in any case both old karyoplasts and old cytoplasts appeared to confer their properties on younger cytoplasts and karyoplasts, respectively. Rao (1976) investigated the capability of young and old fibroblasts to reinitiate DNA synthesis in dormant chick erythrocyte nuclei when fused with fibroblasts of various ages. He found that both RNA and DNA synthesis were resumed in such nuclei when incorporated into younger (phase II) fibroblasts, but DNA synthesis was not resumed (although RNA synthesis occurred) when they were incorporated into old (phase III) cells. Significantly, the same behavior in the chick nuclei was observed when they were introduced into enucleated cytoplasts formed from young and old fibroblasts.

Such investigations as these have been supplemented by extensive ultrastructural and biochemical studies, particularly of senescing and nonsenescing (transformed) cell cultures.

In an ultrastructural study comparing young (early-passage, phase II) cells with senescing (late-passage, phase III) cells from Hayflick's line of human fibroblasts, Lipetz and Cristofalo (1972) reported the following: As the cells senesced, changes appeared in the nuclei, the mitochondria, the Golgi-lysosome-vacuolar system, the endoplasmic reticulum, and the microfibrils. Typically, the nuclei became highly lobar. Although the mitochondrial number did not change significantly, the mitochondria of old cells exhibited changes in their patterns of cristae, as well as a variety of bizarre shapes. There is a sharp increase in lysosomal activity and autophagocytosis, and an increasing prominence of the Golgi apparatus and vacuoles. The endoplasmic reticulum of younger cells tends to appear dilated and "filled," while in old cells the opposite is true; and there are extensive ribosome-poor regions. Finally, the older cells exhibit extensive fibrillar structures throughout the cell; in young cells fibrils

tend to be found near the membranes. Similar results for chick fibroblasts were reported by Brock and Hay (1971).

Lipetz (1973) studied several transformed lines of human fibroblast cells, in comparison with normal fibroblasts. He found that the virally transformed, and hence immortal, lines were ultrastructurally more similar to young than to old fibroblasts. The two lines studied arose from transformation of both young and old fibroblasts; the similarity of the two transformed lines indicates a rejuvenation of the older fibroblasts through transformation (although it cannot be excluded that the transformed line in this case arose from a young cell present in the senescent culture).

However, Robbins *et al.* (1970) showed that the ultrastructural changes occurring in senescing cultures are not found in corresponding fibroblasts taken from aging individuals, and indeed that ultrastructural changes not characteristic of *in situ* fibroblasts take place very soon after cells are placed in culture, regardless of the age of the donor.

Such ultrastructural studies have been paralleled by extensive cytochemical and biochemical analyses seeking to identify biochemical correlates of cell senescence. These studies generally fall into two classes: (1) studies of comparative molecular populations in young, old, and transformed (immortal) cells; (2) studies of the response of such cells to specific culture situations designed to test the fidelity of macromolecular processes.

In the first category, much study has been concentrated on enzymes and protein synthesis. Typical of the enzyme studies is that of Wang *et al.* (1970), refining earlier studies by Cristofalo and his collaborators (e.g., Cristofalo and Kritchevsky, 1967). In this study of the enzymic properties of different doubling levels of cells from normal human fibroblast cultures, three classes of enzymes were considered: (1) phosphatases, considered typical of lysosomal activity; (2) dehydrogenases, associated with carbohydrate metabolism; (3) transaminases, involved in amino acid metabolism. No really dramatic changes were found, though acid phosphatase activity seemed significantly elevated in phase III cells. Yamanaka and Deamer (1974) reported that superoxide dismutase, an enzyme associated with protection of the cell against peroxidation (and associated free-radical damage) did not change in such cells, either as a function of age or of viral transformation. Srivastava (1973) assayed nuclear and cytoplasmic enzymes associated with nucleic acid metabolism (RNase, DNase) and protein synthesis in senescing cultures; no significant changes in the cytoplasmic enzymes were found with increasing age, but nuclear RNA-synthesizing activity declined while nuclear RNase, DNase, and so on, increased; see also Ryan and Cristofalo (1972). Turk and Milo (1974) studied the effect of cortisone on fibroblast cultures and found that treatment with this compound reduced acid phosphatase levels in older treated cells to those found in untreated younger cells.

Many other such studies are available in the literature; we consider some further aspects in Section II,B.

Another cellular system which has been widely studied in this context is the DNA repair system. Typical of the results in this area are those of Painter *et al.* (1973), who measured repair replication in ultraviolet-irradiated fibroblasts at different passage levels. These investigators found reductions in repair levels only at the very last passages and concluded that the integrity of the DNA repair system was maintained through senescence. However, Epstein *et al.* (1974) showed that cells taken from patients with progeria exhibited significantly less ability to repair single-strand breaks than did normal fibroblasts. Wheeler and Lett (1974) investigated the DNA repair system in postmitotic cells (cerebellar cells of dogs of various ages) and showed that there was no age-associated decline in DNA repair (although, perhaps significantly, there was a decline in the sizes of the DNA molecules extractable from these cells with age). Hart and Setlow (1974) examined DNA repair capabilities in fibroblasts of several species and argued that these repair capabilities were directly correlated with the life spans of the organisms from which the cells were taken.

Several other lines of investigation bearing on protein syntesis in cell cultures are of interest here. Cristofalo and his co-workers (Ryan *et al.*, 1974) found that nontoxic concentrations of amino acid analogs such as *p*-fluorophenylalanine incorporated into the culture medium of fibroblast cultures had no effect on proliferative capacity, even though they were incorporated into cell protein. Holland *et al.* (1973) and Tomkins *et al.* (1974) found that there was no significant difference between the ability of young and old fibroblasts to support viral infection and viral multiplication. Both of these observations are reflections of the fidelity of the protein-synthesizing processes in these cells and are pertinent to consideration of "error catastrophe" in these cells (see Section III). Another observation pertinent in this regard is that of Holliday and Tarrant (1972), who reported the presence of thermolabile forms of two dehydrogenases appearing in old fibroblast homogenates; they interpreted thermolability as being evidence of an alteration in primary structure in these enzymes.

As far as nucleic acid synthesis and their associated proteins are concerned, Cristofalo and Sharf (1973) argued that DNA synthesis in fibroblast cultures, as measured by thymidine uptake, depends on the number of doublings undergone by the culture; hence the number of nuclei capable of thymidine uptake at any time is an index of the age of the culture. Petes *et al.* (1974) reported that the rate of chain elongation (growth) of DNA in fibroblasts decreases with age.

Various membrane-associated changes have also been reported as cultures age. For instance, the observation of Muggleton-Harris and Hayflick (1976) that cell fusions occur more readily with old cells may indicate such membrane changes. Courtois and co-workers (Courtois and Hughes, 1974; Azencott and Courtois, 1974) report an age-related decline in adhesiveness in chick fibro-

blasts. Several investigators (Niewarowski and Goldstein, 1973; Haslam and Goldstein, 1974; Goldstein and Singal, 1972) have suggested that there is an age-related decline in various hormonal receptors on the cell surface, and also a loss of surface antigens (see also Roth and Adelman, 1974). However, Kritchevsky and Howard (1970) report that lipid composition in fibroblast cultures remains constant as the cultures age. Also, Brautbar *et al.* (1972) observed no decline in HL-A antigens on fibroblast surfaces as a function of culture age.

In his original work, as we have noted, Hayflick showed that culture conditions do not significantly affect proliferative capacity (cf. Hay, 1970). However, several agents do appear to extend the Hayflick limit significantly. For instance, hydrocortisone included in the culture medium appears to extend the limit by about 40% (Cristofalo, 1972, 1974). This effect may arise through stabilization of lysosomal membranes or through release from mitotic arrest. Packer and Smith (1974) and Epstein and Gershon (1972) have reported that the antioxidant vitamin E (DL-$\alpha$-tocopherol) incorporated in the medium extends the limit by 100% or more; this has, however, not been observed by some other investigators (e.g., Hochschild, 1973).

It should also be noted that Franks *et al.* (1970) were unable to maintain normal human epithelium in culture when it was separated from its stroma but could maintain undissociated tissue in culture for years; and Gey *et al.* (1974) report that chick fibroblasts could be maintained for years on a collagen substrate. The independence of the Hayflick limit from conditions of culture must be considered in the light of such results.

### B. Organism Senescence as a Cellular Phenomenon

Several lines of experimental investigation on senescence in multicellular organisms are pertinent to considerations of senescence and programed death at the cellular level. We cannot begin to review the literature on this subject comprehensively in such a limited space; the reader should consult Strehler (1962) and Comfort (1956) for good comparative discussions of the older literature.

Let us begin with lower forms. Many fungi exhibit senescent phenomena (e.g., *Aspergillus*), characterized by cessation of mycelial growth, accumulation of pigment, and ultimate death. The biochemical correlates of mycelial senescence in *Rhizoctonia* have been extensively studied by Gottlieb and his collaborators (e.g., Davies *et al.*, 1974). They found decreased rates of respiration and protein synthesis in aging mycelia and decreases in the activity of soluble enzymes and ribosomes. They also found decreasing levels of RNA with age, although the ratios of the various RNA species appeared to remain approximately constant. Smith and Rubinstein (1973a,b) showed that, in *Podospora*, the rate of senescence was cytoplasmically inherited, while the time of onset of senescence was related to a nuclear gene.

Some types of fungi, such as *Neurospora*, do not normally senesce. Holliday (1969) found a mutant of *Neurospora* which did exhibit senescence; he suggested that the mutant gene acted by increasing the "error rate" in the synthesis of protein. He also showed that cytoplasm from a senescent mycelium could induce senescence in a younger one, paralleling the results noted earlier on the spanning of *Amoeba*, and at least in some of the cell fusion experiments. See also Holliday (1975) and Thompson and Holliday (1973).

Poulter (1969) investigated the acellular slime mold *Physarum*. This organism is interesting because it exists either as a disaggregated population of free-living ameboid cells or as an aggregated syncytium. The ameboid state appears to be immortal, but the syncytium has a definite life span which appears to be genetically determined. Poulter fused syncytia with different life spans and found that the fused syncytia had intermediate life spans, with the exact value depending on the quantity of material from each of the parental syncytia. He also showed that the life span was insensitive to environmental factors such as heat shock, radiation, and starvation; this is quite distinct from the behavior of senescing fungal mycelia. McCullogh *et al.* (1973) reported an increase in nuclear size and DNA content in senescing plasmodia.

A highly suitable lower animal form for the study of senescence has proved to be the nematode *Turbatrix aceti*. This organism has a short life span, of the order of 30 days; it possesses a fixed number of cells after hatching, which (apart from the reproductive system) never divide or further differentiate. Two groups have studied the change in enzyme activity with age in these nematodes; all report progressive loss in the specific activity of such enzymes as aldolase and isocitrate lyase (e.g., Zeelon *et al.*, 1973; Gershon and Gershon, 1970; Reiss and Rothstein, 1974). However, one group, using immunological techniques, attributed the loss of specific activity to the accumulation of totally inactive molecules, while the other, using electrophoretic methods, found rather a spectrum of partial activity in a family of isozymes.

Similar senescence studies have been carried out on *Drosophila* (which, apart from the reproductive system, also shows no cell division in adult flies) and in other similar insects. Harrison and Holliday (1967) fed amino acid analogs to late larval *Drosophila* and found a diminished life span in the resulting adults. However, Dingley and Maynard Smith (1969) found no reduction in life span on feeding such analogs to young adults. According to Maynard Smith (1973), Shmookler (in press) found no difference in the ability of adult flies to discriminate between amino acids and their analogs when fed to flies of different ages. It is also observed that *Drosophila* males, which are haploid for one-fourth of their genome, have the same life span as female flies and are no more sensitive to ionizing radiation (Maynard Smith, 1973). Clark and Rubin (1961) showed that haploid male wasps (*Habrobracon*) have the same life spans as diploids, and although significantly more radiosensitive they are not dramatically so.

Maynard Smith *et al.* (1970) showed that about 80% of the protein molecules in a newly hatched fly are not replaced during adult life; those which do turn over include ribosomal protein but not muscle protein or muscle mitochondria. Tribe and Ashurst (1970) reported a loss of coupling between oxidation and phosphorylation in dipteran mitochondria with age, which therefore cannot be related to aberrant synthesis but may rather be due to conformational changes in the proteins or the structures which carry them. These workers, and several others (cf. Menzies, 1976), have drawn attention to the unique role played by crucial molecular constituents of cells which do not turn over; at the cellular level, these are analogous to postmitotic cells, such as neurons, which do not proliferate and cannot be replaced.

It should be noted that the life spans of at least certain metamorphosing insects can be prolonged by hormonal means. Such prolongation implies a corresponding retardation in implementing the massive cell death which accompanies metamorphosis.

It is impossible to review even the more recent literature on senescence in vertebrates (particularly in mammals), and its cellular correlates, in this limited space. Moreover, observations of this kind are notoriously difficult to interpret (cf. Adelman, 1975). Perhaps the best compromise is to refer the reader to recent review volumes (e.g., Cutler, 1976) for specific discussions of these topics and extensive references to the vast and contradictory literature.

However, we should note that there seem to exist certain metazoan animal species which do not show evidence of senescence. For instance, Strehler (1962) summarizes observations on sea anemones which did not appear to senesce over a period of 80–90 years. From such observations, it appears that senescence is not a necessary correlate of multicellularity.

## III. The Theoretical Background

As noted earlier, senescence is defined in terms of the probability of death increasing with age. Gompertz was the first to notice that, in humans, survival decreases exponentially with age. This kind of result has been obtained for many other kinds of organisms [cf. Strehler (1962) for a review and for a detailed discussion of research on senescence up to about 1960].

Most of early theoretical approaches to senescence involved the idea of *fluctuation* superimposed upon basic physiological processes in such a way that a Gompertz relationship between age and probability of death is obtained. Typical of these is the Sacher-Trucco theory (Sacher and Trucco, 1962; Trucco, 1963a,b). These investigators argued that the physiological state of an organism could be abstractly represented by a point in some appropriate "configuration space." Fluctuations would cause this representative point to execute a random

walk in this space. Mortality is interpreted as the first exit of the point from some critical region. Mathematically, the problem is one of a random walk with an absorbing boundary. For a detailed discussion of this and similar theories, see Strehler (1962).

Sacher (e.g., Sacher, 1970) has carried such arguments somewhat further. Since, according to his ideas, mortality is directly related to the effect of perturbations in generating a random walk in some physiological space, he argued that organisms exhibiting more effective homeostasis would show a longer life span. Accordingly, he sought to establish a relationship between life span and factors that would measure the effectiveness of homeostasis. In vertebrates, one such factor was relative brain size (index of cephalization), considering the brain as an essential organ of homeostasis. Another was total metabolic rate. On reviewing the data, he proposed an allometric expression of the form

$$L = 26.3E^{0.54}S^{-0.34}M^{-0.42}$$

where $L$ is life span in years, $E$ is brain size, $S$ is body weight, and $M$ is metabolic rate per gram. Such an expression should not properly be regarded as a theoretical relationship; rather it is a representation of comparative data (a species of curve fitting). It should be considered analogous to empirical physical relationships such as the ideal gas law in thermodynamics.

The existence of allometric relationships of this type is closely related to such notions as the principle of similitude in general dynamics and the D'Arcy Thompson theory of transformations (D'Arcy Thompson, 1917). According to these ideas, the phenotypes of closely related organisms can be obtained from one another by a process of continuous deformation. Such ideas were extended in an old article of Lambert and Teissier (1927) to include temporal aspects; if we compare two related organisms at the corresponding homologous instants in their life spans, they are at these instants related to each other by a continuous deformation. This implies in particular that the mortality curve of one species can be deformed into that of a related one in a systematic way, and more generally that related organisms are *models of each other* in space and time. Such considerations suggest that we can reasonably expect common senescence mechanisms in these organisms, an idea which has been suggested on other grounds by Danielli (personal communication), among others. The interrelationships among these ideas, including the concept of structural stability recently proposed by Thom (1975), are discussed in Rosen (1977).

In most of the older theories of senescence, both the *source* of the imposed fluctuations and their precise physiological *effects* were unspecified. They were indeed extraneous to these theories; much of the interest of these theories arose precisely from the fact that they were independent of such specifications. However, this very generality served to make the theories largely untestable by specific experiment; the best that could be done was to show that a proposed

mechanism was compatible with existing data, such as the Gompertz curves or relations between longevity and physiological parameters.

More recent work on such theories of senescence, however, has tended to concentrate more on (1) specifying detailed mechanisms for generating the fluctuations and (2) specifying the detailed physiological response resulting from a particular fluctuation process. A modern theory of senescence typically involves both objectives in an integral fashion. However, for our purposes, it is convenient to consider them separately.

Let us first discuss the proposed mechanisms for generating fluctuations. These are typically thought to arise at the intracellular level and indeed to involve specific macromolecules or macromolecular systems. There are several possibilities which can be considered.

### 1. *DNA and DNA Transcription*

In such theories, the ultimate source of the fluctuations lies in the genetic material itself and in its role as a template. Collectively, they represent the *somatic mutation theory* of senescence. The idea was first explicitly proposed in the context of senescence by Danielli in 1956 and later, in a mathematical form, by Szilard (1959). The basic idea here of course is that the cell genome accumulates "errors" (i.e., stable and cumulative deviations from the initial genome) through the action of random processes; such theories had long been considered in connection with oncogenesis. Somatic mutations could be generated through a variety of mechanisms: (1) radiation; (2) mutagens; (3) free radicals created through metabolism; (4) intrinsic causes, such as quantum-theoretic proton tunneling, causing tautomeric shifts in the DNA bases (Löwdin, 1964); (5) transcriptional errors, involving misreading of the base sequences in DNA replication.

Such a theory is attractive because somatic mutation involves a universal cellular phenomenon. However, by itself, it is hard to account for the observed results, for example, on senescence in fibroblast cultures. For instance, if such mutations occur at random throughout the genome, it is hard to understand why such a sharp limit to replicative capabilities is found under given culture conditions. Such difficulties can be obviated to some extent by assuming a kind of multiple-target theory—that certain essential genes are present in multiple copies, and that viability is not affected until a definite number of them has mutated (e.g., Maynard Smith, 1965). More recently, Medvedev (1972) proposed that there is a direct relation between genetic redundancy and the senescence rate. Such multiple-hit theories are characterized by a *threshold* below which viability is unimpaired and above which death becomes progressively more likely with time. For a detailed discussion of somatic mutation as a theory of senescence, see, for example, Burnet (1974).

2. *Translation and Protein Synthesis*

Another popular mechanism for generating fluctuations in physiological processes involves the translation step in protein synthesis. Orgel (1963) pointed out that this step itself involves protein (e.g., synthetase enzymes intimately involved in attaching individual amino acids onto the correct species of transfer RNA). If such an enzyme should function erroneously, errors of primary structure would be imposed on *all* proteins subsequently synthesized by the cell (including the synthetases themselves, which might be more erroneous than the original ones). The protein-synthesizing mechanism would then serve to amplify the original erroneous behavior through the establishment of a positive feedback loop, ultimately resulting in the loss of all function by all proteins in the cell. This is the *error catastrophe hypothesis*.

Such a hypothesis seems to be immediately testable experimentally; it should simply be observed that aberrant protein accumulates in senescent cells. Some experimental evidence seems to support the hypothesis, particularly the work of Holliday and his collaborators previously referred to. Other work does not, for example, the observation that amino acid analogs incorporated into protein in fibroblast cultures do not affect proliferation capabilities, or that viral infection in old cells is unimpaired. Besides, it is difficult to understand how transformed cells, malignant cells, or germ cells escape the error catastrophe, or even how a genetic code could have evolved in the first place. In fact, mathematical analysis has shown (Hoffman, 1974; Goel and Ycas, 1975) that an error catastrophe can only occur under unusual and unlikely conditions; in most situations the translation machinery is actually intrinsically *error-correcting*. Some of the assumptions underlying these analyses were challenged by Kirkwood and Holliday (1975), but a further paper of Goel and Ycas (1976) appears to meet these challenges completely. Indeed, Orgel himself (1973) has independently substantially revised his earlier views.

One consequence of the mathematical analysis which is worth special mention is the following: The rate of error accumulation in any protein of a cell depends (1) on its own primary structure and (2) on the primary structures of the cell synthetases. Thus different cell proteins can accumulate errors at vastly different rates, and one cannot generalize about cell proteins from an observation that a single protein species does (or does not) accumulate errors with age.

3. *Viral Invasion*

It has been observed (cf. Hotchin, 1972) that certain forms of latent and chronic viral infections mimic senescence, both at the cell and organism levels. Thus it has been suggested that cellular senescence arises from the incorporation of extraneous DNA into the cell genome.

It is perhaps interesting to note that all the mechanisms suggested above for the generation of senescent behavior in cells have also been advanced in the study of malignancy, which seems to require an opposite set of properties.

We have considered some of the influential ideas concerned with the *generation* of random fluctuations. When we turn to the problem of the *effects* of such fluctuations, we find that they are of the widest possible latitude, both intracellularly and intercellularly. Indeed, this results in a blurring of the mechanisms we have discussed, and, at least so far, in an inability to suggest any kind of unequivocal experimental test to distinguish among the innumerable possibilities. For instance, at the intracellular level, an accumulation of errors in a replicase or transcriptase molecule causes somatic mutations in dividing cells, as well as the appearance of aberrant proteins. Conversely, a somatic mutation in a gene coding for a synthetase may conceptually give rise to an error catastrophe. Changes in membrane proteins, from whatever cause (e.g., in permease molecules), could alter the flow of metabolites into the cell and/or of toxic products out of the cell. Mutations could occur in operator regions of the genome, modifying the susceptibility of single genes, or sets of genes, to repression or induction. A mutation affecting DNA repair enzymes (or an error in the primary structure of these enzymes) could give rise to a burst of somatic mutation and/or an error catastrophe. So could a systematic error introduced into the catalases and peroxidases which protect against free radicals. In fact, the catalog of possible intracellular effects of any of the sources of fluctuation we have mentioned is practically endless.

The situation is compounded when we consider matters at the intercellular level in the intact organism. Perhaps the simplest possibility involves the loss, through one or another of the intracellular mechanisms we have discussed, of one or more essential stem cell populations, as in the hemopoietic system or in the brain. Or a change in cell surface protein may involve a change in receptor populations, vastly altering, say, the maintenance of endocrine control. New antigens appearing on cell surfaces may cause such cells to be recognized as "foreign" by immune surveillance mechanisms, and destroyed. Conversely, such changes appearing in the hemopoietic system itself could give rise to an attack on normal cells. Either or both of these possibilities would constitute the autoimmune theory of aging (Burnet, 1972; Walford, 1969). Here too, the catalog is potentially infinite. We can thus generate a new theory of aging by combining any possible source of fluctuations with any possible deleterious effect which it might have at the cellular or the organism level.

Indeed, such a view regarding senescence, at least at the level of the organism, was proposed by Medawar (1945), using a natural selection argument. Medawar observed that genetic defects manifested at any time prior to reproduction tend to be eliminated from a population through selection mechanisms. However, there

is no way for selection to eliminate defects which are manifested only after reproduction has occurred. According to this view, then, senescence is simply the sum of all the accumulated genetic defects in a species which can only show up in the postreproductive period, hence which are neutral to selection. Such a view is attractive, but it implies that a change in selection pressure could extend life spans, and there is no evidence that this is so.

So far, we have considered theories in which a random element (i.e., a source of fluctuation) is regarded as the primary cause of senescence. We turn now to a consideration of *program theories,* which represent the logical alternative. These theories are alike in asserting that the primary cause of senescence is not of a random character; rather, senescence represents the accurate, causal expression of genetic programs similar to (and indeed, extensions of) the programs expressed during development. It is plausible to consider such theories for a variety of reasons, prime among which is the fact that at least most species possess quite sharply defined life spans which can be regarded as specific phenotypic characters. Such phenotypic characters generally are, at least in large part, under genetic control. Furthermore, rather closely related species may have quite different life spans (Strehler, 1962); an explanation of this fact on the basis of a random theory is not impossible, but certainly requires a sizable number of additional post hoc assumptions.

As with the random theories, the program theories of senescence can be classified in terms of the intracellular primary events they postulate, and the intra- and intercellular effects of the primary event. Just as before, then, the concept of programed senescence can give rise to a large number of particular theories of senescence.

Perhaps the simplest of the primary events postulated in terms of programming is the activation of a gene, or group of genes, which synthesize a toxic substance which kills the cell, such as an enzyme which activates lysosomes. The signal for the activation of this gene could be intracellular (e.g., the completion of a prior sequence of gene activations in their proper sequential order, somewhat like the opening of a combination lock) or intercellular (e.g., a hormonal signal from another cell population in the organism or from the environment). Conceptually, such a theory is not very different from the multiple-target theories considered earlier, except that *programmed* events, rather than random events, are involved.

An obvious variant of this kind of theory involves the *repression* of some gene essential for the maintenance of viability.

It should be noted that, depending on the gene or genes involved, the effects of such a programed event could be indistinguishable from those expected from a random theory. For instance, if the genes which are repressed are those coding for DNA repair enzymes, the *effect* of the program would be an increase in mutations, with all its attendant possibilities. Another example is the proposal of von Hahn (1973a,b), who suggests that the (programed) synthesis of protein

capable of binding firmly and nonspecifically to DNA would randomly block transcription and thereby also act like random somatic mutations. There is indeed evidence (Courtois, 1974) that the chromatin in old cells is much less labile than that in younger ones.

It should also be noted that a crucial element in all such theories is the concept of *sequence,* rather than elapsed time. Cells expressing the same sequence could nevertheless exhibit vastly different *life spans,* as measured in clock time, depending on the specific *rates* at which the sequence is expressed in the different cells. To illustrate the distinction, we observe that the same game of chess, say, can be played in any length of time whatsoever; the rates at which the moves are made determines the duration of the game, but the sequence of specific moves which define the game is quite independent of this. The same holds true for any algorithmic or recursive process, of which gene expression is often regarded as an example (Rosen, 1968). We have already seen above that, for instance, Hayflick's fibroblast cultures can be arrested at any passage level for an arbitrary time without affecting their ultimate decline.

The interrelation of rate-independent (sequential or algorithmic) processes and rate dependent (real-time) ones, and its significance for basic biological phenomena, has been extensively discussed by Pattee (1974).

Other types of program theories intimately involve the concepts of *clocks* and *counters,* which are also important in understanding development. Any periodic event can serve as a clock; most organisms seem to possess internal periodicities (*Zeitgebers*) which serve both to coordinate temporally organized processes (Goodwin, 1963) and to signal for various kinds of rhythmic behavior (e.g., Bünning, 1967). If we suppose that associated with such a periodic process is a counter, which remembers how many cycles have occurred, it is easy to imagine that some threshold value of the counter is a signal for the initiation of a programmed sequence leading to cell death. One specific proposal in this connection, for which we cannot provide a reference, is of the following type. We imagine a structural gene transcribed into a particular mRNA which is initially nonfunctional; at each cell division, a codon is cleaved from this mRNA; after a fixed number of divisions, the remaining strand of mRNA can be translated into a protein whose presence is lethal to the cell.

Another extension of the program hypothesis was suggested by Hayflick (1975). In this view, a cell proceeds to express sequentially a genetically coded program. At some point, the program is completed. But instead of triggering some specific lethal event, as in the mechanisms described above, the cell simply drifts along, by momentum, as it were, until it ultimately becomes inviable through one mechanism or another. As Hayflick has put it: "Cells can be programmed simply to run out of program."

Various combinations of program and error theories are possible, since the accumulation of errors can also serve as a clock. We have already seen an

example in the hypothesis of Medvedev, in which a particular level of genetic redundancy is itself genetically programmed; when some level of somatic mutations has reduced this redundancy below some threshold level (itself genetically programmed), senescence occurs. Similarly, some threshold level of aberrant protein could be the signal to activate a lethal gene or repress an essential one.

Most of these intracellular program theories possess natural counterparts at the intercellular or organism level. For instance, we can imagine a biological clock established in some "pacemaker" region of the organism, whose properties are genetically programmed. Such a clock could be coupled to various effector systems, such as the endocrine, circulatory, and immune systems, in such a way as to cause progressive deterioration of these systems. Such deterioration may itself be lethal, or it may render the organism susceptible to characteristic pathologies (e.g., malignancies) which are the immediate cause of death. Such proposals, in one form or another, have been made by many investigators (e.g., Denckla, 1975), motivated in part by the observation that, in mammals at any rate, most organisms appear to die from the same final diseases, which are either circulatory failures or failures of the immune system. Situations of this type seem to be characteristic of higher plants (Woolhouse, 1974).

We can also consider programmed counterparts of the various immunological theories of aging, such as the "forbidden clone" theory (cf. Burnet, 1972; Burch, 1969; Hirsch, 1974). According to such a view, a programmed change in cell antigens in some target organ (e.g., in the circulatory system) causes these cells to be attacked immunologically, thus giving rise to an autoimmune response.

For other discussions of the relation between the immune system and senescence, see, for instance, Bellamy (1973), Robert and Robert (1973), Walford (1974), Good and Yunis (1974), and Stutman (1974).

Clearly, there are no fewer variations of programmed senescence theories than there are of error theories. There are also numerous combinations and intermediate theories involving both error and programming, which may be plausibly entertained.

The situation is not improved by comparing any of the theoretical models described above with the results of experiment and observation. There is sufficient flexibility, on both the experimental and theoretical sides, to conclude that at present *every experimental observation can be made compatible with every theory*. We have cited the experimental literature at some length primarily so that the reader will be satisfied on this point. We also note its contradictory and rather featureless character, which is a corollary of the fact that no mechanism is excluded, nor is any specifically suggested, by a systematic consideration of the experimental results. It is not clear that this situation will be improved by further experimental work alone; it is more likely that quite the opposite will occur. This is a most unsatisfactory state of affairs; we have to consider whether the experi-

mental literature truly reflects a totally heterogeneous biological situation about which no generalizations can be made, or whether something essential is missing from past conisderations of senescence. We now turn our attention to such questions.

## IV. General Problems of Senescence in the Cell and Organism

Let us begin by recalling the three basic problems posed in Section I: (1) Do individual cells undergo senescence? (2) If so, does cellular senescence suffice to explain senescence of the organism? (3) If not, what cellular properties are involved in the senescence of the organism? We now reconsider these questions in light of the experimental and theoretical material we have reviewed, and in light of several other conceptual considerations.

We first ask: Do individual cells undergo senescence? Recall that we have provisionally defined senescence as an increase with age in the probability of death per unit time. We have already seen that this kind of definition is fraught with ambiguities. For instance, is age to be measured in clock time, or in terms of the number of steps in some sequential program through which the organism has already passed? We have seen that there need be no simple relation between the two, since such a relation depends entirely on the rates at which the individual steps in the program are actuated. Leaving this crucial point aside for the moment, we would in any case expect any senescing system to show an increasing probability of mortality with chronological age, manifested throughout the life span.

Now it is reasonably clear what is meant by the life span of a multicellular organism, at least as measured in chronological time. It is not so clear what the life span of a cell is. The reason for this of course is that cells *proliferate*, through a process of mitosis. Strictly speaking, the life span of a cell, as a continuously identifiable individual, is the period from M to M, or from M to death, whichever is first. Indeed, as Danielli (1957) has pointed out, only nonproliferating, postmitotic cells can be regarded as chronologically old.

Now let us observe that the concept of senescence, by its very nature, is a population concept. To see this, let us ask how we can empirically determine the probability of death per unit time, which enters into the definition of senescence. We must envision a large population of *similar* individuals which can be observed over time and for which we can count the number of deaths per unit time as a function of the ages of the individuals which have died. In such a way, we can form ratios which approximately can be regarded as the probability that a *representative* individual in the population will die at a particular age. If these probabilities increase with age, we say that the *individuals* in the population exhibit senescence. Indeed, this is the way in which the Gompertz relation,

which dominates conceptual work on senescence in organisms, was originally developed.

On this basis, we must conclude that *there is not a shred of experimental evidence that cells exhibit senescence,* at least in the same sense that organisms exhibit senescence. Just as for multicellular organisms, such evidence must consist in the gathering of statistics of mortality, in a population of *similar* individuals, which would permit one to conclude that the probability of death of a representative individual is an increasing function of the age of the individual. No such evidence presently exists; the overwhelming bulk of experimental work we have reviewed here does not even bear on this question. The closest approach is to be found in protozoology and in the genealogical studies of fibroblast cells (Absher *et al.*, 1974). As it stands, however, the question whether cells in general can or do senesce is entirely open. Indeed, it seems more than likely that (1) *proliferating cells do not senesce* and (2) the only cells for which a strict concept of senescence is at all meaningful are cells which do not proliferate, that is, *postmitotic cells*.

Thus, if the concept of senescence is to be meaningfully applied to proliferating cells, we must modify its definition in a way which has no counterpart for individual multicellular organisms. Instead of asking that mortality increase with age for individual cells, it appears that *we must require that the mortality of daughter cells be greater than the mortality of mother cells,* over the course of the life span of each. That is, it must be more probable that a daughter cell will die, per unit time of its life span, than that the mother cell will die. Clearly, we are now speaking not of an individual cell but of a *clone;* we must assess the senescence of a single cell by assessing the mortality characteristics of successive generations of *descendants* of the cell.

Note that this is not the same as saying that a clone of cells must possess a Hayflick limit, or that a cell is spanned in the sense of Danielli's hypothesis. The concept of spanning, or of a Hayflick limit, represents in general a different kind of phenomenon which Hayflick was careful to designate by a specific term—*clonal aging*. In order for clonal aging to qualify as a manifestation of *cellular* senescence, according to the definition in the preceding paragraph, it would have to be established that successive generations of cells possess a greater probability of dying per unit time. This has not been established, and it is not unreasonable to conjecture that it is not true in general.

We have seen therefore that the answer to our first basic question, concerning whether individual cells exhibit senescence, is quite complex. All that has been established experimentally is that *cells can exhibit clonal aging*. But as we have seen, this phenomenon is far removed from the senescence of multicellular organisms, and is not analogous to it.

Thus we must modify question 2 and ask: Is *clonal aging* sufficient to explain senescence of the organism? This implies that an understanding of the *mechanism* of clonal aging would also provide the mechanism for whole-

organism senescence. Indeed, this is the basis on which Hayflick's observations have come to play a dominant role in senescence research. We must thus seek a relationship between clonal aging and whole-organism senescence to see how this kind of question can be answered.

The most obvious connection is to recognize that a multicellular organism is itself a clone, typically derived from a zygote. The question then is: Can we look upon the life span of an organism as a kind of Hayflick limit of such a clone? Or, more generally, is a Hayflick limit of one or more subclones of an organism directly related to its senescence?

To attack such questions, we confront the general problem of expressing a property of a population (in this case, the entire clone) in terms of properties of the individual members of the population. Such problems lie at the very root of the concept of multicellularity and in fact underlie any attempt to understand the properties of a system through a (reductionistic) decomposition of the system into more elementary subsystems.

To help fix ideas, let us begin with a familiar nonbiological example. Let us consider a quantity of radioactive material regarded as a population of individual atoms. The atoms are noninteracting, and each of them has a fixed probability $p$ of decaying per unit time. Starting with $n(\mathrm{O})$ atoms at some initial time, the quantity of atoms which remain undecayed after a time interval $T$ has elapsed is

$$n(T) = n(\mathrm{O})e^{-pT}$$

This is a Gompertz-type relation for the population, and indeed it describes the way any chemical property characteristic of the original radioactive element is lost with time. Furthermore, the continuous change in such properties with time can be looked on as a kind of "differentiation" of the original material. The point is that the Gompertz-type relation which holds for the population, and which can therefore be looked upon as exhibiting senescence for the *population,* is generated by a population of elements which do not senesce (since their probability of decay per unit time is *constant*). Furthermore, if the individual atoms did senesce (i.e., if the probability of decay per unit time itself were an increasing function of time), the population could not exhibit a Gompertz-type senescence. In such a case, we see that Gompertz senescence of a population occurs *if and only if* the individual members of the population do not themselves senesce but merely have a fixed probability of dying per unit time.

Suppose now that instead of a fixed population we allow the individual elements to *proliferate* at a fixed rate $\alpha$. In this case, the number of elements remaining after a time $T$ has elapsed is

$$n(T) = n(\mathrm{O})e^{(\alpha-p)T}$$

which is still a Gompertz-type relation. However, there are now three possibilities, depending on the *sign* of $\alpha - p$: (1) If $\alpha - p$ is negative, the material

will still senesce, but the time required for senescence will be extended; the proliferation makes the population behave as if it were composed of elements with a *smaller* probability of mortality per unit time. (2) If $\alpha - p = 0$, the population will be *stationary* and will appear to be *immortal*. (3) If $\alpha - p$ is greater than zero, the population will actually grow exponentially and will again appear to be immortal. This very simple line of reasoning is actually of great power and importance and basically underlies the rigorous discussion of Orgel's error catastrophe given by Goel and Ycas (1975, 1976) in terms of a population of proliferating macromolecules.

Let us also note that, if the rate of proliferation is a *decreasing* function of time, then if $\alpha - p$ is initially positive, the population will initially grow and then senesce. This, crudely, is the behavior characteristic of Hayflick's clonal senescence, or Danielli's spanning. In all cases, it occurs without any senescence of the individual elements; it only requires a temporal decrease in the rate of proliferation, not an increase in the rate of mortality. Paradoxically, we note that the decrease in proliferation rate, with its attendant *increase* in the life span of the individual elements in the population, actually implies extinction of the population as a whole; the greater the increase in individual life span, the sooner the population will die out.

In the light of these considerations, it is tempting to think of redefining cellular senescence in terms of a declining rate of proliferation rather than in terms of an increasing probability of mortality. However, this seems unsatisfactory for several reasons; for instance, (1) it is not analogous to the senescence of multicellular organisms and (2) it automatically classifies all postmitotic cells, such as neurons, as senescent regardless of their age.

Let us also note that the simple arguments just presented are entirely *mechanism-independent;* in this respect they are more like the older ideas of, say, Sacher and Trucco rather than the more recent approaches. In particular, we find no meaningful application of such concepts as clocks, counters, errors, or genetic programs at this level. All the detailed experimental work reviewed above also is not directly relevant to these arguments.

We should also point out that proliferation serves in general to create *redundancy*. As is well known (e.g., Shannon and Weaver, 1949; von Neumann, 1956), redundancy is conceptually the only way to defeat error in communication and in algorithmic systems. In such literature, much attention is given to the artful application of redundancy to increase *reliability*, that is, to make the error-free life span of a composite system greater than those of the fallible components which comprise it. It is one of the ironies of senescence research that cellular theories of senescence often seek to make the composite system have a *shorter* life span than its constituent parts. The trivially simple considerations outlined above are the one way of expressing the war between proliferation (the creation of redundancy) and failure. Another good discussion, in the context of

automata theory (but conceptually not too far removed from our simple method) can be found in Lofgren (1961).

We must now observe that even our simple arguments exhibit the complexity of the basic problem of relating a population to the individual elements which comprise the population. To proceed further, and to place such arguments, the theories, and the experimental work we have described above into a common perspective, we must retreat to a general theoretical consideration of what is ultimately involved in any relation of populations to their elements.

If we consider any individual system (such as a single multicellular organism or a single cell), we can describe the system, as a whole, in terms of its directly observable behavior or properties. Thus we can describe a multicellular organism by its rate of senescence, or a cell by its rate of proliferation.

However, such an individual can also be regarded as a population of subsystems. Thus a multicellular organism is viewed as a population of cells, and a cell is viewed as a population of molecules. The whole point of such an analytic decomposition of an individual system is to attempt to reconstruct its properties and behavior from those of the subsystems.

Now in general, any description or representation of any system involves two ingredients: (1) an assessment of the *state* of the system at an instant of time; (2) a specification of how the state of the system is changing in time (cf. Rosen, 1971).

What determines the state of a *population?* Generally, the state of a population is specified by:

1. The *states* of the individual elements in the population
2. The *interrelation* of the elements.

We must briefly discuss what is meant by the term "interrelation." We can do this most clearly by citing some specific illustrations.

1. If we want to study, say, a crystal, we must specify the structure of the *crystal lattice*. This tells us how the molecules which comprise the crystal are arranged in space, and therefore also *how they are arranged with respect to each other*. We need to know this because the spatial arrangement specifies the manner in which the constitutent molecules can interact. We usually assume that molecules which are contiguous in the lattice interact strongly; those which are remote in the lattice interact weakly. In such situations, interaction is determined by spatial contiguity. *A change in the lattice, even if the molecules remain the same, changes their interaction.* Hence a specification of the state of a crystal, considered as a population of molecules, must involve not only the states of the molecules but also a specification of their spatial arrangement (which in turn, as we have seen, determines their physical interaction).

2. Suppose we want to study a neural network. To specify the network, we must of course specify the manner in which the individual neurons in the network are interconnected; that is, we must specify the topology of the network. Just as in the case of the crystal, this topology specifies the interaction among the individual neurons which comprise the network. This time, however, it is not so much the arrangement in physical space but rather the pattern of axonal interconnections which determines the character of the interaction. Thus the *state* of a neural network is determined not only by the states of its elements but also by their pattern of interconnection. A change in this pattern alters the state of the network, since it modifies the character of the interaction among the elements.

From these two simple examples, we see that the interrelation of the elements of a population involves specifying the manner in which these elements interact with each other. *Each mode of interaction among the elements converts the population into a kind of network* characteristic of that mode of interaction. In turn, the network itself is characterized by a topological structure which can be interpreted as a specification of the proximity or contiguity of the elements with respect to that interaction.

If the elements of a population are capable of interacting in more than one mode (e.g., if the neurons in a neural network can interact through diffusing chemical signals as well as through the electric signals propagated axonally), each mode of interaction will impose a network structure upon the population. These network structures can be totally dissimilar (i.e., the contiguity relations can be utterly different) even though the elements are the same.

Finally, the interactive modes must be considered as being *coupled* to one another, since the interactions occurring in any one mode can affect any of the others.

In sum, then, the term "interrelation" refers ultimately to a specification of contiguities (in interactive terms) among the elements of a population. The state of a population cannot in general be specified without such information; only if the elements in the population are totally noninteracting (as in the example of radioactive decay considered above) is the state of a population represented *only* by the states of the constituent elements.

What is it that causes the state of such a population to change? Evidently such a change in state depends on:

1. A change in the state of the individual elements in the population, both as a result of intrinsic dynamic processes in the elements and as a result of interelement interactions
2. A change in the patterns of interaction among the elements
3. Sources and sinks (proliferation and death) of the elements
4. Interactions with the environment

*All* these factors enter crucially into the dynamics of a population. It is from this dynamics that we attempt to interpret the properties of the population considered as a single system in its own right. And senescence is certainly a dynamic process of this character.

We recognize further that this analytic procedure can be further iterated. The elements in a population can themselves be regarded as populations in their own right, so that their intrinsic dynamic behavior can be referred to a family of subsystems. Conceptually this process can continue ad libitum or until such time as we reach subsystems which can be regarded as having no internal structure, that is, which can exist in only one state. The dynamics of a population of such ultimate units then depends *only* on interactions, that is, only on contiguity. Of course, we do not imagine pressing the analysis to such a limit in most biological contexts.

We thus have an idea of some of the complexity involved in attempting to relate the properties of a system as a whole to the properties of an interacting population of subsystems. This is true even for systems we commonly think of as simple. It is magnified for systems which are complex (by which we mean, roughly, that they are decomposable into units capable of many interactions, and therefore must be resolved simultaneously into many networks of interaction).

Now let us draw some general conclusions from this state of affairs, which are relevant to the concept of senescence.

1. A system may appear to be in a steady state (i.e., unchanging in gross properties) when considered as a whole. At the same time, considered as a population of interacting elements, we may find that the states of the elements themselves are changing widely, that new elements are entering the population and old ones are leaving.

2. Conversely, we can have a radical change in the state of the population *without* an apparent radical change in the states of any of the *elements* of the population. This can happen because the topologies of the interaction networks, which as we have seen form a crucial determinant of the state of the population, can themselves be radically changed by apparently trivial state changes in the elements. For instance:

a. A trivial change in frequencies in the elements of a population of coupled oscillators can force the population as a whole into an entirely new state; it can cause a previously coherent behavior to break down and conversely can establish a coherent pattern from a previously chaotic one.

b. In many complex systems, such as traffic-flow systems, time-sharing computer networks, and communication systems, apparently trivial changes in *interactions* among the elements can cause massive failures or system *crashes*.

c. We have shown elsewhere that in general the pattern of interaction between systems can be far more sensitive to perturbation than the intrinsic dynamics of the elements (Rosen, 1976).

These considerations show that *it is possible for each element in a population to appear perfectly normal by itself, but that the population as a whole can nevertheless fail dramatically.*

Now let us return to the problem of senescence in light of the general observations we have just made. Let us first consider the question of the bearing of cellular processes on whole-organism senescence. It is easy to see that *the overwhelming bulk of the biochemical and ultrastructural investigations reported above have to do almost entirely with the states of individual cells.* As we have seen, state information regarding the elements in an interacting population is an essential ingredient in determining how the population as a whole will behave, *but it is not the only ingredient.* It is also easy to see that the corresponding preponderance of theoretical approaches to senescence, particularly those which emphasize random events, are likewise almost exclusively concerned with the states of individual elements. The view of senescence implicit in all this work is that it is a consequence of *localized failures of specific elements* in a population, with all the attendant consequences which may follow upon such failures. The idea is that we can point to a specific kind of *lesion* which is the primary cause of senescence. Such work necessarily ignores the other essential ingredient in interacting populations, namely, the network aspects. And as we have seen, network aspects suggest very strongly that *it is possible for a system of interacting elements to fail in some global sense without any component subsystem failing.* Stated another way, we can say that senescence studies concentrating entirely on cell states view the phenomenon entirely in terms of local subsystem failures; taking network considerations into account suggests that the study of senescence *cannot* be simply a study of such local failures.

Ideas of this kind have a bearing on the point raised earlier—that the extensive experimental literature on cells and senescence appears to be so contradictory and seems to be devoid of any clue which might point to a basic mechanism for senescence. For if these considerations are valid, the mechanisms sought may not lie in the individual cells at all, but rather in their networks of interaction.

Another pertinent remark is that, in networks, it is difficult to disentangle cause from effect. Indeed, in any system with a feedback loop, in which a sample of the present behavior is fed back into the system, an initial cause itself becomes an effect, and conversely. It is clear that any mode of analysis which ignores the loop will be hard put to understand the observed behavior of the system as a whole. This is perhaps the ultimate reason why we observe the phenomenon mentioned above, that every experimental observation can be made compatible with every theory; in a system with many loops, any pair of processes may eventually become coupled, so that either can be regarded as a cause of the other.

Networks are difficult to study experimentally, because conventional approaches to cells begin precisely by destroying network structures and retain only the individual elements in the network. Hence an empirical approach to network

or interaction properties of cell populations is likely to require techniques, and conceptual basis, entirely different from those currently employed. We believe that such systems cannot be understood without extensive use of carefully chosen *model systems,* perhaps initially even of a nonbiological character. For instance, a study of the establishment and failure of coherent behavior in populations of coupled oscillators should be most instructive in determining the respective roles of local lesions (i.e., gross changes in state in individual elements) and global network modifications in the loss of population characteristics. The growth of intuitions concerning network aspects in populations arising from the study of such model systems may help us understand what to look for in seeking to understand senescence of organisms in terms of the cell theory, or understanding clonal aging in terms of biochemistry.

## V. Conclusions

It is appropriate to sum up the various conclusions we have reached in concise form.

1. There is no evidence that individual cells senesce in the sense in which whole organisms senesce (i.e., the probability of death per unit time increases with some measure of age).
2. Clonal aging and spanning of cells in culture are likewise phenomena distinct from organism senescence.
3. Evidence indicates that there is a correlation between clonal aging and whole-organism senescence, but there is no evidence indicating a causal relationship between them.
4. On balance, the experimental literature points to no specific mechanism either for clonal aging or for senescence; it does not refute any of the specific theories currently being advocated and can be interpreted as supporting any of them equally well.
5. The problems of senescence intimately involve the problem of expressing properties of populations in terms of their individual elements. This problem involves both the study of the elements themselves and of their interactive networks to an equal degree.
6. Both the experimental and theoretical literature have tended to stress the properties of individual elements and to neglect network aspects. We suggest that it is precisely the absence of network considerations which is responsible for the inconclusive character of senescence research up to this time.
7. An experimental study of network aspects is likely to require a set of concepts and techniques different from those currently being employed. It is

suggested that a detailed study of carefully chosen model systems will be necessary to guide experiments in this area.

8. It is reasonable to suppose, on various grounds, that a comprehensive theory of senescence and spanning is possible despite the apparently unlimited number of possible mechanisms which can be entertained, and that the mechanism of senescence is similar in similar organisms.

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