The studies on the membrane receptors and the turnover of transmitters[18-20] have contributed greatly to the explanation of the neuronal reactions. Ultimately, the structural and metabolic changes in nerve cells make their 'contribution' to an organism's aging by changing the functions of neurons and nervous structures. Therefore the studies on molecular mechanisms of the disturbance of neuronal function in aging should determine the key direction which neurobiology of aging will follow in the future. Isn't there a redistribution of various pathways of energy provision of the neuronal function with aging? Couldn't many disturbances of metabolism of the nerve cell be explained by its partial dereception? In what measure are the changes in the membrane receptors and enzymes of the plasmatic membrane related with the shifts of its phospholipid composition during peroxide oxidation reactions? Is it possible to treat the change in protein synthesis of the neuronal membrane, changes in the relationship between protein biosynthesis and electric properties of the nerve cell, as one of the fundamental mechanisms of aging of the neuron?

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Genetics

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Longevity: A trait under genetic control?

In considering the enormous differences in life spans which distinguish the species of the animal kingdom, gerontologists have repeatedly affirmed that longevity is a genetically determined trait. What does that assertion exactly mean? Does it mean that the interspecific differences in life span are genetic in origin? Or does it mean that the potentially large intraspecific differences in life span are genetically determined? Or does it mean that both the inter- and intraspecific differences are under genetic control?

Inter- and intraspecific differences in life span

If one considers the respective sizes of the mayfly and of the elephant one may claim that because these sizes are so different they are genetically determined. However, does one, by that claim, really gain any insight into the mechanism of the genetic control of size in mayflies and in elephants? For an individual to reach the normal life span characteristic of the species to which he belongs implies a proper development of the zygote and of the embryo, a harmonious growth and, once the adult stage is reached, accurately balanced maintenance activities. All these phases of development, growth and life maintenance may be affected by genetic accidents which ultimately may result in death. For example, in humans, a supernumerary chromosome may lead to an impaired development of the embryo; mutations at the loci responsible for chondriodystrophy or for progeria will considerably modify growth; the mutation of the gene responsible for insulin production will significantly impair the maintenance activities. These genetic interferences with development, growth and maintenance activities will eventually result in a premature death. Such accidental deaths, although due to genetic causes, have no relation whatsoever with the normal life span of the human species. They certainly do not demonstrate that human life span is genetically controlled. Neither do they demonstrate that the difference in life span between man and *Drosophila*, for instance, is under genetic control.

In a way which is typical, we believe, of the actual approach to the research in gerontology, Sacher and

Hart[1] defined two basic systems of research on aging and longevity, which they called the ontogenetic and the phylogenetic approaches. The former should attempt to define the parameters of aging at the molecular and cellular level in cells and tissues from an organism during its life span, while the latter should seek to correlate genetic and biochemical parameters to differences in longevity observed between species.

There is no doubt that these two approaches are highly attractive, but one may feel that they are not fully developed. Indeed at the present time no definite answer has been given to the following very simple and basic question: are the variations in normal life span - this excludes the accidental deaths - present among the individuals of a definite population (or a definite species) ascribable to a genetic variation present in that population (or species)? Even if the molecular and cellular variations that an individual goes through during its life time could be perfectly described, no answer could be given about the random or genetically controlled nature of these variations. Furthermore, as far as the phylogenetic approach is concerned, geneticists have repeatedly demonstrated that a difference in the average phenotypes of two populations (or species) need not have a genetic basis even though similar phenotypic variations within either or both populations are largely genetic in origin[2].

A genetic trait

A genetically controlled trait is essentially the advanced stage, sometimes the endpoint of a developmental sequence, the individual step or steps of which are controlled by one or numerous genes. Discontinuous or qualitative traits are primarily controlled by one or a few genes, the so-called major genes, each of which makes a large contribution to the process of character formation. On the contrary, continuous or quantitative traits are supposed and sometimes have been shown to be controlled by many genes, the so-called minor genes or polygenes, with small individual contributions.

Considered as a phenotypic character, longevity may appear as a very peculiar trait. It could indeed constitute the most advanced stage, the real endpoint of innumerable developmental sequences. Any interference with any biosynthetic pathway at any stage of life could, to some extent, have an effect on longevity. The question of the genetic control of longevity could therefore be a spurious one. In other words, does any specific gene, or genes, exert some type of control on longevity, or does the entire genome do so?

What is at the present time the evidence for the hypothesis that life span is genetically controlled? The data concerning the influence of both the major and the minor genes on the intraspecific differences should be considered.

Intra-specific differences: Major genes

In *Drosophila* there are a number of studies showing that longevity may be affected by major genes. The first of these were made by Pearl and Parker[3], the most recent one by Ünlü and Bozcuk[4]. (For a recent review see Lints[5]). In man, too, there is a wide spectrum of mutations affecting life span[6], which produce sometimes a phenotype resembling premature aging. Martin[7] has recently undertaken an analysis of McKusick's catalog of Mendelian inheritance in man[8] and has selected 21 phenotypes which are possibly associated with senescence. Such an approach will probably yield interesting data; it should also be applied to *Drosophila.* Indeed, in the fruitfly, the major genes which have been studied in relation to their eventual control of life span are all genes which mainly affect morphological traits. If one accepts that there are many good reasons to consider aging as the ultimate expression of development, and such reasons do exist (see below), then, obviously, major genes with evident effects on the expression of traits linked to development should be analyzed with respect to life span. In *Drosophila* such mutants do exist.

However, it is significant, in our opinion, that all the major gene mutations which have been analyzed affect life span in a negative way, namely they shorten it. We do not know of a mutation, neither in *Drosophila,* nor in man, which prolonges life span. Therefore we feel that the evidence resulting from the analysis of major genes is not conclusive in favor of specific longevity genes but rather indicative of totally nonspecific mutations with lethal or sublethal effects. Such mutations pertain to genetic accidents, not to the genetic control of life span. The study of these mutations is, however, not useless since such studies may indeed throw some light onto some of the molecular mechanisms proper to aging.

Minor genes with additive action

What about minor genes? At the outset, one should note that gerontologists are not always sensitive to (or do not consider as being relevant?) the distinction between the additive and the non-additive action of minor genes. The study of both these aspects of polygenic action requires of course different approaches. In this context it may be useful to quote from a recent book on the genetics of aging[9] containing two interesting studies on the genetics of longevity in man. The first of these studies[10], originated by Pearl and Parker[11], investigates the relationship between the life spans of a large group of parents and offspring, thus analyzing essentially the action of minor genes with additive action. The author shows that there is a positive but small correlation between parents and offspring life spans. However, a careful

analysis of his data leads him to the unequivocal conclusion that: 'There is no clear evidence to suggest whether ... [the] clear and almost uniform parental component in the length of life ... is due to genetic factors ... or to purely cultural and environmental factors'. The second of these studies[12] concerns a longitudinal study of aging in human twins, originally started by Kallman and Sander[13]. That type of analysis includes the consideration of both the additive and non-additive action of minor genes. The conclusion of the authors is equally unequivocal: 'There is a strong support of the hypothesis that heredity is a significant factor in determining the human life span'.

The continuous variation exhibited in a definite species or population suggests that like other continuously varying traits, life span could be controlled by an array of minor genes with additive action. The existence of such a genetic control might be demonstrated either by selection or by a significant degree of heritability. We know of no experimental results showing a successful selection for a prolonged life span. The work of Strong[14] on selection for life span in mice which for years has been abundantly quoted as demonstrating the existence of a certain amount of genetic variability affecting life span among an experimental mouse colony by no means, as it was recently demonstrated[5], resists a critical reading of the original paper. Lints et al.[15] selected towards a higher longevity for eight successive generations in a wild strain of *Drosophila melanogaster.* Absolutely no response to selection could be observed. The impossibility of selecting towards a higher longevity and the total absence of relation between parental and offspring longevities demonstrate that the very large phenotypic variability displayed by life span in a wild strain of *Drosophila melanogaster* does not depend on a precise set of polygenes with additive action. Using a different method of analysis Flanagan[16] recently claimed to have confirmed these results.

Minor genes with epistatic interaction

Do these results mean that the entire phenotypical variance in longevity exhibited by a particular strain of *Drosophila* is of nongenetic origin, i.e. environmental or intangible? In fact, a part of that variance could be due to non-additive genetic variance, i.e. variance due to the epistatic interaction between genes. Indeed longevity has been shown to be a trait exhibiting both inbreeding depression and heterosis. In that respect the clearest results have been provided by Maynard Smith in *Drosophila subobscura*[17,18] (for review[5]). The precise causes of inbreeding and heterosis are not perfectly understood. However it is generally assumed that inbreeding depression is due either to increased homozygosity for deleterious genes or to the break up of balanced polygenic systems. On the other hand heterosis is assumed to be the phenotypic result of gene interaction in heterozygotes. Thus a trait exhibiting either inbreeding depression or heterosis is clearly under some type of genetic control. However, both these phenomena imply all the phenotypic traits observed and thus the entirety of the genome. That evidence, therefore, is not conclusive; indeed it may not be concluded that specific genes for life span, and not the entirety of the genome, are affected by increased homozygosity or heterozygosity.

Another approach to the problem of the existence of epistatic genetic variance in natural populations could be developed through the analysis of subpopulations issued from a definite population and submitted during a certain period of time to different environments. Such subpopulations should adaptively diverge through genome remodelling and subsequent selection. Such a remodelling could eventually affect life span.

Such an experiment has been in progress in my laboratory for the last two years. Two different populations of *Drosophila* have been kept in cage populations in different environments. A gradual and significant divergence in life span has been observed in these subpopulations. Appropriate crosses between them stronly suggest that the divergence is genetic in origin[19].

Life span and development

Perhaps more important is the fact that parallel to the observed differences in life span, the subpopulations also diverged in size, duration of development and growth rate. In 1963, Lints[20], from an experimental approach, and Muller[21], from a review of the mechanisms of life span shortening, drew attention to the existing relations between development and life span. The latter author argued that development is a continuous process of which senescence forms the last stage, or, in other words, that aging is a built-in consequence of differentiation. Considered from that point of view, life span appears as an epigenetically controlled trait, i.e. a trait whose expression is linked to the regulation of gene function, of differentiation, or of the topographic distribution and function of proteins.

There are a few instances, both in homeotherms and in poikilotherms, in which a close link between development and life span has been shown. There are, for instance, the important experiments of Ros et al.[22] on rats. These authors measured 16 variables related to dietary habits and growth rate in a cohort of 120 rats. Using the technique of the conditional multiple regression analysis the authors were able to estimate the individual life span of the measured rats by employing parameters related mainly to growth rate and accessorily to dietary practices.

A study on mice as well (an older work by Roberts[23] which was not given the attention it deserved) demon-

strated a close genetic link between growth, development and life span. Roberts studied the life span of four strains of mice, two of them selected for fast and two of them for slow growth rates. The slow growing mice live longer and wean twice as many offspring as the fast growing mice. Thus the selection for a rapid growth - not for a slow growth (!) - has an adverse effect on reproductive fitness. Now, in a population in genetic equilibrium, any changes in the array of gene frequencies, such as those eventually caused by selection in either direction, must result in a reduction of fitness - except if the traits considered are genetically correlated. Indeed, correlated responses of two characters - in Roberts' case, growth rate and life span - to selection for one of these characters cannot arise in the absence of a genetic correlation between these traits. A genetic correlation implies either that some genes affect both characters, i.e. are pleiotropic, or that genes affecting both characters are very closely linked.

In poikilotherms the environmental conditions in which the preimaginal period of life of hybrid *Drosophila melanogaster* is spent have been manipulated[24,25,26]. The induced modifications of growth rate are reflected in parallel modifications of imaginal life span: the slower the growth rate, the longer the life span. Recently the same relation was confirmed in two natural populations, two selected lines and two mutant strains of *Tribolium castaneum*[27].

In that last experiment a surprising and probably important phenomenon was observed. When growing *Tribolium* are submitted during their entire life to constant temperatures, the relations between temperature, growth rate and life span are almost linear. The lower the developmental temperature, the slower the growth rate, the longer the adult life span. However, when growing beetles are submitted to alternating temperatures (35 and 25 °C, for instance, the mean being 30 °C) the growth rate and the adult life span are much higher than expected on the basis of the mean temperature to which the growing beetles were submitted. This suggests that the individual submitted during its development to various environments is provided with a larger norm of reactions vis-à-vis the potential stresses of its later imaginal life.

Life span: organismic and cellular approach

Thus, in poikilotherms, an induced decrease in growth rate results in an increase in life span, and vice-versa. Here, one must ask the question, to what an extent may growth rate be associated with mitotic division rate? In other words, when growth rate is accelerated or decelerated, may it, as a first approximation, be contended that the same happens for mitotic division rate? It has been shown from studies carried out *in vivo* in higher organisms that when a cell enters the aging pathway it has a life expectancy that is characteristic of the mitotic rate of the tissue (for full details, see Bullough[28]).

Even before Hayflick's discovery of the limited life span of fibroblast cells in culture[29] the study of aging at the level of the cell and the study of aging at the level of the organism, have been two almost totally independent fields of research. Bozcuk has demonstrated that tissues of holometabolous insects like *Drosophila,* show no mitoses after emergence of the imago[30]. The death of the organism must thus, to a certain extent, coincide with cellular death. Would *Drosophila* therefore not constitue the ideal tool to bridge the gap between the studies of aging at the levels of the cells and of the organisms? Although the mitotic division rate of *Drosophila* has been studied[31] the relationship between growth rate, mitotic division rate and life span has never been analyzed in that organism. This should be done for various mutants affecting traits related to development.

Conclusion

Two main points emerge from this short review. First: one does not know to what extent, either by major genes, or by minor genes with additive action, or by minor genes with epistatic action, life span is genetically determined. Secondly: although there are indications of an existing link between developmental processes and the aging processes, one does not know precisely how these processes are linked and more precisely if and how they are genetically controlled by an identical or by a different set of genes.

At a first level, development is a system of selectively and epigenetically precisely timed repression and derepression of different genes. At a second level, when a gene or a set of genes is switched on, regulatory systems exist which control the assembling of the polypeptides synthesized. A third control of the development exists at the level of the regulatory genes controlling and modifying the relative activities of the different enzymes present in the organism. The existence of such regulatory systems, about which one has already theorized for some time[32], has recently been demonstrated in the case of the alcoholdehydrogenase gene system in *Drosophila melanogaster*[33].

We therefore feel that development, aging and life span must be considered as epigenetically controlled and suggest the following model. As a function of and parallel to the successive stages of development - epigenetically controlled by the genotype, but which may be conditionned, regulated or modulated by various environmental factors - genes are switched on and off. The derepression and/or repression of certain genes or groups of genes, acting either on the synthesis, or on the assembling or on the functioning of given polypeptides and enzymes must be permanent and therefore affect the entire life span of the individuals concerned.

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.