We feel that as a first step it will be necessary to understand, for a single or for a few species, the genetic and epigenetic mechanisms which control the natural variations in life span proper to each species. As a second step one may then try to understand how genetic mechanisms are eventually responsible for the enormous differences in life span which characterize the living species, as, for instance, the mayfly and the elephant.

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Tissue culture in aging research: present status and prospects

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The purpose of this paper is not to make an exhaustive review of the contributions of tissue culture to aging research but to point out its most relevant achievements and cast a glance into the future.

Rationale for the utilization of tissue culture

Before describing the main achievements, it should be stressed that several investigators consider unjustified the use of tissue culture for the study of aging; they claim that the causes of aging in an animal cannot be approached by the study of cells in an artificial environment. We believe however that the situation is similar to what happened in the field of cancer. Although one should not extrapolate completely to conditions in vivo from the studies of cells in vitro, an immense amount of knowledge has been accumulated from the studies of changes induced by carcinogens on cells in culture; this knowledge has been extremely useful in understanding the basic mechanisms involved in oncogenicity and would have been impossible to gather by other methods. But not only cancerology has benefited from tissue culture techniques; an almost endless list of contributions to the understanding of human pathological conditions can be gleaned from works involving in particular the cultivation of human fibroblasts. Thanks to the ease with which one can grow this type of cell, it was possible to identify the cytogenetic[1] as well as the metabolic[2] defects of inherited diseases, to study membrane functions in metabolic disorders[3] or investigate cellular metabolism in rheumatic processes[4], to mention just a few examples. Thus we cannot see why the field of aging should not benefit from tissue culture as human pathology already has.

This does not mean that fibroblasts are the only cells that can express in vitro the defects involved at a cellular level in different conditions. They have been useful because they were the first cells that could be easily grown in vitro, but as new techniques develop and cells from other tissues are cultivated, they will also be useful tools to study changes at the cellular level.

Main achievements

One of the methods that should obviously yield results in gerontology and which is presently used is the comparison of the behavior of cultivated cells from young and old donors. The first attempt, however, to approach animal aging with tissue culture techniques compared the effect of serum from young and old donors on cultivated cells[5]. It was found that the serum from young chickens better supported the growth of homologous fibroblasts than did that of old animals. The same year, Ebeling[6] described a slower migration of cells from explants originating from older chickens. This parameter was later analyzed again by Soukupovà and Holeckovà[7] who found that the latent period between explantation and cell migration increased in tissues from older donors.

The use of tissue culture in experimental gerontology was greatly stimulated by the work of Hayflick and Morrhead which dealt with the potential number of population doublings of serially cultivated human fibroblasts[8]. It was known from the work of Swim and Parker[9] that fibroblasts from certain species (e.g. human) stop growing after a period of rapid proliferation while those of other species are prone to acquire the capacity to divide indefinitely. Hayflick and Moorhead suggested that human embryonic fibroblast-like cells are endowed with a fixed number of potential doublings (approximately 50) and that the loss of these cells after exhaustion of their doubling capacity is an expression of senescence at a cellular level. Although it is known now that changes in the nutrient medium can increase the number of cell generations[10-17], nevertheless, according to the experience of hundred of laboratories, the lifespan of these cells seems indeed to be limited.

Hayflick's hypothesis was buttressed by his own finding[13] that fibroblasts from adult human donors have a smaller division potential than cells of embryonic origin. These results were rapidly followed by other attempts to test the hypothesis. It was ascertained that one of the manifestations of aging in cell culture, the prolongation of the G_2 period[14], is also found in tissues of old animals[15]; that cells obtained from human adults early during their lifespan in vitro are kinetically similar to cells obtained from embryos during the last stages of their in vitro lifespan[16]; that the potential number of doublings in vitro is inversely proportional to the age of the donor[17]; that cells originating from individuals with premature aging have a reduced division potential[18]; and that early-passage fibroblasts from adult donors have the same radiosensitivity as embryonic cells aged in culture[19].

All these works favor the hypothesis that there is an analogy between aging in vivo and in vitro, in other words, that during serial population doublings cultivated human fibroblasts go through changes identical to those occurring in situ. A similar relationship has thus far been described only in another species (tortoise) by Goldstein[20]. The same type of phenomenon was found with cells from other human tissues[21-24] besides fibroblasts.

An obvious question which has been raised refers to a relationship between the lifespan of the species and the doubling potential of the respective fibroblasts in vitro. According to some investigators, there is none[25]; however, in some instances this might be difficult to assess because of the lack of knowledge concerning the real lifespan of the species. This is particularly true concerning domestic animals. Chicken fibroblasts for instance last about 35 doublings[26] and bovine fibroblasts 70 doublings in vitro[27] but the reported lifespan of both species is 30 years[28].

Anyway, the lack of correlation between species lifespan and doubling potential of cells in vitro does not invalidate the hypothesis since the relevant correlation is the one between the lifespan of cells and the donor's age within one species. In those species where the fibroblasts easily become permanent cell lines, the phenomenon is more difficult to assess although it can be analyzed up to the moment when division slows down before resumption of growth and acquisition of an infinite lifespan.

The age of the donor, however, is not the only parameter that determines the doubling potential of fibroblasts in vitro; indeed, it seems that some pathological conditions can either prolong or shorten the lifespan of the cells. Fibroblasts from some patients with neoplastic diseases for instance have been found to have a longer lifespan[29,30]. On the other hand, fibroblasts from donors with some cytogenetic defects have shorter lifespans[31]; a decreased doubling potential has also been described for cells originating from diabetic patients as well as from the respective parents where the disease is not expressed[32]. These findings again raise the problem of the mutual influences between aging and pathology. Cytogenetic defects as well as diabetes can accelerate in vivo aging and it may be difficult to distinguish between the changes due to disease and those due to aging[33]. However, we feel that in this respect tissue culture could make a valuable contribution in the future. Through careful selection of healthy donors, analysis of cellular changes due to physiologic aging can be compared with those occurring in pathological conditions. The comparison between the two processes might be difficult to approach otherwise.

Since the parameter used to measure the age of a cell population was the number of population doublings completed, the first studies attempting to understand the mechanisms involved analyzed the changes occurring in the kinetics of cell proliferation[34,35]. They led to the conclusion that aged cell populations become strongly heterogeneous and that the cells show a spectrum between the two extremes, i.e., complete

inhibition and a normal division cycle. This view was at first difficult to admit because of established concepts of the kinetics of cell proliferation which were plagued by the tendency to define clear-cut limits between the behaviors of cells. Investigators in the field predominantly thought in terms of compartments instead of gradients, leading to over-simplified ideas by considering for example only cycling and noncycling cells, or by believing that the rates of entrance into the cycle are constant. However, the concept of a spectrum of cell behaviors and the idea that during aging there is a shift within the spectrum and that cell cycling becomes increasingly erratic[36] eventually prevailed. This view was reinforced by the observation with indirect[37] and direct[38] methods that there is a periodicity in cell behavior, the probability of reversion to the cycling state decreasing with age. Experiments of lifespan prolongation through maintenance of cells in a nonmitotic state[39] suggest that reversion could be due to some kind of regeneration taking place during the resting phase. Thus in regard to cell division, aging appears as a progressive shift to a new equilibrium where the main change is not the creation of an irreversible new state but rather the probability for a cell to be in a given state. This concept might very well be extended to other parameters in the future.

Implications for in vivo aging

The final event of the fibroblast's lifespan, i.e., the creation of a cell where the probability of reverting into the cycle becomes very small could be an in vitro phenomenon which does not occur in vivo. This had led some people to consider the fibroblast model as unappropriate for experimental gerontology. However, from the point of view of aging, the relevant finding with the fibroblast model is not that division eventually stops but rather that at each division something has changed, i.e., that the daughter cell is not like the mother cell. It is these changes occurring at each cell division that have to be studied rather than the final event, if one wants to understand the aging process.

The lifespan in vitro of a human embryonic lung fibroblast cell population has been divided into three phases[8]. We suggest in addition a final phase IV corresponding to this terminal event extending rapidly to most of the cells during the last 3–4 doublings, where the probability of reverting into a cycling state becomes very low. This final event has been interpreted in terms of the concept that aging is a slow progression towards terminal differentiation[40].

How can the decline of the division potential of mesenchymal tissue influence aging at the level of the organism? One aspect of course concerns regeneration of tissues, and the well-known decline of normal healing during aging probably reflects at least to a certain extent this phenomenon. Another interesting aspect has been raised by Martin[40]. He suggested that some disorderly proliferations leading to local hyperplasia during aging and playing a role in different conditions (e.g. atherosclerosis) are due to a desequilibrium between cellular systems, the decline of the growth potential of one leading to the overexpression of another.

Finally, it should not be forgotten that the capacity to divide is just one cell function among others and that if it is impaired, it must mean that other functions are also impaired. Concerning mesenchymal tissue which is responsible for the synthesis of supporting structures like collagen, proteoglycans and elastine and in general for the creation of a microenvironment, a shift in its functions will certainly disturb the mutual relationships with other cell systems. Although the number of cell divisions has been useful as a starting point to measure changes due to aging, the time is ripe to start looking into metabolic functions that evolve with the number of cell generations completed. Indeed a progressive decline in protein synthesis was previously reported[41] as well as changes in the cell membrane[42,43]. Another interesting function that has also been analyzed is the capacity to repair DNA after UV-radiation. A relationship between this parameter and the number of doublings does not seem to exist, but it has been reported (albeit recently questioned[44]) that there is a relationship between the capacity of fibroblasts in vitro to repair DNA after UV-radiation and the lifespan of the respective species[45]. It has been speculated that the need for this type of repair evolved at a time when the planet was not protected by an ozone shield and only organisms capable of repairing the lesions induced by UV-light could develop[46].

New vistas

To finish this brief review of the implications of the work with tissue culture for gerontology, we should mention a new theoretical approach which originated from the work with cultivated fibroblasts[47]. It concerns the interpretation of the changes occurring at each cell division consistent with present concepts in cell genetics. According to this paradigm, the trigger for a progressive shift in cell behavior could be the DNA strand switching, sister chromatid exchanges and displacement of transposable elements occurring during cell division. Transcription from the reorganized DNA strands could depend on the efficiency of repair and on the mutual influence between the chromatin regions that come together. Deficiency of some repair enzymes or the presence of a non repairable matrix could cause weak points or gaps after cell division, leading to changes in chromatin and subsequently to changes in the activity of different genes. On the other hand, new influences can be created

depending on the fitness between different chromatin regions and on the presence of transposable elements acting as activators or suppressors. This genome reorganization could cause the slow down of transcription in certain regions and activation in others with a progressive decline and/or a shift in cell functions, i.e., a progressive increase in the entropy of the system. This interpretation of aging that originated from the studies performed with the fibroblast model offers a new concept that can be approached experimentally and could be applied to other cell systems.

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Pharmacological aspects of gerontological brain research

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One of the cardinal features of aging is polymorbidity. This is not a feature of aging in man alone. An increased incidence of disease is a well-known phenomenon of aging in laboratory animal colonies, as is reflected, to cite but one example, in the age-related increase in tumour frequency[1,2].

The Nestors of descriptive and experimental gerontology, Max Bürger in Leipzig and later Fritz Verzar in Basle, frequently raised the question of whether it might be possible to influence aging processes directly with drugs. No conclusions were reached aside from general assertions to the effect that disease in elderly people is of major concern to the pharmacologist, since aging is a predisposing factor in the development of diseases. Up until now, the experimental pharmacology of aging diseases of the brain has been a field based directly on the results of descriptive gerontology. With increasing age the human brain loses some of its ability to adapt to increased metabolic and functional demands made upon it. The cause is to be sought mainly in a disturbance of glycolytic turnover capacity[3,4] and respiratory-chain oxidation under conditions of increased metabolic demand[5,6].

Changes in transmitter metabolism take the form of a