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CURRENT PRIORITIES IN THE BIOLOGY OF AGING

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Summary. Aging is an involutive phenomenon progressively affecting all levels of the biological organization: molecular, cellular, tissular, organic and organismic. The aging process does not develop uniformly in time and space, its rate being different for each species, individual, organ and tissue. Between the different phenomena occurring at the same or at different levels of organization, or between mechanisms and effects, there is a feed-back interconditioning. Mention is made of modern concepts on the genetic mechanisms of aging, with emphasis on those which seem to allow a better understanding of the aging process. The author also reports on the contribution of the researches carried out in the National Institute of Gerontology and Geriatrics, Bucharest, in the field of the biology of aging and biotrophic therapy with Gerovital H_3 and Aslavital, products developed by Ana Aslan. A description is given of the research-work on: the increasing degree of collagen polymerization with advancing age; decreasing Fc^+ receptor synthesis in the rat splenocyte with advancing age and its stimulation following therapy; neuronal depletion in the cerebellum; age-induced reduction of structural glycoproteins in the intercellular matrix and their increase in the treated animals; decreased rat peritoneal macrophage migration with advancing age; the increased activity of monoamineoxidase in elderly tissues and B type inhibition induced by Gerovital H3; the protective effect of the treatment in coldstressed, old animals, etc.

Due to the advance made after 1940 in the control of infectious diseases by means of bacteriostatic and bactericidal substances, physicians and scientists were able to focus their attention on the period of organismic involution, old age, frequently accompanied by chronic degenerative diseases, a main factor in shortening the life span.

The progress recorded during the last 30 years in the study of the biology of aging is to a great extent the result of the close collaboration between specialists from different branches of medicine and other sciences, which has led to the elaboration of new and valuable concepts and use of modern techniques.

In the field of the biology of aging the comprehensive scientific data which have accumulated reflect the different gerontological orientations of the various schools throughout the world; a systematic presentation of such data would be thus difficult. Based on the unanimously admitted opinion that aging is a process which affects the entire organism, we shall try, as other authors did, to review the most significant data on the main levels of biological organization: molecular, cellular, tissular, organic and organismic.

The feed-back interconditioning between the various phenomena occurring both at the same and at different levels of organization, should be taken into consideration when interpreting the data presented (Fig. 1).

The mechanisms involved at the molecular level of aging are either genetic or nongenetic [1].

Considering only the mechanism of aging which occurs in the nucleus, there are two possible alternatives of interpreting aging: 1) the result of a well ordered genetic programme, which develops without considerable errors and 2) a biological process which results from the deficiencies occurring in the control of the genetic programme, throughout the life span. Among the causes inducing the defective genetic control mention should be made of: the progressive crosslinking of DNA; somatic mutations; code errors in DNA replication; chromosomal aberrations; chemical changes in DNA, produced by the free radicals, etc.

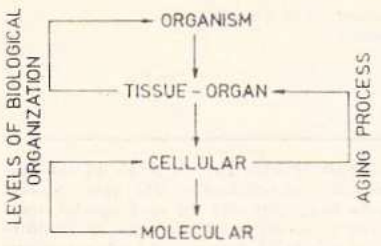

Fig. 1. — Levels of biological organization.

The theories according to which cytochemical aging is attributable to increased errors in translation and in protein synthesis is supported by a possible accumulation of mutagene changes [2].

Greater attention has been given to the coding errors in replication [3], the accumulation of which is dependent on the number of generations; the more active the doubling of the cell, the higher the number of errors.

The somatic mutations seem more certainly involved, because they affect all the molecules synthesized by a certain gene. It is important to know whether aging is the result of either an intrinsic mutagenesis, dependent on the number of generations, or time-dependent mutations which occur in postmitotic cells.

Researches have been conducted in the Institute on the role of the free radicals as endogenous source of mutations. The first stage of the research, conducted by Brazdeș, Crăescu and Rusu [4], was focused on the increase with age in serum nuclear paramagnetic centres; at present, the possibility is studied by Bueșa of capturing the free radicals, which have too short a life span to be evidenced by means of common chemical methods.

Among the other possible molecular mechanisms of aging mention should be made of the progressive crosslinking of the protein macromolecules, protein partial hydrolysis or denaturation, etc.

Aslan, Vrăbiescu [5] made an original contribution to the study of these mechanisms by pointing out the gradual increase in collagen polymerization with advancing age (Fig. 2). Other researches [6] were focused on the increase with age in the mechanical resistance of tendon collagen fibres (Fig. 3).

To the above changes induced by molecular aging, we may also add: the physico-chemical progressive inactivation of DNA; blocked gene accumulation and progressive loss of genetic information; production of wrongly-synthesized inactive or rapidly degrading proteins with antigenic potential; changes in the physico-chemical properties of protein macromolecules; intra- or extracellular accumulation of macromolecules; deficiencies in the synthesis of intercellular matrix macromolecules, etc.

Concerning the ageing at the cellular level, mention should be made of the following mechanisms: the structural and functional changes of biological membranes; diminished membrane receptor synthesis; decreased cell doubling potential, genetically programmed limitation of cell life span, differing *in vitro* for each species; intensification of MAO activity; intensification of proteolytic enzyme acti-

vity; accumulation of pigments with age; functional disturbances in specialized cells, particularly nervous, endocrine, immuno-competent; decrease of intracellular potassium amount, etc.

The researches on cell membrane changes have recently pointed out the reduction of receptor-synthesis in the course of the aging process.

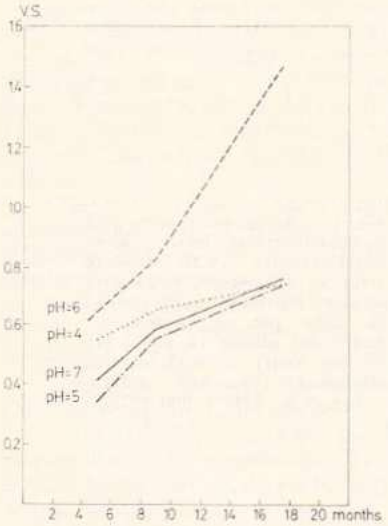

Fig. 2. — Specific viscosity of collagen solutions.

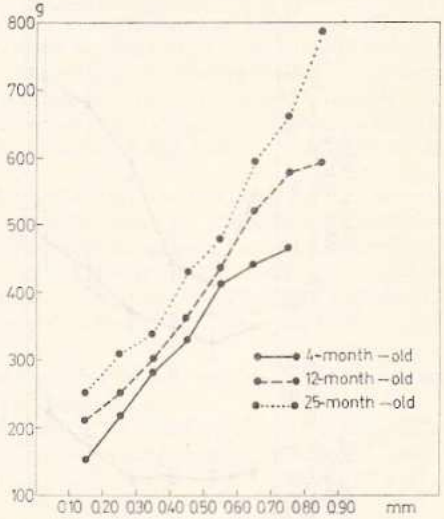

Fig. 3. — Mechanic resistance of tendon fibres in relation to their diametre and the age of the animals.

In the course of aging, deficiencies were found for instance in the receptivity of hormones, mediators, amino acids, etc.

Manciulea, Gheție, Ionescu [7] studied the age dependent variation of Fc^+ receptors on the surface of rat splenocytes. The decreased interaction was noticed between the Fc^+ receptors and heterogeneous immunoglobulin as a result of the diminished receptor synthesis.

The decrease with age of the steroid hormone receptors has been tested for glucocorticoids by Roth [8], in researches on old rat brain. The same author [9] found a 30% reduction in the glucocorticoid membrane binding sites on the neuronal pericardium isolated from old rats.

Other studies pointed out: the decrease of sex steroid hormone receptors in old rat brain [10], the reduction of tissue receptivity for sex hormones neurotransmitters, etc., as a result of advancing age [11].

Due to the importance of the required amount of membrane receptors involved in an efficient biological reaction, the necessity for developing such studies is emphasized for a better understanding of the cell mechanisms.

Recently, greater attention has been given to Hayflick's concept [12] according to which the life span of cells is limited and genetically determined. This

concept was based on studies of human embryo fibroblast cell cultures, which, after 40—60 doublings died.

The author also found a direct relationship between the donor's age and the longevity of the cultures, as the doubling potential of adult human fibroblasts is only 10—30, below that of embryo fibroblasts.

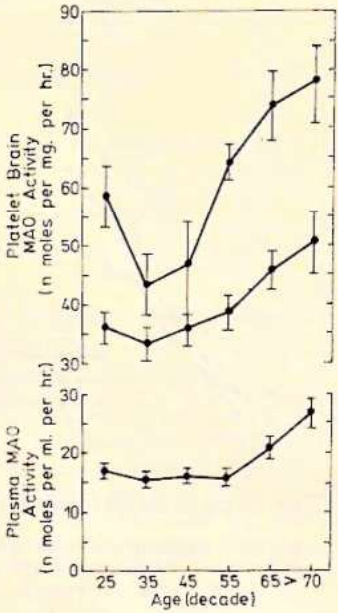

Fig. 4. — Aging, monoamines and monoaminoxidase levels. Mean MAO activity (with standard error of the mean S.E.M.) of human hindbrain and platelet (n moles per mg protein per hour) and plasma (n moles per ml per hour) for each decade. (Robinson, D.S., and co-work, Lancet No. 7745, p. 290, 1972)

The subsequent researches on embryo fibroblasts isolated from different species determined Hayflick to advance the hypothesis according to which the different longevity in different species is dependent on the genetically determined cell doubling potential.

The relationship between monoamineoxidase (MAO) and aging was pointed out by Robinson and coll. [13], who showed that MAO activity increases after 45 years of age in the brain, serum and thrombocytes (Fig. 4).

Due to the importance of the role played by this enzyme in the biochemical modification occurring in the course of aging, Robinson's researches were the corner-stone for numerous recent pharmacological studies in geriatrics and psychiatry.

The accumulation of age pigments, noticed long ago (Stübel, 1911) is about to receive a new interpretation, because recent researches have pointed out the presence of such pigments close to the intracellular membranes, therefore in relation with their aging. Due to the presence of age pigments in lysosomes [14] the tentative inference was drawn, according to which they would result from the incomplete digestion of different intracellular organelle membranes in the lysosomal

autophagic vacuoles. According to other authors the pigments would result from the polymerization of oxidized unsaturated lipids [15].

According to Strehler and coll. [16] lipofuscin granules accumulate linearly in the human myocardium at a rate of 0.6% per cell volume and age decade, up to 6—7% per cell volume at the age of 90. Nevertheless, there are aged individuals with few pigments and young ones displaying intensively pigmented cells. Pigment accumulation was found to increase in the nervous system cells under stress, in certain pathological conditions, as a result of cortisone administration, etc.

Among other changes in the cells some are part of the mechanisms of aging and others occur as a result of these mechanisms: progressive reduction in cell functions; decreased ability of the cell to react to stress; prevalence of catabolic processes; cell involution and death; decreased cell regenerating potential; autoimmune reactions triggered by specialized cells; changes in the form and volume of the cells, etc.

The main mechanisms and modifications in tissues and organs occurring with advancing age are: the decreased tissue regeneration; the decrease in the number of parenchymatous cells, replaced by fibrous and adipose tissue; thickening of capillary basal membranes; diminished blood supply to organs and tissues; structural and functional changes in the intercellular matrix; increased activity of the lytic enzymes in the intercellular spaces; decreased water amounts in different organs and tissues, etc.

The data concerning the decreased number of cells with different tissues are particularly important for the central nervous system.

Brody [17, 18] pointed out the decrease in the number of cells within different cortical areas, sometimes up to 45%; Colon [19] found a substantial loss of neurons in the frontal lobe cortex with the ninth decade; Shefer [20] found a 20% decrease throughout the cortex, and a 28% decrease in the frontal lobe.

The loss of neurons does not seem to occur linearly, as Burnns suggested, at a rate of 100,000 cells daily; the loss is more rapid in the course of certain periods of life and slow in others. For instance: Brody [18] found the greatest loss of cells in the frontal gyrus during the fifth decade of age.

Hall, Miller and Corsellis [21] found a 25% decrease in the number of Purkinje cells from the brain, between the sixth and tenth decades.

Researches conducted by Simion, Costiniu, Bălăceanu [22] pointed out the neuronal loss from the cerebral neocortex, hippocampus cortex, thalamus and cerebellum cortex. A daily loss of 2,700 cells was calculated for Purkinje cells and 26,000,000 cells for granular cells.

The loss of cells is obvious only in the cerebral formations with a complex structure, playing an integrative part (neothalamus, cerebral cortex and cerebellum).

As known, the age-dependent cell loss occurs in other tissues and organs as well. The emphasis was laid on the nervous system, because the neuronal loss is one of the factors which influence the disturbances triggered by advancing age on the regulation, coordination and trophic functions of the central nervous system.

Recently, special attention has been given to the age-induced changes in the intercellular matrix of the connective tissue. Qualitative and quantitative modifications of collagen and elastin fibres have been described. Their synthesis seems to take place according to a certain "programme" in which the genetic, hormonal and environmental factors are decisive.

With advancing age, the collagen fibres are less affected by external influences, the collagen network loses its flexibility [23], the thermic stability of the col-

lagen fibres increases [24], the amount of soluble collagen decreases, the reaction to mechanical stress diminishes.

The cross-binding within and between the collagen molecules accounts to a great extent for the changes in the collagen network of the intercellular matrix.

Concerning the elastin, in the course of embryogenesis only elastin microfibrils are identifiable; the amorphous component appears with advancing age, thus, when mature, the elastic fibre is made up of the amorphous component and a thin microfibrillary cover, where cross-bindings occur.

The basal membrane of the intercellular matrix was found to thicken with age, doubling sometimes.

The age-changes in the other two components of the intercellular matrix, proteoglycans and hyaluronic acid were scarcely investigated. An ever increasing interest has been focused on the structural glycoproteins, because they are responsible for the integrity and trophicity of the tissues. The glycoproteins are also present in the cell membrane and within the cells where they control certain phenomena related to the cell development.

Researches carried out in the Institute by Cofaru and Vrăbiescu [25] pointed out the decrease in the amount of structural glycoproteins from old rat muscle, which is probably due to the increased catabolic activity of the lysosomal enzymes.

To conclude the chapter on the mechanisms of aging in tissues and organs, mention should be made of other changes occurring in the course of aging at these levels: the increased ratio connective tissue/parenchyma (fibrosis, cirrhosis, sclerosis); structural and functional involution of different organs and tissues (with hypo- and atrophy) changes in the form and volume of organs and tissues; reduced exchanges between blood and organs; progressive diminution of metabolic exchanges; decrease or cessation of tissue / organ specialized functions (hormones, enzymes, mediators, antibodies, etc.); disturbances in the intercellular matrix functions, etc.

At the level of the organism mention should be made of the following aging mechanisms and changes: the reduction or blockage of communications between different organs, tissues and cells (hormones, receptors, mediators); progressive impairment in the main systems of integration: nervous, endocrine, cardiovascular; diminished trophic function of the nervous system; cessation of the reproductive functions; diminution in the immune functions; intensification of autoimmune activity; diminution of the psychic functions of metabolic exchanges; onset and progress of chronic and degenerative diseases; unequal involution of the organismic structure and functions; the decreased ability to adapt to the environment, etc.

The immune function becomes progressively deficient in the course of aging. The disturbances which occur lead to the diminution of the immune function, hence the decreased resistance to infectious diseases, and trigger the autoimmune reactions, phenomenon which is considered a possible mechanism of aging.

Among the main age-changes affecting the components of the immune system, mention is made of: the decrease in size and weight of the thymus and mainly the atrophy of the cortex; the atrophy of the IgM level with advanced age; the impaired functions of immune B and T cells; the impaired proliferative response of T cells to phytohemagglutinin, the decrease with age of flagellin antibodies and the increase in nucleus autoantibodies.

In close correlation with the above-mentioned changes, the incidence of infections, autoimmune diseases and cancer was found to increase with age along with the impairment of immune functions [26, 27]. This relationship is supported by the higher mortality rate among the aged with cell mediated deficient immunity.

Cardiovascular and cancer mortality is also higher in subjects with antinuclear antibodies than in those without antinuclear antibodies [27, 28].

The studies carried out by Ionescu and coll. [29] have pointed out the increase in antinuclear and antialbumin autoantibodies with advancing age. Studies have also been developed on cell mediated immunity and particularly on the lymphocyte-macrophage relationship within the mechanisms responsible for the impaired immune functions in the aged.

Recently, Răchită, Vrăbiescu and Constantinescu [30] have studied the behaviour of macrophage under the influence of certain factors secreted by sensitized lymphocytes. The inhibition of rat peritoneal migration decreases with age. If this phenomenon were reproducible in human subjects, the mechanisms responsible for the greater frequency of chronic infections diseases in the aged would be understood.

Aging of the organism involves certainly many other modifications as well, among which: the decreased response to training, metabolic uptake, etc.; impaired ability to react to environmental stress; increased sensitivity to the action of risk factors; diminished resistance toward environmental nocuous factors; reduced physical and psychic working capacity; decreased respiratory exchanges; anthropometrical changes; shortening of life span due to premature or accelerated aging.

The changes which occur with advancing age at all the biological levels show how complex the aging process is. This tallies with Ana Aslan's opinion that aging is a generalized dystrophic process induced by multiple mechanisms.

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Progress in the understanding of the aging mechanisms, obtained on the basis of modern concepts and research methods has allowed more thorough investigations in the field of geriatric pharmacology.

The correlation of researches in the field of the biology of aging with the pharmacological studies has yielded quite interesting results concerning the mechanisms of action of the biotrophic products Gerovital H_3 and Aslavital, elaborated by Ana Aslan [31, 32, 33].

Mention should be made of the most important ones:

As shown above, the increase of MAO activity plays an important part in the biochemical changes, correlated with aging and depressive states.

As early as 1940, Philpot [34] showed that procaine inhibits MAO. In 1972, Hrachovek [35] from Los Angeles University, published the results of some comparative studies which proved that Gerovital H_3 has a stronger inhibitory effect than procaine hydrochloride, on rat brain, liver and heart MAO. Those studies were conducted *in vitro* on homogenate and mitochondrial fractions after intraperitoneal shots.

Significant differences between the two substances were described also by Mac Farlane [36]. According to the amount administered, Gerovital H_3 induced the inhibition of MAO up to 87.7%.

In 1974, the American scientist Yau [37] claimed that Gerovital H_3 was a weak, reversible and competitive inhibitor of MAO. The substance was an antidepressant, because it influenced monoamine levels in the brain.

The studies on Gerovital H_3 ability to inhibit MAO were carried out in the Institute by Ana Aslan and Rusu [38] on rat liver heart and brain mitochondria. Aslavital in much smaller amounts (4 mg/kg body weight) than those used by the American researchers has yielded similar results. The inhibitory effect was prolonged as a result of chronic treatment, lasting 24 hours after the last shot.

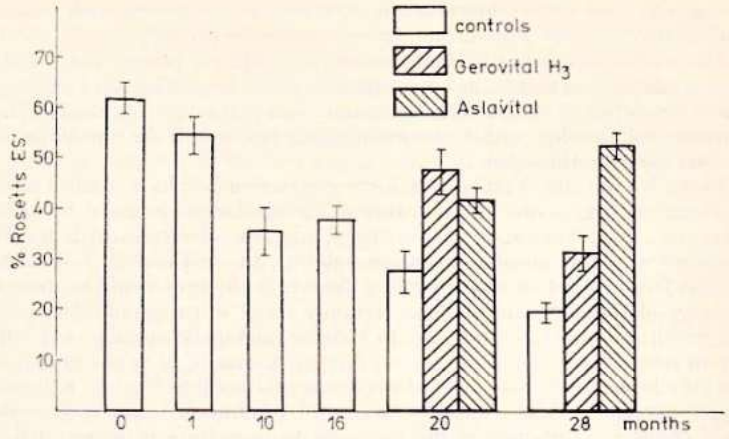

Fig. 5. — Variation of Fc receptor carrier rat splenocyte percentage in relation to age. Influence of Gerovital H_3 and Aslavital treatment.

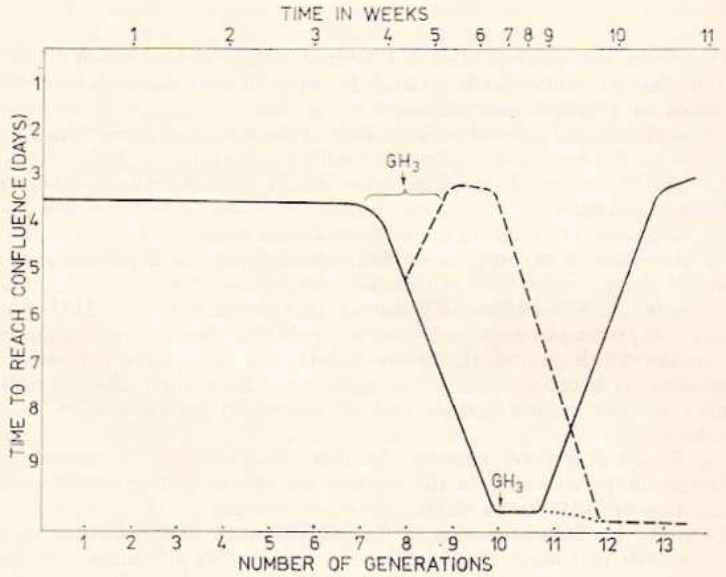

Fig. 6. — Wild mouse embryo cell cultures subcultured when confluent. Gerovital, (GH_3), added at the 8th or 9th generation, renewed cell proliferation, and continued for an additional 2 generations. GH_3 added to cells in the stationary phase maintained the cells in that condition and did not spontaneously transform (J.E. Officer, Symposium on Theoretical Aspects of Aging, Feb. 7—8, 1974, Miami, Florida).

Recently, Stroescu, Gane, Constantinescu, Vrăbiescu [39] studied Gerovital H_3 action as antidepressant and MAO inhibitor based on certain pharmacodynamic tests: antagonizing the conditioned behavioural changes induced by reserpine, hypothermal action, prevention of reserpine-induced hyperthermic changes and palpebral ptosis in rats.

The results obtained support the clinical data showing Gerovital H_3 antidepressant effect and suggest that this substance has a B type action on MAO.

The immunologic studies conducted by researchers from our Institute and "Dr. V. Babeș" Institute [7] have pointed out new elements in the understanding of the mechanisms of action of the biotrophic products:

— increase in the percentage of rosettes formed by the rat spleen lymphocytes after Gerovital H_3 and Aslavital treatment, pointing to the stimulated synthesis of Fc receptors (Fig. 5);

— the decrease of antinuclear and antialbumin autoantibodies in patients under long-term treatment (Table 1).

Table 1

Incidence of autoantibodies

	No. of cases	Mean age	AAN %	AAA %
Controls	299	64.7	5.7	32.1
Gerovital H3	116	65	4.8	22.6

Quite interesting data on the mechanisms of action of the biotrophic substances have recently been communicated by a group of researchers from the Biological Research Centre Cluj-Napoca (Romania). The studies conducted on rats by Rusu, Abraham and Manciulea have shown: 1) age-differences in the reaction of the stressed adrenal gland with animals exposed to cold; 2) the return of most of the adrenal morpho-functional indicator values to those of the control after repeated Aslavital administration, which points to a protective effect against stress; 3) the complete adaptation to stress of the treated animals after 30 days, more obvious with young ones, as against controls.

Related to the age-changes which take place in the intercellular matrix, Cofaru and Vrăbiescu [25] showed that structural glycoprotein level increases with Gerovital H_3 treated old animals as against old controls, reaching values close to those specific to the adult animals (Table 2). These results point to the delayed onset of the dystrophic processes in the course of aging.

Officer's researches [40] should also be mentioned; based on Hayflick's observations concerning the limited life span of cell cultures, Officer noticed that the multiplication rate accelerates as a result of Gerovital H_3 administration during the second passage; once the division had ceased, the treated cells had a longer life span than the controls (Fig. 6).

Aslan and coll. [41] pointed out an average increase in postmitotic cell life span by 16% in treated cultures (Table 3).

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Based on the results of the above-mentioned researches, the following inferences may be drawn:

Table 2

Structural glycoproteins from rat muscle — protein, total hexose, sialic acid contents —

Rat group	Proteins (mg/g wet tissue)	Total hexose (mg/g wet tissue)	Sialic acid (mg/g wet tissue)
Young	84.4±5.5	0.816±0.019	0.168±0.004
Adult	70.9±1.2	0.684±0.017	0.136±0.002
Old	60.4±1.14	0.603±0.017	0.110±0.003
Old + Gerovital H3	66.8±1.23	0.640±0.014	0.124±0.002

Table 3

Postmitotic lifespan of primary tissue cultures of kidney under the influence of Gerovital H3

Average life span, in days		Percentage difference as compared to the control	p
Control	Gerovital H3 0.4%		
62.3	72.4	16	<0.01

— Aging, the process common to all living beings, affects all the biological levels of organisation: molecular, cellular, tissular, organic and organismic;

— The onset and progress of the involutive aging phenomena differs from species to species. For each species, each organ, tissue and cell has its own aging clock; thus, the aging of the whole organism is neither uniform nor linear in time;

— There is a reciprocal influence between the process of aging and chronic degenerative diseases;

— Although the factors that trigger the multiple mechanisms of aging are not known, the numerous observations, experimental data and concepts accumulated agree that the starting point would be within the cell nucleus, the events of the vital cycle occurring either according to a normal programme of differentiation with the end result of aging, or as a process resulting from the accumulation of deficiences in the control of the genetic programme throughout the life span;

— The experiments done at present, according to the different orientations in gerontology, will certainly allow a better understanding of the aging process and possibilities of influencing it;

— Because the rhythm of aging is affected by the interrelationship with internal and external factors, the aging process and the associated pathology can be actively influenced by removing or reducing the effect of the nocuous environmental factors and stimulating the processes involved in maintaining the good trophic condition of the organism. The results yielded by the use of Ana Aslan's treatment are an eloquent proof;

— As a corollary of this new orientation, the new geriatric pharmacology developed, has been based on the study of the mechanisms of action of the biotrophic substances elaborated by Ana Aslan, closely correlated with those underlying the aging process;

— The clinical use of the differently conditioned biotrophic products and their maximum efficiency in geriatric therapy and prophylaxis is thoroughly studied by pharmacologic investigations.

Résumé. Le vieillissement est un phénomène involutif qui affecte de façon progressive tous les niveaux d'organisation biologique: moléculaire, cellulaire, tissulaire, l'organe et l'organisme. Le vieillissement se développe de façon non uniforme en temps et étendue ce qui fait que la vitesse de vieillissement soit différente pour chaque espèce, individu, organe et tissu. Entre les divers phénomènes qui se passent au même niveau ou aux niveaux différents d'organisation, ainsi qu'entre mécanismes et effets, il y a un interconditionnement du type feed-back. On mentionne les conceptions actuelles sur les mécanismes génétiques ou non génétiques du vieillissement, tout en insistant sur les conceptions qui présentent les plus grandes perspectives de progrès dans la connaissance approfondie du processus de vieillissement. On présente également les principales contributions apportées par l'Institut National de Gérontologie et Gériatrie de Bucarest par les recherches entreprises dans le domaine de la biologie du vieillissement et de la thérapie avec des substances biotrophiques Gérovital H_3 et Aslavital, élaborées par Ana Aslan. On expose les recherches concernant: l'augmentation avec l'âge du degré de polymérisation du collagène; la diminution avec l'âge de la synthèse de récepteurs Fc+ de la surface des splénocytes de rat et la stimulation de la synthèse sous l'influence du traitement; la dépopulation neuronale au niveau du cervelet; la réduction avec l'âge des glycoprotéines de structure de la matrice intercellulaire et leur croissance chez les animaux vieux et l'action inhibitrice de type B du Gérovital H_3; l'action protectrice du traitement par rapport au stress par le froid chez les animaux vieux et autres actions.

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