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# FOREWORD

Between 1949—1951, the residents of the "I. C. Frimu" Home for the Elderly — grown in 1952 into the Institute of Geriatrics for the treatment and prevention of aging — were subjected to a procaine treatment in the case of certain old age diseases, e. g. degenerative rheumatism and atherosclerosis. Beside the positive effect on these diseases, a general improvement was observed in the psychic and physical conditions of the patients. In view of it, I suggested to the committee charged with the distribution by treatment groups of the then 201 aged of the Home, to have one group given a procaine treatment. Based on the results obtained until then, scientist C. I. Parhon agreed. It is quite certain that those results facilitated the establishment of the first Institute of Geriatrics worldwide.

The aged subjected to treatment have been followed up in the Stationary of the Institute for thirty years now, often for as long as 10—15—20 years; this has led to the longest longitudinal research in gerontology.

The beginning was certainly difficult. The research was obviously original, because no similar approaches existed and hence, we could not find comparative data in the literature. When the experiment was started on May 5, 1951, the double-blind research had not yet become a prerequiste for the acceptation of a drug. Along a period of three years, we used three therapeutical methods, namely: procaine, vitamin E and epiphyseal extract, injected at the same interval since, at that time, we did not know that procaine would yield the best results.

Several circumstances supported the establishment of the Institute: C. I. Parhon's outstanding personality, the understanding for the problem of old age shown by the Romanian Government, the first results yielded by the biotrophic therapy, the devotion of the small group of researchers, too small in number for the vast research work that lay ahead.

As a result of the treatment given, the condition of the aged from the Institute improved widely compared to other Homes' residents. The therapeutic researches carried out at the Stationary of the Institute pointed out the beneficial effect of the procaine management on the residents' health: they became more active, enjoyed living and working, their memory was stimulated and depression alleviated.

Since our aim was to keep the aged alive as long as possible, we could not immediately comply with other aged persons' requests for hospitalization. Although now everything seems smooth, it was not so at that time. It was not so easy for the Government to fund this research, but I believe everybody's efforts proved worth while.

Another opportunity for the progress of gerontology in Romania, beside the establishment of the Institute, was the setting up of the Romanian Gerontological Society in 1956. This also required efforts and perseverance. During the same year, the Board of the International Association of Gerontology granted affilation to the Romanian Gerontological Society. The president of the Society was co-opted on the leading committee of the IAG. The exchange of ideas promoted by the Romanian Gerontological Society, with the participation of researchers from

the Institute, scientists and physicians from other Institutes has also been a factor of progress for Romanian gerontology.

The Romanian Gerontological Society has been supporting international contacts and, since 1957, the Romanian delegates have participated in all the international congresses of the IAG: Italy (1957), San Francisco (1960), Copenhagen (1963), Vienna (1966), Washington (1969), Kiew (1972), Israel (1975), Tokyo (1978), as well as in many other national and international meetings.

We are looking forward to this Journal publishing many valuable papers authored not only by gerontologists, but also by workers from other medical and related fields interested in this humanitarian problem. Foreign contributions to this Journal are welcome.

We would like to extend our gratitude to the Academy of the Socialist Republic of Romania for accepting the publication of the Romanian Journal of Gerontology and Geriatrics by its Publishing House. As a matter of fact, since 1951, the papers elaborated by the researchers of the Institute have been published in the Scientific Bulletin of the Academy and in other publications of the Academy. Other articles have appeared in the Journal of Normal and Pathological Physiology of the Union of Societies of Medical Sciences.

We wish to thank all those who have helped to the publication of this first issue and we hope to have the scientific value of the Journal enhanced through contributions from an increasingly greater number of scientists.

*ANA ASLAN*

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# THEORETICAL BASES OF PROCAINE THERAPY (GEROVITAL $H_3$ AND ASLAVITAL) IN THE PROPHYLAXIS OF AGING

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**Summary.** The reduced number and functional capacities of the cells during the aging process induce numerous characteristics of aging. As regards the neurons, the main object is to extend the cellular resources, with the help of psycho-pharmacology. The author proved, 25 years ago, the influence of procaine on the nervous system, by psychological and functional, clinical and experimental investigations. Recently the procaine pharmacology has developed by means of some American authors' researches, among which double-blind studies, concerning the favourable effect of Gerovital $H_3$ on the depressive moods of the elderly. The connection was proved between the aging process and the intensification of monoaminoxidase activity in the brain as well as procaine and especially Gerovital $H_3$ capacity to inhibit MAO and to reduce the aging marks at the level of the central nervous system. The anabolic action of procaine was proved in researches on cell cultures as well as on the animals finding, among others, an improved general trophicity and a prolonged life span. Other results obtained in the field of procaine action mechanisms refer to: the improved nitrogenous balance, favoured ATP synthesis, antioxidating action, intervention in the oxidative phosphorylation, the fat metabolism disturbances of atherosclerosis, the increased speed of nerve condition, the excretion of urinary steroids and metabolites, etc. The ultrastructural studies revealed the contribution of Gerovital $H_3$ to the stabilization of membranes and of the main cellular organelles, the intensification of cell metabolic activity in the treated cultures. All these lie at the basis of the prophylactic action and therapy of the aging phenomenon. In order to intensify the lipotropic action as well as that on the cerebral aging, the author elaborated a new product, Aslavital. The results of this treatment obtained in the first 9 years of application prove its efficacy in the involutive processes, predominantly cerebral, and in atherosclerosis (especially in the disorders of blood coagulability and lipid metabolism) as well as in the prevention or treatment of complications.

The decrease in the functional capacity, rendered more evident by the action of a stress factor, is one of the characteristics of the aging body. The decrease is closely correlated with the lowering number and functional ability of the active cells. With the progress of life, the rhythm in which mitotic cells regenerate slows down, thus the number of active cells decreases, as against the total body mass. This decrease induces the numerous characteristics of aging, such as: asthenia, physical disabilities, diminished muscular strength, impaired heart and kidney function, etc. The unbalanced adaptative mechanisms are also common.

Up to the present, there has been no proof of neuronal regeneration. Nevertheless, in our opinion, the possible prolongation of the brain cellular resources is a main objective of current gerontological research, that is of the psycho-pharmacology of aging.

Thirty-four years ago we published the first article on procaine action at the level of the respiratory centres in humans [1]; since 1949 our researches have

pointed out procaine action in the process of aging [2]. The stimulation of hair growth and repigmentation in humans [3], improved fur trophicity in rats [4], positive action on memory and depression have also been pointed out. The clinical and physiological investigations carried out in collaboration with C. I. Parhon and Al. Vrăbiescu [5] pointed out procaine influence on the brain (studies based on conditioned reflexes).

Lüth [6] emphasized that Parhon and Aslan had been the first scientists who mentioned the psychic effect of procaine ("procaine influence on the patient's psychic condition was signaled for the first time in the medical literature by the Romanian authors").

During the last 23 years our investigations of the biotrophic action of this substance, as well as studies carried out by foreign researchers have contributed to the improvement of procaine pharmacology. In the present paper I shall present the investigations of procaine action on the central nervous system and metabolic processes, with emphasis on the study of the regeneration phenomenon: in other words, I shall speak about the theoretical bases of this therapy in the process of aging and the prophylaxis of premature aging.

Our study on conditioned reflexes in human subjects were confirmed by Tsobkallo and Kutcherenko's experimental investigations [7]; they noticed the stimulating effect of 1—2 mg/kg body weight (amounts we used in human subjects) on the higher nervous activity as against the inhibitory effect of 20 mg/kg body weight. The above-mentioned authors pointed out that the upper area of the brain is mostly influenced by procaine. (The experiments were carried out on dogs and rabbits, and procaine was administered subcutaneously). Certain authors noticed that the products resulting from procaine hydrolysis were less active than procaine itself. This is a proof that the effect on the central nervous system is due to the intact procaine molecule.

Injecting procaine intravenously in dogs, Genovese and Garrattini [8] noticed the fixation of the substance in the central nervous system rather than in other organs.

S. Mora's electroencephalographic study [9] revealed significant differences between the 139 subjects investigated, the average age being 66, and younger subjects, the average age being 27. The combination of alpha and beta waves was pointed out in 67.7% of the aged subjects. The corresponding percentage with the younger subjects was 32%. Better EEG tracings were pointed out in 1/3 of the treated cases; no modification was noticed in the subjects with beta waves prevalence. The author establishes a possible correlation between the EEG tracings and the psychic reactivity.

The investigations conducted in collaboration with Broşteanu and C. Enăchescu [10] pointed out normal, unimpaired electric tracings under intermittent luminous stimuli in 75% of the Gerovital $H_3$ chronically treated subjects (the average age 85); in aged untreated subjects, normal tracings were evidenced only in 20% of the cases.

Subsequent to our investigations on the psychic effect of procaine (the results of which were published in 1956) pharmacological studies were carried out in 1957 on dimethylaminoethanol (DMAE) action by Pfeiffer and coll. [11], who noticed a higher improvement in muscular tonus and sleep, as well as a stronger mental stimulation with DMAE rather than with amphetamine. This study placed emphasis on the relations existing between DMAE and acetylcholine. Subsequently, Groth, Bain and Pfeiffer's comparative researches [12] based on $^{14}C$ DMAE and choline action proved that DMAE breaks through the blood-brain barrier, takes part

in the metabolic process of the nervous cells fixing their proteic and lipid fractions and is instantaneously changed into choline and acetylcholine. Unlike DMAE, labelled choline is removed through urine and respiration. DMAE may be looked upon as an acetylcholine precursor.

Numerous biochemical studies on the animal metabolism were carried out with an emphasis on DMAE transformation into choline. The cycle is the following: serine, aminoethanol, monoaminoethanol, DMAE [13]. Eicholtz's investigations [14] *in situ* of striated muscle also revealed DMAE transformation into choline. Beside the above-mentioned pharmacological, biochemical and biophysical data, procaine stimulating action on the central nervous system was pointed out by experimental investigations in white rats. In collaboration with Al. Vrăbiescu and coll. [15] we studied the learning and memorizing capacity with 24-month-old rats, according to Verzar and McDougall's maze method [16]; the results obtained in Gerovital $H_3$ treated animals were significant.

Studies based on our method were conducted on psychic disturbances and brain syndromes in old age. Bucci and Saunders [17] emphasised the favourable effect of the treatment in elderly schizophrenic subjects: the disappearance of hallucinations and the new contact with the environment up to the recovery of the intellectual activities. These authors consider procaine an energizing substance and correlate its action with monoaminoxidase (MAO) inhibition. Besides us, Tsobkallo, Bucci and Saunders found procaine more active than DMAE.

Significant data were communicated in 1972 on the existing relationship between MAO brain level and the aging phenomenon. Robinson and coll. [18] pointed out the marked increase in MAO brain level after 45, as well as the possible connection between this increase and the aging phenomenon.

MacFarlane [19] has appreciated Robinson's important contribution to the understanding of a biochemical modification connected with the aging process. Based on his data he emphasised that Gerovital $H_3$ induces a stronger MAO inhibition than the normal procaine hydrochlorate; its action is reversible and competitive.

The results of such studies pointed out Gerovital $H_3$ favourable effect on the aged subjects, due to the inhibition of the MAO increased levels; biogenous amines levels are also brought to normal values. The results of the clinical researches on the effect of Gerovital $H_3$ on the elderly suggest that the normalization of MAO levels has a positive influence on aging associated symptoms.

Long [20] investigated procaine effect on orientation, memory, attention and body weight in the aged. Double-blind studies according to our method were conducted for 1 year, on 60 subjects with orientation troubles. A special procedure for measuring orientation, attention and memory was used; it pointed out better scores in the treated group. Memorizing ability improved in the treated subjects, whereas a decrease was noticed in controls; body weight increased in the treated subjects and decreased in controls.

Beside positive results, certain negative facts were noticed in Great Britain [21, 22, 23]. Three experiments were conducted on a small number of subjects with severely impaired general condition. The treatment was applied neither with our product nor according to our method.

In our first investigations we used a procaine solution with pH = 4.2. Since 1957, the solution had been buffered at pH = 3—4, in order to obtain a higher stability. Our solution (Gerovital $H_3$) contains: procaine 2%, benzoic acid 0.12%, potassium metabisulphite 0.10%, dinatrium phosphate 0.01%. Potassium salt was added in order to intensify the effect on the nervous system and the heart.

Mention should be made of Holland's investigations (quoted by Giotti [24]) which pointed out the slowing down of potassium loss in a potassium-lacking environment subsequently to procaine administration.

Alfonskaia [25] noticed that potassium prolongs and intensifies procaine action.

The fact is also known that potassium ion potentiates acetylcholine activity; it is also known that procaine acts at the cellular level. The hypothesis may thus be advanced that the potassium ion facilitates procaine penetration into the cell.

The studies carried out by Benetato and coll. [26] pointed out the contribution of the researches on potassium mechanism of action to the cellular biophysics, particularly in the aged.

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The long-term treatment with Gerovital $H_3$ administered to aged subjects resulted in skin revitalization, hair growth stimulation, development of muscles, increased resistance to stress, quicker consolidation of fractures.

Mortality was 5% in the chronically treated subjects, as against 16% in controls. The anatomo-pathological evaluation of the deceased subjects [27] focussed on the analysis of cell division; the multiplication of certain myocardial fibres and nuclei against the atrophy of others was thus noticed. The authors suggest that this fact represents the reactivation of myocardial nuclei correlated with the metabolic process. Another morphological finding was the absence of sclerosis consecutive to parenchymatous atrophy in the liver, heart, brain and endocrine glands.

The experimental researches, conducted in collaboration with Al. Vrăbiescu, C. Domilescu and I. Nicea, consisting in the clipping of the sciatic muscle, pointed out both physiologically and morphologically the stimulation produced by Gerovital $H_3$ in the regeneration of the striated muscle. This effect is significantly stronger with Gerovital $H_3$ than with the common procaine solution. A study on the regeneration of the peripheral nerves pointed out the positive effect of Gerovital $H_3$ on the continuity of the sciatic nerve; it delays the occurrence and the progress of neurodystrophic processes and results in the regression of sural triceps muscle atrophy. The regression of the atrophy was pointed out in 71% of the Gerovital $H_3$ treated cases, in 26.6% of the controls and in 36% of the animals subjected to the procaine treatment.

Statistically speaking, the studies on nervous dystrophy (Speransky's method) and experimental fractures pointed out more significant effects of the drug when applied before the dystrophy or fracture, thus supporting its prophylactic utilisation.

The experimental studies pointed out procaine anabolic action noticed previously. In studies on Infusoria (Colpidium colpoda and Vorticella) we noticed the stimulated cell proliferation as a result of a weak procaine solution, this pointing to the anabolic action of the drug [28]. The investigations on rats drew the attention on procaine anabolic influence noticed in the animals' weight increase and improved quality of the hair [4]. In a study on 3-month-old rats, Berger [29, 30] obtained similar results with 6 mg/kg body weight procaine. A 5-month-prolongation of the life span was noticed.

On the other hand, Verzar [31] used 25 mg/kg body weight procaine (amount which inhibits the oxidoreduction) and did not notice any modification.

In order to solve the problem of these contradictory results we initiated [15] a study on 1800 white rats treated with Gerovital $H_3$ since the age of 3 months,

that is before the onset of aging. The results pointed out a 21% increase of the life-span in Gerovital $H_3$ treated animals; an improved general trophicity was also noticed, as well as an increased resistance to bronchial-pulmonary diseases. Less myocardial lesions were recorded on ECG (coronary irrigation disorders in 30% of the treated animals, as compared to 80% of the controls). Fewer spontaneous tumors occurred in the treated group as against the controls.

Histological examination of the treated animals revealed a less important connective invasion in the myocardium, the striated muscle, and less degenerative lesions in the renal tubule.

Konieczny [32] noticed the weight increase and the diminution of the nitrogen catabolism in the procaine treated rats. Nicolae and Dumitrescu investigated the influence of procaine therapy on the nitrogen metabolism in aged. The treatment resulted in the decreased nitrogen excretion, the improvement of the nitrogenous balance even in quite aged patients. These authors emphasized the importance of the above mentioned therapy in dystrophic states associated with proteic metabolic troubles.

In a previous study [34] we noticed the improvement of the restricted diffusion as a result of procaine administration; the intensified albumin supply was thus ensured. Procaine intervention in the carbohydrates metabolism was experimentally evidenced in our researches. The study on aloxanic diabetes in rats [35] pointed out procaine intervention in balancing metabolic disturbances as well as its action in preventing aloxanic aggression (insulin-like effect).

The investigations of isolated homeothermal and poikilothermal organs evidenced the stimulation of the vital processes produced by procaine. The studies were conducted by Teitel and coll. [36]. The authors noticed that low procaine amounts have a trophic and stimulating effect on the dorsal muscle of the leech prolonging its spontaneous activity and increasing the amplitude of the contractions. The researchers noticed procaine to be more sensitive to potassium action.

In other studies the emphasis was placed on procaine intervention in the intermediate metabolism, favouring ATP synthesis [37, 38]. This hypothesis was based on the discrepancy between the favourable trophic effect on one hand, and on the reduced $O_2$ consumption on the other. This finding seems to point out procaine similarity with the antioxidative substances. Fichez and Klotz also insist on the procaine antioxidative effect [39].

Other studies carried out on yeast [40] pointed out procaine action on the enzymes involved in oxidoreduction. Researches on liver homogenate pointed out procaine intervention on oxidative phosphorylation of glucidic metabolism [41, 42].

Analysing the relationship existing between aging and atheromatosis we realised that procaine effect on lipid metabolism required further investigation.

The studies on experimentally induced atheromatosis revealed the slowing down of the process in the animals simultaneously treated with Gerovital $H_3$ [43]. From a humoral standpoint the breaking down of lipoproteins shift to beta macromolecules was noticed. These results were confirmed by Tchernov [44]. Sidorovitch [45] noticed a similar action and suggested a possible synergism between procaine and female hormones.

The effect on atherosclerosis was investigated by Litovchenko and coll. [46] in 145 patients; the oscillometric evaluation subsequent to 2—4 series of procaine injections revealed a normal reaction to cold stimuli. The capillaroscopic investigation pointed out the acceleration of the blood flow subsequent to 3—5 series of injections. A similar effect on the capillaries was communicated also by Schulze.

Litovchenko emphasized that procaine influenced the neurotic and circulatory disturbances in atherosclerotic subjects [46].

Kurth's studies [47] on procaine action on the lipid metabolism recorded favourable results in atherosclerotic subjects in whom the function of the cell membrane was corrected. Also 3/5 of the arteriosclerotic dysproteinemias became normal as a result of procaine administration. The author also noticed the quicker serum clearing as well as the decrease of cholesterol levels.

In order to point out the antiatherogenous action of Gerovital $H_3$ we carried out a study on 25 subjects aged 72—90, subjected to procaine treatment for 4—11 years; an equal number of patients were used as controls. The author studied lipoproteinlipase activity *in vitro* as well as different fractions after both heparin injections and the activation of the endogenous lipoproteinlipase. Lipoproteinlipase activity reached the average values $13.01 \pm 2.01$ in the treated subjects, as against $8.75 \pm 1.77$ in the controls (the normal value is $15 \pm 1.6$).

An obvious dislocation of the lipoproteic fractions was noticed after the heparin injection, with the modification of the beta / alpha-lipoprotein gradient (80% in the treated subjects, as against 15% in the controls). The modifications of the coefficient beta / alpha specific to the post-heparin lipoproteins tallied with the lipoproteinlipase enzymatic activity *in vitro*.

The inference may be thus drawn that one of the important links in the atherosclerotic dyslipoidosis chain is also subjected to the biotrophic influence. These data may be correlated with the reduced number of thrombotic accidents in aged subjects treated with Gerovital $H_3$. These observations confirm the results of our previous studies [48] on Gerovital $H_3$ based prophylaxis of atherosclerosis.

Comparative studies on Gerovital $H_3$ and procaine action, carried out by Greppi and Scardigli [49] pointed out the higher efficiency of Gerovital $H_3$.

Gordon and coll. conducted comparative studies on Americane procaine and Gerovital $H_3$. The experiments on rats revealed higher procaine levels subsequent to intramuscular Gerovital $H_3$ injections as against American procaine.

The "rejuvenating" effect of Gerovital $H_3$ on the excretion of the urinary steroids and metabolites, as well as on the conduction speed of the stimuli to the peripheral nerves were pointed out in the double-blind comparative researches [50].

The above-mentioned authors carried out psychic and social studies on 60 patients aged 68—90, subjected to the treatment based on Gerovital $H_3$ and American procaine. The general and psychic conditions improved more with the Gerovital $H_3$ treated group [51].

Friedman [52] communicated the results obtained with the Gerovital $H_3$ treatment in mental disturbances (confusion of organic origin). The clinical alleviation of the senile symptoms present in 1/3 of the treated subjects was noticed. In the author's opinion, senile confusion may be correlated with the metabolic disturbances, which can be alleviated through a biochemical reaction.

The favourable influence upon the depressive psychical states of the aged was also noticed [37]. The antidepressive effect of procaine has also been pointed out by Bucci and Saunders [17], Siggelkow [53], Cambel [54] and other researchers.

Together with Parhon [3] we found an improvement of memory, attention and sleep in patients chronically treated with procaine.

Recently, a double-blind research, Zung and coll. [55] using placebo and imipramine, tried to evaluate the efficiency of Gerovital $H_3$ therapy in depressive troubles, on 30 elderly patients. The clinical observations and psychometrical tests performed

before and after the four week treatment pointed out the superiority of Gerovital $H_3$ over imipramine.

Recently published data [18, 19, 56] draw attention to the modification induced by aging and depressive states in the enzymatic activity of the nervous cell as well as to the intervention of procaine at this level. The increased MAO activity could play an important part in the biochemical modifications induced by aging and depressive states. As a matter of fact, depressive states have been correlated with the reduction of central amines [57], which is due, as recently shown, to the increase of monoaminoxidase.

All these data point to procaine action on the cell metabolism beside that on the cell membrane. They also show the stronger effect of Gerovital $H_3$ (potentiated procaine solution) at cellular level particularly on the central nervous system as against common procaine.

We should also emphasize Officer's research [58] which, based on Hayflick's observation on the limited life-span of culture cells, pointed out that Gerovital $H_3$ introduced during the second passage accelerates cell doubling rate and, after the division has ceased, provides a longer life-span in the treated as against the control cells; it also prevents the transformation of the cell into a permanent cell line.

Our studies carried out on secondary cellular cultures from monkey kidneys showed that the average cell postmitotic life span was 72.4 days in cultures treated with Gerovital $H_3$ having undergone 14 passages and 62.3 days in nontreated cultures having undergone 12 passages.

The ultrastructural study revealed the contribution of Gerovital $H_3$ to the stability of main cellular organelles during the cultivation of cercopithecus kidney cells. A significant increase in the number and size of autophagic vacuoles was detected in untreated, control cells. The cytoplasmic reticulum was constricted and empty with few polyribosomes and prominent vacuoles. Large cytoplasmic damage was seen.

The ultrastructural findings correlate with stimulation and increase of the metabolic activity in Gerovital $H_3$ treated cells.

Based on the clinical and experimental results of the research work in our Institute, we have started since 1954 the prophylaxis of aging based on eutrophic medication (Gerovital $H_3$).

During the last years, 144 gerontological centres have been organised in industrial and agricultural enterprises.

Under the supervision of the Institute of Geriatrics in Bucharest, the workers aged 45 to 60 were subjected to medical examination; the physicians and the local social workers also drew up the social anamnesis of the patients. The complex evaluation of the health and social condition of the population is associated with a series of functional tests based on which the subjects' biological state is assessed. Gerovital $H_3$ therapy was thus administered to a group of subjects, as against a control group. The evolution was recorded every 6 months. (Prophylactic therapy: 5 series each including 12 Gerovital $H_3$ injections — 5 ml — annually, with one-month break between the series). The improvement of the psychic condition and work capacity was noticed in 70% of the cases; the appetite as well as the body weight increased; morbidity figures dropped and the resistance to stress increased.

Beside the anaesthetic effect, procaine also acts as an eutrophic agent in the process of aging. The clinical and experimental investigations pointed out this prophylactic efficacy. According to Soehring [59] the anaesthetic effect is but a small part of the procaine general effect. Both as an intact molecule and through

DEAE, PABA and the folic acid it intervenes in the metabolic regulation. It favours acetylcholine action and prevents epinephrine oxidation. According to Laborit and coll. [60] the substances having this type of action play an important part in the cellular reactivation.

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In order to intensify Gerovital $H_3$ lipotropic action, as well as that on the nervous system, a lipotropic factor and another one with stimulating effect on the neurons were added to the basic formula. The new product, called Aslavital, was studied 10 years both experimentally and clinically.

Since 1961, the Aslavital ampoules and dragées have been administered to 182 patients hospitalized at the Institute of Geriatrics and 1230 outpatients. The effects of the treatment were studied comparatively with a group of patients that were not subjected to treatment. The patients were examined clinically, physiologically and by Xrays; functional, biochemical and hematological investigations were also carried out.

The clinical and laboratory data pointed out improvements in the general biological condition, tissue revitalization, the slowing down of the involutive process, the alleviation of the chronic degenerative diseases and the prevention of the complications they might induce. 50%—80% of the cases with predominantly cerebral aging, particularly in initial stages, improved. Physical and psychic asthenia was alleviated in 82% of the cases, the intellectual over-exertion (disturbed memory, concentration and attention) in 67% of the cases, depressive state in 86% of the cases and anxiety in 56% of the cases.

The EEG tracings pointed out the increased amplitude of the waves, a better modulation and frequency of the alpha rhythm in 72% of the cases; the frequency of the slow elements decreased in 22% of the cases and remained unchanged in 30.5%.

Favourable results were obtained in cases of atherosclerosis, either generalized or cerebral, coronary or peripheral as well as in the prevention and control of thromboembolic accidents and posthemorrhagic sequelae.

The results of the clinical and functional investigations pointed out the normalization and the stabilization of the blood pressure, the improvement of hemodynamics in 63% of the cases and a better vascular reaction in all the subjects that presented deviations from the normal values.

The EKG pointed out the improvement in the S-T sequence and T wave in subjects with coronary pathology.

The improvement or the correction of global plasma hypercoagulability, hypofibrinolysis, thrombocytic hyperfunctionality, endogenous hypoheparinemia and hyperlipoproteinemia in the treated atherosclerotic aged patients, also pointed out the antiatherogenic therapeutical properties of Aslavital.

Positive results have also been obtained on sugar, lipid, cholesterol and beta-lipoprotein levels.

Important improvements have been noticed in the psychosomatically deficient children, as well as in those with postencephalomyelitic sequelae.

We could not include in this paper all the studies recently conducted on Gerovital $H_3$ therapy in old age and the prevention of premature aging. Those data were mentioned which are the theoretical basis of procaine therapy and emphasize its action on the central nervous system and the regulation of the metabolic processes.

**Résumé.** La baisse du nombre et de la capacité fonctionnelle des cellules au cours du vieillissement induit de nombreuses caractéristiques du vieillissement. En ce qui concerne les neurones, le principal objectif est le prolongement des ressources cellulaires, fait rendu possible grâce à la psychopharmacologie. L'auteur a démontré, il y a 25 ans déjà, l'influence de la procaïne sur le système nerveux par des investigations psychologiques et fonctionnelles, cliniques et expérimentales. Récemment la pharmacologie de la procaïne s'est développée grâce aux recherches de certains auteurs américains, parmi lesquelles des études double-blind aussi, sur l'effet favorable du Gérovital $H_3$ dans les états dépressifs des vieillards. On a démontré la relation entre le phénomène de vieillissement et l'intensification de l'activité de la monoamineoxydase dans le cerveau, ainsi que la capacité de la procaïne et spécialement du Gérovital $H_3$ d'inhiber la MAO et de réduire les signes de vieillissement au niveau du système nerveux central. L'action anabolique de la procaïne a été démontrée par des recherches sur des cultures de cellules, mais sur des animaux aussi, en constatant parmi autres, l'amélioration de leur état trophique général et la prolongation de leur durée de vie. Autres résultats obtenus dans le domaine des mécanismes d'action de la procaïne se réfèrent à: l'amélioration du bilan azoté, la favorisation de la synthèse de l'ATP, l'action anti-oxydante, l'intervention dans la phosphorylation oxydative, dans les troubles du métabolisme lipidique de l'athérosclérose, dans la croissance de la vitesse de conduction nerveuse, l'excrétion de stéroïdes et métabolites urinaires, etc. Les études ultrastructurelles ont relevé la contribution du Gérovital $H_3$ à la stabilisation des membranes et des principaux organites cellulaires, l'intensification de l'activité métabolique des cellules des cultures traitées. Tous ces faits constituent la base d'action de la prophylaxie et de la thérapie du phénomène de vieillissement. Afin d'intensifier l'action lipotrope, ainsi que celle agissant sur le vieillissement cérébral, l'auteur a élaboré un nouveau produit, l'Aslavital. Les résultats obtenus les 9 premières années d'application de ce traitement attestent son efficience dans les processus involutifs, à prédominance cérébrale et dans l'athérosclérose (surtout dans les troubles de coagulabilité sanguine et du métabolisme lipidique), ainsi que dans la prévention ou le traitement des complications.

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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.

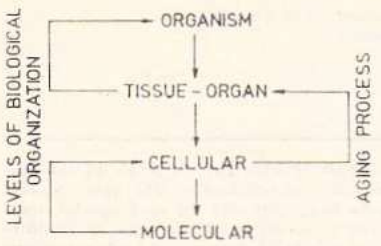


Fig. 1. — Levels of biological organization.

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.

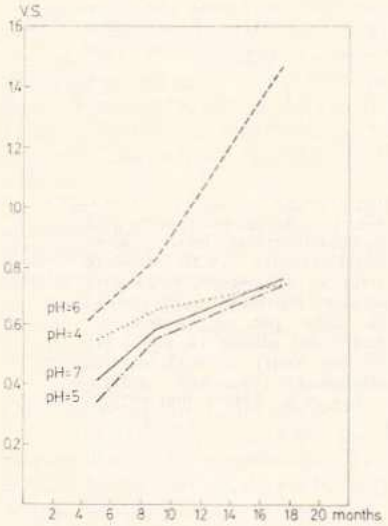


Fig. 2. — Specific viscosity of collagen solutions.

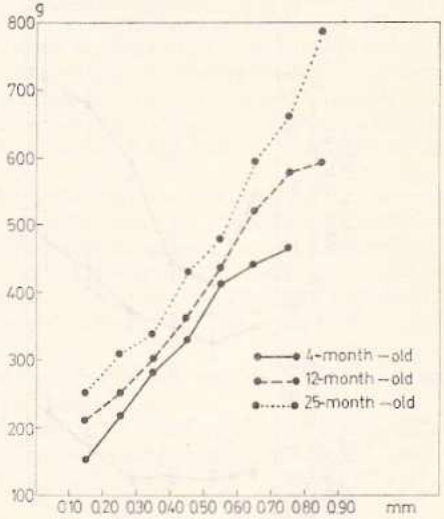


Fig. 3. — Mechanic resistance of tendon fibres in relation to their diametre and the age of the animals.

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.

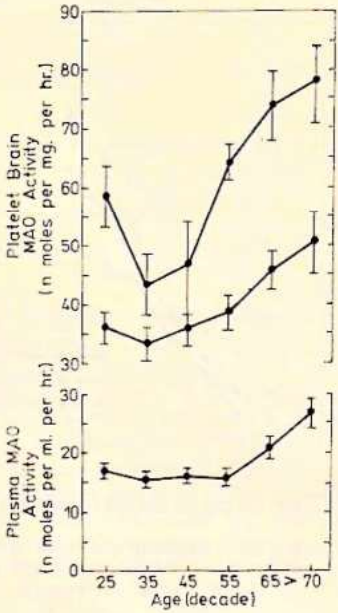


Fig. 4. — Aging, monoamines and monoaminoxidase levels. Mean MAO activity (with standard error of the mean S.E.M.) of human hindbrain and platelet (n moles per mg protein per hour) and plasma (n moles per ml per hour) for each decade. (Robinson, D.S., and co-work, Lancet No. 7745, p. 290, 1972)

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.

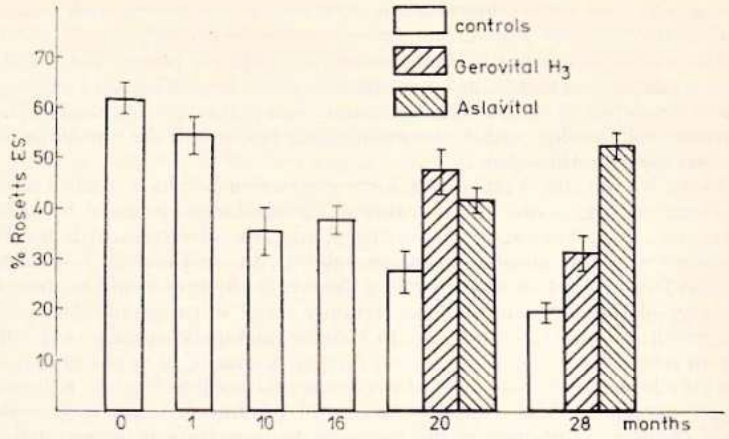


Fig. 5. — Variation of Fc receptor carrier rat splenocyte percentage in relation to age. Influence of Gerovital $H_3$ and Aslavital treatment.

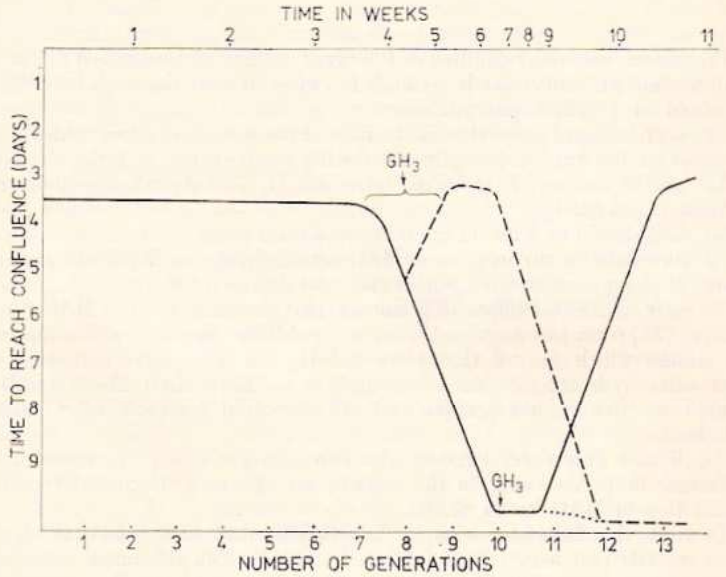


Fig. 6. — Wild mouse embryo cell cultures subcultured when confluent. Gerovital, ($GH_3$), added at the 8th or 9th generation, renewed cell proliferation, and continued for an additional 2 generations. $GH_3$ added to cells in the stationary phase maintained the cells in that condition and did not spontaneously 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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# THE LONGITUDINAL OUT-PATIENT TREATMENT WITH GEROVITAL $H_3$

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**Summary.** The results are presented of the therapy with Gerovital $H_3$ administered to out-patients by the gerontological centre of the National Institute of Gerontology and Geriatrics.

The effects of the treatment, based on the indicators of the functional capacity of some apparatus and systems (dynamometric indices, ventilation tests, Schneider test, stature and weight indicators a.s.o.) were observed in different groups of subjects under treatment for 3 to 5, 6 to 10 and over 11 years.

The observation of the patients under common life and working conditions allowed us to make an accurate evaluation of the resistance to stresses, work efficiency, resistance to intercurrent diseases.

The statistic evaluation of the data and their dynamic evolution allowed a longitudinal representation of the efficiency of the biotrophic therapy with each and every case, as well as the global estimation of the effect in relation to the duration of the treatment.

The positive results confirm the efficacy of Gerovital $H_3$ treatment; the longer the treatment and the earlier its setting up, the more conclusive the data.

Recent researches brought about new proofs of the beneficent effects of the Gerovital H3 treatment. The positive results point out the metabolic implications in the regulation of certain mechanisms involving the biochemical components [1].

The researches also pointed out the positive influence upon interoceptors, glucide, lipid and protein metabolism, as well as an improved oxidative balance.

These multiple implications account for the anabolic and eutrophic effects, the maintenance and stimulation of regeneration, the positive plastic, metabolic and tissular changes [2].

The double-blind studies [3], pointed out the efficiency of the Romanian product, the procaine levels subsequent to Gerovital H3 administration were much higher — the elimination of metabolites was superior as against plain procaine, the nervous influx conduction speed in the peripheral nerves increased. The metabolic role and the antidepressive effect of the biotrophic products, administered according to Prof. Dr. Aslan's method were also evidenced [4].

The present paper aims at evaluating the efficacy of the Gerovital H3 treatment longitudinally administered, on certain somatometrical and functional parameters. The dynamics of these parameters was particularly assessed under the long-term administration of Gerovital H3.

## MATERIAL AND METHOD

A group of Gerovital H3 treated patients were subjected to the study. The treatment, administered at the Polyclinic of the National Institute of Gerontology and Geriatrics consists in 4 annual series of 12 ampoules each, 3 per week, adminis-

tered to out-patients. The subjects were divided into 3 subgroups, as shown by Table 1:

*a)* persons subjected to the treatment for at least 3 years (the group 3—5 years) — 36 women, 26 men;

*b)* persons subjected to the treatment for at least 5 years (the group 6—10 years) — 38 women, 32 men;

*c)* persons subjected to the treatment for at least 10 years (the group over 11 years) — 16 women, 12 men.

*Table 1*

**Distribution of subjects by duration of treatment, age and sex groups**

| | | Women | Men |
|---|---|---|---|
| | | absolute figures | |
| 3—5 years | 40—59 years | 23 | 2 |
| | 60—69 years | 32 | 37 |
| | 70+ years | 21 | 27 |
| Total | | 76 | 66 |
| 6—10 years | 40—59 years | 19 | 2 |
| | 60—69 years | 28 | 33 |
| | 70+ years | 21 | 27 |
| Total | | 68 | 62 |
| 11+ years | 40—59 years | 9 | 2 |
| | 60—69 years | 21 | 19 |
| | 70+ years | 16 | 21 |
| Total | | 46 | 42 |

Table 1 also shows the distribution of subjects by age and sex.

The parameters under study:

*a)* somatometric: — weight
— height
— height in sitting position
— thoracic perimeter both in inspiration and expiration;

*b)* functional: — dynamometry
— Broca index
— induced apnoea test
— Schneider test
— arterial pressure

## RESULTS AND DISCUSSION

Among the parameters studied, particular attention was paid to dynamometry, arterial pressure and weight.

*Dynamometry.* The positive effect of the Gerovital H3 treatment was easily noticed (Diagram 1). For the age groups 40—59 and 60—69 years, the effect is represented by an annual ascending curve even since the end of the first year;

it continues until the 6th year for the 5th and 6th age decades and until the 6th year for the 8th age decade. With the subjects under treatment for over 10 years, the dynamometric values were constantly superior to the initial ones. With the subjects in the 8th decade and over, the line remained at the same level, fact which is also significant. Comparatively, by age decades, the improvement is evident during the first years of treatment with the younger ages. The positive effect was stronger in women as against men in the 8th decade and over.

*Arterial pressure*. Diagram 2 points out clearly the constant effect upon the mean arterial pressure values. A slight tendency of the values to increase without reaching the pathological limit was noticed with all the age-decades, as a result of the biotrophic treatment; the mean values remained within the limits of physiological involution. The values of the arterial pressure did not exceed the figure which requires the use of a major hypotensive agent when risk factors with a certain negative effect were present before initiating the treatment and subsequently continued to act.

No coronary and cerebral accidents, either minor or major, occurred in the studied persons.

*Body weight* (Diagram 1). Values improved particularly in the slightly underweight persons. The highest positive values, exceeding the average values throughout the country were noticed with the age groups in the 5th and 6th decades. Over the age of 60, the results were not conclusive during the first 2 years; starting with the 3rd up to the 8th year of treatment, the weight gain was remarkable, particularly in men.

The age-group in the 8th decade and over benefitted by the treatment, particularly in the interval of 60—80 years.

*Vital capacity, thoracic perimeter, Schneider test* (diagrams 2, 3). Although appearing as slight deviations from the straight line they pointed out the efficacy upon certain mechanisms and processes which are usually constantly descending.

Mention should also be made of the following facts:

— Osteoarticular allergic phenomena either improved or were totally controlled in 80—85% of the cases. The uninfluenced cases did not worsen.

— Seasonal osteoarticular painful recurrences were controlled, the psychic condition and sleep improved, the resistance toward seasonal intercurrent diseases increased.

The number of sick-leave days subsequent to the administration of the treatment decreased particularly in subjects aged 40—59 (23 women and 2 men, most of them employees — 19).

Among the laboratory investigations, the EKG revealed no important modification even with the most advanced age-groups.

The cholesterol level tended to become normal in the subjects under treatment for 3—5 years. In the other age-groups, cholesterol levels ranged between the admitted limits (160—260 gr%) with no tendency of the previous values to increase.

## CONCLUSIONS

1. The long-term treatment with Gerovital H3 had a positive influence on the functional and somatometric parameters in all the subjects studied.

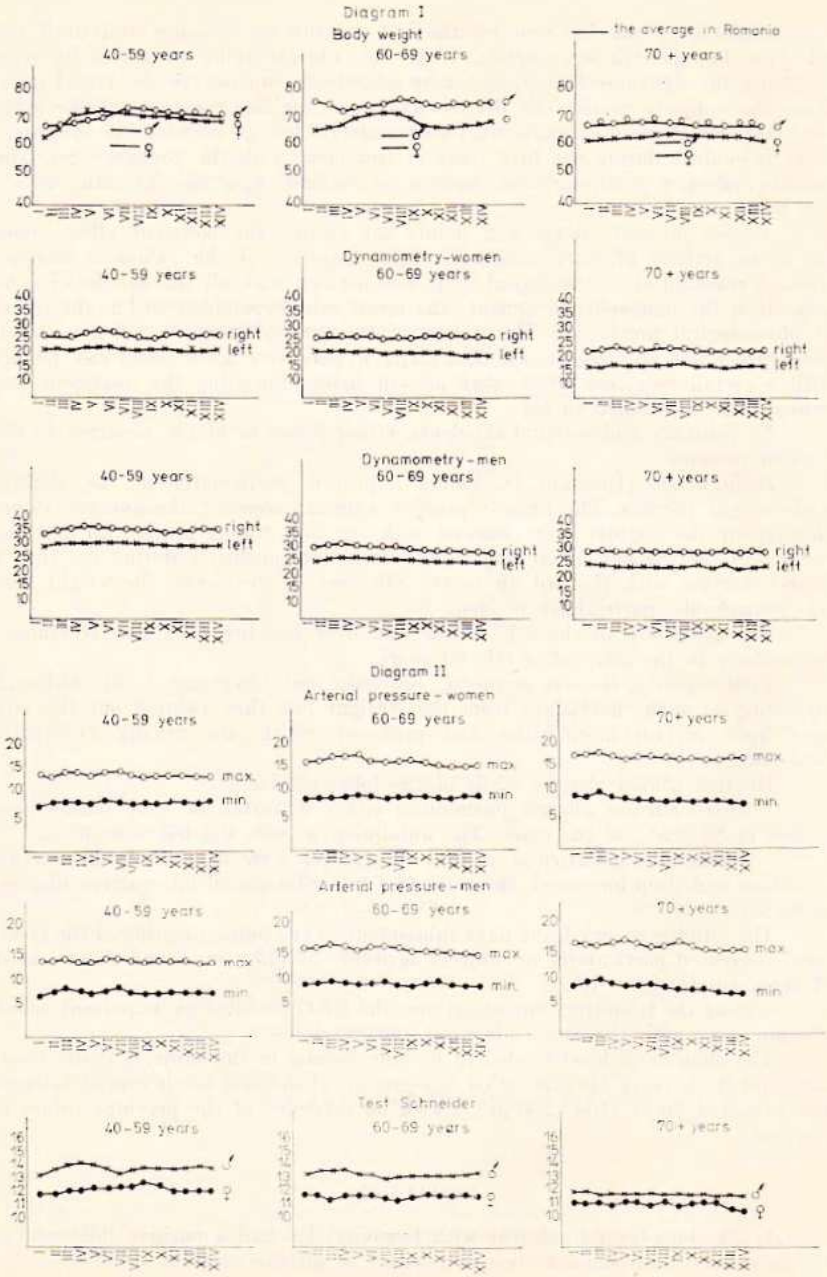
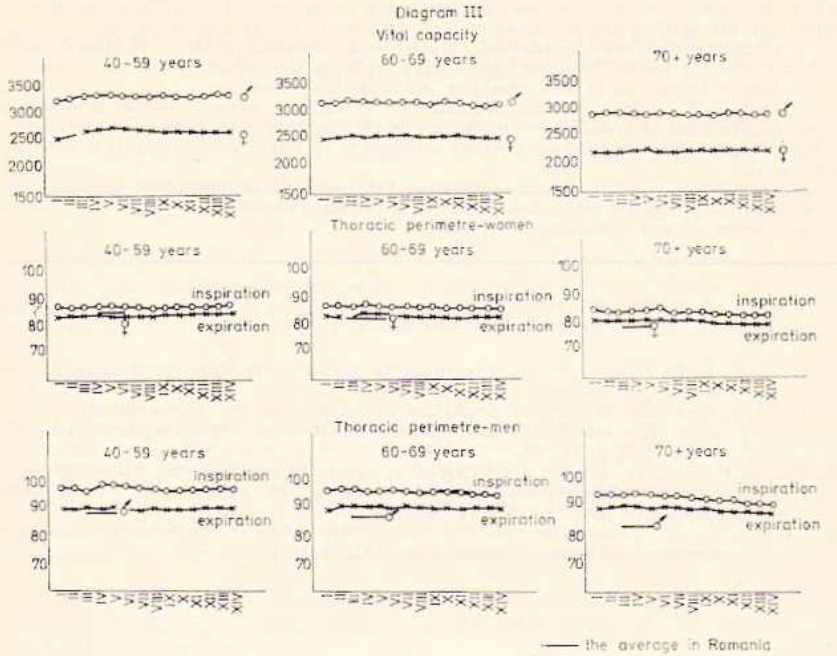


Diagram I
Body weight
the average in Romania
40-59 years
60-69 years
70 + years
Dynamometry-women
Dynamometry-men
right
left
Diagram II
Arterial pressure-women
Arterial pressure-men
max.
min.
Test Schneider




2. The efficacy is remarkable from the first year of treatment; it follows an ascending curve and maintains itself at the highest level during the second until the sixth year of treatment, after which it keeps constantly above the initial values.

3. The dynamometry is a parameter which points out the efficacy of Gerovital H3 in the 5th — 6th age-decades.

4. Gerovital H3 proved to be one of the chemiotherapeutical products with a complex influence on the mechanisms involved in the regulation of the arterial pressure; our results indicate that Gerovital H3 can be prophylactically used for maintaining the physiological involution.

5. The major weight gain is constantly distributed by age-decades, the highly underweight subjects' gain in weight taking place particularly from the second until the eighth year of treatment.

6. According to the results of our study, the improvement of the preservation of functional and somatometric parameters requires the long-term administration of Gerovital H3 treatment.

7. The out-patient treatment is widely accepted since the patient is not obliged to remain in the hospital. The results of the out-patient treatment are as good as those obtained with hospitalized patients.

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**Résumé.** On présente certains résultats obtenus par la thérapie au Gérovital $H_3$ dans les conditions ambulatoires au centre gérontologique de l'I.N.G.G.

Les effets du traitement évalués à partir d'indicateurs de la capacité fonctionnelle de certains appareils et systèmes (dynamométrie, épreuves de ventilation, épreuve de Schneider,

indices de hauteur et de poids, etc.) ont été suivis de manière différentielle sur groupes de sujets qui ont suivi le traitement entre 3—5 ans, 6—10 ans et plus de 11 ans.

L'observation directe des cas dans les conditions habituelles de vie et de travail nous a donné aussi la possibilité d'une appréciation réelle de la résistance au stress, du rendement dans le travail, de la résistance aux maladies intercurrentes.

Le travail statistique des données dans leur évolution dynamique permet la représentation longitudinale de la thérapie biotrophique de chaque cas à part, mais aussi l'estimation globale du phénomène du groupe de sujets soumis à la recherche en fonction de la durée du traitement.

Les résultats positifs obtenus confirment l'efficience du traitement au Gérovital $H_3$, les données étant d'autant plus concluantes, que la période d'administration a été plus longue et le moment de son initiation plus précoce.

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# RESEARCHES ON THE IMMUNE REACTIVITY OF THE ORGANISM UNDER THE ACTION OF GEROVITAL $H_3$

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**Summary.** Previous observations made by Ana Aslan during a severe influenza epidemic at the home of the Institute, as well as other researches conducted on larger numbers of subjects have pointed out the increased resistance to intercurrent diseases of Gerovital $H_3$ treated subjects, as against untreated ones.

The increased resistance to acute and chronic infections has been noticed also in researches on treated rats, in which a 18–21% prolongation of the life span was possible.

The subsequent investigations have been focussed on the mechanisms by which Gerovital $H_3$ stimulates the defense reactions of the organism.

Aslan, Bălan and coworkers studied endocytosis in treated rat peritoneal histiomacrophages *in vitro*. Colloidopexic and phagocytic indexes increased by 11.9–20.2%, as compared to controls.

Other researches pointed out a 16% increase in human leukocyte phagocytosis as against inactivated *Staphylococcus aureus* and *Saccharomyces cerevisial* strains. In treated rats, the phagocytic capacity to these germs was by 16.8–18.2% higher than in untreated ones.

Emphasis is laid on the role of catecholamine in the stimulation of phagocytosis; catecholamine levels increase as a result of monoaminoxidase inhibition induced by Gerovital $H_3$.

Other researches envisaged the ability of lymphocyte Fc receptors to bind immunoglobulins. In collaboration with Ghetie and Manciulea we noticed the percentage of Fc receptor carrier lymphocytes to increase by 48% in treated old rats, as against 13% in controls.

These results were correlated with the researches on the action of Gerovital $H_3$ in maintaining low autoantibody levels in aged subjects.

A series of researches have pointed out the stimulating activity of procaine and Gerovital H3 on the defence reactions of the organism against infectious diseases.

A. Aslan and coll. [1] have made the first observations and have found out that, during a severe epidemic of influenza that occurred in the home for elderly people in 1959, mortality was 2.7% in the group treated with Gerovital H3 and 13.9% in the nontreated group.

From this point of view very interesting are the researches of Enăchescu and David [2] pointing out a significant increase in the isoautoantibodies as well as the increase in the percentage of complement in patients actively immunised with influenza antigens, and treated with Gerovital H3.

Investigations made by Aslan and Vrăbiescu [3] on 1800 rats showed a 18–20% increase in the life span of treated rats, as well as a higher resistance to morbidity and mortality by chronic infections and degenerative diseases.

Morbidity and mortality occurred much later in treated subjects in comparison with controls.

Rascova and coll. [4] have demonstrated that repeated injections with procaine produce an increased resistance of mice to the toxins of *Shigella Shigae* susceptible to be maintained by periodical injections.

*Table 1*

**Percentage values regarding the phagocytic index of histiomacrophages treated *in vitro* with Gerovital H3 in different concentrations**

| Variants \ Experiment | 1 | 2 | 3 | 4 | 5 | 6 | Average | Percentage difference as compared to the controls | Statistical significance |
|---|---|---|---|---|---|---|---|---|---|
| Controls | 62.8 | 59.0 | 54.0 | 64.0 | 60.0 | 58.5 | 59.7±1.44 | – | p |
| Gerovital $H_3$ $10^{-2}$ | 49.9 | 41.8 | 50.0 | 46.0 | 45.1 | 42.7 | 45.9±1.43 | 13.8 | <0.01 |
| Gerovital $H_3$ $10^{-3}$ | 59.9 | 67.3 | 76.8 | 78.9 | 79.0 | 62.0 | 70.6±3.14 | 10.9 | <0.05 |
| Gerovital $H_3$ $10^{-4}$ | 72.7 | 66.0 | 79.0 | 72.2 | 75.0 | 59.8 | 70.5±2.79 | 10.8 | <0.02 |
| Gerovital $H_3$ $10^{-5}$ | 73.1 | 59.3 | 69.5 | 72.3 | 71.0 | 58.7 | 67.3±2.67 | 7.6 | <0.05 |

*Table 2*

**Percentage of the phagocytic index of the histiomacrophages, obtained from rats treated with Gerovital H3**

| Variants \ Experiment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Average | Percentage difference as compared to the controls | Statistical significance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls | 58.0 | 45.0 | 45.2 | 38 | 55.2 | 60.7 | 46.8 | 59.6 | 44.9 | 51.0 | 44.3 | 49.9 | 49.8±2.6 | – | – |
| Gerovital $H_3$ | 72.0 | 59.0 | 73.0 | 58 | 74.0 | 59.0 | 69.0 | 76.0 | 55.0 | 49.0 | 54.0 | 75.0 | 64.5±2.79 | 14.4 | p <0.01 |

Pop and coll. [5] found an increase in serum gammaglobulins in dogs as a result of procaine treatment.

On the basis of these observations we tried to elucidate the mechanism by which Gerovital $H_3$ increases the defence mechanisms of the cell.

The endocytosis of histio-macrophages of the peritoneus was studied by Aslan, Bălan and coll. [6] in cell cultures treated with Gerovital $H_3$.

Peritoneal histiomacrophages were submitted to this research. They were obtained from six-month-old male Wistar rats. The colloidopexic activity was evaluated by the capacity of these cells to incorporate particles of China ink and by the capacity to phagocytose *Mycobacterium muris*.

Two experimental models were used: cultures treated *in vitro* with Gerovital $H_3$ in concentration of $10^{-2} - 10^{-5}$ and cells from animals treated with Gerovital $H_3$ in doses of 4 mg/kg body weight during 4 months.

The results obtained pointed out the increase by 20.2% in the colloidopexic index of the histiomacrophages treated *in vitro* with Gerovital $H_3$, in concentration of $10^{-2}$. The same index records an increase by 11.9% in animals treated *in vivo* with Gerovital $H_3$.

The increase in phagocytic index with 13.8% of the histiomacrophages treated *in vitro* with Gerovital $H_3$ in concentrations of $10^{-2}$ was observed. In treated animals the index increased by 14.4% in comparison with controls (Tables 1 and 2).

In other researches we have followed the phagocytic capacity of human leukocytes, in subjects submitted to a long treatment with Gerovital $H_3$.

One group of 20 subjects, 70 to 85 years old, treated during 5 years with Gerovital $H_3$ and another group of 20 subjects were investigated as control.

Leukocytes were obtained by venous puncture, then put on slides and the phagocytic capacity tested with inactivated *Staphylococcus aureus* and *Saccharomyces cerevisiae*.

The average phagocytic index for *Staphylococcus aureus* was 51.7 in control leucocytes and 68.4 in those treated with Gerovital H3; that means a 16.7% increase (Table 3).

In the researches on *Saccharomyces cerevisiae* the phagocytic index was 43.1 in controls and 61.3 in the treated group, the increase being 16.2%.

In the case of *Staphylococcus aureus*, rat leukocytes phagocytic index was 33.1% in controls and 71.3 in the treated group, with a 18.2% increase (Table 4); with *Saccharomyces cerevisiae* phagocytic index the values obtained were 48.7 in controls and 63.6 in the treated group, the increase being 16.8%.

These results are in agreement with the results of other authors who have studied leukocytes phagocytosis after procaine administration. Bakos [7] has found that procaine stimulated the leukocytic phagocytosis of *Salmonella typhi* in man and dog. Vilardo [8] finds an increase in phagocytosis in rabbits subsequent to procaine administration in parallel to the increase in serum complement.

The stimulation induced by Gerovital H3 in the activity of phagocytic cells could be at least partially explained by the inhibition of the MAO activity, so that the blood level of catecholamine increases. Other authors such as Baciu [9] have already shown that catecholamine increases the phagocytic activity.

Hrachovek [10] has recently demonstrated that Gerovital $H_3$ is a stronger MAO inhibitor than procaine.

Mac Farlane [11] has also found significant differences between Gerovital H3 and procaine.

*Table 3*

**Percentage of the phagocytic index of human leucocytes**

| Variants | Number samples | Average | Diff. % | p |
|---|---|---|---|---|
| A. Germs susceptible of phagocytosis: *Staphylococcus aureus* | | | | |
| Controls | 20 | 51.7±1.22 | — | — |
| Treated with Gerovital $H_3$ | 20 | 68.4±1.47 | 16.7 | < 0.01 |
| B. Germs susceptible of phagocytosis: *Saccharomyces cerevisiae* | | | | |
| Controls | 20 | 45.1±1.06 | — | — |
| Treated with Gerovital H3 | 20 | 61.3±1.61 | 16.2 | < 0.01 |

*Table 4*

**Percentage of phagocytic index of rat leucocytes**

| Variants | Number samples | Average | Diff. % | p |
|---|---|---|---|---|
| A. Germs susceptible of phagocytosis: *Staphylococcus aureus* | | | | |
| Controls | 15 | 53.1±0.84 | — | — |
| Treated with Gerovital $H_3$ | 15 | 73.3±1.10 | 18.2 | < 0.01 |
| B. Germs susceptible of phagocytosis: *Saccharomyces cerevisiae* | | | | |
| Controls | 15 | 48.7±1.26 | — | — |
| Treated with Gerovital $H_3$ | 15 | 65.5±1.37 | 16.8 | 0.01 |

Our research was focussed on the possibility of pointing out Gerovital H3 influence on the capacity of the lymphoid Fc receptors to bind immunoglobulins because of their importance in the mechanisms involved in mediated immune responses.

With Gheţie and Maneiulea [12] a research was carried out in spleen lymphocytes of Wistar rats treated since the age of two months with i.m. Gerovital H3 injections in amounts of 4 mg/kg body weight, series of 12 injections in 4 weeks, with a break of two weeks between the series.

The percentage of Fc receptor-bearing lymphocytes increased by 44% in 16-month-old male rats treated with Gerovital H3 in comparison with the controls in which the percentage was only 20%.

In 20-month-old female rats treated with Gerovital $H_3$ the percentage of Fc receptor carrier lymphocytes was 43% in comparison with 21% in controls. At the age of 28 months the percentages were 48% in the treated group and 13% in the control one.

Our results showed that the treatment with Gerovital $H_3$ maintained the lymphocytic reactivity involved in the cell mediated immune responses.

According to these data the assumption can be advanced that the long term treatment with Gerovital $H_3$ decreases the deterioration of membrane structures, susceptible to become antigenic producers of autoantibodies.

These data could also account for the low level of autoantibodies in subjects treated with Gerovital $H_3$, in comparison with the controls.

Thus, Gerovital $H_3$ acts as a factor susceptible to prevent autoaggression in aged subjects.

## CONCLUSIONS

The defence capacity of the organism increases under the influence of Gerovital H3, fact demonstrated by the stimulation of the endocytic capacity of peritoneal histiomacrophages and the phagocytic ability of leukocytes in man and animals.

The favourable influence of Gerovital H3 was also pointed out by the rat spleen lymphocytes immunoglobulin binding capacity.

The effect of Gerovital H3 on the maintenance of low incidence of autoantibodies was evidenced in elderly subjects.

**Résumé.** Des observations antérieures effectuées par Ana Aslan, pendant une épidémie sévère de grippe dans le dispensaire de l'Institut, ainsi que d'autres recherches ayant des groupes plus nombreux de sujets, ont mis en évidence la résistance accrue envers les maladies intercurrentes des sujets traités au Gérovital $H_3$ en comparaison des sujets non traités.

On a aussi observé la résistance accrue envers les infections aiguës et chroniques dans les recherches effectuées sur les rats; la durée moyenne de vie des animaux traités a été prolongée de 18—21%.

Les investigations ultérieurement effectuées s'efforcent à élucider les modalités par lesquelles le Gérovital $H_3$ stimule les mécanismes de défense de l'organisme.

Aslan, Bălan et les coll. ont étudié l'endocytose des histiomacrophages péritonéaux traités *in vitro* ou provenus des rats traités. L'indice colloïdopexique et de phagocytose a augmenté de 11,9—20,2% en comparaison des témoins.

On a constaté, dans d'autres recherches, une augmentation de 16% de la capacité de phagocytose des leucocytes humains par comparaison aux souches inactivées de *Staphylococcus aureus* et *Saccharomyces cerevisiae*. La capacité de phagocytose chez les rats traités a été de 16,8—18,2% plus grande par rapport aux rats non traités.

Il est à remarquer le rôle des catécholamines dans la stimulation de la capacité de phagocytose, leur niveau croissant comme conséquence de l'inhibition de la monoaminoxydase par le Gérovital $H_3$.

D'autres recherches ont porté sur les capacités des récepteurs Fc lymphocytaires de joindre les immunoglobulines.

On a constaté, avec la collaboration de Gheție et Manciulea, que le pourcentage de lymphocytes porteurs des récepteurs Fc a augmenté de 48% chez les rats âgés traités, par rapport à 13% chez les témoins.

Ces résultats sont en corrélation avec les recherches concernant l'activité du Gérovital $H_3$ de conserver une incidence diminuée des autoanticorps chez les sujets âgés.

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# RESEARCHES ON MONKEY RENAL CELLS TREATED *IN VITRO* WITH GEROVITAL $H_3$

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**Summary.** The behaviour of monkey renal cells (Rhesus) in long-term cultures was studied, having as indicators: cellular proliferation and viability, aspects connected with cellular aging (vacuolisation, pycnosis, cytolysis, fat loading, acid phosphatase and esterase activity) as well as cells postmitotic life-span.

Researches were made in control cultures and cultures treated with Gerovital $H_3$ (0.4 ml% concentration).

The obtained data point out that Gerovital $H_3$ activates cellular proliferation and delays aging signs with the reduction in acid phosphatase and esterase activity.

The postmitotic life-span of the cells treated with Gerovital $H_3$ during 72.4 days was 10.1 days longer (16%) than that of the nontreated cultures (62.3 days). The number of passages as well as the cellular adhesiveness increased within the treated cultures.

The obtained results are correlated with the known data regarding the action of this biotrophic substance on the cellular metabolism.

Many experimental pharmacological researches concerning the mechanism of Gerovital H3 action, have pointed out the intervention of this substance at the level of biochemical cellular activities.

Aslan and coll. [1] have ascertained the stimulation of DNA synthesis induced by Gerovital H3. They used tritiated thymidine in studies on monkey renal cells.

The intensified synthesis of DNA induced by Gerovital H3 has also been evidenced by Rusu and Naum [2] in studies on hepatic regeneration in old rats.

Other researches at cellular level have pointed out that the activation of oxidative phosphorylation processes influence the carbohydrate metabolism at mitochondrial level as well as the increase of oxygen demand [3, 4, 5].

Consecutive to Robinson's data [6], which pointed out that the monoaminoxidase activity increases with aging, researches of Hrachovec [7], MacFarlane [8, 9], Yau [10] certify that Gerovital H3 has a stronger inhibiting effect on MAO than hydrochloric procaine [11].

Another characteristic of Gerovital H3 action on cells is the effect of procaine in restoring and maintaining the cellular membrane physiological potential.

In this respect we had in view the proliferation, postmitotic life-span and the aging of renal cells under the action of Gerovital H3 treatment.

## MATERIAL AND METHOD

The researches were made in monkey cells (Rhesus)* obtained by the disintegration of the renal tissue in trypsin solution 0.25%. The cultures were made in tubes 180/18 and in Kolle plates both with and without lamellae.

* Cellular suspension prepared at the Cantacuzino Institute, Bucharest, Romania.

As culture media we used: I.C. 65+2% calf serum for the initial culture and DP + 1% calf serum for the successive cultures. Gerovital H3 was added to these media in a final concentration of 0.4 ml/100 ml tissue culture medium.

The cellular density in the culture medium was $1.5 \times 10^4$ in tubes and $2 \times 10^6$ on the Kolle plates.

The temperature of culture incubation was 37°C.

The researches were focussed on the following aspects:

— Cellular proliferation and viability. In the logarithmic stage of cellular increase, the density and viability of the cells were studied by countings in the hemocytometre, using as dilution liquid 1% eosin solution. Countings were made at 2, 3, 4, 5 and 7 days' interval in 6 tubes for each control and treated groups.

Cell viability was calculated after the relation between the total number of cells and the number of dead cells, according to Paul John's formula* [12].

— Postmitotic life-span of the cells was determined in accordance with the cellular adhesiveness and the number of passages undergone by cells.

Cellular adhesiveness was studied by observing directly in the microscope the cellular monolayer on the culture vessels' walls.

— The aging of the cells was observed in the microscope after staining the monolayer with violet crystal and Giemsa. The indicators were: cytoplasmatic vacuolisation, pycnosis and cellular cytolysis.

— The cytochemical aspects were studied on coverships with adherent cells from culture vessels after staining with Scharlach [13], Nile blue [14] for lipid material, or treated in accordance with Burstone's method [15, 16] for the acid phosphatase and esterase.

The enzymatic activity was evaluated by positivity scores, after examining 100 cells from each culture separated in 4 groups according to the different degrees of activity. On the basis of the obtained values, the enzymatic positivity percentage was simultaneously determined.

The researches were conducted on control cultures and cultures treated with Gerovital H3. 300 cultures were prepared for each group within 10 series of experiments.

## RESULTS

The average cellular density varied, depending on the age of the culture, between 218.9 cells/ml and 712.5 cells/ml in the group treated with Gerovital H3 and between 163.5 and 480.3 cells/ml in the control group. The data point out the intensification of cellular proliferation in the treated cultures (Fig. 1).

The maximum density is achieved on the fourth day in both groups.

The differences are statistically significant (Table 1, Fig. 2).

Cellular viability percentage = 85.1—88.4 in treated groups and 84.0—86.2 in controls.

The postmitotic life-span of the cells was of about 72.4 days for the cultures treated with Gerovital H3 which underwent 14 passages and of 62.3 days for the nontreated cultures with 12 passages.

The difference of 10.1 days represents a statistically significant prolongation of the life-span, of 16% for the cells in the treated cultures as compared to the control group (Table 2).

* $\frac{\text{Total number of cells} - \text{number of dead cells}}{\text{Total number of cells}} \times 100.$

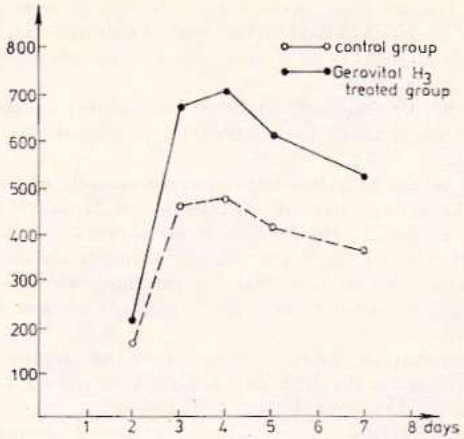


Fig. 1 – Renal cells density during the first 7 days in control and treated groups.

*Table 1*

**Gerovital H3 effect on the density and viability of monkey renal cells**

| Age of culture (days) | Cellular density (thousands of cells/ml medium) | | | | Cellular viability % | | |
|---|---|---|---|---|---|---|---|
| | Control | Gerovital $H_3$ | Difference % as compared to the control | P | Control | Gerovital $H_3$ | Difference % as compared to the control |
| 2 | 163.5 | 218.9 | 33.8 | $< 0.01$ | 86.0 | 88.1 | 2.1 |
| 3 | 463.0 | 671.5 | 45.0 | $< 0.01$ | 86.1 | 87.1 | 1.0 |
| 4 | 480.3 | 712.3 | 48.3 | $< 0.01$ | 86.2 | 88.4 | 2.2 |
| 5 | 418.0 | 610.6 | 46.0 | $< 0.01$ | 85.1 | 86.1 | 1.0 |
| 7 | 363.6 | 529.7 | 45.6 | $< 0.01$ | 84.0 | 85.1 | 1.1 |

*Table 2*

**Gerovital $H_3$ effect on postmitotic life-span of monkey renal cells**

| Average life-span (days) | | Difference as compared to the control % | P* |
|---|---|---|---|
| Control | Gerovital $H_3$ | | |
| 62.3 | 72.4 | 16 | $< 0.01$ |

* $$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{\Sigma\varepsilon_1^2 + \Sigma\varepsilon_1^2}{n_1 + n_2 - 2}}} \cdot \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

The signs of cellular aging were noticed to appear after 40—45 days in the control groups and 8—10 days later in the treated cultures. Such signs are characterized by: decrease of density in living cells, cellular anisomorphism with vacuolisation, pycnosis and cytolysis.

Frequently, the increase of the treated cells adhesiveness materialised in the occurrence of reduced areas of the monolayer detachment from the culture vessels' walls (Fig. 3).

With respect to the cytochemical aspect, marked differences between the control groups and the groups treated with Gerovital $H_3$ have been noticed.

In the nontreated cells, the lipid material appears as confluent grains which gradually invade the whole cytoplasmatic mass leading to the early aging of the cells.

In the cells treated with Gerovital $H_3$, the lipid content during cell cultivation appears as isolated granulations, rarely confluent, with a frequent perinuclear disposition (Fig. 4).

The acid phosphatase activity decreases in the treated cells starting with the 30th day, reaching on the 50th day a positivity score of $2.98 \pm 0.31$ as compared to $3.68 \pm 0.29$ in the control groups (Table 3).

Researches on esterase activity indicate also a decrease in the treated cells as compared to the control cells (Table 4).

*Table 3*

**Gerovital H3 effect on the acid phosphatase activity in renal cells from cultures of different age**

| Age of cultures (days) | Control | | Gerovital $H_3$ | |
|---|---|---|---|---|
| | Positivity score | Positivity % | Positivity score | Positivity % |
| 6 | $0.68 \pm 0.06$ | 34 | $0.79 \pm 0.08$ | 38.3 |
| 10 | $1.21 \pm 0.10$ | 39.8 | $1.33 \pm 0.31$ | 41.0 |
| 16 | $1.37 \pm 0.12$ | 46.8 | $1.42 \pm 0.13$ | 50.0 |
| 20 | $1.99 \pm 0.16$ | 51.9 | $1.89 \pm 0.37$ | 54.0 |
| 25 | $2.17 \pm 0.09$ | 67.0 | $2.08 \pm 0.15$ | 65.9 |
| 30 | $2.33 \pm 0.10$ | 79.0 | $2.16 \pm 0.08$ | 69.9 |
| 40 | $2.42 \pm 0.09$ | 84.2 | $2.29 \pm 0.10$ | 76.4 |
| 50 | $3.68 \pm 0.29$ | 91.0 | $2.98 \pm 0.31$ | 80.0 |
| 60 | $4.20 \pm 0.39$ | 96.4 | $3.61 \pm 0.28$ | 95.0 |
| 70 | — | — | $4.39 \pm 0.47$ | 98.3 |

*Table 4*

**Gerovital H3 effect on the esterase activity in renal cells depending on the age of the cultures**

| Age of cultures (days) | Control | | Gerovital $H_3$ | |
|---|---|---|---|---|
| | Positivity score | Positivity % | Positivity score | Positivity % |
| 5 | $0.32 \pm 0.03$ | 16.0 | $0.40 \pm 0.02$ | 16.7 |
| 11 | $0.82 \pm 0.08$ | 19.3 | $0.80 \pm 0.10$ | 17.1 |
| 15 | $1.19 \pm 0.13$ | 26.0 | $1.13 \pm 0.09$ | 22.3 |
| 21 | $1.65 \pm 0.26$ | 30.0 | $1.58 \pm 0.16$ | 31.2 |
| 26 | $1.73 \pm 0.25$ | 34.2 | $1.70 \pm 0.14$ | 33.5 |
| 32 | $1.88 \pm 0.40$ | 35.4 | $1.81 \pm 0.06$ | 34.9 |
| 41 | $2.66 \pm 0.22$ | 60.2 | $2.58 \pm 0.26$ | 57.8 |
| 51 | $3.00 \pm 0.30$ | 74.0 | $2.89 \pm 0.11$ | 69.0 |
| 58 | $3.69 \pm 0.36$ | 88.0 | $3.06 \pm 0.29$ | 79.1 |
| 68 | — | — | $3.88 \pm 0.16$ | 89.4 |

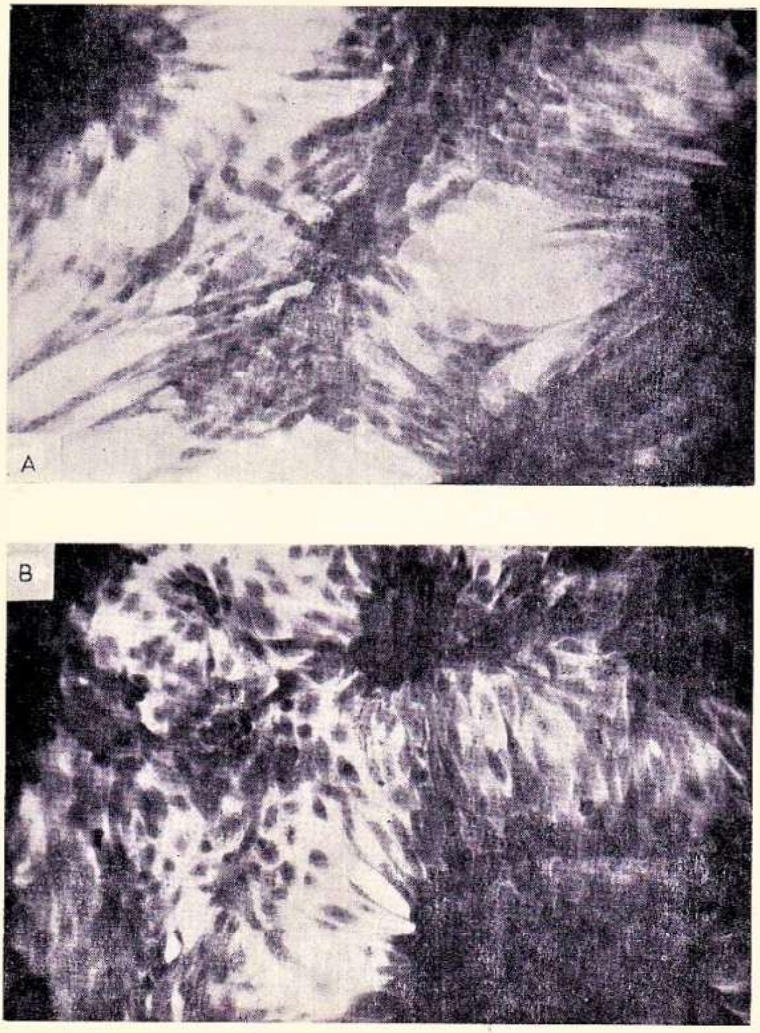


Fig. 2. — Microphotograph of a 4-day-old-cellular area: A — in the control group; B — in the group treated with Gerovital $H_3$. Increased cellular density in the treated culture. Staining violet crystal. 400 ×.

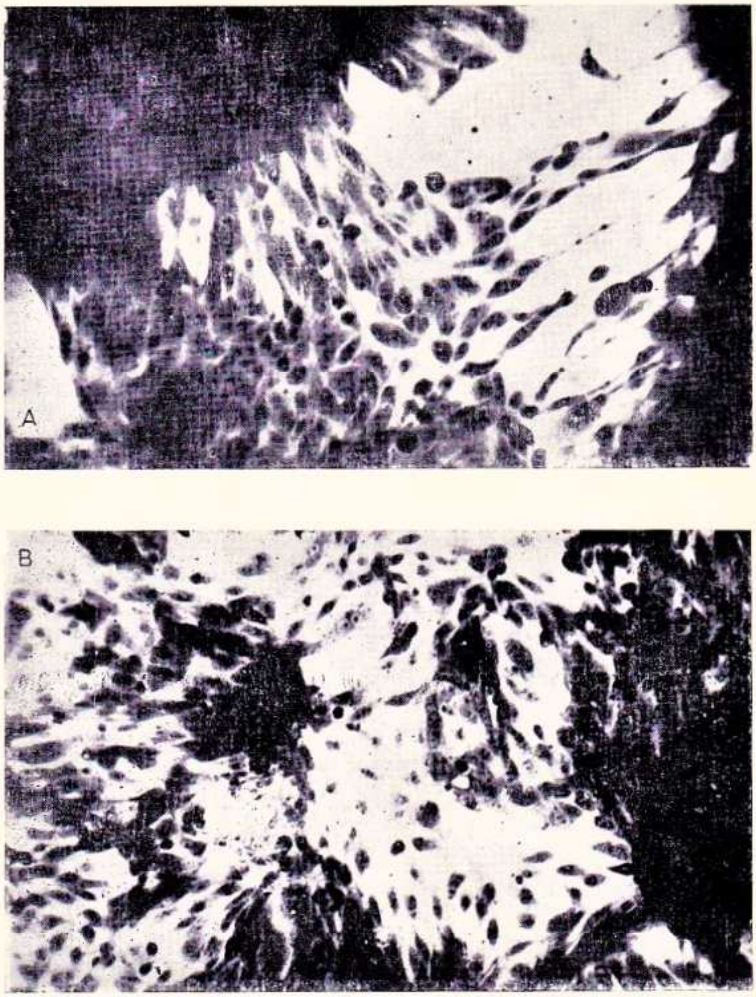


Fig. 3 — Microphotograph of 25-day-old-cellular areas; A — in the control group; B — in the group treated with Gerovital $H_3$. The areas are slackly populated as the cells fall from the vessel walls.
Staining violet crystal. 400 ×.

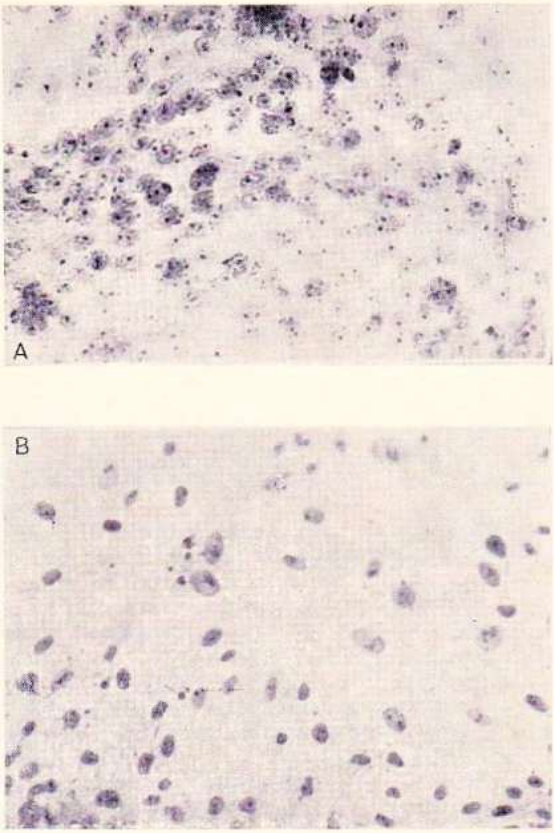


Fig. 4 — Microphotograph — 14-day-old renal cell in the control group (A) and in the group treated with Gerovital $H_3$ (B). Marked fat loading in the control group. Staining Nile Blue. 200 ×.

## DISCUSSION

The above data show that in cultures of monkey renal cells Gerovital $H_3$, in concentration of 0.4 ml/100 ml tissue culture medium determines an increase in the postmitotic life-span, the activation of cellular proliferation and the delay of cellular aging.

We recall that 0.4 ml Gerovital $H_3$ per 100 ml tissue medium proved most favourable in former researches which used different concentrations of Gerovital $H_3$.

With regard to the increase in the postmitotic life-span evidenced by us since 1972 on monkey kidney cells, we mention that Polet [17] attributes it to the stimulation of the cellular metabolism and to the maintenance of the physiological state of the cellular membrane. We would remind that Eicholtz [18] noticed in 1957 that procaine acts on the density and stabilization of the cellular membrane, increasing its physiological potential.

Officer [19] considers that the procaine from Gerovital $H_3$ transforms the irreversible bind of calcium accumulated in the cellular membrane into a reversible one, thus permitting the aged cells to function normally. As Gerovital $H_3$ is added to cell cultures in later passages, 7—9, (when normally cellular multiplication decreases) it determines an immediate decrease in the time necessary for cell doubling and the life of the cells continues for 2—3 generations beyond that of the control groups.

We also noticed in our researches a delay in the appearance of cellular aging signs under Gerovital H3 action. We underline particularly the signs referring to the decrease in the acid phosphatase and esterase activity — the intensification of which in the process of aging is well known [20—23].

Similar results have been reported by Officer [19] in cultures of mouse embryo fibroblasts. When Gerovital H3 is added to the cultures after the cells had ceased to multiply, it maintains them for a longer period of time, delays aging signs and prevents them from transforming into a continuous line.

Cell proliferation stimulation in cultures was evidenced by us [24] not only in monkey renal cells but in chicken embryo heart and liver cells as well.

Similar data regarding the stimulation of cellular proliferation have been signaled by Parhon, Aslan and Cosmovici [25] in researches on infusorians.

All these data underline the complexity of the mechanism of Gerovital H3 action, its eutrophic influence on cellular structures affected by the aging process.

## CONCLUSIONS

Researches in monkey renal cells point out that Gerovital H3 in a concentration of 0.4% activates cellular proliferation, extends postmitotic life-span, delays cellular aging with the reduction in acid phosphatase and esterase activity.

**Résumé.** Le comportement des cellules rénales de singe (Rhesus) dans les cultures de cellules de longue durée a été étudié par rapport aux paramètres suivants: prolifération cellulaire, viabilité des cellules, différents aspects concernant le vieillissement cellulaire (vacuolisation, pycnose, cytolyse, chargement graisseux, activité des phosphatases acides et des estérases), de même que la durée de vie postmitotique des cellules.

Les recherches ont été effectuées aussi bien sur des cultures de cellules traitées au Gérovital $H_3$ (concentration 0,4%) que sur des cultures de cellules témoins.

Les résultats ont démontré que le Gérovital $H_3$ stimule la prolifération cellulaire et retarde les symptômes du vieillissement cellulaire diminuant aussi le niveau de l'activité de la phosphatase acide et de l'estérase.

La durée de vie postmitotique des cellules traitées au Gérovital $H_3$ pendant 72,4 jours a été de 16% plus longue (voire 10,1 jours plus longue) que celle des cultures de cellules non traitées (qui a été de 62,3 jours). Le nombre des passages, de même que l'adhésivité cellulaire s'est accru dans les cultures soumises au traitement.

Les résultats obtenus sont en corrélation avec les données déjà connues concernant l'action de ce médicament eutrophisant sur le métabolisme cellulaire.

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# STUDY OF GEROVITAL $H_3$ ACTION ON MITOCHONDRIAL FRACTION IN RAT LIVER AND BRAIN

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**Summary.** The present paper discusses mitochondrial fraction in rat liver and brain by studying the following functional aspects: $GH_3$ action on oxidative phosphorylation, binding of sodium ions and of procaine hydrochloride to the mitochondrial membrane and the effect on its structural integrity at various concentrations.

The supplementary release of protons caused by the presence of $2.10^{-5}$ and $8.10^{-5}$M procaine hydrochloride per mg protein, dependent on the ionic strengths, was at a maximum at 80 mM NaCl. The procaine molecule, positively charged at pH 6.5, is able to form ionic bonds with the proteins or phospholipids of the mitochondrial membrane. An important increase of oxygen and phosphorus consumption was produced at the lower $GH_3$ concentrations of $1.5.10^{-5}$ and $3.10^{-5}$M per mg mitochondrial protein. Higher $GH_3$ concentrations ($10^{-3}$M per mg protein) produced an inhibitory effect, blocking ADP phosphorylation to ATP.

It is possible that $GH_3$ in lower concentrations of $10^{-5}$M per mg mitochondrial protein, produces the activation of the respiratory chain enzymes.

As clinical researches and pharmacologic investigations were undertaken with a view of detecting the mechanism of action of the therapy based on procaine, the studies carried out on beer yeast, as well as on rat liver homogenate with the Warburg method [1, 2, 3], showed that the presence of procaine in the test tubes determined an increase of the oxygen consumption due to the activation by procaine of the processes of oxidative phosphorylation.

In 1972, Hrachovec published the results of comparative researches which showed that Gerovital $H_3$ ($GH_3$), (procaine hydrochloride 2%, benzoic acid 0.12%, potassium metabisulphite 0.10%, and disodium phosphate 0.01%) had a more pronounced inhibitive action than procaine hydrochloride, upon the monoaminoxidase in the brain, liver and heart of rats [4]. These researches were carried out both upon homogenates *in vitro* and upon mitochondrial fractions, after the animals had been injected intraperitoneally. In contrast to the properties of other classical MAO inhibitors, it was demonstrated that $GH_3$ is a weak, reversible and competitive inhibitor of MAO [5, 6].

We have used the mitochondria to assess the effects of $GH_3$ on oxidative phosphorylation, binding of sodium ions and procaine hydrochloride to mitochondrial membrane and the effect on its structural integrity.

## MATERIALS AND METHODS

We used female Wistar rats, the groups being divided as follows: young rats (2—3 months), adults (6—8 months) and old rats (22—24 months).

Liver and brain mitochondria were isolated essentially as described by Schneider and Hogeboom [7] in 0.25 M sucrose, 0.05 M TRIS-HCl, pH 7.4.

*Oxidative phosphorylation* was measured by classical Warburg manometric methods at 38°C [8]. The main compartment reaction mixture (the final pH 7.5) consisted of the following components in the Warburg flask: 1.2 ml of a solution containing 0.02 M TRIS, 0.06 M potassium phosphate, 0.5 M sucrose; 0.15 ml of a 5% solution of bovine serum albumine (BSA); 0.6 ml of 0.05 M $MgCl_2$ solution; 0.30 ml of a solution of hexokinase (12.5 mg/ml); 0.6 ml of the mitochondrial suspension (8—10 mg protein) and 0.1 ml of various $GH_3$ (procaine) concentrations, ranging from $1.5.10^{-5}$ to $1.10^{-3}$ M/mg protein. The side arm contained: 0.15 ml of a solution of 0.45 M glucose, 0.15 ml of 0.05 M ADP solution and 0.15 ml of 0.1 succinate. The central well contained a small wick of filter paper and 0.2 ml of 20% KOH. The reaction was stopped by the addition of 0.65 ml of 10% trichloroacetic acid. Inorganic phosphate was determined by the method of Fiske-Subbarow [9].

*The binding of $Na^+$.* Mitochondrial fraction from the liver of adult rat was suspended in 0.25 M sucrose at 50 mg protein per ml. The suspension was kept at 0°C and used within 4 hours. The pH changes in the mitochondrial suspensions were followed with a combination glass electrode linked to an Orion pH-meter. From the initial and final pH values the amount of $H^+$ produced in medium was evaluated. In this experiment pH units were transformed in concentration of hydrogen ions by logarithmic calculation. The binding of $Na^+$ was measured according to the procedure of Gear [10]. Mitochondrial suspension containing 2.5 mg protein per ml in 0.25 M sucrose, was mixed with different NaCl solutions (80 mM, 160 mM, 240 mM, 320 mM) at three pH values: 6.5,7 and 8. A constant osmolarity of 160 mosM was maintained with sucrose except for the final 320 mM NaCl.

*Binding of procaine molecule.* Rat liver mitochondria were added (5 mg protein) to make a total volume of 2 ml containing increasing NaCl concentrations from 80 to 320 mM.at pH 6.5. The net ejection of $H^+$ was monitored. In another experiment the medium contains $2.10^{-5}$ and $8.10^{-5}$ M procaine hydrochloric per mg protein. Results are expressed as nanomoles of $H^+$ ejected per mg mitochondrial protein. Mitochondrial protein was determined by the Lowry procedure [11].

*The influence of different concentrations of $GH_3$* on mitochondrial protein concentration in supernatant was studied at six levels of $GH_3$ (M/mg protein) added to 5 ml aliquots of mitochondria: $1.5.10^{-5}$, $3.10^{-5}$, $1.5.10^{-4}$, $3.10^{-4}$, $1.10^{-3}$, $2.10^{-3}$. These were incubated at 25°C for 10 minutes and then centrifuged at 15000 g for 10 minutes. The protein content was estimated in the supernatant and the variations were expressed in per cent.

## RESULTS

Mitochondrial fractions of rat liver suspended in salt-free isotonic sucrose with buffered media containing NaCl of various concentrations, release $H^+$ ions into the medium. The amounts of $H^+$ released increase with pH and with the salt concentrations. At pH 6.5, 7 and 8 the increase of salt concentrations is accompanied by a large release of $H^+$ into the medium (Table 1).

Measurement of proton release by the mixture of mitochondria with different NaCl concentrations (80 mM, 160 mM, 240 mM, 320 mM) is thus a sensitive

*Table 1*

**H+ ejection during procaine binding to rat liver mitochondria**

| | NaCl, mM | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH 6.5 | | | | pH 7 | | | | pH 8 | | | |
| | 80 mM | 160 mM | 240 mM | 320 mM | 80 mM | 160 mM | 240 mM | 320 mM | 80 mM | 160 mM | 240 mM | 320 mM |
| Procaine $2.10^{-5}$M per mg prot. | 16 | 17 | 21 | 24 | – | – | – | – | – | – | – | – |
| Procaine $8.10^{-5}$M per mg prot. | 15 | 16 | 20 | 22 | – | – | – | – | – | – | – | – |
| Without Procaine | 13 | 16 | 20 | 23 | 19 | 23 | 26 | 30 | 25 | 30 | 31 | 36 |

means of following a cation binding. Procaine-hydrochloride was therefore added to a sodium-containing medium at pH 6.5 and the proton ejection monitored during one minute. The supplementary release of proton caused by the presence of $8.10^{-5}$ M and $2.10^{-5}$ M procaine hydrochloride per mg protein, dependent on ionic strengths, was maximum at 80 mM NaCl.

Since $GH_3$ appeared to be bound to mitochondria, it was expected that the effects would be more closely related to the ratio of drug: mitochondrial protein, than to the initial molarity of drug in the incubation medium. The results revealed the variation between 1% and 70% of protein concentrations in supernatants with the $GH_3$ concentration comprehended between $1.5.10^{-5}$ M and $2.10^{-3}$ M

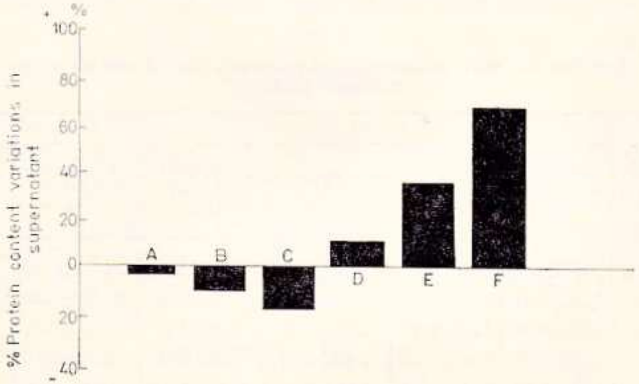


Fig. 1. — The influence of different concentrations of $GH_3$ on mitochondrial protein concentrations at six levels of $GH_3$ (M/mg protein) added to 5 ml aliquots of mitochondria: $1.5.10^{-5}$ (A), $3.10^{-5}$ (B), $1.5.10^{-4}$ (C), $3.10^{-4}$ (D), $1.10^{-3}$ (E), $2.10^{-3}$ (F).

per mg of mitochondrial protein (Fig. 1). Having in view this aspect, the effect was studied of the treatment with $GH_3$ *in vitro*, at various concentrations, on oxidative phosphorylation. For this purpose the determinations were performed on rat liver and brain mitochondrial preparations.

The effects of $GH_3$ on oxygen consumption and P/O ratio are shown in Tables 2, 3 and 4. The results demonstrate that the treatment with $GH_3$ *in vitro* at two levels of concentration ($1.5.10^{-5}$ M and $3.10^{-5}$ M per mg of mitochondrial

*Table 2*

**Effect of $GH_3$ *in vitro* on oxidative phosphorylation and respiration in liver and brain mitochondria of young rat (μatoms/mg protein/30')**

| $GH_3$ concentration (M/mg protein) | Liver | | | Brain | | |
|---|---|---|---|---|---|---|
| | Oxygen consumption | Phosphorus consumption | P/O | Oxygen consumption | Phosphorus consumption | P/O |
| — | 2.08±0.08 | 3.84±0.11 | 1.84±0.08 | 2.34±0.08 | 4.40±0.25 | 1.85±0.04 |
| $1.5.10^{-5}$ | 6.71±0.16 | 13.50±0.13 | 2.00±0.10 | 7.06±0.10 | 13.90±0.21 | 2.03±0.06 |
| $3.10^{-5}$ | 3.88±0.08 | 7.55±0.09 | 1.94±0.06 | 4.09±0.08 | 7.89±0.16 | 1.91±0.08 |
| $4.5.10^{-5}$ | 2.50±0.09 | 4.58±0.10 | 1.81±0.04 | 2.80±0.12 | 6.04±0.14 | 1.80±0.04 |
| $6.10^{-5}$ | 1.88±0.06 | 3.46±0.18 | 1.84±0.02 | 2.04±0.08 | 3.55±0.10 | 1.74±0.03 |

* Each value given is an average by five animals (mean ± S.E.M.)

*Table 3*

**Effect of $GH_3$ *in vitro* on oxidative phosphorylation and respiration in liver and brain mitochondria of adult rat (μatoms/mg protein/30')**

| $GH_3$ concentration (M/mg protein) | Liver | | | Brain | | |
|---|---|---|---|---|---|---|
| | Oxygen consumption | Phosphorus consumption | P/O | Oxygen consumption | Phosphorus consumption | P/O |
| — | 2.05±0.08 | 3.89±0.25 | 1.87±0.04 | 2.40±0.09 | 4.58±0.18 | 1.87±0.09 |
| $1.5.10^{-5}$ | 6.39±0.16 | 12.64±0.24 | 1.98±0.07 | 7.10±0.18 | 13.63±0.25 | 1.92±0.06 |
| $3.10^{-5}$ | 4.10±0.05 | 7.96±0.19 | 1.94±0.10 | 5.38±0.12 | 9.68±0.22 | 1.80±0.04 |
| $4.5.10^{-5}$ | 2.79±0.08 | 3.26±0.17 | 1.80±0.10 | 3.24±0.06 | 6.76±0.16 | 1.78±0.08 |
| $6.10^{-5}$ | 1.81±0.10 | 3.12±0.23 | 1.74±0.08 | 2.16±0.04 | 4.18±0.10 | 1.83±0.10 |
| $1.10^{-4}$ | 1.80±0.09 | 2.92±0.14 | 1.64±0.07 | 1.80±0.11 | 3.00±0.06 | 1.60±0.07 |
| $1.10^{-3}$ | 0.90±0.08 | — | — | 0.45±0.03 | — | — |

* Each value is an average by five animals (mean ± S.E.M.)

*Table 4*

**Effect of $GH_3$ *in vitro* on oxidative phosphorylation and respiration in liver and brain mitochondria of old rat (μ atoms/mg protein/30')**

| $GH_3$ concentration (M/mg protein) | Liver | | | Brain | | |
|---|---|---|---|---|---|---|
| | Oxygen consumption | Phosphorus consumption | P/O | Oxygen consumption | Phosphorus consumption | P/O |
| – | 2.01±0.09 | 3.78±0.19 | 1.89±0.08 | 2.38±0.11 | 4.42±0.24 | 1.85±0.05 |
| $1.5.10^{-5}$ | 6.45±0.12 | 13.11±0.20 | 2.03±0.07 | 6.08±0.18 | 12.30±0.22 | 2.04±0.07 |
| $3.10^{-5}$ | 4.06±0.13 | 7.99±0.18 | 1.97±0.04 | 5.90±0.10 | 11.32±0.31 | 1.92±0.02 |
| $4.5.10^{-5}$ | 3.10±0.14 | 5.60±0.22 | 1.80±0.12 | 3.10±0.08 | 5.58±0.20 | 1.80±0.05 |
| $6.10^{-5}$ | 1.80±0.11 | 3.21±0.16 | 1.78±0.07 | 2.08±0.13 | 3.75±0.18 | 1.78±0.04 |

Each value is an average for five animals (mean ± S.E.M.)

protein) produce an increase of oxygen and phosphorus consumption with a slight modification of the P/O ratio. $GH_3$ concentration at $6.10^{-5}$ M/mg protein slightly decreases oxygen consumption and ADP phosphorylation to ATP. Phosphorylation activity and mitochondrial respiration in the brain were increased in young, adult and old rats at two levels of $GH_3$ concentration: $1.5.10^{-5}$ M and $3.10^{-5}$ M per mg of mitochondrial protein. These effects decrease at the concentration of $4.5.10^{-5}$ M $GH_3$. $6.10^{-5}$ M $GH_3$ per mg of mitochondrial protein slightly inhibits oxygen consumption and decreases the P/O ratio. Studies of oxidative phosphorylation were performed on mitochondrial preparations from the liver and brain of adult rats in the presence of $GH_3$ at the ratio of $1.10^{-4}$M per mg of protein and $1.10^{-3}$ M per mg of protein. It was observed that $1.10^{-4}$ $GH_3$ mg of mitochondrial protein inhibits oxygen and phosphorus consumption with a change of the P/O ratios, and $1.10^{-3}$ M $GH_3$ per mg of protein blocking ADP phosphorylation to ATP.

## DISCUSSION

The release of $H^+$ ions in the medium by the mixture of mitochondria with different salts solutions represents a sensitive means of following this effect with procaine hydrochloride added to the medium. The process might be caused by displacement of $H^+$ from protonated anionic groups of the mitochondria by the binding of $Na^+$ in good agreement with the data reported by Gear and Lehninger [10]. Procaine hydrochloride in forming the salt, in aqueous solutions, yields the positively charged quaternary amine ion, $R \equiv NH^+$ for short. Dissolved in water, the cation is in dissociation equilibrum with the base according to the following:

$$R \equiv NH^+ \rightleftarrows H^+ + R \equiv N$$

The direction of this dissociation depends on the prevailing concentration of hydrogen ions. At pH 6.5 procaine hydrochloride ($pK_a = 8.9$) is positively charged and releases $H^+$ ion on binding. The supplementary release of protons caused by the presence of $8.10^{-5}$ M and $2.10^{-5}$ M procaine hydrochloride per mg

protein dependent on ionic strengths, being at a maximum at 80 mM NaCl, suggests an analogy between the $Na^+$ behaviour and that of quaternary amine specific to the procaine molecule at pH 6.5.

In many respects mitochondria suspended in sucrose solutions behave like particles of a cation exchange resin in the protonated form, which can release $H^+$ to the media in exchange with a variety of different cations, like quaternary amine of the procaine molecule.

Water lysed rat liver mitochondria which have lost about 50% of the total mitochondrial protein in soluble forms, were found to release $H^+$ when mixed with NaCl medium [12]. In agreement with the data reported by Gear [9], dissociating groups could be contributed by either the protein or phospholipids of the membrane, the latter source appears more likely.

Quaternary amine of the procaine molecule positively charged is able to form ionic bond with oppositely charged ($COO^-$, $PO_4^{3-}$). After Feinstein [13], procaine molecules compete with calcium for charged regions on the polar tails of phospholipid molecule. In Fig. 2 is represented a model of bridge complex formation between one procaine molecule and two phospholipid molecules. The polar aromatic amine ($+\delta$) and aliphatic amine groups are shown oriented toward the oppositely charged phosphate groups. Procaine molecule could displace $Ca^{2+}$ bound to a variety of phospholipids, in agreement with the relative nonspecificity of salts in promoting $H^+$ release from mitochondria.

$GH_3$ with 2% procaine hydrochloride was tested *in vitro* for its effects on oxidative phosphorylation at various concentrations. The increase of oxygen and phosphorus consumption was produced at the lower $GH_3$ concentrations of $1.5.10^{-5}$ M per mg protein and $3.10^{-5}$ M per mg protein. Higher $GH_3$ concentrations ($10^{-3}$ M per mg protein) produced inhibitory effects and damaged membrane integrity.

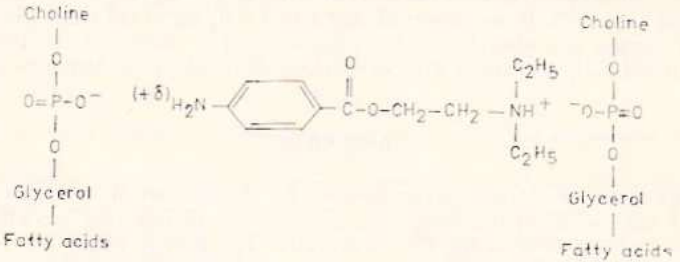


Fig. 2. — Proposed model of bridge complex formation between one procaine molecule and two phospholipid molecules.

These results agree with the studies carried out on beer yeast as well as upon rat liver homogenate by the Warburg method [1]. Research on yeast suspension shows that, depending on the dose, the action of procaine upon respiration was threefold: small doses (0.001%) had a stimulating effect, large doses (1%) had an inhibitory effect and average doses (0.01%) did not affect the oxygen consumption.

Previously, we have reported [14] the effect of $GH_3$ on succinate dehydrogenase activity in liver and brain mitochondria. An important increase of enzyme

activity at lower $GH_3$ concentrations was found ($1.10^{-5}$ M per mg protein), especially in the nervous tissue. The brain mitochondria are in this respect much more sensitive than those extracted from the liver. It is possible that $GH_3$ in lower concentrations stabilises the mitochondrial membrane through fixation on it, preserving its integrity and, at the same time, influencing the membrane transport process and the activity of the respiratory chain enzymes.

**Résumé.** Les recherches ont été effectuées sur la fraction mitochondriale du foie et du cerveau de rat, en abordant les aspects fonctionnels suivants: l'action du Gérovital $H_3$ sur la phosphorylation oxydative, le rattachement des ions de sodium et de la procaïne hydrochlorique à la membrane mitochondriale, ainsi que l'action exercée par le Gérovital $H_3$ sur l'intégrité structurale mitochondriale aux diverses concentrations.

La libération supplémentaire de protons, produite par $2.10^{-5} - 8.10^{-5}$ M procaïne hydrochlorique par mg de protéine, dépend de la teneur ionique, en présentant un maximum à 80 m M NaCl. La molécule de procaïne chargée positivement à pH = 6,5 est en mesure de former des liaisons ioniques avec les protéines ou les phospholipides de la membrane mitochondriale. Gérovital $H_3$, à de petites concentrations, de l'ordre de $1{,}5.10^{-5} - 3{,}10^{-5}$M par mg. de protéine mitochondriale, détermine une consommation augmentée d'oxygène et de phosphore.

Les concentrations plus grandes de Gérovital $H_3$, de l'ordre de $10^{-3}$M par mg de protéine, ont un effet inhibiteur, en bloquant la phosphorylation de l'ADP à l'ATP.

Il est possible que le Gérovital $H_3$, en concentration de l'ordre de $10^{-5}$ M par mg de protéine mitochondriale, produise une activation des enzymes de la chaîne respiratoire.

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# BIOCHEMICAL AND PHARMACODYNAMIC ARGUMENTS TO SUPPORT PROCAINE IMAO "B" TYPE ACTION

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**Summary.** The improvement of neuro-psychic functions and particularly of depressive states in the procaine-treated aged was pointed out for the first time by Ana Aslan. Subsequently numerous clinical and experimental researches have demonstrated the action of this substance on the central nervous system. The psychological and electroencephalographic investigations have completed the clinical observations.

In procaine biochemical mechanism of action an important part is played by its ability to inhibit monoaminoxidase. Based on a series of pharmacodynamic tests, the authors tried to define the type of inhibition. Researches in rats pointed out that procaine antagonises the worsening of conditioned behavior, intensifies and prolongs hyperthermia, delays reserpine-induced hypothermia and does not prevent reserpine-induced palpebral ptosis. These data suggest a B-MAO selective inhibitory effect. Contrary to the classical MAO inhibitors, procaine-based products are well tolerated by the aged patients.

Relatively recent data published in the field literature point out procaine depressive action.

Lüth [1] showed that Parhon and Aslan were the first scientists who mentioned procaine action on psychic states, in other words, the psychic effect of procaine. Aslan and coll. evidenced procaine eutrophic effect on the nervous system, manifest as improved memory, concentration and perception abilities, diminished insomnia and fatigue, increased desire to enjoy living and working, increased ability to adapt to the environment. A favourable influence upon the depressive psychical states of the aged was especially noticed [2–5]. Bucci and Saunders' study [6] conducted on 24 chronic psychotic patients followed up from 2 to 25 years pointed out that procaine (100 mg procaine hydrochloride 3 times per week for 6 months) improved depressive states and diminished psychotic symptoms associated with schizophrenia. In an experiment done on 32 hospitalized patients and 20 outpatients, aged 72–81, with senile and arteriosclerotic psychoses, Kral and coll. [7] found a procaine-induced transient improvement of depression accompanied by increased physical and psychical activity [9]. A double-blind study carried out by Smigel and coll. [8] on 60 patients with arthritis, nervous and senile disturbances pointed out significant improvements in 25 out of 29 patients treated with procaine for 5 months, whereas in the control group, the condition improved in only 9 out of 21 patients. The authors noticed procaine favourable effect particularly in patients with chronic nervous disturbances and less in those with chronic cerebral syndrome.

Lewicki and coll. [9] reported improvements in memory, thinking and associations in 75% of the aged subjected to the long-term treatment with Gerovital $H_3$ [10]. In a comparative study on 42 patients aged 73—80 suffering from arteriosclerotic senile dementia [38], alcoholic dementia with Korsakov syndrome [3], alcoholic senile dementia [1], Schneeberger [10] found that the procaine-based chronic treatment resulted in obvious improvements in one third of the treated cases. Cheerfulness, behavioral disturbances, morbidity improved. If prior to the treatment the patients had been completely indifferent to the environment, subsequently they reached an almost normal psychic level. None of these changes was noticed in the controls (quoted by [11]). Siggelkov (1966) subjected to the chronic treatment 74-year-old patients with cerebral atherosclerosis and deficient cerebral arterial circulation and found in 51.5% the alleviation of symptoms, expressing a better general reactivity of the organism: improvement of depressive states, irritability, motor and sensorial disorders, psychic deficiencies and mental confusion. Cohen and Ditman [12] carried out an open study on 41 patients: 17 normal, 17 psychiatric and 7 depressive patients with or without anxiety; they found that the administration of 100—200 mg Gerovital $H_3$ daily, 3 times a week for 4 weeks, resulted in the improvement of the general condition, energy, libido, motivation and somatic disturbances. S. Mora [13] noted important EEG differences between 139 subjects, mean age 66 and younger subjects, mean age 27. Combinations of alpha and beta waves or prevalence of beta waves were found in 67.7% of the aged subjects. The corresponding percentage in the younger subjects was 32%. Improved EEG tracings were pointed out in one third of the treated cases, whereas no modification was noticed in subjects with beta waves prevalence. The author inferred a possible correlation between psychic reactivity and cerebral electric activity.

The EEG investigations carried out in 1962 by Aslan and coll. pointed out normal electric tracings with no abnormality induced by the luminous intermittent stimulation in 75% of the cases (mean age 85) subjected to a long-term treatment with Gerovital $H_3$; in controls (untreated subjects) normal tracings were revealed in only 20% of the cases [4, 5].

Research efforts were further directed toward pointing out procaine biochemical mechanisms of action on the central nervous system. In 1940, Philpot [14] showed that procaine induced *in vitro* the inhibition of rat and guinea pig liver monoamineoxidase (MAO) by 60—100%. In 1950, Aslan and Vrăbiescu also noticed the inhibition of MAO and showed that procaine i.v. injections resulted in increased arterial pressure as a reaction to epinephrine [5]. In experiments conducted *in vitro* on rat liver, brain and heart, Hrachovec [15] pointed out that Gerovital $H_3$ induced a stronger MAO inhibition than procaine (Table 1). MacFarlane and Besbris [16] found procaine to act as a rapid and reversible inhibitor of brain MAO, which was proved *in vitro* by the decrease in the catecholamine and kynuramine metabolisms. The authors found that a 50% inhibition of MAO, requires amounts over 1 mg/ml procaine. They used MAO from rat brain mitochondrial preparations or homogenates and much higher procaine concentrations than the maximum serum levels *in vivo*. Yau [17] found the optimum procaine concentration required by MAO inhibition *in vivo* to be $10^{-6}$ M. Such concentrations induce *in vitro* only a 20 to 40% inhibition of mouse brain MAO. The same author, injecting i.p. quite large procaine amounts (90—180 mg/kg body weight) found a weak, but significant increase in cerebral serotonine and no significant change in dopamine or norepinephrine, indicating the weak or inexistent MAO inhibition. The same

*Table 1**

**The inhibitory effect of Procaine HCl on MAO in rat brain, liver, heart and platelets**

*In vitro experiments*

| Inhibitor | Concentration of inhibitor | Percent inhibition | | | |
|---|---|---|---|---|---|
| | | Brain | Liver | Heart | Platelets |
| Procaine HCl | $1.10^{-4}$ M | 31.5±2.3 | 37.8±1.7 | 36.4±1.4 | 28.9±0.9 |
| Procaine HCl | $1.10^{-3}$ M | 64.7±2.8 | 58.5±2.9 | 59.4±2.1 | 51.5±0.9 |
| Gerovital $H_3$ | $1\times10^{-4}$ M | 51.4±2.6 | 45.7±2.9 | 46.8±1.4 | 41.6±1.0 |
| Gerovital $H_3$ | $1\times10^{-3}$ M | 87.4±1.9 | 74.2±2.1 | 69.9±2.3 | 69.1±1.2 |

*In vivo experiments*

| Inhibitor | Dose of inhibitor | Percent inhibition | | |
|---|---|---|---|---|
| | | Brain | Liver | Heart |
| 2% Procaine HCl | 1.0 ml | 16.7±0.9 | 13.6±1.2 | 14.3±1.2 |
| 2% Procaine HCl | 2.0 ml | 24.9±1.2 | 34.8±0.5 | 28.1±1.0 |
| Gerovital $H_3$ | 1.0 ml | 27.3±0.9 | 24.7±0.9 | 26.9±1.2 |
| Gerovital $H_3$ | 2.0 ml | 39.4±0.6 | 43.8±1.0 | 45.1±0.8 |

* (after Hrachovec J. P., The Physiologist, 1973, **15**, 3)

author did not find any significant changes in the above-mentioned amines subsequent to the chronic administration of 90 mg/kg body weight. Some MAO preparations were also found to be more sensitive to procaine.

These results called for an attempt to explain procaine antidepressive action, at least in part based on its IMAO effect which seems justified at present by the innumerable data accumulated that reveal the role of MAO in the function of the nervous system. The following facts were pointed out: cerebral MAO activity reaches quite high values; there is an ununiform distribution of MAO through the different areas of the brain, the enzyme prevailing in the hypothalamus; the independent and differentiated enzyme formation is possible in different cerebral structures; there is a significant variation of MAO concentration through the nervous tissue in the course of the ontogenetic evolution; there is a substantial change in the cerebral enzyme concentration and specific activity with advanced ages and certain psychic diseases.

Based on the use of specific inhibitors (chlorgyline and deprenyl), two major types of enzymatic activity have been pointed out: A-MAO and B-MAO. A-AMO, also called 5-hydroxytriptaminoxidase acts on 5-HT-norepinephrine (preferentially), thyramine, dopamine and is selectively inhibited by chlorgyline, which has a marked affinity for 5-HT-minergic receptors, the blockage of which is incomplete.

B-MAO, also called phenylethylaminoxidase acts on phenylethylamine bensylamine (preferentially) thyramine, dopamine and is selectively inhibited by deprenyl (isomer). Gascoigne and coll.'s histochemical investigations ints rat brain [18] pointed out a MAO variant which metabolized 5-HT, but was not sensitive to chlorgyline; they considered it a new form of enzyme, named C-MAO. Recent data have suggested the existence of a new MAO, with dopamine as favourite substratum [19].

As far as procaine inhibitory effect is concerned, although Young and coll. postulated the existence of certain MAO types particularly sensitive to procaine inhibitory effect, no mention has been made of the type of inhibition.

Starting from the fact that procaine IMAO effect is pointed out particularly *in vitro* and because of the lack of data in the field literature referring to the type of procaine IMAO action, we decided to use a battery of pharmacodynamic tests in order to check procaine antidepressive effect and point out *in vivo* procaine IMAO effect for specifying the type of inhibition. Procaine antidepressive effect was studied by means of the test of reserpine-induced conditioned behavioral changes in rats; IMAO effects were investigated by means of pharmacodynamic tests indirectly pointing out actions such as those attributed to MAO inhibitors (tests of hypothermic action, prevention of reserpine-induced hyperthermia and palpebral ptosis in rats).

Our experimental results [20] showed that 5 mg/kg body weight procaine antagonized reserpine-induced worsening of the conditioned behaviour in rats pointing out an antidepressive action comparable to a certain extent with that specific to tricyclic antidepressives (Table 2); on the other hand the same procaine amount strengthens and prolongs hyperthermia, delays reserpine-induced hypothermia and does not hamper reserpine-induced palpebral ptosis; these results support the clinical data which grant procaine IMAO properties and suggest the B type MAO inhibiting action (Tables 3 and 4).

The data are numerous which support procaine antidepressive effect due to MAO inhibition. The therapeutic use of specific MAO inhibitors in psychic diseases [21] characterized by changes in MAO concentration (schizophrenia, Parkinson's disease, certain forms of depression) as well as in aging, which results in the alleviation of psychiatric symptoms and age-related nervous disturbances, also support procaine antidepressive action through its IMAO effect.

## CONCLUSIONS

Procaine antidepressive properties are all the more interesting as the substance is better tolerated by aged and ill persons than the classical MAO inhibitors which are potential therapeutic risk factors, because of their much too stronger effect, unspecific character and inhibition of a large spectrum of drug metabolized enzymes.

The study of procaine antidepressive action should be a current research priority because of its fundamental implications and clinical applicability. As we have already mentioned, our results suggest that procaine induces the selective inhibition of B-MAO.

*Table 2*

**Influence of procaine and reserpine on the conditioned behaviour of rats**

| Lot of experiment | Period 0—30 minutes | | | | Period 0—60 minutes | | | |
|---|---|---|---|---|---|---|---|---|
| | Electrical stimuli | Differences | Tactile stimuli | Differences | Electrical stimuli | Differences | Tactile stimuli | Differences |
| | | | Group A* | | | | | |
| Control group | 0 | | 0 | | 0 | | 0 | |
| Procaine 5 mg/kg.b.wt. i.p. 5 days | 0 | | 0 | | 0 | | 0 | |
| Reserpine 2 mg/kg.b.wt. i.m. (24 hrs before recording) | 0 | | 106.2 (±17.04) | | 0 | | 251.1 (±38.22) | |
| Procaine 5 mg/kg.b.wt. i.p. 5 days + reserpine 2 mg/kg.b.wt. i.m. (24 hrs before recording) | 0 | | 30 (±23.43) | −76.2 p∼0.02 | 0 | | 73.3 (57.04) | −177.8 p∼0.02 |
| | | | Group B** | | | | | |
| Control group | 0 | | 87.6 (±34.38) | | 36. (±34.61) | | 213.6 (±85.35) | −100.8 |
| Procaine 5 mg/kg.b.wt. i.p. 5 days | 0 | | 48 (±36.54) | −39.6 p∼0.5 | 88.8 (±73.62) | 52.8 p∼0.5 | 112.8 (±84.64) | p∼0.4 |
| Reserpine 2 mg/kg.b.wt. i.m. (24 hrs before recording) | 43.2 (±40.37) | | 160.8 (±22.05) | | 76.8 (±68.34) | | 418.8 (±87.37) | |
| Procaine 5 mg/kg.b.wt. i.p. 5 days + reserpine 2 mg/kg.b.wt. i.p. (24 hrs before recording) | 15.6 (±9.99) | −27.6 p∼0.6 | 102 (±53.40) | −58.8 p∼0.3 | 50.4 (±36.32) | −26.4 p∼0.7 | 196 (±100.48) | −222.8 p∼0.1 |

The mean number of tactile and electrical stimuli is given in relation to the conditioned reflex of the 1st and 2nd order, respectively (the figures in parentheses indicate standard error; significance of differences was calculated by the "t" test).

* 21 rats, which easily developed an optimal behaviour (well-balanced)

** 15 rats, incapable of having a proper conditioned behaviour (agitated, aggressive).

*Table 3*

**Effect of procaine and reserpine, administered separately or in association, on the rectal temperature of rats**

| | $T°_m$ | 30′ | 60′ | 90′ | 120′ | 190′ | 240′ |
|---|---|---|---|---|---|---|---|
| Single procaine dose 5 mg/kg.b.wt., i.p. | 37.5(±0.24) | 36.8(±0.86) dif. =0.7 p <0.005 | 36.9(±0.45) dif. = 0.6 p >0.025 | 37.26(±0.44) dif. =0.3 p = N | 37.49(±0.11) dif. =0.01 p = N | | |
| Single reserpine dose 2 mg/kg.b.wt., i.m. | 37(±0.20) | 37.78*(±0.55) dif. = 0.78 p >0.001 | 37.92**(±0.59) dif. =0.92 p >0.001 | | 36.4(±0.35) dif. =0.6 p >0.05 | | |
| Single reserpine dose after procaine | 37.53(±0.48) | 38.07±0.92 dif. = 0.6 p <0.005 | 38.25±0.67 dif. =0.72 p <0.005 | | 38±0.55 dif. =0.47 p <0.005 | | 36±0.8 dif. =0.63 p = N |
| Reserpine after chronical administration of procaine (5 days). | 37.7±0.61 | 37.4±0.78 dif. = 0.3 p = N | 37.6±0.52 dif. =0.1 p = N | 37.6±0.12 dif. =0.3 p = N | 37.4±0.54 dif. =0.3 p = N | 37.2±0.63 dif. =0.5 p = N | 36.1±1.1 dif. =1 p <0.05 |

The figures in parentheses indicate standard error; significance of differences (p) was calculated by the "t" test.
* Mean value of t° in the 7 rats which presented hyperthermia.
** Mean value of t° in the 5 rats which presented hyperthermia.

*Table 4*

**Influence of procaine on palpebral ptosis induced by reserpine in rats (3 lots of ten rats each)**

| | Total scores | | | Modification of reserpine effect on ptosis (%) | | |
|---|---|---|---|---|---|---|
| | 30′ | 60′ | 90′ | 30′ | 60′ | 90′ |
| Reserpine 2 mg/kg.b.wt., i.m. | 15 | 25 | 30 | | | |
| Reserpine 20′ after a single procaine dose—5 mg/kg.b.wt., i.p. | 11 | 25 | 32 | −9 | 0 | +6 |
| Reserpine 20′ after the last dose of procaine administered chronically (5 days). | 11 | 27 | 32 | −9 | +8 | +6 |

Differences are statistically non-significant.

**Résumé.** L'amélioration des fonctions neuro-psychiques et, spécialement, des états dépressifs chez les personnes âgées traitées au Gérovital $H_3$, a été mise en évidence pour la première fois par Ana Aslan. Ultérieurement, de nombreuses recherches cliniques et expérimentales ont démontré l'action de cette substance sur le système nerveux central. Les investigations psychologiques et électroencéphalographiques ont complété les observations cliniques. Du point de vue biochimique, dans le mécanisme d'action de la procaïne un rôle important est détenu par sa capacité d'inhibition de la monoaminoxydase.

Les auteurs ont essayé de définir le type d'inhibition en utilisant une série de tests pharmacodynamiques. Les recherches sur les rats ont révélé que la procaïne est antagoniste à l'aggravation du comportement et qu'elle intensifie et prolonge l'hyperthermie, retarde l'hyperthermie induite par la réserpine et ne prévient pas la ptose palpébrale induite par la réserpine. Ces données suggèrent un effet inhibiteur du B-MAO. Contrairement aux inhibiteurs classiques MAO, les produits à base de procaïne sont bien tolérés par les patients âgés.

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# L'ACTION LOCALE DU GÉROVITAL $H_3$ DANS LE VIEILLISSEMENT EXPÉRIMENTAL DU TISSU CUTANÉ

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**Résumé.** Les recherches électronomicroscopiques effectuées sur les téguments des rats soumis à l'action des rayons ultraviolets, dont un groupe a été protégé par le Gérovital $H_3$, appliqué sous forme de crème, ont révélé que la détérioration des structures tégumentaires est beaucoup moins importante chez le groupe traité.

L'action protectrice s'est exercée aussi bien au niveau des couches cellulaires biologiquement actives de l'épiderme qu'au niveaux des tonofilaments et des mitochondries.

Du point de vue clinique on a noté chez les sujets traités au Gérovital $H_3$, une amélioration évidente de la trophicité des téguments. Le Gérovital $H_3$, sous forme de crème de beauté, assure la trophicité et la régénération de la peau et donne aussi des résultats remarquables dans les brûlures du premier et du second degré [1].

Le but de cette étude a été de vérifier l'action topique du Gerovital $H_3$ en applications locales contre les lésions dermiques provoquées par l'action des rayons ultraviolets. (r.UV).

## MATÉRIEL ET MÉTHODE

L'étude a porté sur 18 rats blancs Wistar âgés de 6 mois divisés en trois groupes: A — animaux traités à la crème à base de Gérovital $H_3$; B — animaux traités à une crème semblable mais qui ne contient pas le Gérovital H3 et groupe C — témoin. Les deux crèmes avec et sans Gérovital $H_3$ ont été préparées à la pharmacie de l'Institut.

Après l'épilation de la face dorsale des animaux on a provoqué des lésions par exposition à l'action des r.UV, chaque jour pendant trois semaines. Nous avons utilisé un appareil de radiation UV-IR au domaine de radiation: 280–314 nanomètres.

La durée de chaque séance: 20 minutes, à 100 cm de distance de la source UV. Avant et après chaque exposition aux r.UV on a appliqué la crème à base de Gérovital $H_3$ au groupe A, la crème sans Gérovital $H_3$ au groupe B, tandis que le groupe C est resté sans aucune application locale.

Les biopsies cutanées pour l'étude électronomicroscopique ont été faites le 21e jour de l'expérience. Les fragments de peau ont été fixés en glutaraldéhyde et incorporés dans Epon 812; le contraste des images a été obtenu avec le citrate de plomb Reynolds [2]. Les blocs ont été sectionnés avec un Ultrotom LKB. L'examen et la photographie ont été réalisés à l'aide d'un microscope Opton E $M_9$ F.

## RÉSULTATS ET DISCUSSIONS

Le derme des rats adults est constitué de faisceaux collagènes denses et serrés dans la zone profonde et d'un réseau de fibres élastiques fines. Des fibroblasts actifs s'observent surtout dans le derme moyen et profond [3], [4]. Le réticulum endoplasmique est bien développé, avec de nombreuses granulations ribosomales.

Par conséquence de l'application des r.UV, le matériel élastique disparaît progressivement et les cellules fibroblastiques présentent des détériorations exprimées par l'apparition de larges formations vacuolaires et des signes de sécrétion altérée.

L'étude électronooptique a démontré aussi qu'à la suite de l'action des r.UV se produit une destruction des myofilaments, une abondante vacuolisation cytoplasmique, avec les membranes cellulaires parfois détruites. Le réticule rugueux est faiblement représenté, pauvre en ribosomes. Les mitochondries sont dilatées, avec les crêtes fragmentées, dégradées. Les cellules basales et surbasales sont relativement applatives avec œdème prononcé (figs 1 et 2).

Chez les animaux qui ont reçu le traitement local au Gérovital $H_3$ on a remarqué la préservation des structures normales, qui n'ont pas été puissament influencées par les r.UV. Le réticule rugueux est bien représenté avec de nombreux ribosomes attachés. Sur un fonds lysosomal abondant, les myofilaments se présentent sous forme de tonofilaments. Les espaces intercellulaires et les desmosomes ont un aspect normal. Les mitochondries sont rondes, en général avec une disposition périnucléaire, avec des crêtes courtes et inégales, traduisant une activité physiologique intense, parfois avec de discrètes vacuolisations.

Dans la jonction dermo-épidermique la membrane basale est indigne du point de vue ultrastructural, sous forme d'une bandelette continue. Les jonctions desmosomales sont normales, avec quelques aspects de vacuolisation. Les tonofilaments sont en faisceaux, la couche granuleuse avec de la kératohyaline et des kératinosomes (figs 3 et 4).

On n'a pas trouvé une différence saisissable entre les groupes B et C.

La stimulation de la régénération des tissus par le Gérovital H3, confirmée par des données cliniques et expérimentales, est évidente, surtout au niveau de la peau où il produit un aspect de rajeunissement [5]. La cicatrisation plus rapide de plaies à la suite du traitement au Gérovital $H_3$ a été remarquée aussi.

Indiqué dans les dystrophies généralisées ou localisées [6], [7], le Gérovital $H_3$ assure et entretient la nutrition de la peau sèche et donne aussi des résultats remarquables dans les brûlures légères [1].

En conclusion, notre expérimentation apporte des données de morphologie électronooptique qui révèlent l'action protectrice du Gérovital $H_3$ envers les lésions produites par les r.UV.

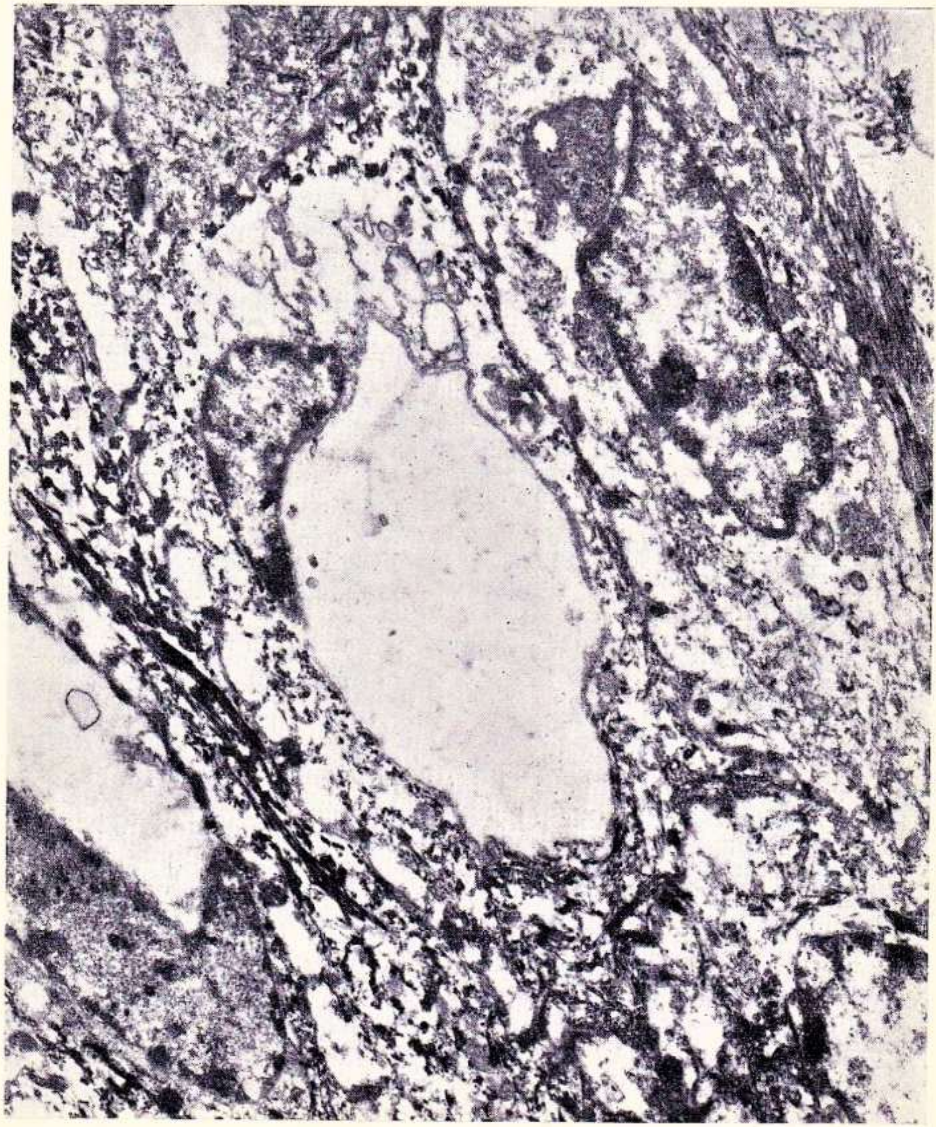

Fig. 1. — *Peau de rat témoin.* À la jonction dermoépidermique la membrane basale sous forme de bandelette discontinue. Importantes vacuolisations intracytoplasmiques. Mitochondries dilatées, détériorées, cellules basales et surbasales relativement applaties, quelques-unes à œdème prononcé. Fragmentation et destruction des tonofilaments.

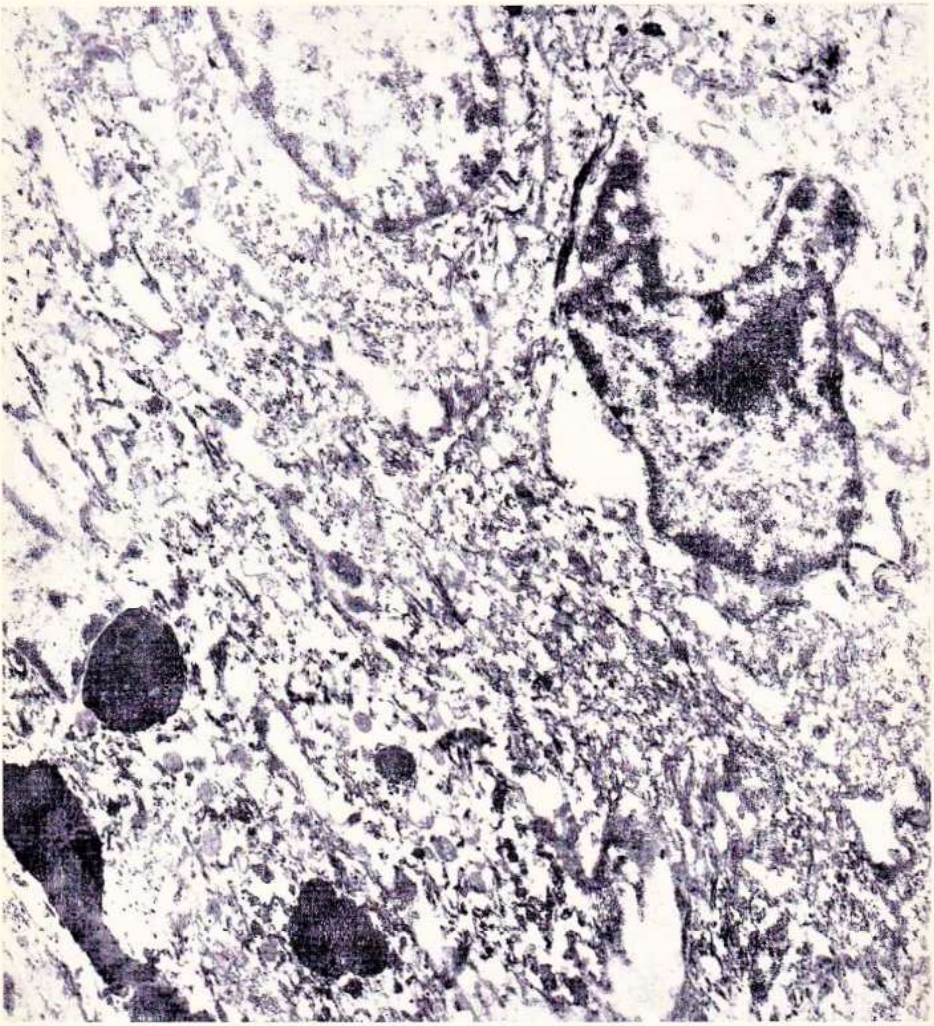

Fig. 2. — *Peau de rat traitée au Gérovital $H_3$.* Couche malpighienne granuleuse et cornifiée. Réseau endoplasmique rugueux bien représenté. Petites mitochondries à courtes crêtes inégales. Nombreux ribosomes. Tonofilaments en faisceaux. Lysosomes. Couche granuleuse et à kératohyaline. Lamelle cornéuse et kératinosomes.

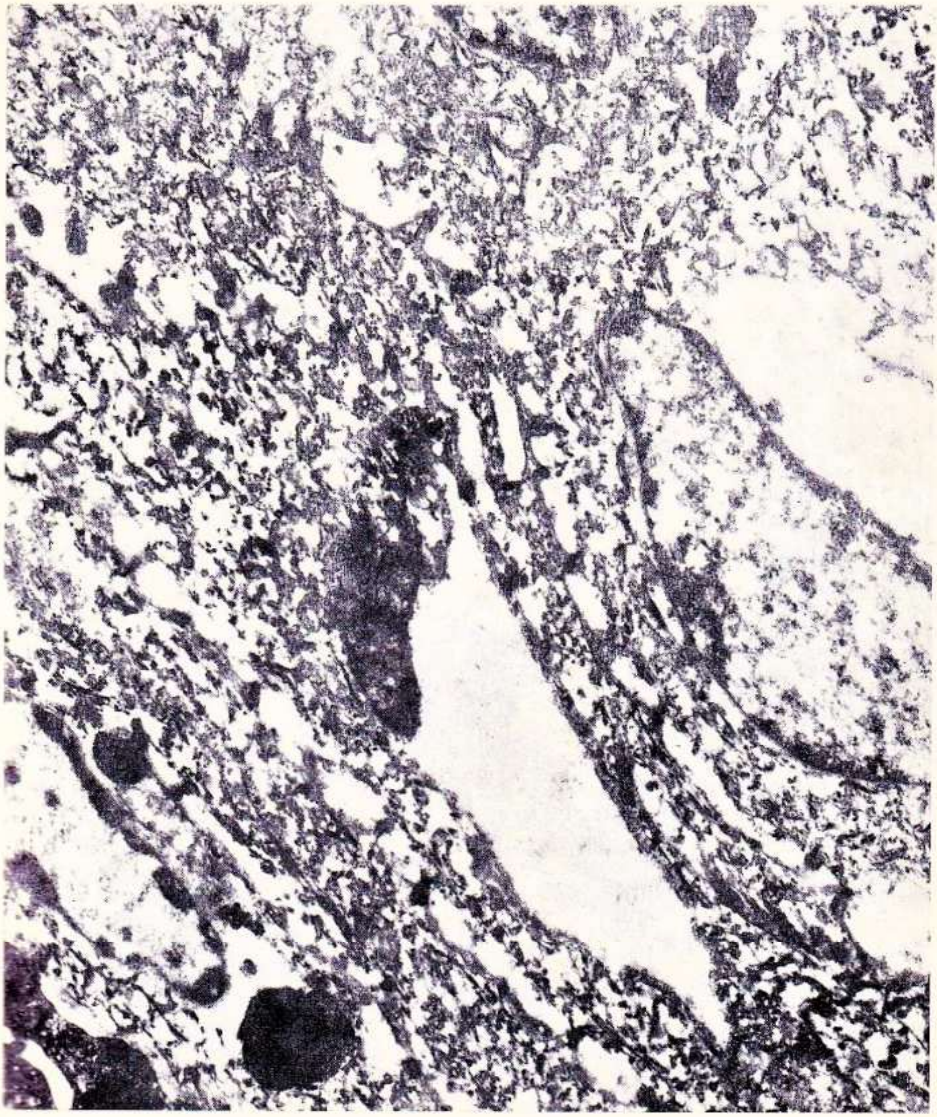

Fig. 3. — *Peau de rat témoin.* Grandes vacuolisations intracytoplasmiques et périnucléaires. Mitochondries à crêtes fragmentées quelque-unes à vacuolisations, ratatinées. Tonofilaments et agglomérations de kératine. Réseau endoplasmique un peu tuméfié. Rares ribosomes isolés. Lamelle cornifiée.

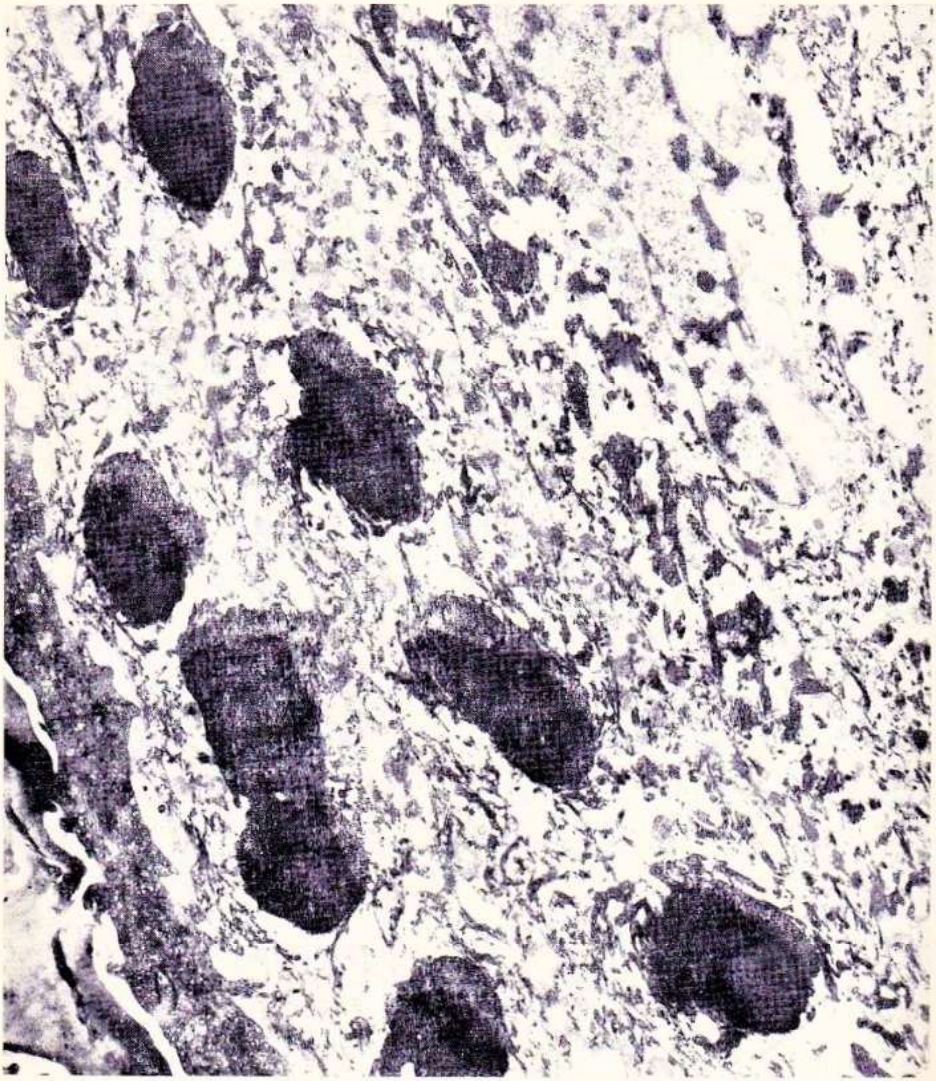

Fig. 4. — *Peau de rat traitée au Géroctol* $H_3$. Couche malpighienne supérieure, granuleuse et cornifiée. Espace intercellulaire à desmosomes normaux. Réseau endoplasmique rugueux, ribosomes attachés au réticule endoplasmique. Faisceaux et tonofilaments. Couche granuleuse avec de grands blocs de kératohyalin à forme ovale et irrégulière. Fréquents lysosomes, jonctions desmosomales normales. Cellule cornifiée. Mitochondries à crêtes courtes et inégales, kératinosomes.

**Summary.** Electron microscopic studies performed on the rat skin submitted to U.V. rays revealed a less severe deterioration of the skin structures in the animal group whose skin was protected by Gerovital $H_3$ cream in comparison with the control group.

The protective action of Gerovital $H_3$ was observed at the level of the biologically active cellular layers of the skin as well as in tonofilaments and mitochondria, proving an intensive physiological activity of these cell organelles.

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# ÉTUDE R.E.S. SUR LE SÉRUM ET LE SANG TOTAL HUMAIN EN RELATION AVEC LE VIEILLISSEMENT, LA PATHOLOGIE ET LE TRAITEMENT BIOTROPHIQUE AU GÉROVITAL $H_3$

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**Résumé.** On a déterminé la concentration de centres paramagnétiques par des mesures R.E.S. effectuées sur 110 épreuves de sérum et de sang total humain prélevées à 30 sujets jeunes (20–39 ans), 36 sujets âgés orthogères (60–85 ans) et 34 sujets âgés (60–90 ans) aux tares pathologiques (affections cardio-vasculaires). Les épreuves de sérum provenant des sujets âgés orthogères présentent une concentration de centres paramagnétiques manifestement plus grande ($p < 0{,}02$) que celle du sérum des sujets jeunes. Entre la concentration de centres paramagnétiques du sérum des sujets âgés aux tares pathologiques et celle du sérum des sujets âgés aux tares pathologiques soumis à un traitement de longue durée au Gérovital H3, la différence est aussi significative du point de vue statistique ($p < 0{,}02$).

## INTRODUCTION

Le vieillissement des organismes vivants représente un phénomène très complexe, consistant dans une décroissance continuelle de la capacité intégrative et adaptative de l'organisme jusqu'il atteint un stade incompatible à la vie. Cette définition du processus en tant qu'une totalité couvre un grand nombre de changements structuraux et fonctionnels spécifiques des paramètres aux niveaux moléculaire et surmoléculaire.

Une telle complexité des faits explique les difficultés rencontrées dans les essais d'élaborer une théorie unique, scientifique, hypothétique-déductive du processus de vieillissement.

Un de ces essais, « la théorie des radicaux libres », permet l'interprétation de certains aspects du vieillissement, en supposant que le nombre des réactions des radicaux libres de l'organisme et les effets qu'ont sur celui-ci, augmentent avec l'avancement en âge. Les radicaux libres, du fait de leur grande réactivité chimique, peuvent induire des processus d'altération fonctionnelle et structurale de l'intégrité de l'organisme [1], [2].

L'augmentation avec l'âge des lipo-peroxydes qui s'accumulent dans les membranes cellulaires et dans des formations spéciales (dénommées pigments d'âge) [3], la décroissance du taux de mortalité des rats soumis à une diète aux acides gras non saturés [4] et l'augmentation de la durée moyenne de vie à la suite de la médication aux anti-oxydants, tels que vitamine E, 2-mercapto-éthylamine, hydroxytoluène butylate [5] constituent quelques épreuves expérimentales à l'appui de la théorie du rôle des radicaux libres dans le processus de vieillissement.

La cellule vivante contient du fer, du cuivre, du manganèse et autres ions paramagnétiques, qui, par leur action de diminution de l'énergie d'activation facilitent la production de radicaux libres par la décomposition des produits peroxydiques. Les ions paramagnétiques et les radicaux libres organiques, qui constituent les centres paramagnétiques dans les épreuves biologiques, peuvent être détectés et évalués quantitativement par la technique de la résonance paramagnétique de spin (R.E.S).

Ce travail expose les résultats d'une recherche expérimentale effectuée sur sérum humain normal et sur sérum humain prélevé aux sujets ayant des tares pathologiques (affections cardiovasculaires); on présente aussi les implications du traitement au Gérovital $H_3$ sur la concentration de centres paramagnétiques du sérum.

## MATÉRIAUX ET MÉTHODES DE TRAVAIL

Les mesures de spectroscopie électronique de spin (R.E.S.) ont été effectuées sur 110 épreuves de sérum et de sang total prélevée à 30 sujets jeunes (20—39 ans), 36 sujets âgés orthogères (60—85 ans) et 34 sujets âgés (60—90 ans) aux tares pathologiques (affections cardio-vasculaires). De plus, on a effectué 10 mesures sur des épreuves provenues des sujets âgés ayant des affections cardio-vasculaires, soumis à un traitement de longue durée au Gérovital $H_3$.

Les épreuves prélevées ont été introduites et conservées en azote liquide et ensuite lyophylisées. La lyophylisation des épreuves a été effectuée en petits flacons de pénicilline, quatre-cinq heures étant nécessaires pour une épreuve. Après la lyophylisation, les épreuves ont été pesées et introduites dans des tubes de quartz. La lyophylisation a été effectuée à l'aide d'un appareil du type SECFROID LYO-LAB-C-3021. Pour éviter le contact à l'air, l'installation a été lavée et remplie d'azote-gaz. Pour l'enregistrement des spectres R.E.S. des épreuves biologiques on a utilisé un spectromètre R.E.S. du type JES-ME-3X à cavité de résonance cylindrique, la fréquence de résonance $f_0 = 9455$ MHz et la modulation intérieure du champ magnétique statique (100 kHz). Les spectres R.E.S. ont été enregistrés à la température ambiante et dans des conditions de travail identiques pour l'entier ensemble. Pour la détermination du nombre « N » de centres paramagnétiques de l'épreuve, on a utilisé une méthode relative de détermination de la concentration, par comparaison au nombre de centres paramagnétiques « *Ne* » d'une épreuve-étalon — DPPH (diphénylpicrylhydrazyl)

$$N = N_e \cdot \frac{m}{m_e} \cdot \frac{H^2_{pp} \cdot A \cdot \rho}{H^{e2}_{pp} \cdot A_e \cdot \rho_e} \cdot \frac{H^2_{mod}}{H^2_{mod}}$$

L'indice *e* se rapporte à l'épreuve-étalon. On a noté par $m$, la masse de l'épreuve exprimée en grammes. $\rho$ est la densité de l'épreuve en g/cm³, $H_{mod}$ — l'amplitude du champ magnétique de modulation, $H_{pp}$ — la largeur de la ligne de résonance mesurée entre les sommets de la déviation, et $A$ — l'amplitude de la ligne de résonance.

$N_e = 1{,}52.10^{21}$ centres paramagnétiques.

## RÉSULTATS ET DISCUSSIONS

Les spectres R.E.S. obtenus des épreuves de sang total diffèrent des spectres R.E.S. obtenus du sérum des mêmes personnes, à cause, probablement, des produits d'oxydation ou de scission de l'hémoglobine.

*Pour les épreuves de sérum* (fig. 1), les spectres R.E.S. sont composés, pour la majorité des épreuves, d'une ligne de résonance caractérisée par un facteur de

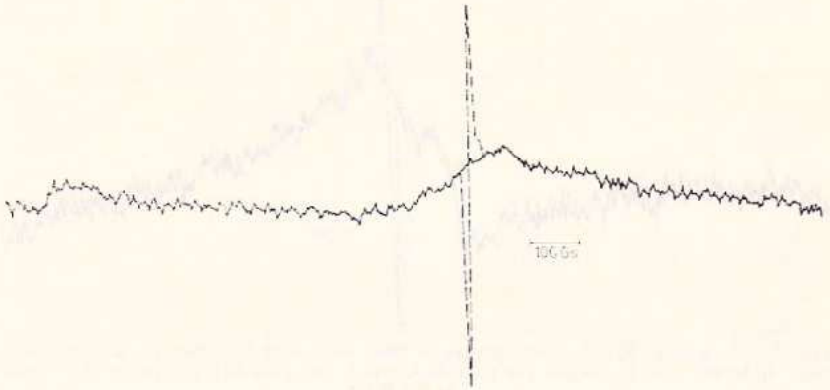

Fig. 1. — Le spectre R.E.S. d'une épreuve de sérum dans la région $g = 2{,}0$. Conditions de travail: modulation: 10 gauss; puissance de micro-ondes: 20 mW; amplification: 2000; temps de balayage: 10 minutes. (en pointillé: le spectre de l'étalon: D.P.P.H.)

scission spectroscopique $g_{eff} \approx 2{,}003$ (valeur moyenne). La largeur de la ligne de résonnance est d'environ 20 gauss. Certaines épreuves présentent en plus dans le spectre R.E.S. une autre ligne de résonance caractérisée par un facteur de scission spectroscopique $g_{eff} = 4{,}00$, la largeur de la ligne étant de 100—200 gauss. On attribue cette ligne à la présence de l'ion $Fe^{3+}$ dans l'épreuve. La ligne de résonance à $g_{eff} = 2{,}003$ est attribuée à la présence dans l'épreuve de certains centres paramagnétiques complexés [6]. Nous supposons que dans cette ligne de résonance est incluse encore une ligne de résonance qui est attribuée à un radical libre, ligne qui est caractérisée par un $g_{eff}$ rapproché de celui de l'électron libre; la largeur de la ligne a la valeur de 2—10 gauss.

De ce fait, ces deux lignes de résonance se superposent et, de la sorte, il n'a pas été possible, en partant des mesures effectuées, de mettre en évidence la ligne correspondante du radical libre. Dans l'hypothèse de l'existence des radicaux libres dans l'épreuve, leur mise en évidence par la méthode R.E.S. pourrait être réalisée en mesurant le temps de relaxation spin-réseau ($T_1$) des centres paramagnétiques et du radical libre. Ces mesures supposent l'utilisation de la méthode de la saturation progressive de la ligne de résonance, à de basses températures et à de grands niveaux de puissance des micro-ondes. La techniques est laborieuse et exige une installation annexe au spectromètre de résonance standard.

On n'a mis en évidence la présence dans 27% des épreuves du sérum d'aucuns centres paramagnétiques.

*Pour les épreuves de sang total* (fig. 2), les spectres R.E.S. se composent d'une ligne de résonance à $g_{eff} \approx 2$, qui apparaît à 97% des épreuves.

Une deuxième ligne de résonance est caractérisée par $g_{eff} \approx 4$. Ces deux lignes de résonance sont attribuées à la présence dans l'épreuve des ions paramagnétiques complexés, respectivement, $Fe^{3+}$. Les largeurs des lignes de résonance sont d'environ 20 gauss, respectivement 100—200 gauss.

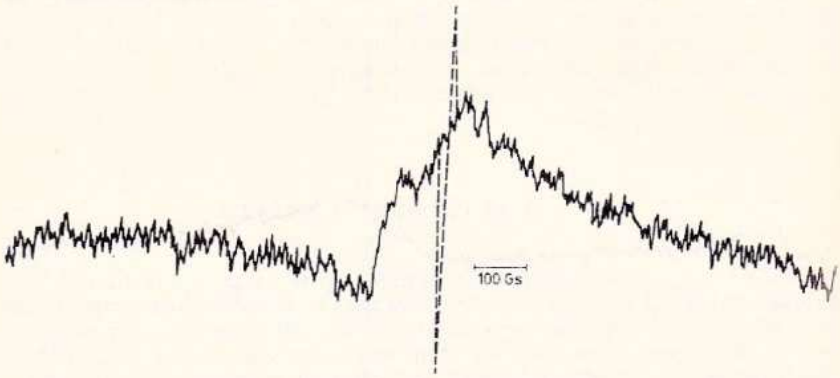


Fig. 2. — Le spectre R.E.S. d'une épreuve de sang total dans la région $g = 2{,}0$. Les conditions de travail sont les mêmes que pour la figure 1 (en pointillé: le spectre de l'étalon: D.P.P.H.)

Nous supposons également qu'au-dessus de la ligne de résonance caractéristique aux ions paramagnétiques ($g_{eff} \approx 2$) se trouve superposée la ligne caractéristique d'un éventuel radical libre, qui a la largeur de la ligne comprise entre approximativement 2—10 gauss.

Pour les spectres de résonance des épreuves de sang total est caractéristique le fait que la majorité des épreuves présente un spectre composé de 2—3 lignes, tandis que les épreuves de sérum présentent, dans leur grande majorité, un spectre de résonance composé d'une seule ligne. Le nombre moyen de centres paramagnétiques des épreuves du sérum (tableau 1) provenues du groupe de sujets âgés orthogères est manifestement plus grand ($p < 0{,}02$) que la concentration de centres paramagnétiques du sérum provenu du groupe de sujets jeunes.

Entre la concentration de centres paramagnétiques du sérum des sujets âgés du groupe orthogère et celle du sérum des sujets âgés aux tares pathologiques il existe une différence non significative ($p > 0{,}05$).

Entre la concentration de centres paramagnétiques du sérum des sujets âgés appartenant au groupe aux tares pathologiques et celle du sérum des sujets âgés aux tares pathologiques qui suivent un traitement de longue durée au Gérovital $H_3$, la différence est significative du point de vue statistique ($p < 0{,}02$).

La concentration de centres paramagnétiques des épreuves de sang total des sujets âgés orthogères ne diffère pas de façon significative de celle du sang total des sujets jeunes ($p > 0{,}05$); entre le groupe de sujets âgés aux tares pathologiques et le groupe de ceux ayant des tares pathologiques, qui ont subi un traitement de longue durée au Gérovital $H_3$, il n'existe non plus de différences significatives du point de vue statistique ($p > 0{,}1$); entre le groupe de sujets âgés orthogères et

*Tableau 1*

**Le facteur de scission spectroscopique et la concentration de centres paramagnétiques dans les épreuves de sang total et sérum en diverses conditions d'âge, pathologie et traitement (on donne, entre parenthèses, les dispersions des moyennes calculées pour chaque groupe)**

| Sujets | Nombre de cas | $g$ | | Nombre des centres paramagnétiques ($10^{16}$ spin/g) | |
|---|---|---|---|---|---|
| | | sang | sérum | sang | sérum |
| Jeunes | 10 | | 1,996 (0) | | 0,335 (0,063) |
| | 20 | 1,972 (0,041) | | 2,476 (0,558) | |
| Âgés orthogères | 12 | | 1,992 (0,022) | | 0,847 (0,377) |
| | 13 | 2,001 (0) | | 2,135 (0,321) | |
| Âgés traités au $GH_3$ | 10 | 2,004 (0) | 1,996 (0) | 2,678 (0,413) | 0,258 (0,141) |
| Âgés aux tares pathologiques | 16 | 2,009 (0,004) | 1,995 (0,003) | 1,779 (0,584) | 0,661 (0,327) |

le groupe de ceux ayant des tares pathologiques il n'y a pas une différence significative du point de vue statistique en ce qui concerne la concentration de centres paramagnétiques des épreuves de sang total.

Les données exposées nous suggèrent la corrélation nécessaire qui existe entre la présence des oligoéléments paramagnétiques et les radicaux libres des produits biologiques, ainsi que l'influence du traitement au Gérovital H3 sur la concentration de centres paramagnétiques, influence qui peut être attribuée à certaines propriétés anti-oxydantes de la médication biotrophique.

**Summary.** The concentration of paramagnetic centres was evaluated by means of R.E.S. measurements on 110 serum and blood specimens from 30 young (aged 20–39), 36 orthogerous elderly (aged 60–85) and 34 elderly subjects (aged 60–90) with pathological problems (cardiovascular affections). The serum concentration of paramagnetic centers is significantly higher ($p>0.02$) in orthogerous elderly as against young subjects. The difference is also statistically significant ($p>0.02$) between the serum concentration of paramagnetic centres in the elderly with pathological problems and that of the elderly subjected to the long-term treatment with Gerovital $H_3$.

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# MODIFICATIONS DU SYNDROME HUMORAL CHEZ LES ATHÉROSCLÉREUX ÂGÉS SOUS L'INFLUENCE DU TRAITEMENT BIOTROPHIQUE À L'ASLAVITAL

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**Résumé.** En appliquant à un groupe d'athéroscléreux âgés la thérapie intensive de durée relativement courte (90 jours) à l'Aslavital, les auteurs démontrent l'influence favorable par ce produit procaïnique sur la plupart des troubles appartenant au syndrome humoral.

Les propriétés antiathérogènes de cette médication se sont manifestées par une action antithrombogène, démontrée par l'augmentation de l'héparinémie endogène, l'activation de la fibrinolyse, la réduction de la fibrinogénémie, la diminution de l'hyperfonction thrombocytaire et le redressement de l'activité lipoprotéinelipasique.

Des effets plus réduits ont été obtenus sur les modifications dyslipémiques et dysglycoprotéinémiques.

On arrive à la conclusion de la nécessité d'appliquer systématiquement le traitement biotrophique à l'Aslavital dans le traitement et la prophylaxie de l'athérosclérose.

Les nombreux faits d'observation clinique et expérimentale qui ont fait l'objet des recherches des récentes années ont documenté l'utilisation large des substances biotrophiques dans la thérapeutique du vieillissement et des affections dégénératives liées à l'involution de l'organisme [1]–[8]. La supériorité des résultats obtenus par la chimiothérapie à la procaïne, d'après le procédé du prof. Ana Aslan — en utilisant les produits biotrophiques originaux, Gérovital $H_3$ et Aslavital — ont justifié l'extension de l'application de ces préparations à l'échelle internationale. [9]–[17].

La reconnaissance des troubles métaboliques comme facteurs de base dans la pathogenèse de la dystrophie d'involution et surtout par des affections dégénératives justifie l'utilisation des produits biotrophiques à action métabolique dans le traitement et la prévention de l'athérosclérose [18]–[21].

En ce domaine il faut souligner le fait que les effets eutrophisants du Gérovital $H_3$ et de l'Aslavital, au niveau des organes plus fréquemment atteints par la pathologie dégénérative (appareil cardio-vasculaire, système nerveux, etc.) sont révélés surtout par des paramètres cliniques et des essais fonctionnels, et moins par des modifications humorales. On connaît aujourd'hui l'importance de l'identification des déviations humorales chez les athéroscléreux, ainsi que la possibilité d'utiliser les éventuelles corrections qu'on peut obtenir par la médication anti-athérogène, comme indicateur de l'efficience de la substance respective.

Étant donné la pathogénie complexe de la maladie, les constantes du milieu interne circulant — reflétant les corrélations entre les principaux facteurs pathogéniques et les corrections éventuelles produites par la médication anti-athérogène — peuvent donner des indications précieuses concernant l'efficience de ce traitement.

Nous nous sommes proposé d'étudier en ce sens les modifications du syndrome humoral par les indicateurs complexes, sur un groupe d'athérosclérotiques âgés, sous l'influence de la thérapie biotrophique de courte durée à l'Aslavital.

## MATÉRIEL ET MÉTHODE

Les effets thérapeutiques de l'Aslavital ont été étudiés sur 43 sujets âgés, 24 ♂ et 19 ♀, entre 60 et 85 ans (la moyenne d'âge étant 70 ans), sélectionnés du point de vue clinique et par des explorations fonctionnelles, comme athérosclérotiques en un état manifeste de maladie, à différentes localisations prédominantes du point de vue clinique.

Le tableau 1 présente la répartition des patients en rapport avec le sexe, l'âge, l'état de l'évolution et la localisation clinique prédominante de la maladie athéromateuse.

*Tableau 1*

**Répartition des patients par rapport au sexe, à l'âge au stade évolutif et à la localisation clinique prédominante de la maladie**

| Nombre de cas | Athérosclérose généralisée | Athérosclérose coronaire | Athérosclérose cérébrale | Athérosclérose périphérique |
|---|---|---|---|---|
| ♂ | 6 | 7 | 7 | 4 |
| ♀ | 9 | 4 | 5 | 1 |
| Total | 15 | 11 | 12 | 5 |
| Nombre de cas à accidents vasculaires | — | 4 | 5 | — |

Outre l'examen clinique et fonctionnel, on a effectué un examen humoral complexe, par une série représentative de tests de laboratoire, en vue d'apprécier les modifications de type thrombophilique, la fonctionnalité thrombocytaire, la dyslipidémie et la dysglycoprotéinémie:

1. Évaluation de l'activité héparinique du sang — exprimée en unités internationales/ml — d'après la méthode Pieptea [22].
2. Détermination de la coagulabilité globale du sang par:
   *a)* enregistrement thrombélastographique (TEG)
   *b)* épreuve de tolérance à l'héparine « in vitro » (Soulier).
3. Détermination de l'activité thrombodynamique thrombocytaire (ATT), calculée en partant de la différence entre les amplitudes maximales de 2 diagrammes TEG, effectuées simultanément sur le plasma de sédimentation (riche en plaquettes) et sur le plasma rigoureusement dépourvu de plaquettes par centrifugation.
4. Détermination de l'activité fibrinolytique plasmatique [22] par le test de lyse des euglobulines (TLE).
5. Bilan lipidique:
   *a)* dosage du cholestérol dans le sérum [23];
   *b)* dosage de la lipémie totale [23];

*c)* appréciation turbidimétrique des bêtalipoprotéines du sérum par le test Burstein (à l'héparine) [23].

6. Détermination de l'activité lipoprotéine lipasique (LPL) « in vitro » (méthode turbidimétrique).

7. Dosage des hexoses totales et des mucoprotéines du sérum [24].

Les investigations cliniques et humorales ont été effectuées sur les patients athéroscléreux, autant avant, qu'à la suite de l'application du traitement à l'Aslavital, pendant 90 jours, d'après le schéma suivant: 5 séries de 12 ampoules d'Aslavital (5 ml), chaque jour une injection intramusculaire, avec une pause de 5 à 7 jours entre les séries. Pendant l'application du traitement on a évité d'associer d'autres médicaments à effets anti-athérogènes ou lipoconvertisseurs.

## RÉSULTATS

Le bilan statistique général de la fréquence des troubles humoraux — métaboliques ou thrombophiliques — signalés avant le traitement dans le lot d'athéroscléreux étudiés, est représenté schématiquement dans la fig. 1. On remarque le grand pourcentage de cas à hypohéparinémie endogène (75%), les autres déréglages humoraux se succédant en ordre décroissant: hyperfonctionnalité thrombocytaire (69%), diminution de la LPL « in vitro » (67%), hypercoagulabilité et/ou hypofibrinolyse (65%), hyperbêtalipoprotéinémie (62%), hypermucoprotéinémie (56%), hyperhexosémie (51%), hyperlipémie (41%), hypercholestérolémie (25%).

À la suite du traitement à l'Aslavital on a mis en évidence des modifications significatives du syndrome humoral concernant la plupart des troubles signalés au début.

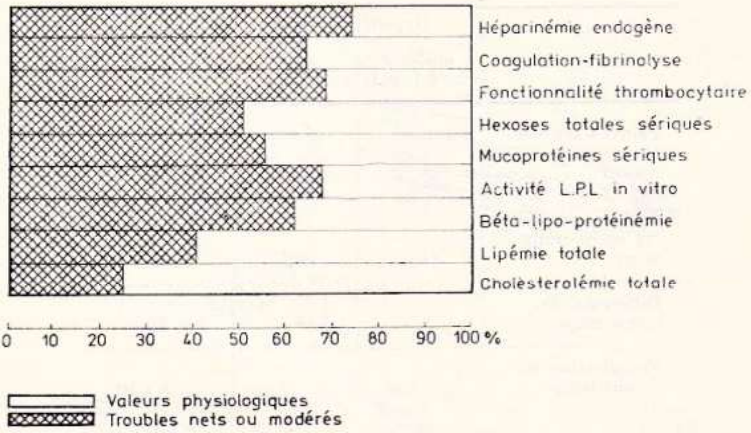


Fig. 1. — Pourcentage des troubles métaboliques et thrombophiliques avant traitement, dans le groupe des athéroscléreux investigués.

1. L'activité anticoagulante de type héparinique du sang exprimée en unités/ml — diminuée chez la plupart des patients, apparaît nettement influencée par la thérapie à l'Aslavital. Après 5 séries de traitement on constate une diminution

significative du nombre de cas d'hypohéparinémie nette ou modérée, ainsi que l'accroissement significatif des valeurs moyennes d'héparinémie endogène (fig. 2, tableau 2).

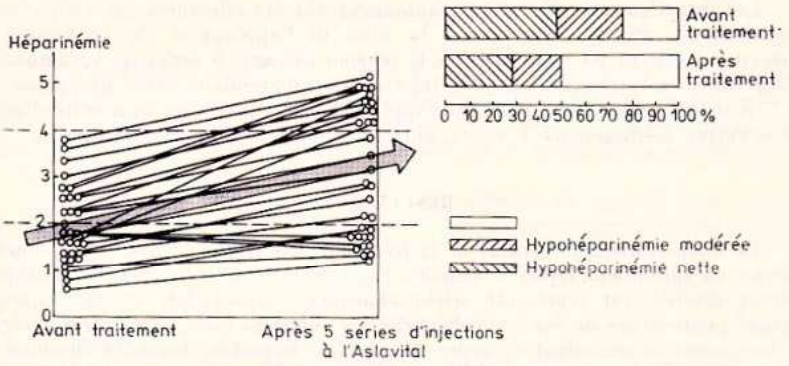


Fig. 2. — Valeurs individuelles et moyennes de l'héparinémie endogène chez les athéroscléreux, avant et après le traitement biotrophique à l'Aslavital.

*Tableau 2*

**Évaluation statistique de l'effet de la thérapie sur l'héparinemie endogène chez les athéroscléreux**

| | Hypohéparinémie | | Héparinémie normale (> 4 U/m.l.) |
|---|---|---|---|
| | modérée (2—4 U/m.l) | nette (< 2 u/m.l.) | |
| Pourcentage des cas avant traitement | 28 | 46,5 | 25,5 |
| Pourcentage des cas après traitement | 18,5 | 28,5 | 53 |
| Différence de pourcentage | −9,5 | −18 | +27,5 |
| Signification de la différence | $p < 0{,}01$ | $p < 0{,}01$ | $p < 0{,}01$ |

2. L'action favorable de l'Aslavital sur les troubles thrombophiliques chez les athérosclereux s'est concrétisée d'une part par la diminution remarquable du nombre des sujets à hypercoagulabilité plasmatique et/ou hypofibrinolyse, après l'application du médicament (tableau 3, fig. 3), et d'autre part par la diminution significative de l'hyperfonctionnalité thrombocytaire, pour un pourcentage élevé de cas (tableau 3, fig. 4).

On remarque tout spécialement la diminution significative des valeurs moyennes concernant le TLE, ainsi que la diminution des valeurs moyennes de l'ATT. L'hyperfibrinogénémie apparaît aussi favorablement influencée dans un nombre important de cas, fait démontré par la réduction de l'amplitude maximale de la valeur du TEG sur le plasma dépourvu de plaquettes.

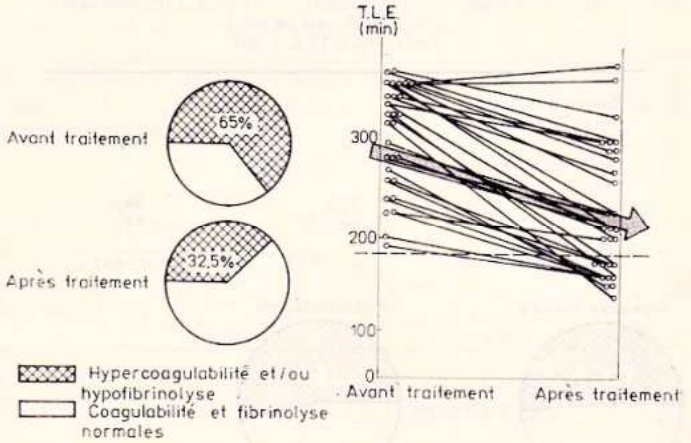


Fig. 3. — Influence de la thérapie biotrophique sur les troubles thrombophiliques chez les athéroscléreux.

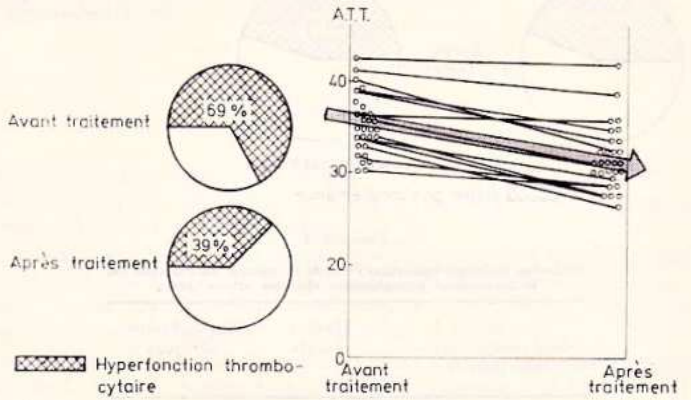


Fig. 4. — Influence de la thérapie biotrophique sur l'activité thrombodynamique thrombocytaire chez les athéroscléreux.

3. Comparativement aux troubles de type thrombophiliques et hypohépariniques, les modifications humorales dysmétaboliques (dyslipémie et dysglycoprotéinémie) apparaissent moins influencées par la thérapie de courte durée à

*Tableau 3*

**Évaluation statistique de l'effet de la thérapie à Aslavital sur la trombophilie et l'hyperfonctionnalité thrombocytaire chez les athéroscléreux**

| | Hypercoagulabilité plasmatique globale et/ou hypofibrinalyse T.E.G. r + k 20 mm et/ou am > 60 mm TLE > 180' | Hyperfonctionnalité thrombocytaire A.T.T. > 30 mm |
|---|---|---|
| Pourcentage des sujets avant traitement | 65 | 69,5 |
| Pourcentage des sujets après traitement | 32,5 | 39,5 |
| Différence % | −50 | −44 |
| Signification de la différence | $p < 0{,}01$ | $p < 0{,}01$ |

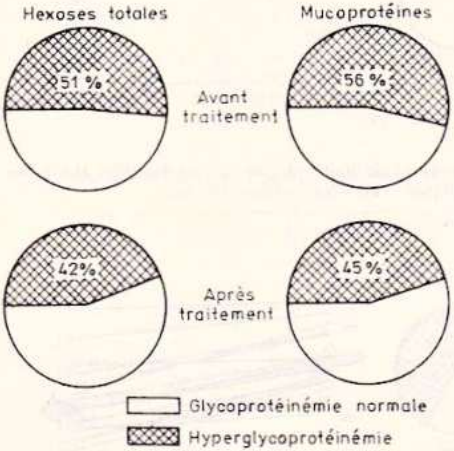


Fig. 5. — Effet de la thérapie à Aslavital sur le métabolisme glycoprotéique chez les athéroscléreux.

*Tableau 4*

**Évaluation statistique concernant l'effet de la thérapie biotrophique sur le métabolisme glycoprotéique chez les athéroscléreux**

| n = 29 cas à disglycoprotéinémie initiale | Hexase totale | Mucoprotéines sériques |
|---|---|---|
| Valeurs moyennes avant traitement | 143 mg% | 19,2 mg% |
| Valeurs moyennes après traitement | 123 mg% | 14,7 mg% |
| Différence % | −14 | −20 |
| Signification de la différence | $p < 0{,}10$ | $p < 0{,}10$ |

l'Aslavital. Quoique l'on constate une diminution du nombre de cas à niveau élevé d'hexosémie totale et de mucoprotéinémie (fig. 5), ainsi qu'une diminution des valeurs moyennes et individuelles de ces constantes, les modifications mentionnées ne paraissent pas significatives au point de vue statistique (tableau 4). De même, bien que les valeurs moyennes et individuelles concernant 11 patients à hypercholestérolémie et 15 cas à hyperlipidémie totale aient présenté des améliorations à la suite du traitement, la différence par rapport aux chiffres initiaux n'est pas significative au point de vue statistique (tableau 5).

4. Enfin, il faut remarquer l'effet nettement favorable de l'Aslavital sur l'activité lipoprotéine-lipasique « in vitro » chez les athéroscléreux. À la suite du traitement on constate une augmentation significative de l'activité de clarification plasmatique (tableau 5).

*Tableau 5*

| n =32 cas de dislipidémie initiale | Hypercholestérolémie totale n =11 cas | Hyperlipémie totale n = 17 cas | Hyperbétalipoprotéinémie (Burstein) n= 17 cas | Hypolypoprotéine lipasémie n =29 cas |
|---|---|---|---|---|
| Valeurs moyennes avant traitement | 278 mg% ±8,50 | 815 mg% ±11,30 | 52 U. ±0,90 | $k$ =1,15 |
| Valeurs moyennes après traitement | 245 mg% ±7,30 | 780 mg% ±10,20 | 47 u ±0,85 | $k$ =1,95 |
| Différence % | −11,7 | − 4,3 | −10 | +69,5 |
| Signification de la différence | non significatif | non significatif | non significatif | $p < 0,01$ |

## DISCUSSIONS

En étudiant l'ensemble des résultats obtenus, on remarque l'effet net du traitement biotrophique de courte durée sur les multiples troubles dans le cadre du syndrome humoral athéroscléreux.

La stimulation de l'activité héparinique du sang, ainsi que le freinage de la tendance thrombophilique et de l'hyperfonctionnalité thrombocytaire ont constitué — pour le lot d'athéroscléreux considérés — les critères majeurs pour apprécier l'action antiathérogène de l'Aslavital.

L'importance de l'accroissement du niveau de l'héparinémie endogène, sous l'influence de la thérapie biotrophique, doit être appréciée à la lumière de l'étroite corrélation entre l'hypohéparinémie et l'âge avancé [20], critère d'âge biologique qui explique le développement du syndrome dysmétabolique chez les patients âgés, même en l'absence de l'athérosclérose manifeste.

D'autre part, le problème intéresse aussi la prévention et le traitement des accidents vasculaires thrombotiques chez les athéroscléreux, et l'utilisation de la thérapie anticoagulante.

Étant donné le temps relativement court d'application du traitement, l'accroissement de l'héparinémie s'explique plutôt par la mise accrue en liberté des dépôts intracellulaires existants, que par une stimulation hémato-poïétique des héparinocytes.

La tendance vers la normalisation de l'hyperfibrinogénémie, signalée chez certains patients, ayant comme conséquences la diminution de l'hyperpolymérisation, de la coprécipitation de l'héparine et de la formation des complexes « héparine-fibrinogène » peu solubles, pourrait également expliquer la normalisation de l'héparinèmie endogène, au moins dans certains cas.

L'équilibrage du status thrombophilique s'est réalisée surtout par l'accroissement de l'activité fibrinolytique plasmatique et moins par la normalisation de l'hypercoagulabilité plasmatique.

La tendance au redressement de l'équilibre des protéines du sérum — remarquée depuis longtemps pendant le traitement au Gérovital $H_3$ — peut expliquer la stimulation de l'activité LPL chez les athérosclérotiques traités.

L'efficience plus réduite de la thérapie à l'Aslavital sur les modifications dyslipémiques et dysglycoprotéiques (pour le groupe étudié) nous paraît être due au pourcentage plus réduit des dérèglements de ce type, ainsi qu'à leur degré réduit d'intensité. La reprise de l'étude sur un groupe de patients sélectionnés en ce sens (avec prédominance des troubles métaboliques) s'impose.

## CONCLUSIONS

L'expérimentation du produit procainique activé — l'Aslavital — sur un groupe d'athéroscléreux âgés, par une thérapie intensive de durée relativement courte (90 jours) a démontré la possibilité d'influencer favorablement la plupart des troubles appartenant au syndrome humoral athérosclérotique.

Les propriétés thérapeutiques anti-athérogènes de l'Aslavital se sont manifestées par une action antithrombogène, démontrée par l'accroissement du niveau de l'héparinémie endogène, la stimulation de l'activité fibrinolytique plasmatique, la réduction de l'hyperfibrinogénémie et la diminution de l'hyperfonctionnalité thrombocytaire. À celles-ci on doit ajouter l'effet de redressement de l'activité LPL « in vitro » chez la plupart des patients athéroscléreux.

Nous estimons que les effets plus réduits obtenus sur les modifications dysmétaboliques — la dyslipémie et la dysglycoprotéinémie — dans le groupe étudié nécessitent des études nouvelles sur des lots de patients sélectionnés à cet effet.

L'application systématique de la procainothérapie, avec le produit biotrophique roumain, s'impose dans la pratique courante comme une méthode supérieure de prophylaxie et de traitement de l'athérosclérose.

**Summary.** The authors applied the intensive therapy with Aslavital for a relatively short period of time (90 days) to a group of aged atherosclerotic patients and proved the favourable influence of this procaine — based product on most of the troubles specific to the humoral syndrome.

The antithrombogenous effect was pointed out by the increased endogenous heparine levels, activation of fibrinolysis, reduction of fibrinogenemy, diminution of thrombocytic hyperfunction and normalisation of lipoproteinlipase activity; the antiatherogenous properties of this medication were thus proved.

The dyslipemic and dysglycoproteinemic changes were less influenced by this therapy. The authors concluded that the biotrophic treatment with Aslavital should be systematically used in atherosclerosis, both curatively and prophylactically.

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# CONTRIBUTIONS À L'ÉTUDE DES FACTEURS THROMBOPHILIQUES DE RISQUE ET DE LA THÉRAPIE PRÉVENTIVE DES ACCIDENTS THROMBOEMBOLIQUES CHEZ LES GENS ÂGÉS

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**Résumé.** L'importance du diagnostic précoce du syndrome thrombophilique et de la prophylaxie des accidents vasculo-thrombotiques chez les vieux, s'explique par deux particularités majeures clinico-biologiques, du grand âge: le dysmétabolisme du vieillissement et la fréquence bien connue des affections à potentiel thrombogène: athérosclérose, cardiopathies, fibrillation atriale, insuffisance respiratoire chronique, azotémie, néoplasies, etc.

Pour apprécier correctement le status thrombophilique et, par suite, le risque de thrombose, l'auteur préconise certaines constellations propres de tests humoraux, poursuivant l'investigation simultanée de l'hypercoagulabilité plasmatique, de l'activité fibrinolytique, des anticoagulants physiologiques et de la fonctionnalité thrombocytaire.

On y discute aussi les aspects de l'efficacité réelle du traitement anticoagulant, chez les vieillards, avec référence spéciale à l'héparine et aux antivitamines K, ainsi que sur les effets prophylactiques antithrombophiliques de la thérapie de longue durée avec des substances biotrophiques comme l'Aslavital.

Les syndromes cliniques groupés actuellement sous le nom de thromboembolisme représentent pour les praticiens de toutes les spécialités, un problème quotidien, les manifestations fréquentes de thrombophlébite, de phlébothrombose — avec ou sans complications emboliques — et, surtout, d'oblitérations artérielles impliquant, presque toujours, l'utilisation correcte de la thérapie anticoagulante et thrombolytique [1], [2].

Dans le domaine de la prophylaxie et du diagnostic précoce des thromboses artérielles — dont la plupart ont un fond athéroscléreux, dû surtout à l'inconstance des signes cliniques prémonitoires — la notion de syndrome humoral thrombophilique a pris une importance considérable, mais non exclusive [3], [4], [5]. A côté des facteurs favorisants hémodynamiques, des altérations de la paroi vasculaire, de l'hématocrite, de la viscosité sanguine, des modifications des protéines sériques, des agressions allergiques locales, des prédispositions constitutionnelles, etc., le rôle des troubles humoraux d'hypercoagulabilité, de l'hypofibrinolyse, ainsi que de l'hyperfonction thrombocytaire dans l'étiopathogénie des thromboses, est évident [6]–[15].

Théoriquement réactualisée et remaniée ces dernières années sur la base de nouvelles observations sur le « turnover » du fibrinogène marqué, l'hypercoagulabilité de la maladie athéromateuse, considérée comme une cause principale favorisante des dépôts pariétaux de fibrine « intra vitam », se situe, aujourd'hui, dans le cadre d'un véritable syndrome de coagulation intravasculaire ou de coagulo-

pathie de consommation similaire à l'hypercoagulabilité des états thrombophiliques propres à d'autres entités, comme la maladie carcinomateuse, la polyglobulie, etc.

La mise en évidence, chez les patients athéroscléreux, de certaines caractéristiques propres aux coagulopathies latentes de consommation, à savoir, l'accélération significative du « turnover » du fibrinogène, semblable à celle qui suit l'administration de fibrinolytiques, ainsi que la normalisation du « turnover » sous l'influence des anticoagulants de type héparinique, associée ou non aux substances antifibrinolytiques, démontre l'existence, dans ce type de thrombophilie, d'un syndrome de coagulation intravasculaire latente et continue, associée à une activité lytique locale accélérée, même si ce dernier phénomène se produit sur un fond apparemment paradoxal d'une hypoactivité fibrinolytique globale, unanimement acceptée, surtout dans l'athérosclérose des vieux [14], [16].

L'importance du diagnostic précoce des facteurs thrombophiliques de risque, ainsi que de la prophylaxie des accidents vasculo-thrombotiques chez les vieux est liée à deux particularités majeures du grand âge: 1) le dysmétabolisme du vieillissement, qui, par ses composants dyslipidémiques, dysprotéinémiques et dysglycoprotéinémiques, ainsi que par l'hypohéparinémie endogène, favorisent l'installation de l'hypercoagulabilité plasmatique et de l'hyperfonctionnalité thrombocytaire [17], [18]; 2) une fréquence augmentée des affections à potentiel thrombogène: athérosclérose, cardiopathies, fibrillation atriale, insuffisance respiratoire chronique, azotémie, néoplasies, infections de longue durée, etc., souvent associées au decubitus prolongé [19].

Sur la base de notre expérience clinique et de nos recherches clinico-biologiques sur l'athérosclérose des vieillards, nous préconisons l'application des tests suivants, pour obtenir un diagnostic précoce du syndrome thrombophilique et par conséquent du risque de thrombose:

*a)* détermination de l'hypercoagulabilité plasmatique globale de type chronométrique ou structural, par thrombélastogramme (TEG) et par l'indice de tolérance à l'héparine (ITH);

*b)* détermination de l'activité fibrinolytique plasmatique, en se basant sur le dosage du plasminogène et sur la mesure du temps de lyse euglobulinique (TLE) et de l'indice fibrinolytique du plasma riche en plaquettes (IFPRP) par la méthode thrombélastographique;

*c)* détermination de l'activité thrombodynamique thrombocytaire (ATT) et de l'activité antifibrinolytique thrombocytaire (AAT) par thrombélastographie, ainsi que de l'hyperadhésivité thrombocytaire;

*d)* dosage des inhibiteurs physiologiques de la coagulation: antithrombine III progressive (A III) et l'héparine endogène.

La figure 1 présente les valeurs moyennes de ces indicateurs humoraux thrombophiliques, obtenues à l'Institut National de Gérontologie et de Gériatrie de Roumanie, sur un groupe de 60 personnes de 55 à 70 ans, avec des formes cliniques avancées d'athérosclérose et sur un autre groupe de 40 personnes âgées, sans signes cliniques évidents d'athérosclérose, avec la comparaison des valeurs moyennes de ces indicateurs chez les adultes sains. Par rapport à la zone des valeurs physiologiques chez les adultes sains et à la courbe des valeurs moyennes du groupe témoin de gens âgés, qui se situent, pour la plupart de ces indicateurs thrombophiliques, dans des limites physiologiques, la courbe des valeurs moyennes des patients athéroscléreux indique un déplacement significatif vers l'hypercoagulabilité globale (surtout vers le type structural), une baisse significative des anticoagulants physiologiques et une nette diminution de l'activité fibrinolytique

(hypoplasminogénémie, l'augmentation du TLE et la diminution de l'IFPRP sur le diagramme thrombélastographique).

À remarquer que, dans le diagnostic de laboratoire de l'hypercoagulabilité, le rôle principal doit être réservé non seulement à l'aspect quantitatif (hypercoagulabilité chronométrique), mais surtout aux modifications qualitatives représentant l'hypercoagulabilité de type structural, liée aux fonctions thrombodynamiques thrombocytaires.

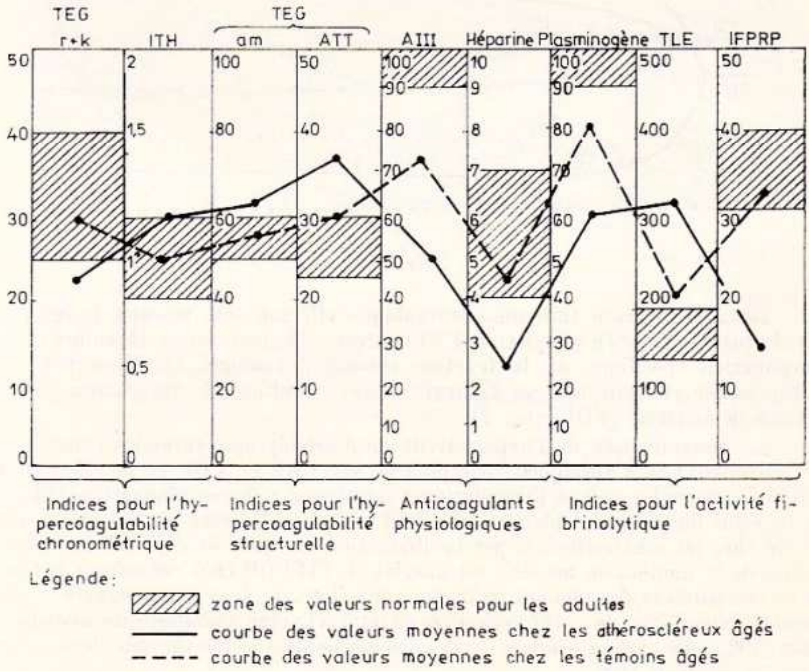


Fig. 1 — Valeurs moyennes des indicateurs humoraux thrombophiliques chez les athéroscléreux et les témoins âgés, par rapport aux mêmes indicateurs chez les adultes sains.

Le principal facteur de risque de la maladie thrombo-embolique est l'hyperactivité thrombocytaire, liée, non seulement aux phases précoces de la thrombogénèse, avant le début de la coagulation plasmatique, par la mise en disponibilité du facteur 3 thrombocytaire et du facteur Hageman adsorbé, mais surtout aux phases ultérieures de la thrombogenèse avec la participation du mécanisme autocatalytique de coagulation, dans lequel la fonction thrombocytaire primordiale n'est pas la fonction thromboplastique, mais la fonction d'ordre structural, thrombodynamique, facteur déterminant de l'hypercoagulabilité de type structural. [20], [21]. Comme la fonction d'adhésivité, la fonction thrombodynamique implique

la présence, dans l'hyalomère et même sous la membrane des plaquettes, de la protéine fibreuse contractile, analogue à l'actomyosine des muscles, à savoir la thrombosténine.

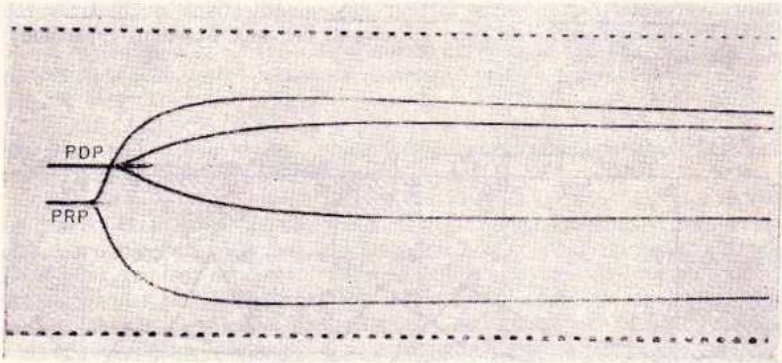


Fig. 2

Devant un tracé thrombélastographique effectué sur du sang intégral ou sur du plasma riche en plaquettes (PRP) suspect d'hyperfonction thrombocytaire, l'exploration spécifique de la fonction thrombodynamique thrombocytaire est indispensable et s'effectue par l'enregistrement simultané du diagramme sur du plasma déplaquetté (PDP) (fig. 2).

La détermination de l'hyperactivité antifibrinolytique thrombocytaire exige des enregistrements thrombélastographiques sur PRP et PDP et le calcul de la différence entre les indices fibrinolytiques du plasma riche en plaquettes (IFPRP) et de celui du plasma déplaquetté (IFPDP). Cette différence est nettement plus élevée chez les athéroscléreux, par la diminution marquée de l'IFPRP en comparaison de la diminution modérée ou discrète de l'IFPDP chez ces mêmes patients et en comparaison des témoins en bonne santé (fig. 3). Nous avons signalé ce phénomène chez 42% des athéroscléreux en état clinique manifeste de maladie et chez 70% des athéroscléreux avec des accidents vasculo-thrombotiques, dans leurs antécédents [17].

En tenant compte des conceptions actuelles, on considère que la relation existante entre le déficit de facteurs et l'hypocoagulation diffère beaucoup de la relation existante entre l'excès de facteurs, l'hypercoagulation et les dépôts intravitaux de fibrine. Tandis que l'hypocoagulation a toujours à la base un déficit de facteurs, dans les états d'hypercoagulabilité les plus marqués, il est difficile de mettre en évidence, à l'exception du fibrinogène, l'excès de tout autre facteur de coagulation. On admet en général que la possibilité d'accélération du temps de coagulation n'est pas produite par l'augmentation quantitative d'un facteur, mais plutôt par la présence d'un facteur activé ou par la diminution des anticoagulants.

Les études réalisées à l'Institut National de Gérontologie et de Gériatrie de Roumanie ont démontré le rôle important de l'hypoactivité de l'antithrombine III dans l'installation du syndrome humoral thrombophilique et surtout de l'hy-

perceoagulabilité de type chronométrique, chez les gens âgés avec une athérosclérose avancée. D'ailleurs la prédisposition aux thromboses vasculaires répétées, ainsi que l'amélioration évidente, à la suite du traitement à l'héparine, sont mentionnées dans la littérature, pour les patients à déficit congénital d'A III.

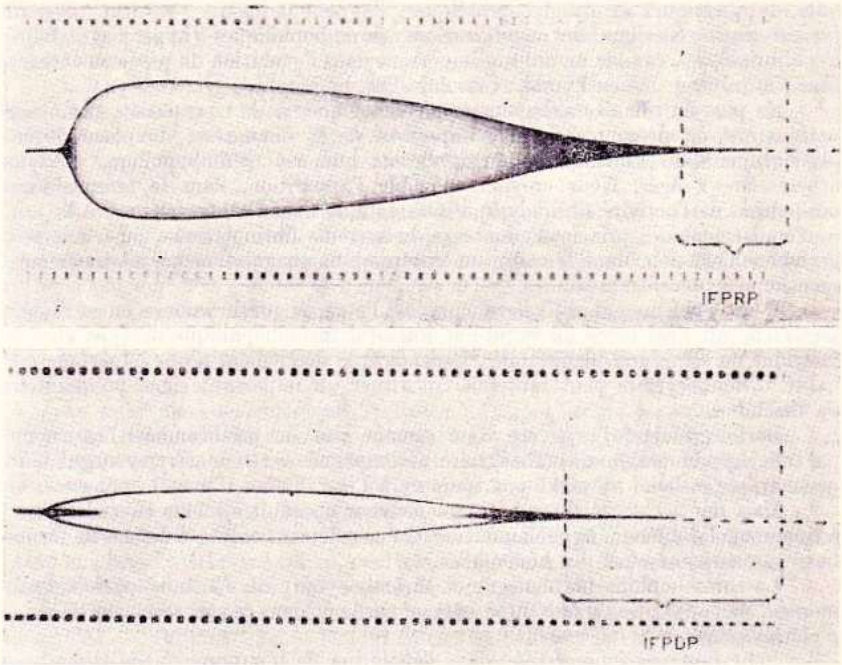


Fig. 3

L'hypohéparinémie des sujets athéroscléreux, accompagnée par l'augmentation des glycoprotéines sériques, doit être considérée comme la réflexion sur le plan humoral, de la réaction mésenchymale vasculaire de type spécial de l'athérosclérose, caractérisée par la diminution de la quantité de substances hépariniques à topographie intimale prédominante superficielle, et par l'augmentation des chondroïtine-sulfates et de l'acide sialique dans la profondeur de l'intima. La clarification du plasma lipémique induit par l'héparine concomitamment avec la réorientation des molécules lipoprotéiques et avec le transfert des lipides des fractions lentes sur les factions rapides à plus grande stabilité, l'effet protecteur de l'héparine sur l'athérosclérose expérimentale, etc. sont des arguments plaidant en faveur de l'origine hypohéparinique des altérations humorales dyslipémiques, dans l'athérosclérose.

Au niveau humoral, l'hypothéparinémie serait, à son tour, intensifiée par l'absorption de l'héparine sur les chilomicrons et les complexes de bêtalipoprotéines-fibrinogènes; d'autre part, l'hyperlipémie primitive et de durée peut conduire à la longue, à l'épuisement de la fonctionnalité des cellules héparino-formatrices. On

sait aujourd'hui que l'hypercoagulabilité peut apparaître par l'activation du facteur Hageman, par les acides gras saturés à longue chaîne, phénomène qui déclenche tout le mécanisme intrinsèque de formation de la thrombine, l'hypohéparinémie constituant dans ces situations, un facteur favorisant.

Par interférence et conditionnement réciproque avec les troubles métaboliques dyslipémiques et dysglycoprotéiques, l'hypohéparinémie constitue ainsi un facteur pathogénétique des modifications thrombophiliques (hypercoagulabilité-hypofibrinolyse), comme un anneau important dans l'évolution du processus athérogène au niveau mésenchymal, vasculaire et humoral.

En plus du rôle des antagonistes physiologiques de la coagulation, nos observations ont pu démontrer le rôle important de la diminution du plasminogène plasmatique dans l'installation du syndrome humoral thrombophilique chez les athéroscléreux âgés. Nous croyions possible l'apparition, dans le temps, d'une diminution de l'activité fibrinolytique plasmatique dans l'athérosclérose, à la suite de l'épuisement des principaux facteurs du système fibrinolytique, dû à une trop grande sollicitation, dans le cadre du syndrome de coagulation vasculaire latente, mentionnée précédemment.

Il est évidemment difficile de prévoir l'état de préthrombose en se basant seulement sur l'existence du syndrome humoral thrombophilique de type chronométrique ou structural plaquettaire. Cependant le décèlement d'une hyperfonctionnalité thrombocytaire peut toutefois constituer un important signe prémonitoire de thrombose.

Parfois, malgré l'existence d'un certain état de préthrombose, caractérisé par une hyperfonction thrombocytaire accompagnée ou non d'hypercoagulabilité plasmatique, celle-ci ne mène pas toujours à l'installation d'une thrombose.

Mais des accidents thrombotiques peuvent apparaître même chez des sujets à hypocoagulabilité et à hypoplaquettose. On a également constaté des cas de thrombose coronarienne chez des hémophiles.

La thrombophilie plasmatique et thrombocytaire ne s'installe parfois qu'au moment de l'apparition d'une thrombose en perdant, dans ce cas, sa valeur de signal d'alarme.

Il ne faut pas ignorer que, dans l'évolution de n'importe quelle thrombose, il y a souvent une période d'hyperactivité thrombocytaire et d'hypercoagulabilité, qui ne peut être décelée, à cause de sa courte durée. De même, dans l'état de préthrombose ou au cours même d'une thrombose, des périodes plus ou moins courtes d'hyperplaquettose et d'hypercoagulabilité peuvent être interrompues par des périodes d'hypocoagulation. Les alternances brutales d'hypo- et d'hypercoagulabilité ont une importance considérable dans l'établissement du diagnostic, en indiquant une instabilité humorale qui peut précéder l'installation de l'hypercoagulation et de la thrombose.

En général, en présence de la suspicion clinique du risque de thrombose ainsi que des images hématologiques d'hypercoagulabilité, d'hypofibrinolyse et surtout d'hyperplaquettose fonctionnelle, il faut administrer le traitement anticoagulant, le grand âge ne constituant pas une contre-indication pour cette thérapie [22].

Si les contre-indications du traitement anticoagulant sont plus fréquentes chez les gens âgés, les indications de ce traitement sont, elles aussi, plus nombreuses.

Nous considérons donc que la susceptibilité des vieux aux anticoagulants, impliquant la diminution des doses, ainsi que les conséquences d'une éventuelle hypocoagulabilité, ne permettent pas d'utiliser la médication anticoagulante d'une manière systématique.

En éliminant les principaux risques de la thérapie (tares évidentes digestives, hépatiques ou rénales) et en tâtonnant correctement la posologie pour obtenir une hypocoagulabilité efficace, avec un minimum de risques d'hémorragie [23], en assurant une surveillance rigoureuse clinique et biologique, et en individualisant attentivement la durée du traitement, c.-à-.d., en prenant toutes les précautions nécessaires, il ne nous paraît pas opportun de priver les gens âgés du triple effet prophylactique de ce traitement, dans le cas où son utilisation est formellement indiquée.

Il faut donc: 1. prévenir l'installation de la thrombose; 2. prévenir l'extension d'une thrombose installée, ainsi que ses complications emboliques dans les cas d'infarctus du myocarde, de thrombophlébite, de thromboses artérielles périphériques, etc. ([24], [25]), et 3. éviter les récidives thrombotiques souvent mortelles chez les gens âgés.

Nous mentionnons ci-après deux indications spécifiques du traitement des vieillards: 1. l'administration du traitement anticoagulant en même temps que celui de la thérapie tonicardiaque, surtout chez les malades avec des troubles de rythme, qui sont très exposés aux embolies artérielles dans le cas d'arythmie complète régularisée par la digitale; 2. l'administration prophylactique pré-et post-opératoire des anticoagulants dans les cas d'interventions avec de grands risques thrombotiques et emboligènes: interventions orthopédiques, sur le petit bassin, etc. [26] — [30].

Au point de vue biologique, l'objectif principal est de placer le malade en hypocoagulabilité chronométrique et structurale qui, pour être efficace, doit être franche. On continuera donc, le traitement jusqu'à la réduction complète de l'hyperplaquettose fonctionnelle et des signes graphiques d'ordre inflammatoire, dénotant le caractère évolutif d'une thrombose.

L'héparine déprime nettement la fonction thrombocytaire thrombodynamique, étant une arme précieuse avec une activité immédiate sur l'hyperplaquettose fonctionnelle, si elle est administrés en perfusion continue [31] — [36].

Dans le traitement par les antivitamines K, l'évaluation du complexe prothrombine — proconvertine, — facteur Stuart ne donnant pas d'indications sur la coagulation intrinsèque, c.-à.-d. sur l'efficacité réelle du traitement, on doit y associer le contrôle thrombélastographique car c'est le seul moyen de savoir de manière précise si le malade est en état d'hypocoagulabilité et d'hypoplaquettose efficaces.

Les antivitamines K ont une faible action dépressive sur l'hyperplaquettose fonctionnelle; les signes graphiques spécifiques restent parfois irréductibles et le TEG indique alors que l'aide de l'héparine est devenue indispensable dans une substitution de courte durée. En cas d'hyperfibrinémie, on y associera avec prudence la thérapie anticoagulante et la médication anti-inflammatoire (corticoïdes, etc.).

Un traitement anticoagulant de plus longue durée ne sera, en général, institué que sous une rigoureuse surveillance clinique et biologique. Nous considérons comme inopportun le maintien d'un traitement devenu inutile, mais nous proposons que l'arrêt du traitement soit fait par doses régressives, étalées sur quelques semaines.

De nombreuses études et observations cliniques et expérimentales ont justifié une large utilisation de la chimiothérapie à la procaïne, d'après le procédé du Professeur Anna Aslan, en utilisant ses produits biotrophiques originaux, le Gérovital H3 et l'Aslavital, dans la thérapie de la vieillesse et des affections chroniques dégénératives liées à l'involution de l'organisme [37].

Les études poursuivies à l'Institute National de Gérontologie et de Gériatrie de Bucarest ont mis en évidence les propriétés thérapeutiques antiathérogènes de

l'Aslavital, et tout particulièrement son action antithrombogène. Celle-ci a été démontrée, au point de vue clinique, par la diminution marquée de l'incidence des accidents thromboemboliques chez les gens âgés en cours de traitement biotrophique de longue durée, et surtout, au point de vue biologique, par son influence favorable sur les déréglages thrombophiliques (fig. 4): augmentation du niveau

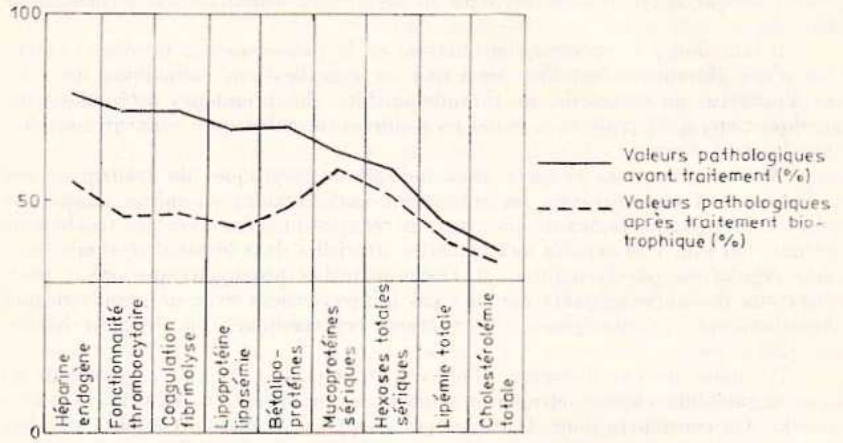


Fig. 4 — Pourcentage des sujets athéroscléreux avec des troubles thrombophiliques avant et après 5 séries d'injections à Aslavital (étude sur 60 sujets athéroscléreux âgés).

de l'héparinémie endogène, stimulation de l'activité fibrinolytique plasmatique, réduction de l'hyperfibrinogénémie et diminution de l'hyperfonctionnalité thrombocytaire. Il faut y ajouter l'effet de redressement de l'activité lipoprotéine-lipasique (LPL) « in vitro », chez la plupart des patients athéroscléreux. L'application systématique de la procaïnothérapie de longue durée avec le produit biotrophique roumain, l'Aslavital, s'impose donc dans la pratique courante, comme une méthode supérieure de prophylaxie des accidents vasculo-thrombotiques, chez les gens âgés athéroscléreux.

**Summary.** The importance of precocious diagnosis of the thrombophilic syndrome and of the prophylaxis of vasculo-thrombotic accidents among the elderly is explained by two major clinical and biological old age characteristics: the ageing dysmetabolism and the wellknown frequency of diseases with thrombogenic potential: atherosclerosis, cardiopathies, chronic breathing insufficiency, azotemia, neoplasias, etc.

For appreciating correctly the thrombophilic state and consequently thrombosis risks, the author recommends certain humoral tests constellations following the simultaneous investigations of plasmatic hypercoagulability, fibrinolytic activity, physiological anticoagulants and of thrombocytary functionality.

There are also discussed the aspects of the real efficiency of the anticoagulant treatment for the elderly, with special reference to heparin and K antivitamins and also to the antithrombophilic prophylactic efficiency of a long therapy with biotrophical substances like Aslavital.

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# THE ASLAVITAL TREATMENT IN THE RECOVERY OF MENTALLY-DEFICIENT CHILDREN*

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**Summary.** This research was conducted on 44 school children, males aged 7 to 14, with the diagnosis of slight or moderate mental deficiency. The double-blind technique was used; thus 23 subjects were treated with Aslavital and 21 subjects received injections with Placebo (physiological serum). All the subjects received i.m. equal amounts of solution (4 ml) according to the same schedule (6 days per week); each subject thus received 75 injections. The psychological examination included tests aimed at assessing level, memory, attention, motor activity. The psychological tests prior to the treatment did not reveal significant differences between the two groups. The tests subsequent to the Aslavital treatment pointed out obvious improvements both in the dynamics of the intellectual activity and its projection on the motor plan. The teachers noticed better school results with the children belonging to this group. The children in the Placebo group did not show significant changes as compared to the initial tests.

Aslavital improves the metabolism of the nervous cell and stimulates the trophic functions of the central nervous system [1].

Due to its properties, Ana Aslan started to use the Aslavital therapy not only in geriatrics, but for improving the psychic state of mentally deficient children.

A study was thus conducted in order to assess the efficacy of the Aslavital therapy under the above-mentioned circumstances; it included two stages: a screening research and a double-blind study.

During the first stage, the research consisted in the comparative analysis of the results obtained with a group of children both before and after the Aslavital treatment.

The study was conducted on a group of 34 children, from a school for mentally deficient children, boys and girls aged 8 to 12 whose main diagnosis was slight and moderate mental deficiency.

Aslavital was administered as pills, 2 per day in 24 day-series, with 10 days breaks between the series, over a period of 6 months.

The research consisted in the investigation of mental deficiency, attention, memory, psychopedagogical level and behaviour. Clinical and neurological examinations were also performed.

The results pointed out the improvement of the psychological tests after the Aslavital treatment.

* Research carried on in collaboration with the School for mentally deficient children no. 6.

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The teachers noticed significant changes such as conscious realisation of school activities, assessment of will in interschool relations, development of initiative, sociability and better school results in children who initially showed apathy, slowliness, superficial inhibition, inertia, passivity, tendency to isolate from the class.

Due to the results of the screening research, the action of Aslavital on mentally deficient children was further investigated in a double-blind study.

## MATERIAL AND METHOD

This study was conducted on a group of 44 boys from a school for mentally deficient children, aged 7 to 14 with the diagnosis of slight or moderate mental deficiency.

The diagnosis, the etiology and the socio-cultural environment to which the subjects belonged were taken into consideration in selecting the cases. The encephalopathic sequelae, obstetrical cranio-cerebral traumatisms, psycho-somatic underdevelopment, ethylic or mentally deficient parents were prevalent in the etiology of the investigated subjects.

The following peculiarities of the family environment were prevalent in most of the subjects:

— disorganized family;
— conflicts between the parents;
— inadequate hygienic, cultural and instructive conditions;
— behaviour disturbances in the parents.

The Raven-coloured progressive matrices were used for the psychologic examination of the mental level. The research was conducted on the children whose IQ ranged from 0.44 to 0.70 [2].

The clinical neuropsychiatric examination was also performed.

The children of the two groups subjected to the double-blind study had similar ages and IQ's.

The following aspects made the object of the psychological examination:

*a)* memory, using the number-memory test from WISC [3];

*b)* attention, using the double-checking test (A.d.c.) by R. Zazzo [4];

*c)* motor style and level with R. Zazzo's tests for the child's psychological examination [4];

— circle outline (C.O.n.s.m.)
— circle cutting (C.C.n.s.m.)
— tapping lines (T.L.n.s.m.).

The major objectives of the psychological examination were the various aspects of the subjects' personality [5, 6].

Consequently, the tests used pointed out the dynamics of the intellectual activity, perception, thought and their projection on the motor plane through the assessment of the coordination of movements, manual ability, speed.

All the psychological tests were applied both before (day 0) and after the treatment (day 75).

The research was conducted during the school year, the children being permanently supervised by the medical staff and the teachers.

Similar amounts of solution (4 ml) were injected i.m. to all the subjects following an identical schedule (daily, 6 days per week). Each subject received thus 75 shots, because the treatment was not administered during the holidays.

The subjects' tolerance to Aslavital was tested in the first place.

The Aslavital treated group counted 23 subjects; whereas that receiving Placebo (physiological serum) counted 21 subjects.

## RESULTS

The statistic analysis (test t) of the results of the psychological examination before the treatment pointed out the equivalence of all the psychological tests in both groups (Table 1).

*Table 1*

**Statistic comparison of the psychological tests of the two groups prior to treatment**

| Tests | Aslavital | Placebo | Statistic significance |
|---|---|---|---|
| N.M. | 3.60 | 3.61 | N.S. |
| | 2.56 | 2.76 | N.S. |
| A.d.c. | 0.15 | 0.16 | N.S. |
| | 0.67 | 0.51 | N.S. |
| C.O.m.l.s. | 29.82 | 38.70 | N.S. |
| C.C.m.l.s. | 2.43 | 2.04 | N.S. |
| | 1.43 | 1.04 | N.S. |
| T.L.m.l.s. | 199.21 | 188.14 | N.S. |

*Abbreviations:*

N.M. = number-memory
A.d.c. = attention, double-checking
C.O.m.l.s = circle outline — motor level and style
C.C.m.l.s.: = circle cutting — motor level and style
T.L.m.l.s = tapping lines — motor level and style

No significant modification of the measured variables (A.d.c., N.M., C.O., C.C., T.L.) with the Placebo group was revealed by the statistic analysis (Table 2).

*Table 2*

**Comparative data of the psychological tests prior and consequent to the placebo**

| Tests | Before Placebo | | After Placebo | | |
|---|---|---|---|---|---|
| | m. | d.c. | m. | d.c. | |
| N.M. | 3.61 | 0.11 | 3.76 | 0.11 | N.S. |
| | 2.76 | 0.12 | 2.90 | 0.09 | N.S. |
| A.d.c. | 0.16 | 0.05 | 0.07 | 0.02 | N.S. |
| | 0.51 | 0.07 | 0.42 | 0.07 | N.S. |
| C.O.m.l.s. | 38.70 | 6.19 | 34.47 | 7.58 | N.S. |
| C.C.m.l.s. | 0.43 | 0.12 | 0.52 | 0.10 | N.S. |
| | 2.04 | 0.22 | 1.80 | 0.18 | N.S. |
| | 1.04 | 0.15 | 1.09 | 0.14 | N.S. |
| T.L.m.l.s. | 188.14 | 13.33 | 179.95 | 11.11 | N.S. |

*Abbreviations:*

N.M. = number-memory
A.d.c. = attention, double-checking
C.O.m.l.s. = circle ouline — motor level and style
C.C.m.l.s. = circle cutting — motor level and style
T.L.m.l.s. = tapping lines — motor level and style

After the individual interpretation of the results of the psychological tests prior and subsequent to the treatment, the results were statistically analysed on group, according to test t.

The comparison of all the variables measured before and after the treatment pointed out significant improvements in the Aslavital treated group (Table 3):

— A.d.c., variant I with $p < 0.01$ and variant II with $p < 0.02$;
— N.M., variant I with $p < 0.02$ and variant II with $p < 0.01$;
— C.O.n.s.m., with $p < 0.05$;
— C.C.n.s.m., variant I with $p < 0.2$ and variant II with $p < 0.01$;
— T.L.n.s.m., with $p < 0.05$.

*Table 3*

**Comparative data of the psychological tests prior and consequent to Aslavital**

| Tests | Before | | After | | p |
|---|---|---|---|---|---|
| | m. | d.c. | m. | d.c. | |
| N.M. | 3.60 | ±0.12 | 4.04 | ±0.13 | <0.02 |
| | 2.56 | ±0.10 | 3.21 | ±0.12 | <0.01 |
| A.d.c. | 0.15 | ±0.03 | 0.05 | ±0.01 | <0.01 |
| | 0.67 | ±0.10 | 0.35 | ±0.07 | <0.02 |
| C.O.m.l.s. | 29.82 | ±3.47 | 21.17 | ±3.13 | <0.05 |
| C.C.m.l.s. | 1.08 | ±0.15 | 0.60 | ±0.12 | <0.02 |
| | 2.43 | ±0.27 | 1.47 | ±0.15 | <0.01 |
| | 1.43 | ±0.18 | 0.69 | ±0.11 | <0.01 |
| T.L.m.l.s. | 199.21 | ±12.79 | 166.78 | ±9.94 | <0.05 |

*Abbreviations:*

N.M. = number-memory
A.d.c. = attention, double checking
C.O.m.l.s. = circle outline — motor level and style
C.C.m.l.s. = circle cutting — motor level and style
T.L.m.l.s. = tapping lines — motor level and style

The above-mentioned data pointed to the positive influence of the Aslavital treatment on the mentally deficient children.

## DISCUSSIONS

Aslavital is a procaine-based solution which influences the psychic functions, as pointed out by Ana Aslan as early as 1954 [7]. Procaine and diethylaminoethanol action on the metabolism of the neurons account for the above-mentioned influence [8, 9, 10, 11, 12].

Subsequently, Ana Aslan added potassium glutamate to the solution of Gerovital H3, preserving the pH. An intensified acetylcholine production, as well as a stronger influence of the glutamic acid on the energy metabolism of the neurons, were thus obtained. The researches pointed out that Aslavital had a stronger action on the central nervous system than Gerovital H3.

The researches on the new product Aslavital carried out by Ana Aslan, evidenced its effectiveness on the precocious aging of the central nervous system.

The present double-blind study revealed the effectiveness of the Aslavital treatment on a group of mentally deficient children.

The results of the research have been interpreted as follows:

The number memory tests points out the improvement of the voluntary memorizing ability in the treated children.

The double-blind checking test of attention revealed the significantly improved ability of the subjects to carry on a mental activity with twofold perceptive discrimination: checking of one sign and checking of two signs.

The results of the tests checking the motor style and level point out the higher motor abilities of the children in the treated group as well as the coordination of the movements of the two hands.

The higher qualitative index of the results of the circle outline test points out the favourable change induced by the treatment through the intellectual factor, whereas in the tapping lines test through the motor style.

Mention should be made of the relatively short period of treatment (three months); consequently, the influence of the growth and maturation process can not be taken into consideration.

As the study was conducted during the school year, the teachers noticed significant improvements in the Aslavital treated children, particularly in their ability to learn (reading, calculating, memorizing) and to behave (integration with others, active participation in school activities, desire to be appreciated, decreased psycho-motor instability).

No side effect of the medication was noticed during the treatment.

## CONCLUSIONS

The conditions and criteria of this research proved that, in the recovery of the mentally deficient children, the Aslavital chemo-therapy has an effective influence on the dynamics of the adaptative function of the central nervous system.

Consequently, its great importance should be mentioned for the special instructive steps taken in order to recover these children.

Aslavital thus receives new therapeutical indications.

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**Résumé.** L'étude a été effectuée sur un lot de 44 élèves, garçons ayant entre 7 et 14 ans avec le diagnostic de déficience mentale légère ou modérée.

On a utilisé la technique du double insu; ainsi, 23 sujets ont été traités à l'Aslavital et 21 sujets ont subi des piqûres à Placebo (sérum physiologique).

On a administré à tous les sujets des quantités égales de substance (4 ml), conformément au même schéma (6 jours par semaine); on a administré à chaque sujet, de la sorte, 75 injections.

L'examen psychologique a inclus des tests pour l'évaluation du niveau mental, de la mémoire, de l'attention, de l'activité motrice.

Les tests psychologiques antérieurs au traitement n'ont pas mis en évidence de différences significatives entre les deux groupes.

Les tests ultérieurs au traitement à l'Aslavital ont mis en évidence des améliorations manifestes, aussi bien de la dynamique de l'activité intellectuelle, que de sa projection sur le plan moteur.

Les professeurs ont constaté de meilleurs résultats chez les garçons appartenant à ce lot.

On n'a pas observé de modifications significatives aux garçons du lot « Placebo » par rapport aux tests initiaux.

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# CHANGES INDUCED BY AGE AND STRESS IN THE BEHAVIOUR AND BRAIN METABOLISM OF FREE AMINO ACIDS IN WISTAR RATS. INFLUENCE OF ASLAVITAL TREATMENT

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**Summary.** The experiments were carried out on 90 Wistar rats, 45 males and 45 females, 1 year old. Each sex-group was made up of three subgroups: control rats, rats under stress treated with Aslavital and untreated rats under stress.

The treatment consisted in intramuscular injections of 0.2 ml/kg body-weight, three times a week, for 30 days.

The stress was applied as electric shocks (50 Hz, 1 mA, 0.5 s), three minutes daily, for 30 days.

All the rats were examined with a complex maze in order to estimate the learning and memorizing capacity.

At the end of the experiments, the rats were sacrificed, and their brains were cut off for biochemical determinations.

The amino acids extracted from the cerebral hemispheres were identified by circular paper chromatography.

As regards the effect of stress on the learning and memorizing processes, the results showed significant differences between the studied groups: the maze was solved by: 80% control females; 7% stressed untreated females; 27% stressed treated females; in male rats, the percentages were 67%, 0 and 13%, respectively.

Under stress the content of free amino acids in the cerebral hemispheres increased.

In stressed animals treated with Aslavital, the amino acid content was lower than in untreated animals under stress, the difference being statistically significant.

With regard to the stress-age relation, some authors [1] consider aging itself the result of stress-induced accumulations in the course of life, whereas others [2, 3, 4] claim that the process of aging is accelerated by repeated exposure to stressing stimuli. The clinical observations and the laboratory investigations have pointed out the age-induced decrease in the organism's adaptability to stress.

The researches into the effect of stress on animals have pointed out behavioural changes, such as apathy, drowsiness, anorexia, phobism, etc. [5–10].

Other studies have investigated the biochemical changes in the brain of stressed animals [7, 8, 11]. Mention should be made of the studies on brain protein synthesis inhibition [12], with the subsequent increase in the amount of free amino acids [13].

Taking into consideration the age-stress interaction we decided to investigate the influence of age and stress on learning, memory and content of glutamic acid, glutamine, gamma-aminobutyric acid in the brain. As known, the first two

amino acids mentioned above play an important part in the metabolic process involved in maintaining the morphological and functional integrity of the nervous cell [14]; the gamma-aminobutyric acid (GABA) is a membrane modulator involved in inhibitory processes.

We studied also the effect of the biotrophic substance Aslavital on the above-mentioned functional and biochemical nervous processes in stressed rats at different ages. Aslavital is a solution containing procaine 2%, glutamic acid and an increased amount of potassium ions (as against Gerovital $H_3$).

As known, Aslavital was elaborated by Ana Aslan in order to potentiate the psychotropic and lipotropic action of procaine. Beside the clinical studies on 1,400 patients [15], which pointed out these characteristics of Aslavital, a number of experimental researches were conducted, having similar goals. Mention should be made of the studies on the effect of Aslavital on learning ability, memory and passive avoiding behaviour in rats [16].

The electroencephalographic investigations have pointed out that the changes resulting from advancing age, such as the slowing down of the basic rhythm and the decrease in the amplitude of the tracings, are less marked in the Aslavital treated rats as against controls, in which slow waves were detected [17].

## MATERIAL AND METHOD

The experiment was conducted on 270 Wistar rats, males and females, equal in number; three age-groups were investigated, each including controls, stressed — untreated and stressed — Aslavital treated animals.

The stress consisted in electric shocks (2—6 mA; 50 Hz) to which the animals were exposed 0.5 sec. every 10 sec. intervals for 3 min. daily, during 30 days.

Verzar Mc Douglas' maze method [18] was used in evaluating the changes induced by stress, age and treatment on rats' learning ability and memory.

At the end of the experiment, the animals were sacrificed in order to make the necessary biochemical determinations on the brain. The glutamic acid, glutamine and gammaaminobutyric acid (GABA) were extracted. The circular paper chromatography for the identification of amino acids and the spectrophotometric method for quantitative determinations [19, 20] were used.

The Aslavital treatment consisted in i.m. injections with 0.2 ml/kg body weight, 3 times per week for 30 days.

## RESULTS

a) *In relation to age.* A significantly decreased ability of solving the maze problem was noticed in 12- and 24-month-old rats, both males (from 67% to 20%) and females (from 80% to 40%; $p < 0.05$), (Table 1).

With regard to the concentration of free amino acids in the brain, a constant and significant increase with age of glutamic acid, glutamine and GABA was pointed out (Tables 2 and 3). For instance, the glutamic acid level which was 64.3 mg/100 g wet tissue in 4-month-old male rats reached 123.4 mg and 297.2 mg in 12- and 24-month-old rats, respectively ($p < 0.01$).

Glutamine levels increased from 14.4 mg in 4-month-old rats to 190 mg and 167.8 mg in 12- and 24-month-old rats, respectively. The corresponding values for GABA were 28.2 mg and 78.3 mg ($p < 0.01$).


Male rats


Female rats

Fig. 1. — Free amino acids in the cerebral hemispheres of control and stressed (treated and untreated) rats.

*Table 1*

**Ability to solve the maze problem**

| Group | Sex | Rats constantly solving the maze % | Rats inconstantly solving the maze % | Rats which do not solve the maze % |
|---|---|---|---|---|
| Control | females | 80±3.63 | 20±3.63 | – |
| | males | 67±3.11 | 13±2.21 | 20±2.64 |
| Stressed, untreated | females | 7±2.21 | 20±2.64 | 73±2.92 |
| | males | – | 13±2.21 | 87±2.21 |
| Stressed, treated with Aslavital | females | 27±2.92 | 27±2.92 | 46±3.29 |
| | males | 13±2.21 | 20±2.64 | 67±3.11 |

*Table 2*

**Free amino acid contents in male rats' cerebral hemispheres**

| Amino acids mg/100 g wet tissue / Group | Controls I | Stressed, untreated II | Stressed, treated with Aslavital III | Statistic significance I–II | Statistic significance II–III |
|---|---|---|---|---|---|
| Glutamic acid | 62.0±0.44 | 117.0±9.87 | 74.0±1.19 | $p < 0.01$ | $p < 0.01$ |
| Glutamine | 9.5±0.25 | 16.9±2.01 | 10.9±0.30 | $p < 0.01$ | $p < 0.01$ |
| GABA | 39.1±1.30 | 70.3±6.58 | 56.5±0.81 | $p < 0.01$ | $p < 0.01$ |

The data were analysed statistically by means of Student's "t" test.

*Table 3*

**Free amino acid contents in female rat cerebral hemispheres**

| Amino acids mg/100 g wet tissue / Group | Controls I | Stressed, untreated II | Stressed, treated with Aslavital III | Statistic significance I–II | Statistic significance II–III |
|---|---|---|---|---|---|
| Glutamic acid | 43.5±0.52 | 77.5±0.96 | 71.0±0.89 | $p < 0.01$ | $p < 0.01$ |
| Glutamine | 1.9±0.03 | 7.3±0.14 | 6.1±0.89 | $p < 0.01$ | $p < 0.01$ |
| GABA | 14.7±0.16 | 44.4±0.75 | 28.0±1.19 | $p < 0.01$ | $p < 0.01$ |

The data were analysed statistically by means of Student's "t" test.

b) *Stress-induced changes at different ages.* The results in solving the maze problem were greatly inferior in all the stressed groups, e.g.: from 60% to 27% in 4-month-old rats, from 67% to 0% in 12-month-old rats and from 20% to 0% in 24-month-old rats (Table 1).

With regard to the 3 amino acids investigated, a more prominent value increase was noticed, sometimes by 80—100, even 200% as compared to the unstressed animals of the same age (Tables 2 and 3, Fig. 1).

c) *The influence of Aslavital treatment on age and stress-induced changes.* Better results were obtained in solving the maze problem (Table 1), such as:

— from 27% to 40% in 4-month-old male rats, from 0% to 13% in 12-month-old rats, from 0% to 30% in 24-month-old rats;

— from 20% to 47% in 4-month-old female rats, from 7% to 27% in 12-month-old rats, from 0% to 40% in 24-month-old rats.

With regard to the concentration of the 3 free amino acids investigated (Tables 2 and 3), a tendency was noticed to reaching the levels pointed out in unstressed rats of the same age; e.g.:

— in 4-month-old male rats = glutamic acid levels decreased from 91.7 mg/100 g wet tissue to 71.2 mg (64.3 mg in controls); glutamine levels decreased from 27.6 mg to 16.9 mg (14.4 mg in controls); GABA levels decreased from 59.4 mg to 30.6 mg (28.2 mg in controls);

— in 12-month-old male rats = glutamic acid levels decreased from 146.8 mg to 126.6 mg (123.4 mg in controls); glutamine levels decreased from 21.7 mg to 18.0 mg (19.0 in controls); GABA levels decreased from 112.6 mg to 81.4 mg (78.2 mg in controls);

— according to the above data, as a result of the treatment, the concentrations of the 3 amino acids reached values close or equal to those pointed out in younger controls.

Similar results were obtained in female rats. (Table 3).

## DISCUSSION

The changes in the process of learning, memory and brain biochemistry, resulting from advancing age and stress were pointed out in the course of the experiment. The fact was expressed by the modifications in the concentration of 3 amino acids (glutamic acid, glutamine, GABA) which play an important part in the development of the brain metabolic processes.

The different aspects of the behavioural changes in old animals have been described by numerous investigators [16, 21, 22].

The stress-related changes in the concentration of certain brain amino acids may be correlated with the well-known sensitiveness to exogenous and endogenous factors, of the brain biochemical mechanisms which incorporate amino acids into the proteins.

According to some authors [23, 24], the adaptability of the brain biochemical mechanisms to stress as well as protein synthesis diminish with advancing age.

Under these circumstances, the use of the biotrophic treatment with Aslavital is particularly significant since it improves learning ability and memory in stressed aged rats. The decrease in the concentration of the investigated amino acids could be due to the stimulated protein synthesis resulting from the treatment.

These data confirm experimentally the results obtained at the National Institute of Gerontology and Geriatrics on aged subjects treated with Aslavital, some of whom had an accelerated aging syndrome due to environmental stress factors (family, profession, accidents, etc.).

Generally, 3—5 months of treatment resulted in a changed attitude of the elderly towards the surrounding environment. They became more optimistic, active, got along better with each other. Memory improved considerably and the behavioural disturbances of some elderly patients either diminished or disappeared. Meanwhile, psychopathic tendencies were reduced, the depressive states disappeared and a good psychic balance was reached.

## CONCLUSIONS

The Aslavital treatment, administered to Wistar rats induced important improvements in some brain functions, such as learning ability and memory, which had previously been diminished by age and stress. It also improved the functioning of certain biochemical brain mechanisms, fact which was pointed out by the decrease in the concentration of free amino acids, probably due to an increased protein synthesis.

**Résumé.** Les expérimentations ont été effectuées sur 90 rats Wistar, 45 mâles et 4 femelles, âgés d'un an. Chaque groupe par sexe a été formé par trois sous-groupes: des rats témoins, des rats stressés et traités à l'Aslavital et rats stressés non traités.

Le traitement a été composé par des piqûres intramusculaires 0,2 ml/kg corps, trois fois par semaine, pendant 30 jours.

Le stress a été appliqué sous forme de chocs électriques (50 Hz, 1 mA, 0,5 s), trois minutes par jour, pendant 30 jours.

Tous les rats ont été testés dans un labyrinthe complexe, pour pouvoir estimer l'apprentissage et la mémoire.

Les rats ont été sacrifiés à la fin des expériences, et le cerveau a été prélevé pour des déterminations biochimiques.

Les amino-acides provenus des hémisphères cérébraux ont été identifiés par chromatographie circulaire sur papier.

En ce qui concerne l'effet du stress sur les processus d'apprentissage et de mémoire les résultats ont montré des différences significatives entre les groupes étudiés; les rats témoins femelles donnent une solution correcte au problème du labyrinthe en proportion de 80%, les rats stressés non traités en proportion de 7%, et les rats stressés et traités, en proportion de 27%; chez les rats mâles, les pourcentages sont 67%, 0 et 13%.

Chez les rats stressés on a observé une augmentation du contenu d'amino-acides libres des hémisphères cérébraux, sous l'action du stress.

Chez les animaux stressés et traités à l'Aslavital, le contenu d'amino-acides étudiés a connu des valeurs diminuées par rapport aux valeurs enregistrées chez les animaux stressés et non traités, les différences étant significatives du point de vue statistique.

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# EVALUATION OF THE BIOLOGICAL AGE

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**Summary.** Based on researches carried out at the National Institute of Gerontology and Geriatrics, a synthesis is presented of the criteria used in the evaluation of the biological age and aging rhythm, which express the fundamental traits of the aging process, the diminution in the adaptability and reactivity of the organism. The age indicators are grouped in organizational and integratory types: molecular, cellular, tissular and organismic.

Molecular aging is pointed out by the intensification of crosslink formation with age. The cellular and tissular aging process is selective, asynchronous. Normal aging is characterized by a marked lability of the general homeostatic balance. At this level, the evaluation of the age indicators under stress is particularly important. Functional age can be evaluated only by means of complex battery of tests. The main criteria are pointed out in the evaluation of the cardiovascular, nervous, renal, immune systems and internal medium.

The researches carried out at the National Institute of Gerontology and Geriatrics have led to the elaboration of certain synthetic indicators used in the determination of the biological age at populational and individual levels. The concluding part of the paper presents the main directions of future researches. The biological age will be most exactly determined by means of: complex tests, including some stress indicators in the program, the study of areas in the longevous, the thorough use of statistico-mathematical method.

The assessment of the age criteria is an obligation of gerontology, aimed at appreciating the biological age and studying the evolution of the aging process. We began this study in 1951 also with a view to objectivizing the therapeutic action.

From this standpoint, the criteria should be representative, easily applicable and, if possible, reversible.

The speed of the biological phenomena — the aging rhythm results in either concordance or discordance between the chronological age and the morphofunctional condition of the organism. The significant differences between the rhythm and manifestations of the biological aging process in different individuals determined thorough gerontological studies with a view to identifying the indicators of the biological age.

The researches on the determination of the biological age carried out recently have defined quite a number of tests which allow a better understanding of the biological aging process at individual and populational levels. The process of aging implies a complex of biochemical and biophysical changes which can be morphofunctionally pointed out at the end of the growth period in different organs and tissues.

The dissimilar aging in different tissues and organs is due, in the first place, to the genetic inheritance, and secondly to the environmental factors — geographic conditions, climate, socioprofessional environment, nutrition — which induce great variations in the aging rhythm of each individual and between the members of

the same population. The pathological conditions injure different systems, induce a faster and severer degradation by stressing the integratory mechanisms.

The evaluation of the biological age is organically dependent on these factors; usually, it does not correspond to the chronological age of the individual, which expresses the morpho-functional condition of the organism in relation to the outlived years.

In the longevous, the chronological age precedes the biological age, whereas in premature aging the ratio is reversed.

The physiological aging defines the normal, slow, continuous, asynchronous, heterogeneous aging and allows the individual to reach advanced ages despite certain adaptative difficulties. The physiological aging, Bürger biomorphosis is clinically known as orthogeria or orthobiosis. The peculiarities of the aging process in humans are generated by the elements specific to the onto- and phylogenetic development of this species. The evolution of the nervous system and psycho-social activity, the early and marked cardiovascular involution [1], [2], are areas where the human aging acquires essential importance and individualizing elements.

The finding of the factors which induce the accelerated involution, or the discovery of the premature aging in a certain function or organ, are of major importance from the medical standpoint.

This implies a complex biological investigation, by means of a battery of tests allowing the evaluation of the biological age, different functions and the comparison between performances recorded and the standard curves.

The morpho-functional involution specific to the human aging becomes obvious at the end of the growth period and results from the decrease in the active metabolic tissues, increase in the adipose and connective tissues as well as the qualitative changes in the function of different tissues and organs.

The involutive process is based in the first place on the physico-chemical and biochemical mechanisms the result of which is the primary aging of the organism.

The secondary aging is particularly dependent on the insufficiency of the autoregulation mechanism of the complex reactive, dynamic and biological systems.

Beside the chronological age, the evidence of other criteria in evaluating the functional age and aptitudes is a medico-social imperative.

At present, in the absence of a comprehensive estimative criterium, the biological age and the aging rhythm are evaluated in terms of numerous tests expressing the fundamental peculiarities of the aging process: the decrease in reactivity and adaptability. The lack of specificity in the data on the physiologic involution entitles us to present a synthesis of the most significant clinical and paraclinical indicators. Undoubtedly the age criteria are not present in each and every individual; nevertheless, because of their statistical value, they compel recognition as age indicators.

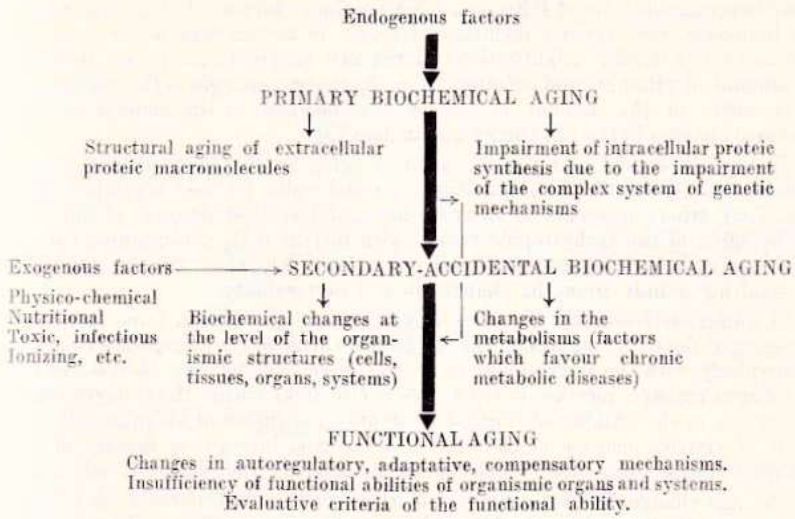
The biological aging can be followed at all levels of organization and integration: molecular, cellular, tissular, organic and organismic.

The modern biochemical, electrochemical and histochemical research methods allowed the thorough examination of the ultra-structure in the living matter and consequently the better understanding of the aging process (Table 1) at all organizational and integrational levels [3, 8]: molecular, cellular, tissular, organic and organismic. This allowed multiple organic and functional parameters (indicators) to be established, which differentiate the young and old organisms.

A large number of morpho-functional indicators (structural, physiological, biochemical, clinical) have been included in the biological evaluation.

*Table 1*

**Biological bases of the pathogenesis of aging**




## CRITERIA OF AGING, BASED ON CHANGES IN THE STRUCTURE OF THE ORGANISM

The molecular aging includes changes in the structure, stability and specificity of the proteic macromolecules, of great biological significance, with slow or absent turnover: nucleic acids (particularly the DNA from the fixed postmitotic cells) and the connective tissue proteins in which the morphologic evolution is greatly dependent on age. The aging of the collagen macromolecule appears as the spontaneous evolution of the adult collagen and is characterized mainly by the increasing number of covalent, intra- and intermolecular crosslinks and the decreasing amount of soluble material (proline, hydroxyproline). With the nucleic acids, the increasing number of stable bindings between histones and DNA triggers the impairment or loss of genetic information, or the ability of synthesizing the proteins with highly significant biological value (enzymes, hormones). The cell genetic control apparatus is known to represent the key of the primary aging process at the molecular and cellular level.

The aging of the cells and tissues should be analysed in relation to their degree of differentiation. With the higher organisms it is not uniform, manifesting an asynchronous evolution in the various structures. A series of quantitative and qualitative changes define the morphologic aging in cells and tissues: the diminished regularity in the distribution of the cells through the tissues, greater variability in the size of the cells, reduction of the nucleo-cytoplasmic ratio, aspects of cellular atrophy and degeneration, as well as compensatory hypertrophy, variation in the size, form and staining of the nucleus, nucleolus and organelle, fatty or pigment infiltrations into the cytoplasm, vacuolar or hyaline degenerations.

The biochemical aspects of the cellular and tissular aging, which are the substrate of the morphological aging consist mostly in the decrease of the cellular proteic nitrogen, reduction of DNA and RNA synthesis, increase in Ca, lipid, cholesterol, lipofuscin, etc., cellular inclusions, increase in the amount of Ca from the cell membrane, complex modifications of the cell enzymatic activities, decrease in the amount of vitamins and cofactors from the tissues, changes in the cell electrolytes (increase in the amount of cellular Na, decrease in the amount of K), biochemical changes in the cell energy metabolism [3].

The morphological and the biochemical aging in the bradytrophic tissues with decreased $O_2$ consumption (cartilages, arterial walls, tendons, crystalline lens, cornea, etc.), where it occurs as dehydration and increased density, is different from the aging of the tachytrophic tissues with increased $O_2$ consumption (brain, muscles, etc.) where it means loss of active metabolic substance, atrophy and sclerosis, resulting mainly from the changes in cell permeability.

In numerous researches the connective tissue is granted an important part in the aging of the entire organism. The biophysico-chemical age changes are connected particularly with the intercellular structures of this tissue: the increase in the total collagen amount, increase in the covalent crosslinks within the collagen molecule, increase in the number of fibrillar structures, expansion of globular collagen, decrease of elastin, increase in the action of elastase, increase in density of the argentaphil fibres, diminution of the basic, amorphous fundamental substance.

The age changes in the structures of the organism considered as a unitary entity are essentially characterised by a linear regression in the number of units which make up the metabolically active cellular mass. The regression of the active cellular mass is asymmetric and asynchronous with different organs or even within the same organ. A nearly 30% regression was estimated in the metabolically active cellular mass (10 to 50% according to the type of cell) in parallel to the decrease in the water amount of the organism and to the increase in total lipid amount.

As an adaptation to the regression of the cellular mass attended, the reduction occurs in the blood globules with the aged (with no obvious functional impairment and a relatively constant plasma volume, directly proportional to the vascular capacity).

## METABOLIC CRITERIA IN AGING

The essential peculiarity of the biochemical framework with normally aging elderly and old individuals is a marked lability of the general homeostatic balance, manifest through the decreased adaptative abilities under stress. The classical pattern of the metabolic aging at the humoral level is characterised by a general tendency to increase during the VI and VII decades of the biochemical values for the factors involved in the lipid metabolism and blood coagulation.

The serum albumins and heparinoid substances tend to decrease; the diminution of the former is part of the general so-called 'anabolic deficit', the decrease of the latter is highly significant for the concomitant total disorders in hypercoagulability, hypofibrinolysis and dyslipemia, similar and closely correlated with the atherosclerotic and thrombotic types of fundamental biochemical lesions (Tables 2, 3, 4, metabolic indicators in normal aging) [3].

*Table 2*

**Biochemical indicators of lipid metabolism in aging**

Main peculiarity: THE DECREASE IN THE RATE OF THE LIPID METABOLISM IN SENESCENCE

DEPENDENT ON:

1. *Impairments in the intestinal absorption of neutral fats*
2. *Abnormalities in plasma lipid transport*
   - abnormalities in the proteic support
   - abnormalities in plasma hydrolysis
3. *Decrease in lipid catabolism*

   dependent on:
   - glucose insufficient metabolism: insufficient amounts of NADP-$H_2$ (through the hexoso-monophosph. cycle) and alphaglycero-phosphate
   - changes proper to the adipose tissue (cytological and of the vessels)
   - changes in the factors of *hormonal regulation* (decrease in the action of gonads and hypophyso-adrenal axis), *enzymatic balance* (L.P.L., elastase, transaminase) and *vitamin balance* (particularly pyridoxine)

MANIFEST BY:

*Dysmetabolism of the adipose tissue*

- increase in the total fat amount of the organism
- decrease in the metabolism of the adipose tissue, pointed out by:
  - incorporation of C14-acetate and C14 palmitate
  - $O_2$ uptake
  - decrease in reactivity to catabolic factors: epinephrine, norepinephrine, growth hormones.

*Plasma lipid dysmetabolism*

- Hyperkilomicronemia
- Increase in most of the lipid fractions levels
- Alteration of the lipid uptake dynamic curve.

*Table 3*

**Biochemical indicators of glucide metabolism in aging**

Main characteristic: DECREASE IN THE TISSULAR GLUCOSE UPTAKE IN SENESCENCE

DEPENDENT ON:

*Primary tissular changes in aging*

- decrease in number of active cells and glucose uptake, for synthesis or energogenetic reasons
- decrease in tissular enzymatic equipment

*Age changes in the efficiency of the glycoregulatory mechanisms*

- deficiency of the precocious insulin response
- decrease in the biological efficiency of circulatory insulin

MANIFEST BY:

- Decrease in the rhythm of extracellular glucose renewal
- Diminished glucose supply to the tissues
- Decreased glucose tolerance, in senescence

*Table 4*

**Biochemical indicators of protein metabolism in senescence**

*Total proteinemia within physiological limits*

*Hypoalbuminemia* (with moderately decreased turnover)

*Hyperglobulinemia*

| | |
|---|---|
| alpha | compensatory |
| beta | or |
| gamma | sum total of accumulated |
| fibrinogen | immune reactions |

*Decrease in the A/G ratio*

- positivity of routine tests in dysproteinemia
- electrophoretic: frequent aspects of secondary dysglobulinemia

*Changes in the content of some free amino acids*
*Hyperpolypeptidemia*
*Increase in oxidated glutathion*
*Blood urea levels at highest upper limit*
*Moderate hyperuricemia*, with decreased uric acid clearance
*Discrete normo- or hypercreatininemia*
*Hypercreatinemia*, with creatinuria (frequent with the age 50–70)

In the normal aging, the entire metabolic activity in close correlation with the structural age criteria is carried on under conditions of slow, progressive regression of the metabolically active cellular mass (advanced as total indicator in the evaluation of age), decrease in $O_2$ consumption and general biologic activity. The age criteria of the intermediate metabolic activities (decrease in total hydration and hydroelectrolytic turnover, tissular and humoral lipid dysmetabolism, decrease in glucide tolerance, frequent onset of a negative nitrogen balance), present particularly under stress, appear largely dependent on the impairment of cell enzymatic equipments, intermetabolic feed-back connections and age-induced morpho-functional peculiarities of the neuro-endocrine system.

## CRITERIA OF FUNCTIONAL AGING

As a result of the structural and metabolic involution, the impairment in adaptative reactions, the functional parametres of the organs and systems change. The functional age can be estimated based on a complex of criteria. The Romanian gerontologists have had a remarkable contribution in pointing out the most characteristic indicators.

1. Cardiovascular criteria. The cardiovascular age indicators reflect the interrelationship of the functional complex heart — circulatory system. They are pointed out by the main clinical, electrocardiographic, metabolic, radiologic, coronarographic, plethysmographic investigations [1, 5, 7]. The evaluation of the cardiodynamic parametres revealed a latent insufficiency in the aging heart. Among the factors which induce the decrease in the cardiodynamic performance with advanced age, the changing in geometry of the left ventricle is one of the most important [6].

2. Criteria of aging in the nervous system. Starting with the VIth decade, a significant decrease was noticed in the conduction speed of the nervous influx along the axons as well as a gradual increase in the latent period. At the level of the cortical neuronal networks, the slowing of the alpha rhythm was found as well as the progressive increase in the percentage of slow waves which become significant with the Vth decade. The neuraxial aging is more obvious in the psychometric tests. The progressive decrease of the subjects' reliability was pointed out, which follows an exponential curve starting with the age of 20. This phenomenon was noticed both with the simplest and the integrated functions (cognitive, creative).

3. Criteria of aging in the respiratory apparatus. The involutive changes which occur in the lungs are in the first place the result of the diminished elastic retraction force. The pulmonary mechanisms display an increased static compliance, dependent on frequency.

4. Kidney age indicators. The elementary, minimum combined and complex combined criteria point out the decrease in the glomerular filtrate, secretion capacity and tissular resorption, decreased ability to adapt to take up tests or to hydric restriction tests.

5. The peripheral hematologic indicators reflect the diminution of the medullary hematopoietic tissue, the decrease in the medullary mitotic and maturation indices.

6. The lability of the internal homeostasis. The decreased adaptative ability leads to the impairment of the acido-basic balance and electrokinetic processes from the internal medium. The changes in the electrokinetic potential of the blood elements are quite important in the case of platelets, because they favour hyperadhesiveness and thrombocytic aggregability. The researches carried out at the National Institute of Gerontology and Geriatrics have pointed out a humoral context characterised by dyslipemia, dysglycoproteinemia, increased concentration of coagulation factors, antithrombine decrease, impairments in the vascular wall, thrombocytic hyperadhesiveness; all these elements reveal the onset of thrombophilia in the aged.

7. The hypofunctionality of the immune system. This involves the ability to detect the non-self and to recognize the self, either normal or changed, which is equivalent with the loss of the born tolerance to some autocomponents. The insufficiency of the 'immune control' on the accumulation of new antigen carrier cells — resulting mainly from the wear of the thymodependent system — is considered by some authors the essential cause of senescence.

Beside the clinical, biological and psychological criteria individually elaborated research efforts aimed at finding new synthetic indicators at populational level [3].

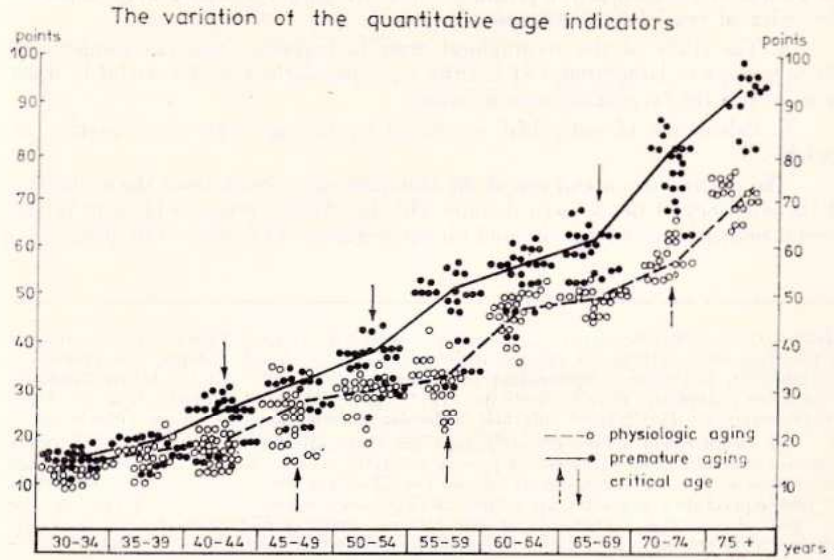



Fig. 1

Such an indicator is the 'mean biological age', which takes into consideration clinical, morphological, physiological, biochemical and morbidity data. The statistical and mathematical analysis of cohort morbidity data resulted in the elaboration of morbidity tables, used in the determination of the mean biological age and aging clock.

The static analysis of the age criteria is the quantitative expression of the age-induced qualitative changes (Fig. 1). The proportional quantification in relation to the importance of each criteria in the aging process allows the evaluation of the biological age both for individuals and communities, by means of sum totals.

In conclusion, the present methodology can be used for the evaluation of the biological age at individual and populational levels. Despite the progress achieved in the elaboration of criteria to define the morpho-functional age, there are still many unknown facts which have escaped researchers' attention.

Nevertheless, there are a few facts to point the way of future researches:

1. The Romanian gerontologists consider that the biologic condition of the individual can be evaluated by means of a large number of tests. The use of such batteries of tests in the course of longitudinal studies — as the one carried out at the National Institute of Gerontology and Geriatrics — will allow a comparison between the indicators specific to individuals with premature or accelerated aging and those of the longevous [2].

2. Elaboration of tests under stress.

3. The tests which proved useful should be maintained and the same prospective indicators included in the program, such as: anthropometric, work capacity, the index of cranial bone osteoporosis, etc.

4. The study of the geographical areas in longevous and the incidence of the longevous in large groups of healthy aged populations is also useful in order to point out the favourable natural factors.

5. Calculation of individual age based on the age regressive equation, as variable.

The determination and use of the biological age criteria allow the evaluation of the efficiency of the modern therapy with Dr. Aslan's products [4] with fundamental contribution to longevity and the prolongation of the active life-span.

**Résumé.** On présente, basée sur les recherches effectuées à l'Institut National de Gérontologie et Gériatrie, une synthèse des critères utilisés pour évaluer l'âge biologique et le rythme de vieillissement. Ces critères représentent les caractéristiques fondamentales du vieillissement, la baisse de l'adaptation et de la réactivité. Les indices de l'âge sont groupés dans les types d'organisation et d'intégration suivants: moléculaire, cellulo-tissulaire et de l'organisme.

Le vieillissement moléculaire est indiqué par l'intensification des « crosslinks ». Le vieillissement cellulaire et tissulaire est un processus sélectif, asynchrone. Le vieillissement normal est caractérisé par une labilité accentuée de l'équilibre homéostatique général. A ce niveau, est très importante l'évaluation des indices de l'âge sous conditions de stress. L'âge fonctionnel peut être évalué seulement par une batterie complexe d'épreuves. On mentionne les principaux critères pour l'évaluation des systèmes cardio-vasculaire, nerveux, rénal, immunologique et du milieu interne.

Les recherches faites à l'Institut National de Gérontologie et Gériatrie ont mené à l'élaboration des indices synthétiques qui servent à la détermination de l'âge biologique au niveau populationnel et individuel.

À la fin de l'article on indique les directions principales des recherches ultérieures. L'âge biologique sera déterminé avec plus d'exactité par des tests complexes, par indicateurs d'effort, l'étude des zones de longévité, en approfondissant les méthodes statistico-mathématiques.

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# PHYSIOPATHOLOGICAL ASPECTS OF CORONARY ATHERO/ARTERIOSCLEROSIS IN THE ELDERLY

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**Summary.** On the basis of personal studies and data from the field literature, the physiopathological peculiarities of coronary athero/arteriosclerosis with the elderly are synthetically presented in this paper.

In the context of the clinical physiopathology of the aged, the term "chronic ischemic cardioangiopathy" – CICA – to which the clinical form of the disease is associated, seems closer to the anatomic substratum and involutive changes of the heart – vascular system. The athero/arteriosclerosis reveals the kind of the process and the morpho-pathological objective condition of the elderly and aged.

The asynergism is one of the basic characteristics of the decreased contractility of the myocardium in chronic CICA elderly patients. The protodiastolic relaxation, the modified distensibility and the impaired kinetics of the ventricular wall are the main elements which account for the change in the left ventricle dynamics in coronary ischemia. The therapeutic approach is dictated by the simultaneous existence of several pathogenic and clinical facts with the aged.

The rapid and early occurrence of parietal changes in the coronary vessels lays a characteristic mark on the aging process in these circulatory areas.

Due to the incidence and clinical consequences of the disease, the coronary circulation represents the main area where athero/arteriosclerosis occur in the elderly.

The coronary failure is the physiopathological substratum of ischemic cardiopathy; its characteristic is the discrepancy between the oxygen and nutrient supply and requirements in the two organs.

The ischemic cardiopathy in the elderly and the aged should be integrated in the wider concept of chronic coronary ischemic cardioangiopathy (CICA), expressing the process of myocardial ischemia and its involutive hemodynamic consequences on the vascular tree and the myocardium [2].

CICA in the elderly represents in fact the geriatric physiopathology: the interference of multiple independent pathogenic mechanisms, either programmed or gained in the course of ontogenesis (Fig. 1), the synergism of which generates numerous clinical, metabolic, hemodynamic, enzymatic consequences.

I. Consequences of the coronary failure.

1. *Clinically*, the elderly and the aged display acute and chronic forms of the diseases.

The clinical consequences in acute forms are represented by separate entities:

– sudden death

– angina pectoris at rest and under stress

— angina Prinzmetal
— preinfarction
— transmural myocardial infarction.

Chronic CICA may occur in aged patients as: clinical syndrome of cardiac failure, rhythm or conduction disturbances, asymptomatic clinical forms.

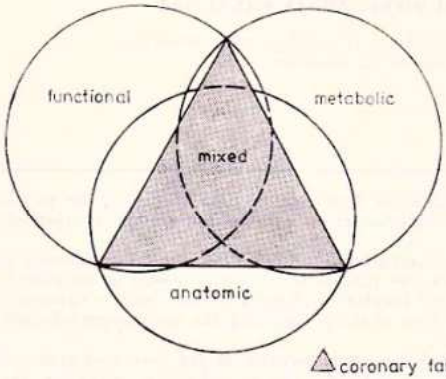


Fig. 1. — Factors that generate and hasten coronary failure in the aged.

2. *Metabolic consequences.* Certain age-peculiarities have been noticed to occur in the three stages of the energetic myocardial transformation [1]; they result from the diminution of the cellular respiration, some changes in the fermentation systems, increased myofibrilar sensitiveness toward humoral factors [3] having an important part in maintaining the functional energetic tonus. For the development of energy the oxidation of the carbohydrates is prevalent in the aged myocardium, to the detriment of fatty acids oxidation [1].

The qualitative and quantitative changes of the protein contraction with the aged, correlated with energo-formative deficiencies raise the problem of the critical elements in the transformation of the chemical energy into mechanical energy.

The studies on the metabolic consequences of chronic CICA have been focussed mostly on pyruvic and lactic acid concentrations. The oxygen myocardial post-capillary saturation was found unchanged [1] and the ischemic disturbances from different areas did not affect the general metabolic aspect. Most opinions tally with the deviation of glycolysis toward anaerobiosis, with the significant increase in lactate amounts.

Schwartz [6] pointed out that ischemia triggers the increase of intracellular $H^+$ concentration, which can have a competitive effect on $Ca^{++}$ in the troponine-tropomyosine complex. A clear decrease has been noticed of the pH from the coronary sinus and the extracellular medium.

3. *Hemodynamic consequences.* In 1894, Porter published the results of his hemodynamic researches on the effects of the coronary arteries obstruction. The consequences of the anterior intraventricular thrombosis would be: decreasing left ventricular systolic pressure, increasing diastolic pressure, progressively decreasing systolic output down to ventricular asystole.

The disturbances in the cardiac rhythm are secondary to the decreasing contractility power and enhance the deficit of the myocardial pump.

The coronary blood flow is not uniform through all myocardial areas, hence a direct relationship with the mechanical efficiency of the ventricular systole.

Thus, the asynergism becomes one of the basic elements in myocardial hypocontractility in elderly and aged patients with CICA.

The coronarographic changes express the severity of the ventricular asynergism [2].

The following elements are responsible for the left ventricular dynamics in ischemia [5]:

*a)* Protodiastolic relaxation. The analysis of the isovolumetric stage of the ventricular systole in aged heart points out the delayed protodiastolic relaxation and velocity.

*b)* Distensibility changes. Closely related to the protodiastolic relaxation, a great variation of the end-diastolic distensibility has been noticed.

The study of the end-diastolic ratio pressure/left ventricular volume during the atrial pacing in patients with CICA, suffering an anginous crisis points out the modification of the ratio, as a result of the decreasing distensibility and residual contraction powers.

*c)* Ventricular wall kinetics. The local ventricular dynamics in atherosclerotic coronaropathy has been studied by means of angio-cardiographic investigations. The data included in these studies point out the asynergism of the myocardial pump as a major element. Alongside areas with normal contractility, there are other areas in which this parameter has been modified and the velocity abated

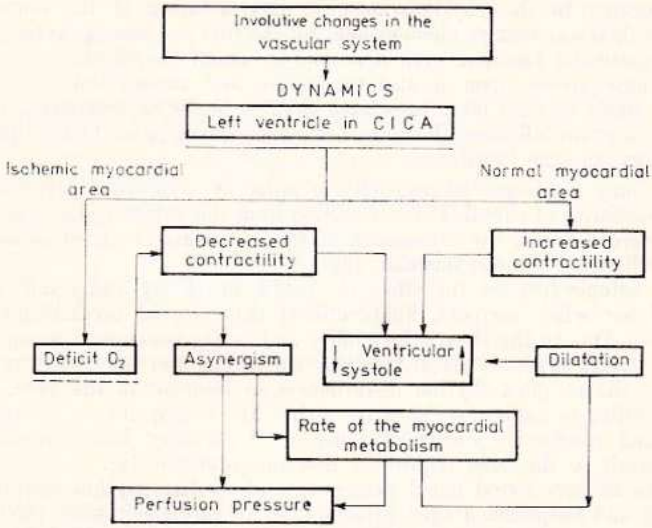


Fig. 2.—The influence of coronary ischemia on the integral pumping function (modified Rutishauer).

and delayed. In this way, ventricular dyskinesia as global concept, accounts for the global changes in ventricular dynamics with CICA patients (Fig. 2).

The filling, contractility and ventricular systolic efficiency are the main elements to determine cardiac performance in myocardial and age ischemia.

In the beginning, the normal myocardial areas try to compensate for the contraction deficiencies in the hypokinetic areas.

Based on the correlation between the hemodynamic and angiocoronarographic investigations, a transient left ventricular failure and an overt chronic deficit of the myocardial pump have been pointed out in aged patients.

The transient ventricular failure lays a characteristic mark on the aged patient with CICA, the cardiodynamic parametres of whom are below the normal limits even under so-called normal circumstances.

The hemodynamic data mark out a category of aged patients the hemodynamic parametres of whom are within normal limits when at rest, and in whom bodily exertion results in volume and end-diastolic ventricular pressure increase with well-adjusted stroke and cardiac output. Such aged persons do not present clinical complaints. The significant decrease of the stroke and cardiac output accompanies the end-diastolic ventricular pressure increase in another category of patients.

These 2 classes of patients can not be differentiated by means of coronarogram or ventriculogram, because these are obviously correlated with all the abated hemodynamic elements.

Mention should be made of the succession in time of the hemodynamic changes correlated with the ECG and the onset of anginous crises.

The timing of these data: the end-diastolic pressure modifies at 30—40 sec; the ECG changes occur within the next 2—3 min; 3—4 min later the patient feels the pain.

According to the physiopathological interpretation of the sequence the anginous pain is a secondary phenomenon, not the first one among the factors affecting the ventricular function, even if it does break off the effect.

4. Consequences upon cardiac conduction and automatism.

The single or most often associated changes in the automatism and conduction of the nervous influx are the main mechanisms involved in the multiple rhythm disturbances in the aged.

The lack of electric homogeneity in adjacent myocardial areas and fibres [2] is a peculiarity of chronic CICA, resulting from the anatomical changes in different myocardial areas, the asynergism of the ventricular contraction and ununiform distribution of catecholamine [2].

The ectopic foci are the effect of electric instability and result from the action of borderline currents, unidirectional decremential mechanisms or focal reexcitation. Due to the electric instability and to the consequent unequal excitability of certain areas, a masked conduction mechanism may start to function generating the complex rhythm disturbances so frequent in the aged.

According to anatomo-clinical researches, the standpoint on the relationship rhythm and conduction disturbances/degree of coronary failure resulting from atherosclerosis in the aged requires a new interpretation [2].

Some authors found equal percentages of cardiac rhythm disturbances in unselected and hospitalized aged persons even in cardiologic units (30%) [4].

Our own observations on 2131 patients hospitalized at the National Institute of Gerontology and Geriatrics (1971—1974) pointed out 67.8% cardiac arrhythmias and conduction disturbances in the coronary athero/arteriosclerosis etiogenesis [2].

Based on Lenègre's conclusions and on some anatomo-clinical data concerning the aged patient, a special part is played by the relationship between the atrio-ventricular blocks and the condition of the coronary vessels.

At present, the coronary ischemic substratum is attributed to the blocks that complicate myocardial necrosis, or that are accompanied by anginous crises. In the genesis of sinus bradycardia and particularly in that of the atrio-ventricular blocks, the role of the Hissiene junction primary degenerative lesions which had been minimized, is given more attention nowadays.

## CONCLUSIONS

1. The physiopathological peculiarities of the major clinical forms of coronary athero/arteriosclerosis in the elderly and the aged prove the complexity of the mechanisms involved and the simultaneous existence of more pathogenic facts with the geriatric age.

2. The understanding of the physiopathological implications of the atherogenous disease in the aged depends to a great extent on all the biological data of the third age and the quasi-constant evidence of the multiple pathology.

3. The consequences of ischemic coronary failure consist in some clinical, metabolic, hemodynamic and enzymatic peculiarities which reflect the morpho-functional substratum and the "milieu intérieur" of the aged.

**Résumé.** L'article présente dans une manière synthétique et basé sur les recherches et résultats des auteurs les particularités physiopathologiques de l'athéro/artériosclérose coronarienne chez les sujets âgés.

Dans la physiopathologie des personnes âgées, le terme « cardioangiopathie ischémique chronique » (CAIC) auquel est associée la forme clinique de la maladie, semble plus approprié aux aspects anatomiques et modifications évolutives du complexe système cardio-vasculaire.

L'athéro/artériosclérose nous révèle le type du processus et la situation objective morphopathologique des personnes âgées et des vieillards.

L'asynergisme est une des caractéristiques fondamentales de la contractilité diminuée du myocarde dans la CAIC des sujets âgés.

La relaxation protodyastolique, la distensibilité modifiée et le kinétisme défectueux de la paroi ventriculaire représentent les principaux éléments déterminant les modifications de la dynamique du ventricule gauche dans l'ischémie coronarienne. La thérapeutique est dictée par l'existence simultanée des divers facteurs pathogéniques et cliniques chez les personnes âgées.

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# ASPECTS NEUROMORPHOLOGIQUES AU COURS DU PROCESSUS DE VIEILLISSEMENT DANS LE CERVELET

## Ire Note

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**Résumé.** Les études ont été effectuées sur les cervelets provenant de deux témoins (de 40 et 57 ans) et de 5 vieillards appartenant aux décennies 8–10, qui ne présentaient aucune symptomatologie de la série cérébelleuse.

On a constaté que le nombre des cellules de Purkinje diminue, depuis l'âge adulte jusqu'à la 10e décennie, d'environ 40%, ce qui représente, pour un taux constant, une perte d'environ 2700 cellules par jour.

Les cellules granulaires diminuent elles aussi de 26–30%, ce qui, pour un taux constant, exprime une perte d'environ 26 000 000 de cellules par jour, les deux groupes de chiffres étant garantis du point de vue statistique.

Les pertes respectives sont uniformes sur le cortex cérébelleux tout entier et n'affectent pas profondément l'activité de celui-ci, du fait de son organisation morphologique extrêment redondante, aussi bien du point de vue du nombre des cellules qu'à cause du principe de la distribution des connexions, soit dans le cadre des sous-systèmes excitateurs (fibres moussues, cellules granulaires, fibres parallèles, fibres grimpantes), soit dans celui des sous-systèmes inhibiteurs (cellules de Golgi, cellules en panier, cellules étoilées).

### INTRODUCTION

L'un des principaux aspects du processus de vieillissement du système nerveux est constitué par une réduction graduelle du nombre des neurones. Le fait a été signalé pour la première fois par Hodge [1] au niveau du cervelet et des ganglions spinaux des mammifères, ainsi qu'au niveau du système nerveux des abeilles. Depuis lors on a accumulé d'assez nombreux travaux qui confirment le phénomène de dépopulation neuronale, les données fournies étant contradictoires seulement en ce qui concerne leur aspect quantitatif. Récemment, on a émis même des opinions qui tendent à minimiser l'importance de la réduction du nombre des neurones avec l'âge (Comfort [2]; Königsmark et Murphy [3]).

Bien que le mécanisme de la dépopulation neuronale ne soit pas bien connu (désorganisation biochimique de la cellule, phénomènes d'auto-immunité, etc.), l'importance théorique du processus même a été soulignée dans un autre travail par l'un des auteurs du présent travail C. Bălăceanu, ([4] avec Gabriela Angel).

Pour obtenir une image plus réelle quant aux dimensions de ce processus, l'Institut National de Gérontologie et Gériatrie de Bucarest a pris l'initiative d'un programme de recherches neuro-anatomiques concernant les aspects morphologiques du vieillissement du système nerveux.

Dans ce travail nous essayerons de fournir quelque données quantitatives concernant la réduction avec l'âge du nombre des neurones du cervelet. Nous nous référerons en premier lieu au nombre des cellules de Purkinje (les plus grands neurones — 30/70μ du système nerveux des vertébrés), nombre qui a déjà été étudié (Hodge [1]; Ellis [5]; Harms [6]; Lojda [7]; Hall et collab. [8],) pour nous créer une opinion propre par rapport aux données contradictoires, mentionnées dans la littérature de spécialité. Nous nous référerons ensuite au nombre des cellules granulaires qui n'ont pas été étudiées jusqu'à présent, et qui représentent la plus vaste et la plus dense population neuronale du système nerveux, constituée par les plus petits neurones du névraxe (5—8 μ diamètre).

## MATÉRIEL ET MÉTHODES

1. On a prélevé: le cervelet de deux témoins, l'un ayant 40 ans, l'autre 57 ans, ainsi que le cervelet de cinq grands vieillards ayant plus de 90 ans et de cinq vieillards, décédés dans la 8e décennie, et qui ne présentaient aucune symptomatologie de la série cérébelleuse.

2. Toutes les pièces ont été fixées au formol 10% pendant un an. On a prélevé des fragments variables de l'hémisphère gauche du cervelet et on les a inclus à la celloïdine.

Les sections ont été effectuées à une grosseur de 15μ. Pour la coloration on a utilisé la méthode de Nissl. Les solutions et les techniques utilisées ont été rigoureusement similaires dans tous les cas et ont été effectuées par la même personne, afin d'éliminer les variations d'un cas à l'autre.

3. Pour le dénombrement des cellules on a utilisé une technique de cytocaryométrie, en projetant sur un plan l'image microscopique.

On a utilisé le microscope IOR M.C. 1 qui dispose d'accessoires nécessaires.

Pour les cellules de Purkinje on a utilisé l'oculaire 7 et l'objectif 10;pour les cellules granulaires — l'oculaire et l'objectif 10.

4. Le dénombrement des cellules de Purkinje a été effectué de façon linéaire le long de la couche respective. La longueur explorée était mesurée à l'aide d'un courvimètre. On a calculé la densité des cellules sur une unité de longueur choisie de façon arbitraire et toujours la même.

Le dénombrement des cellules granulaires a été effectué dans l'espace d'une aire arbitraire mais constante et on a calculé la densité des cellules rapportée à l'aire respective.

Les deux types de dénombrement des cellules ont été effectués sur 150 zones différentes, pour chaque cas séparément.

5. On a comparé les moyennes obtenues dans chaque cas au cours des 150 dénombrements effectués, avec les moyennes obtenues pour les témoins; les résultats de ces comparaisons ont été exprimés en pourcentages (en considérant la situation pour le témoin de 100%).

On a obtenu de cette façon le pourcentage des cellules de Purkinje et granulaires qui ont subsisté et qui ont disparu dans chaque cas, ainsi qu'une moyenne globale du pourcentage des cellules restées ou disparues pour tous les cas étudiés.

## RÉSULTATS ET DISCUSSIONS

Les données obtenues peuvent être résumées dans le tableau 1.

*Tableau* 1

| | Cellules de Purkinje | | Cellules granulaires | |
|---|---|---|---|---|
| | Cellules présentes % | Cellules disparues % | Cellules présentes % | Cellules disparues % |
| Témoin 1 (40 ans) | 100 | 0 | 100 | 0 |
| Témoin 2 (57 ans) | 100 | 0 | 100 | 0 |
| Cas I 93 ans | 69,82 | 30,18 | 89,79 | 10,21 |
| Cas II 94 ans | 61,25 | 38,75 | 68 | 32 |
| Cas III 95 ans | 55,24 | 44,76 | 72 | 28 |
| Cas IV 94 ans | 53,10 | 46,90 | 63,41 | 36,59 |
| Cas V 92 ans | 54,18 | 45,82 | 58,58 | 41,42 |
| Moyenne pour la 10e décennie | 58,7 | 41,3 | 70,36 | 29,64 |
| Cas VI 78 ans | 82,33 | 17,67 | 85,60 | 14,40 |
| Cas VII 72 ans | 76,79 | 23,21 | 77,95 | 22,05 |
| Cas VIII 77 ans | 80,25 | 19,75 | 87,57 | 12,43 |
| Cas IX 72 ans | 82,37 | 17,63 | 75,80 | 24,20 |
| Cas X 71 ans | 59,77 | 40,23 | 66,78 | 33,22 |
| Moyenne pour la 8e décennie | 76,30 | 23,70 | 78,74 | 21,26 |

1. Les données incluses dans le tableau 1 montrent que le processus de vieillissement du cervelet, décelable par le phénomène de dépopulation neuronale, peut être constaté à partir de la 6e décennie.

Les deux témoins (le témoin I de 40 ans et le témoin II de 57 ans) ont la même densité neuronale. Nous avons considéré cette densité comme grandeur de référence, aussi bien pour les cellules de Purkinje, que pour les cellules granulaires.

Nous avons effectué des moyennes séparées pour la période de vieillissement comprise entre la 6e et la 10e décennie (les cas I—V), ainsi que pour la période comprise entre la 6e et la 8e décennie (les cas VI—X), afin de constater si les taux de vieillissement subissent une accélération pendant les dernières décennies. Les chiffres obtenus sont significatifs du point de vue statistique. On constate qu'une telle accélération ne se produit pas, les taux annuels de dépopulation neuronale demeurant relativement constants.

2. Il résulte de l'analyse du tableau que le nombre de cellules de Purkinje diminue d'environ 40% pour les cas de la 10e décennie et d'environ 24% pour les cas de la 8e décennie, par rapport aux témoins. Ces valeurs se situent à un niveau similaire à la majorité des données de la littérature (Lojda 30%; Harms 25%; Hall et collab. 25%). Nos données infirment l'opinion de Delorenzi [9], selon laquelle le nombre des cellules de Purkinje ne se réduit pas avec l'avancement en âge.

On constate aussi que le nombre des cellules granulaires diminue d'environ 30% pour les cas de la 10e décennie et d'environ 21% pour les cas de la 8e décennie. Cela prouve que ce système neuronal, lui aussi, est soumis à la dépopulation du fait de la sénescence.

Il existe un parallélisme, dans les divers cas, entre le pourcentage de la diminution du nombre des cellules de Purkinje et celui des cellules granulaires, ce qui

nous suggère l'existence d'un mécanisme commun. La proportion plus réduite de la dépopulation au niveau des cellules granulaires s'explique, selon nous, par les dimensions plus réduites de ces neurones, ce qui diminue la probabilité de l'impact des facteurs abiotrophiques qui agissent sur le volume de la substance.

3. En partant des estimations effectuées par Lojda [7], qui est arrivé à la conclusion que, dans le cervelet de l'adulte témoin il existe approximativement 13 000 000 de cellules de Purkinje, il résulte que depuis la 6e décennie jusqu'à la 10e le cervelet humain perd 5 000 000 de cellules de Purkinje, dont 3 000 000 de cellules entre la 6e et la 8e et 2 000 000 cellules entre la 8e et la 10e décennie.

Nous pouvons donc admettre comme taux approximatiff une perte annuelle d'environ 1 000 000 de cellules de Purkinje, ce qui nous indique une perte moyenne de 2 700 cellules de Purkinje par jour à partir de l'âge de 50 ans.

En considérant qu'il existe un rapport de 1/10 entre les cellules de Golgi et les cellules de Purkinje, et que les cellules de Golgi ont un diamètre d'arborisations de 1.200 $\mu$ (Eccles et collab. [10]), nous avons calculé la surface de la couche des cellules de Purkinje, obtenant $14695{,}2 \times 10^8$ $\mu^2$. En connaissant que la couche granulaire a une grosseur d'environ 300 $\mu$, il en résulte un volume de $440\,856 \times 10^9$ ou 440 856 mm$^3$. En tenant compte que les recherches de Fox et Barnard [11] ont consigné qu'il y a environ 2 400 000 de cellules granulaires par mm$^3$, il s'ensuit que le cervelet humain dispose d'environ $10^{12}$ cellules granulaires ($1\,058\,054{,}4 \times 10^9$).

En appliquant le résultat de nos recherches au chiffre ci-dessus, il s'ensuit qu'au cours de 4 décennies disparaît un nombre de $3{,}3.10^{11}$ cellules granulaires dont $1{,}8.10^{11}$ disparaissent dans l'intervalle entre la 6e et la 8e décennie, ce qui nous fournit une moyenne approximative de $5.10^{10}-10^{11}$ cellules granulaires perdues par an.

Il en résulte qu'à partir de l'âge de 50 ans le cervelet humain perd en moyenne, par jour, 13 500 000—27 000 000 cellules granulaires.

Le chiffre semble impressionannt, mais il faut tenir compte aussi bien du nombre considérable de cellules disponibles que de la théorie de la fiabilité des groupes de réserve, appliqué aux réseaux neuronaux (C. Bălăceanu et G. Angel [4]).

4. Malgré cette diminution des cellules, prodigieuse du point de vue numérique, les fonctions cérébelleuses se maintiennent à peu près intactes chez les grands vieillards, ainsi qu'il ressort de l'examen clinique. Cela s'explique par l'organisation redondante du réseau neuronal cérébelleux.

En tenant compte que la dépopulation neuronale s'effectue de manière aléatoire et avec une uniformité statistique dans toute l'écorce cérébelleuse, on peut faire une série de constatations et déductions concernant la persistance de l'efficience du sistème cérébelleux chez les personnes âgées.

4.1. Chacune des fibres moussues (qui constituent le principal canal d'entrée du cervelet) transmet, par une seule de ses ramifications, ses informations à un groupe d'environ 600 neurones granulaires. Dans la 10e décennie, conformément au calcul déduit de nos observations, demeurent encore 420, ce qui est suffisant. La structure est encore plus fiable si nous considérons que, fréquemment, une fibre moussue se termine par plusieurs ramifications.

Le contact synaptique entre les fibres moussues et les fibres granulaires se produit au niveau des glomérules, où une ramification afférente entre en contact avec un nombre moyen de 3,15 cellules granulaires, ce qui permet, dans la 10e décennie, la persistance d'une moyenne de 2,80 cellules granulaires par glomérule.

4.2. L'information est transmise par les cellules granulaires aux cellules de Purkinje à l'aide du système des fibres parallèles de la couche moléculaire du cervelet. La fiabilité est maintenue par une double redondance.

4.2.a. Chaque cellule de Purkinje reçoit des afférences provenant d'environ 209 000 fibres parallèles (Eccles et collab. [10]), ce qui lui permet de conserver encore 146 300 fibres dans la 10e décennie.

4.2.b. Chaque fibre parallèle s'étend, en moyenne, sur une longueur de 1—1,5 mm, ce qui lui permet de venir en contact avec approximativement 300 cellules de Purkinje, dont 176 lui restent disponibles dans la 10e décennie.

4.3. Parallèlement à la distribution des signaux excitateurs dans le cortex cérébelleux, on distribue aussi des signaux inhibiteurs par le système des cellules de Golgi, des cellules en panier et des cellules étoilées.

4.3.1. En ce qui concerne les cellules de Golgi, on connaît qu'une telle cellule distribue ses informations à environ 10 cellules de Purkinje, ce qui lui assure encore dans la 10e décennie, une quantité disponible d'environ 5,87 de telles cellules.

4.3.2. Les cellules en panier distribuent leurs axones (qui ont un trajet perpendiculaire aux fibres parallèles) dans la couche moléculaire à environ 70 cellules de Purkinje, ce qui leur permet d'agir, dans la 10e décennie, sur environ 42 cellules de Purkinje.

4.3.3. Les cellules étoilées **a** et **b** ne sont pas susceptibles d'une interprétation quantitative à cause de leur connectivité extrêmement variable.

4.4. En ce qui concerne le système des fibres grimpantes (l'autre canal d'entrée dans le cervelet), nous ne disposons pas de données quantitatives suffisantes. Nous connaissons pourtant qu'aucune fibre grimpante n'entre en contact avec un seul neurone de Purkinje, ainsi que l'on croyait, mais avec un groupe de tels neurones, soit par collatérales directes, soit par collatérales indirectes (Scheibel et Scheibel [12]).

L'analyse de la connectivité de ces fibres aurait été particulièrement significative, parce que toutes ces fibres proviennent du système de l'olive du bulbe rachidien (Szentagothai et Rajhovits [13]) et celle-ci est une structure dont le nombre de neurones ne varie pas avec l'âge, mais reste constant — autour de 364 000 (Brody [14]).

## CONCLUSIONS

1. Le nombre des cellules de Purkinje diminue, à partir de 50 ans jusqu'à la 10e décennie, d'environ 40%, ce qui représente, à un taux constant, une perte d'environ 2700 cellules par jour.

2. Les cellules granulaires diminuent elles aussi de 30%, ce qui, pour un taux constant, exprime une perte d'environ 26 millions de cellules granulaires par jour.

3. Les pertes respectives sont uniformes sur le cortex cérébelleux tout entier et n'affectent pas profondément l'activité de celui-ci, du fait de son organisation morphologique extrêmement redondante, aussi bien du point de vue du nombre des cellules, qu'à cause du principe de la distribution des connexions, soit dans le cadre des sous-systèmes excitateurs (fibres moussues, cellules granulaires, fibres parallèles, fibres grimpantes), soit dans celui des sous-systèmes inhibiteurs (cellules de Golgi, cellules en panier, cellules étoilées).

**Summary.** The study was conducted on human cerebellum from controls aged 40 to 57 years and old persons in the 8th to 10th decades with no cerebellar symptoms.

A 40% decrease in the number of Purkinje cells was found in the adults until the 10th decade, meaning a constant daily loss of 2,700 cells.

The granular cell number also dropped by 26–30%, which means a constant daily loss of 26,000,000 cells; both figures were statistically ensured.

The respective cell loss was uniform throughout the cerebellum cortex; its activity was not deeply affected, because of its extremely redundant morphological organization based on the number of cells and the distribution of the connections either in the excitatory subsystems (mossy fibres, granular cells, parallel fibres, climbing fibres) or inhibitory subsystems (Golgi cells, basket cells, spider cells).

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# CLINICAL IMPLICATIONS OF GLUCOSE TOLERANCE TEST INTERPRETATION IN RELATION TO THE AGE OF THE SUBJECT

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**Summary.** Based on data from personal and field literature on senescence and its possible relationship with the decreased glucose tolerance, the authors discuss the interpretation of glucose tolerance test. Arguments are gathered to support a methodology aimed at standardizing the dosings and the interpretation of the results. Mention is also made of the possible implications of enzyme and radio-immune dosings in explaining metabolic peculiarities with advanced age.

The paper includes essential data on the discovery of diabetes mellitus in the aged. Previous studies (1969) pointed out the increase in fasting glucose levels with each advancing age decade as follows:

| Age of subject (years) | Fasting glucose levels (mg%) |
|---|---|
| 20–29 | 112 |
| 30–39 | 118 |
| 40–59 | 125 |
| over 60 | 130 |

(according to Honigmann and coll. – 1969)

According to West K. M. fasting glucose levels over 100–110 mg % occur in a small number of aged persons [29]. Verifying the changes in glucose levels in relation to advancing age and based on fasting glucose test and on GTT, Nițulescu J., Ornstein I., Sibi Maria [26] found insignificant variations against standard glucose levels in young persons. Mention should be made that the data were not statistically analysed, their significance being estimated per cent. The topic has been taken over by the authors of this paper [15] and fasting glucose levels as well as GTT were calculated based on Student Fischer test on aged subjects. The dynamic checking of glucose tolerance yielded results comparable with those obtained by Nițulescu, Ornstein, Sibi; GTT curves revealed certain peculiarities related to the context habits, in the first place. Neither does our research infer the necessity for special standards with the aged. According to Pyke D. A. (quoted by [25]), the general metabolic disturbance would account for the elevated glucose levels in the aged. The factors inducing decreased glucose tolerance could be: tissue wear off, decrease in number and functional capacity of beta cells.

According to Harrth A. [13], the diagnosis of diabetes mellitus is positive in all the aged persons with fasting glucose levels exceeding 130 mg % (and no glycosuria). The senile hyperthyroidism which can not be discovered clinically, could favor hyperglycemia and glycosuria.

In order to discuss the diagnosis in relation to glucose values during GTT, we shall first give the data reported by Honigmann and coll. (1969).

*Table 1*

| Age (years) | Minutes | | |
|---|---|---|---|
| | 30 | 60 | 120 |
| 20–29 | 155–175 mg% | 145–170 mg% | 105–125 mg% |
| 30–39 | 170–190 mg% | 155–185 mg% | 105–125 mg% |
| 40–49 | 185–210 mg% | 165–200 mg% | 105–125 mg% |
| 50–59 | 185–210 mg% | 185–215 mg% | 105–125 mg% |
| 60–69 | 185–210 mg% | 195–230 mg% | 105–125 mg% |
| 70–79 | 185–210 mg% | 195–230 mg% | 130–160 mg% |

(according to Honigmann and coll. – 1969)

Referred to fasting glucose values, the variation of the figures does not allow a distinction between 'diabetic' and 'normal' persons. According to Mana and Tchobroutsky [21] diabetes mellitus could be suspected when fasting glucose values exceed 1.30 g‰ (capillary blood) and 1.25 g‰ (venous blood). The values close to the above-mentioned ones require an interpretation within the clinical context which is the more difficult as the subject is older. Joshn's criteria of the diagnosis of diabetes mellitus in the aged are the following: glucose levels exceeding 13 mg %, with glycosuria, or glycosuria exclusively (the renal threshold is usually higher in the aged). Diagnosis is rendered more difficult by the uptake of glucose due to the delayed, weaker reaction of the glycoregulatory system in the aged. According to Köbberling J. A. and coll. [20] glucose levels indicating the disease are: GTT 200 mg % at one hour and 150 mg % at 2 hours. The above-mentioned authors consider that 170 mg % at one hour and 130 mg % at 2 hours do not indicate diabetes mellitus. For diagnosing diabetes mellitus the following paraclinical parametres should be used:

*Table 2*

| | Absent clinical signs | | |
|---|---|---|---|
| Fasting glucose levels | increased | increased | normal |
| Glycosuria | + | – | + |
| GTT | pathological | pathological | pathological |

(according to Mana H., Tchobroutsky G., 1968)

Glycosuria is frequently absent in the aged, even when fasting glucose levels have increased; as a peculiarity of diabetes mellitus in the aged, the diminution or even normalization of glucose levels have been noticed to accompany the onset of complications [7, 8]. The frequent glycoregulatory disturbances in coronary patients with overt diabetes mellitus [3, 4, 5] raise certain difficulties of diagnosis.

The interpretation of GTT curves has always been a problem with the aged outside the clinical context of diabetes mellitus disease [6, 15]. As glycosuria is sometimes absent, even with elevated glucose levels reaching 3 g $^0/_{00}$, polyuria and polydipsia are also absent, making the diagnosis of diabetes mellitus even more difficult in these cases [1, 19]. The possibility of diabetes mellitus with normal fasting glucose levels requires the use of the glucose uptake test — carried out with 50—100 g glucose per os (generally 1 g/kg body weight). Some authors consider the aged a virtual diabetic. According to Graff E. S. and coll. [11], the higher incidence of latent diabetes in advanced ages is statistically significant; the data are based on the fact that a certain number of persons with normal oral GTT when young, later presented either latent or clinically overt diabetes; these authors suggested a genetic proneness to diabetes to account for the above-mentioned fact.

Because no standard norms have been established for persons aged over 40—50, the suggestion has been advanced to consider normal glucose values those resulted from adding an average amount of 0.10 g $^0/_{00}$ glucose to each age decade.

*Table 3*

| Author | Year | to add g$^0/_{00}$ | Age decade |
|---|---|---|---|
| Mana-Tchobroutsky [21] | 1964 | 0.10—0.12 | to each age decade over 45 |
| Mana-Tchobroutsky [21] | 1965 | 0.13 | to each age decade over 50 |
| Fajans-Conn [9] | 1965 | 0.10 | to each age decade over 40 |
| Ricketts et al [28] | 1966 | 0.10—0.15 | to each age decade over 30 |

(fasting glucose values resulted from the increase in glucose amounts according to the table)

Mention should be made of the National Health Survey (1960—1962) carried out on 111,000 GTT curves, with 50 g glucose; they pointed out a linear increase by 14 mg % with each age-decade (quoted by [24]). With 100 g glucose, — a study conducted at Tecumseh (Michigan) — the elevation of the venous blood glucose levels in 4,000 persons was also linear and progressive: 100 mg % (16—19 years) and 177 mg % (70—79 years); the increase was thus 13 mg % by age-decade, which is not a significant difference from the GTT curves yielded by 50 g glucose [14]. Butterfield's study conducted on a London community which received 50 g glucose *per os* measured capillary blood glucose levels (the values were thus found to increase by 14 mg % and 7 mg % at 1 and 2 hours, respectively, by age-decade (quoted by [25]). The epidemiologic studies carried out by Mincu and coll. between 1970—1972 aimed at establishing the limits of the normal glucose levels in relation to age, 2 hours after the intake of 100 g glucose, as follows: 122 mg % (25—30 years), 145 mg % (over 60—65 years). Glucose uptake and cortisone stimulation in the aged are considered useful only for research goals [27], which also require enzyme investigations, insulin and radioimmune determinations.

The glucose tolerance test should be repeated because glycoregulation abnormalities can be considered among diabetic risk factors only when they persist. In order to detect diabetes mellitus among larger communities the test should be resumed every 2 years whenever necessary [1]. The above-mentioned observations

should be taken into consideration when reading the curves. According to Justin-Besançon [18], all the curves with glucose levels exceeding 1.20 g ‰ at 2 hours should be considered normal if the other GTT values are within normal limits. He considers diabetic curves only those with an arrow pointing higher than 2 g ‰; the curves with the arrow pointing 1.60 to 2 g ‰ being "paradiabetic".

There are also physiological GTT changes like those occurring during pregnancy (which disappear after birth); GTT is also impaired in other numerous conditions besides diabetes mellitus (digestive, hepatic, cardiovascular diseases, various afflictions with important metabolic dysfunctions). In the same subject, GTT curves can be sometimes "normal", at other times "abnormal" (or suspect) as authorised researches have pointed out [22]. The fluctuations which affect glucose homeostasis by means of an elevated catecholamine secretion during the test may account for the above-mentioned results [10]. The explanations are certainly more numerous [21]. Enzyme, insulin, radioimmune dosings will probably allow a better understanding of this process. Clinically, Fajans and Conn's criteria [9] are applicable to evaluating GTT curves. Nevertheless, the use of different criteria and dosing methods (Fajans and Conn dosing was based on Somogy-Nelson's method) lead to a different evaluation of the incidence of diabetes mellitus among a given population, even among researchers [10]; this requires further discussion in order to establish a uniform evaluation criterion, at least on the national level.

Considering the preservation within normal limits of the functions of glands with hyperglycemic role up to advanced ages, a larger difference between the normally preserved system and the deficient hypoglycemic one would also account for the diminished GTT in the aged. There are numerous possible interpretations of the diminished glucose tolerance among which, peripheral glucose uptake seems particularly important.

In conclusion, mention should be made of the following facts: our researches point out that increased sugar levels in clinically healthy aged subjects are not indicative of diabetes mellitus, their variation remaining within the limits admitted for mature adults; it can be interpreted in terms of an adaptative phenomenon in the course of senescence.

**Résumé.** Les auteurs mentionnent les recherches propres et celles signalées dans la littérature médicale, concernant le processus de vieillissement en relation avec le test de tolérance vis-à-vis de la glucose; les auteurs font une interprétation de ce test. Les auteurs plaident pour une méthodologie susceptible d'uniformiser le dosage et l'interprétation des résultats, dans le domaine clinique et dans celui de la recherche fondamentale. Les perspectives des dosages enzymatiques et radio-immunologiques, dans l'explication des particularités métaboliques aux âges avancés, sont aussi considérées.

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# AUTOIMMUNE PHENOMENA IN THE ELDERLY

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**Summary.** Studies were carried out on 300 apparently healthy subjects (180 women and 120 men) from Bucharest, ranging in age from 40 to 90 years, on the presence of smooth muscle (SMA), antinuclear (ANA) and anticytoplasmic antibodies (ACA) by indirect immunofluorescence, antialbumin antibodies (AAA) tested by agarose gel immunodiffusion with glutaraldehyde polymerized human serum albumin, and immunoglobulin concentrations by single radial immunodiffusion.

The results showed an increase with age in the incidence of autoantibodies, especially in women, while in men a decrease in the prevalance of autoantibodies was registered in the 7th and 8th decades, in agreement with the influence of autoimmune phenomena on the death rate in males.

AAA increased in both men and women as compared to the other categories of autoantibodies, but showed a different association tendency AAA-SMA in women and AAA-ANA in men, suggesting a different etiopathogeny for some subclinical liver diseases.

Slight increase in IgG and IgA concentrations and decrease in IgM values with age were also noticed.

## INTRODUCTION

Studies performed in apparently healthy individuals have revealed an increase with age in the frequency of antinuclear antibodies (ANA) [1—3], smooth muscle antibodies (SMA) and rheumatoid factors [2, 4].

The concomitant raised incidence of infections and cancer described in the elderly, as well as in naturally or therapeutically induced immunodeficient states, lend support to the theory of T lymphocyte deficiency associated with aging [1, 5, 6]. A defect in the suppressor T cell population, however, would ascribe pathogenic significance to the different categories of autoimmune phenomena and to the association of autoantibodies with cardiovascular disease and mortality with age observed by Mackay [3] and by Roberts-Thompson et al. [4]. On the other hand, the increased incidence of autoantibodies in the aged might reflect pathologic alterations of endothelial and parenchymal cells, especially in the arteries, liver and kidney following different metabolic, infectious or toxic aggressions accumulating in time.

It is well known that besides age and sex dependence, the incidence of autoantibodies registers geographical differences correlated with genetic and environmental factors. Hence, we considered it of interest to investigate the frequency

of autoantibodies and immunoglobulins concentration in 300 normal subjects from Bucharest, ranging in age from 40 to 90 years.

## MATERIALS AND METHODS

*Subjects.* Sera were obtained from 180 women and 120 men ranging in age from 40 to 90 years, in whom no overt hepatic or other clinical impairment was observed. Case distribution according to sex and age is presented in table 1. Sera were kept at −20°C till analyses were performed.

### TESTS FOR AUTOANTIBODIES

*The indirect immunofluorescence technique* was used [7] for testing autoantibodies to nuclei (ANA), to smooth muscle (SMA) and to the cytoplasm of gastric parietal and kidney tubular cells, i.e. anticytoplasmic antibodies (ACA) characterized by a fine granular and diffuse cytoplasmic fluorescence.

Rat heart, kidney and stomach were cut in 4 μ thick sections in a cryostat at −20°C. All tissue fragments on the slides were first layered with test serum diluted 1:10 in phosphate buffered saline (PBS) at pH 7.3, and then with rabbit anti-human immunoglobulin serum conjugated with fluorescein isothiocyanate. The sections were mounted in 10% PBS in glycerol and examined in fluorescence microscopy (HBO 200 lamp). Sera which gave off fluorescence at a 1:10 dilution were considered positive.

*Detection of antialbumin antibodies (AAA)* was performed by immunodiffusion (ID) with glutaraldehyde polymerized human serum albumin (ID—HSAP), using the method described by Lenkei and Gheție [8].

An amount of 20 mg human serum albumin (HSA) (Kabi, Stockholm) was dissolved in 0.9 ml PBS 0.1 M pH = 6.8 and 0.1 ml of a 2.5% glutaraldehyde solution was added. The mixture was incubated for 2 hrs at room temperature and further dialysed against PBS for 3 hrs with frequent changes of buffer.

The glutaraldehyde treated HSA solution (HSAP) (20 mg/ml) was mixed with an equal volume of untreated HSA (40 mg/ml) dissolved in 0.2 M carbonate buffer (pH = 9) and further incubated for 1 h at 37°C and overnight at +4°C. This solution of copoli-HSA was used for ID in serial dilutions ranging from 1.250 μg/ml to 19.9 μg/ml. The lowest concentration of copoli-HSA giving a precipitation with the undiluted patient's serum was considered to reflect AAA concentration in the respective serum. Positivity was considered to begin with 625 μg/ml.

*Quantitative determinations of serum immunoglobulins* (IgG, IgA, IgA) were performed by the Mancini technique [9].

*HBs Ag positivity* was determined by the counter current electrophoresis method [10].

*Statistical analysis.* The results were expressed as the arithmetic mean of N values. The standard errors were calculated and the standard errors of the mean (SEM) were used to indicate the statistical significance of the results.

## RESULTS

*HBs Ag positivity.* As can be seen in Table 1, HBs Ag distribution had an irregular aspect probably due to the small number of cases in each group. The

greatest HBs Ag incidence was observed in the 71—80-year-old groups (6.9 % in females and 5.7% in males), no HBs Ag positivity being recorded in the 61 — 70 and 81—90-year-old groups.

*Table 1*

**Age and sex distribution of 300 clinically healthy subjects from Bucharest; HBsAg presence according to sex and age groups**

| Sex | Number of cases by age (years) | | | | |
|---|---|---|---|---|---|
| | 41—50 | 51—60 | 61—70 | 71—80 | 81—90 |
| Females | 25(1)* | 34(0) | 36(0) | 58(4) | 27(0) |
| Males | 13(0) | 26(1) | 28(0) | 34(2) | 18(0) |

* In brackets number of HBsAg positive cases.

*Autoantibodies.* General data referring to the incidence of autoantibodies are presented in Table 2.

*Table 2*

**Autoantibodies and HBsAg incidence in 300 normal subjects grouped according to sex**

| Subjects | No. of cases | Mean age (years) | Autoantibodies (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | ANA | SMA | ACA | AAA | HBsAg (%) |
| Females | 180 | 62.9 | 3.5 | 15.4 | 14.3 | 32.9 | 2.9 |
| Males | 120 | 66.6 | 8.9 | 15 | 9.7 | 31.1 | 2.5 |

Similar AAA and SMA frequencies were recorded in both sexes, significant differences appearing only in the other categories of autoantibodies. Thus, the incidence of ANA was higher in males (8.9 as against 3.5% in females, $p \leqslant 0.02$), and that of ACA higher in females (14.3 as against 9.7% in males; $p \leqslant 0.02$).

The incidence of different autoantibodies according to sex and age groups is given in Fig. 1a, b, c, d.

As can be seen in Fig. 1a, ANA showed in both sexes a continuous increase in incidence between 40 and 60 years. While in males the increase continues in the seventh decade followed by a decrease in prevalence in the eighth decade, in females decrease in the incidence supervenes a decade earlier.

The same parallelism between the curves corresponding to the prevalence of autoantibodies in females and males between the ages of 40 and 60 was noted for ACA (Fig. 1b) with a slightly greater incidence in females. After 60 years a contrary evolution of ACA incidence was recorded: increase in the seventh decade followed by a sharp decrease in the eighth decade, and decrease in the seventh decade followed by a steep increase in the eighth decade in males.

SMA had a fairly constant frequency between 40 and 60 years in both sexes, somewhat greater in males; but an inverse evolution of SMA incidence was noticed (Fig. 1c) in the seventh and eighth decade, i.e. an abrupt rise in females (40% SMA positivity in the 80—90 years age-group) and a corresponding decrease in males (7% in the same age-group).

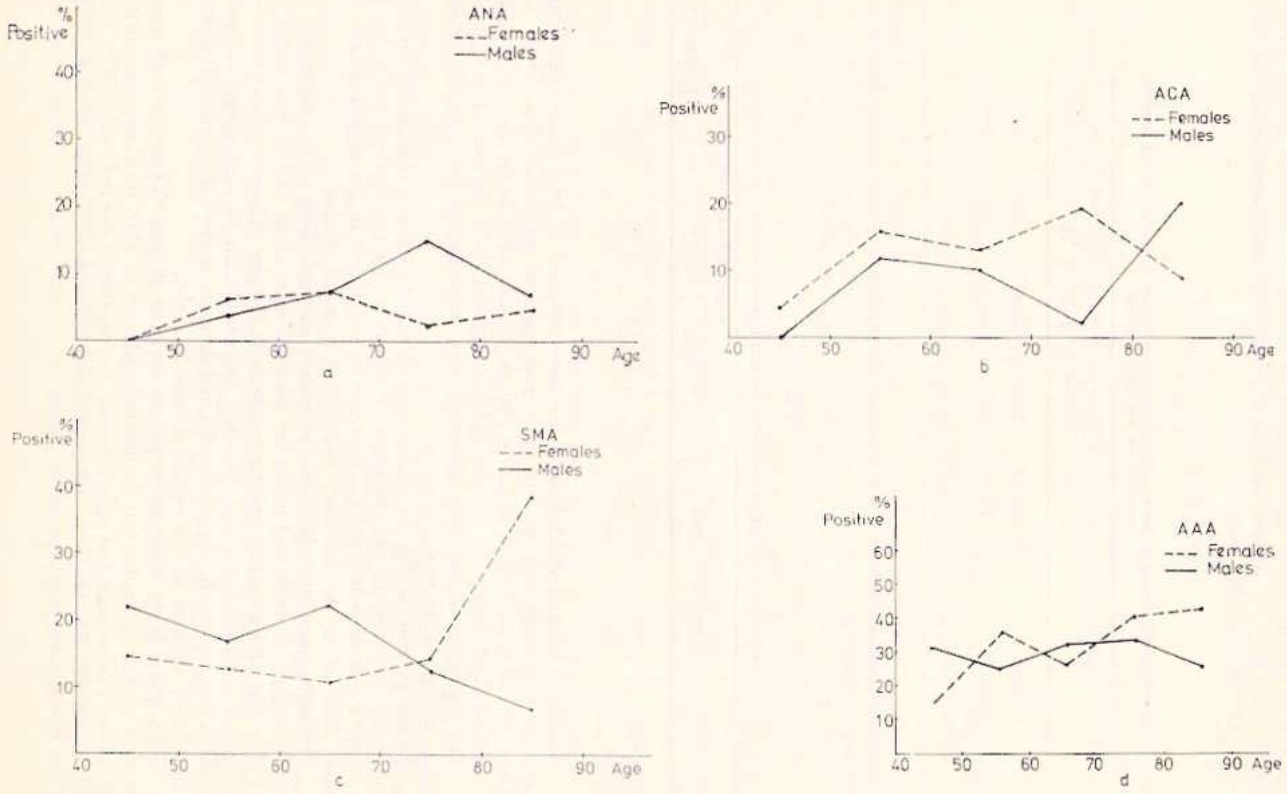

ANA
Females
Males
% Positive
40
30
20
10
40
50
60
70
80
90 Age
a
ACA
Females
Males
% Positive
30
20
10
40
50
60
70
80
90 Age
b
SMA
Females
Males
% Positive
40
30
20
10
40
50
60
70
80
90 Age
c
AAA
Females
Males
% Positive
60
50
40
30
20
10
40
50
60
70
80
90 Age
d

Fig. 1

AAA (Fig. 1 d) had an almost equal incidence in males in all age-groups, while a rise was observed in females from 12% in the 40—50 age-group to 40% after 81 years.

The general incidence of autoantibodies and their association are represented in Fig. 2.

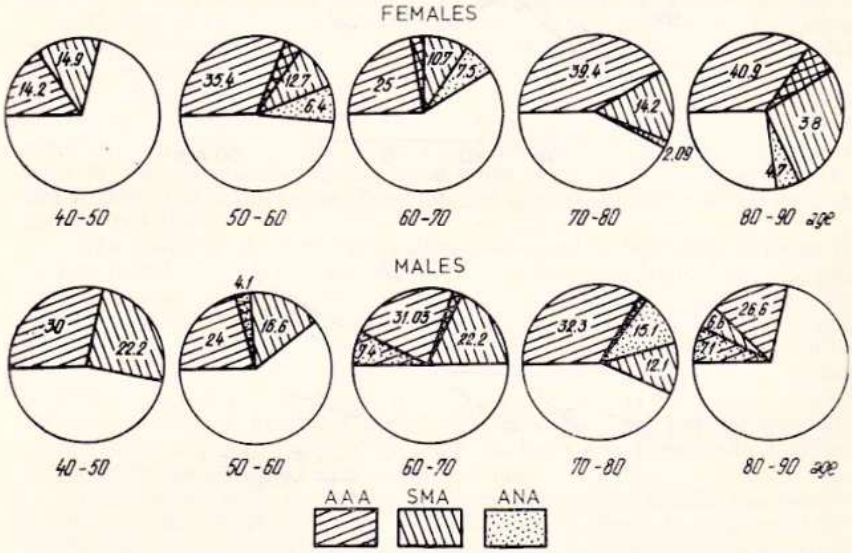



Fig. 2

An increase with age in the prevalence of autoimmune phenomena is evident in females (29.1% in the 41—50 age-group and up to 78.6% in the 81—90 age-group). This phenomenon is not registered in males, where a clear fall in the incidence of autoantibodies was observed between 51—60 and 80—90 years.

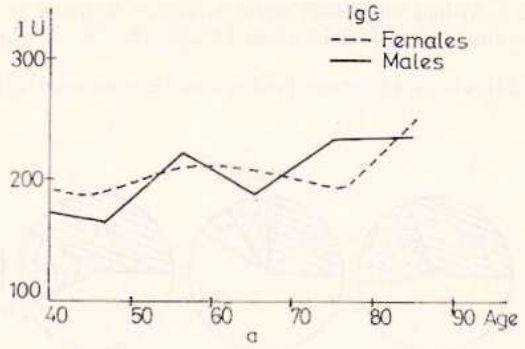
The prevalent association noticed in females was between AAA and SMA while in males AAA were associated with ANA.

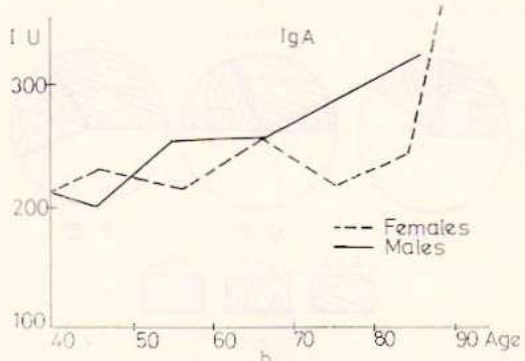
*Immunoglobulins.* In general, serum immunoglobulin concentrations register similar levels in both sexes and each age-group (Fig. 3).

An increase in serum IgG and IgA with age was observed, more marked in the case of IgA (Fig. 3 b). IgM levels presented a constant and significant decrease with age in females.

## DISCUSSION

The general incidence of ANA (5%) obtained in our group of 300 subjects is comparable to that recorded by Hooper et al. in 1969—1972 [11] in the 3492 individuals constituting the Caucasian rural community of Busselton (Western Australia), respectively 5.5%; it differs, however, from this study and from the well-


IgG
I U
300
200
100
Females
Males
40
50
60
70
80
90 Age
a


I U
IgA
300
200
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Females
Males
40
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b

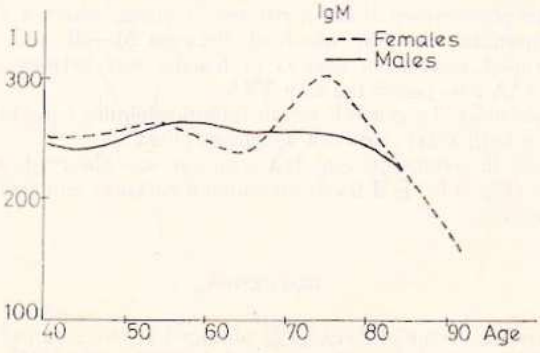
IgM
I U
300
200
100
Females
Males
40
50
60
70
80
90 Age
c

Fig. 3

known tendency of females toward developing autoantibodies and autoimmune diseases [12], in that a significant higher frequency of ANA was recorded in males than in females (8.8 and 3.5% respectively). A possible explanation for this observation will be given further on.

The parallel increase in the incidence of ANA in both females and males in the fourth-to-sixth decade is also in agreement with numerous other studies which show an increase with age in autoimmune phenomena in both sexes [13, 14].

The net sharp decrease registered in the incidence of ANA in octogenarian males may be interpreted as due to a higher death rate of males positive for ANA. Thus, in a follow-up study of the Busselton population continued during 1972—1975 in order to estimate the death rate in subjects with different autoantibodies Mackay et al. [15] observed among men with autoantibodies an excess mortality rate from vascular causes and from cancer among men with rheumatoid factor, these autoimmune phenomena having no death predicting value in women. It is also of interest that the autoantibody reaction associated with nuclei involved the greatest risk [4].

The relatively constant SMA incidence observed in both sexes between the ages of 40 and 70 years is in agreement with the Busselton study. However, SMA were registered with higher frequencies than in the Busselton population. This lack of correlation of SMA incidence with age was interpreted as being possibly due to a masking effect provoked by the induction of SMA in the course of an endemic infection [15]. A terminal, steep rise was noticed in our group of women, concomitantly with the aspect shown in Fig. 2, i. e. the greater number of autoimmune reactions in women in the 81—90 years age-group corresponding to a lower immune response in men, which could also be explained by a pathogenic role of autoantibodies in diseases causing death in men.

Nevertheless, in the present study a sharp terminal rise in SMA was observed in women in the 81—90 age-group. This phenomenon should be interpreted with care in view of the relatively small number of cases forming the latter age-group.

An age and sex dependence was observed in the prevalence of ACA. Thus, the incidence of ACA constantly registered higher frequencies in women than in men, except in the last decade when a divergent evolution was noticed (Fig. 1 b). The incidence of ACA also increased with age in women from the fourth-to-seventh decade, while in men a decrease in ACA prevalence was observed in the preceding decade, concomitantly with a decrease of SMA.

The group with the presence of ACA probably included sera with low antigastric parietal cell and antithyroid cell antibody titers; this appears to be in keeping with the well-known predisposition of females toward developing these categories of autoantibodies. ACA may be assumed to have a certain protective role in women, helping to eliminate the necrotic products resulting from cellular destruction more accentuated in the aged. Thus, the favourable role of ACA was observed in patients with diseases of the liver, in which the presence of ACA was associated with a better hepatocellular function [16].

High AAA titers were registered in females, their prevalence being age-dependent, and an almost flat curve recorded in men. Many studies have revealed the value of AAA in the diagnosis of liver cell dysfunction, these autoantibodies appearing in children and adults strictly as a consequence of liver alterations [17, 18, 19].

As can be noticed in fig. 2, a good correlation was registered between AAA and SMA in women, the phenomenon being also observed in studies performed in

1065 patients and apparently healthy subjects [20]. This association points to the appearance of AAA in women as a result of liver cell dysfunction, accentuated with age. Studies carried out by Thompson and Williams [21] and Skaunic et al. [22] also furnished arguments lending support to liver functional alterations in the elderly; the liver status may be very different in the diseased population, since it depends to a great extent on nutritional habits and environmental conditions. The association of AAA with SMA in the presence of a fairly low HBs Ag incidence in these aged individuals also noticed in other studies [23] argue for the role of autoimmune phenomena in the maintenance of discrete liver cell alterations in females.

The predominant association observed in men was between AAA and ANA, the maximum incidences of both these autoantibodies being in the seventh decade, concomitantly with the highest HBs Ag incidence (Table 1 and Fig. 2). This association of AAA and ANA suggests the possibility of viral and microbial infections as the principal factors involved in the production of these antibodies in men, probably also dependent on liver cell alterations.

The data shown in figure 2 illustrate the general increased incidence of autoantibodies with age in females, and a net decrease in males after the eigth decade, supporting the hypothesis of autoantibodies contribution to the death rate in men.

As regards immunoglobulins, our results are in agreement with the data obtained by Schwick and Mecker [24] who in their study on a group of blood donors found an increase in IgG with age and a significant fall in IgM levels, and with those of Cassidy et al. [25], who observed an increase with age in IgG and IgA concentrations.

**Résumé.** On a testé 300 sujets de Bucarest, âgés entre 40 et 90 ans, sans phénomènes pathologiques cliniques évidents. Par la technique d'immunofluorescence indirecte, on a mis en évidence, dans le sérum des sujets testés, les autoanticorps antinucléaires (ANA), anticytoplasmiques (ACA) et anti-muscle lisse (SMA), et par immunodiffusion avec albumine polymérisée à glutaraldéhyde les autoanticorps antialbumine (AAA). On a aussi testé le niveau des immunoglobulines sériques (IgG, IgA, IgM).

On a souligné l'augmentation de l'incidence d'autoanticorps avec l'avancement en âge, spécialement chez les femmes, pendant que chez les hommes une baisse de la prévalence des autoanticorps a été enregistrée pendant la 7e et 8e décennie, conformément à l'influence déjà connue des phénomènes autoimmuns sur les décès des hommes.

Les AAA ont présenté une prévalence augmentée tant en ce qui concerne les hommes que les femmes comparativement aux autres catégories d'autoanticorps. On a observé une tendance différente en ce qui concerne les associations des autoanticorps, respectivement AAA-SMA chez les femmes et AAA-ANA chez les hommes; cela suggère une étiopathogénie différente concernant les maladies sous-cliniques du foie les plus fréquemment rencontrées.

On a observé avec l'avancement en âge une faible augmentation de la concentration des IgG et IgA et une diminution de la valeur de l'IgM.

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# RECHERCHES CONCERNANT LE VIEILLISSEMENT BIOLOGIQUE DIFFÉRENCIÉ PAR RAPPORT À CERTAINS FACTEURS DE RISQUE ET AUX CONDITIONS SPÉCIFIQUES DE MILIEU

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**Résumé.** En utilisant, dans le cadre de certaines études épidémiologiques et écologiques, une méthode originale roumaine de la quantification proportionnelle de 41 indicateurs cliniques d'âge, on a investigué le processus de vieillissement biologique par groupes d'âge, de façon comparative, par rapport à deux facteurs de risque — l'obésité et l'irradiation — et dans les conditions d'une zone balnéo-climatique sous-montagneuse.

Les résultats de l'étude, concrétisés dans les valeurs de l'âge biologique moyen des lots, ont mis en évidence l'intensité et le mode différencié par lesquels les facteurs de risque interviennent dans le processus de vieillissement biologique de l'organisme, des systèmes et des appareils.

Ainsi, tandis que les facteurs de milieu de la zone balnéo-climatique favorisent un vieillissement physiologique, exprimé par un âge biologique moyen de 54,8 points, les deux autres facteurs de risque analysés dans l'étude déterminent une accélération du processus de vieillissement, tout particulièrement des systèmes cardio-vasculaire, digestif et ostéo-articulaire, avec un nombre de points de l'âge biologique moyen de 67,9 chez les personnes irradiées et de 106 chez les obèses.

On constate, dans la pratique du terrain, qu'il y a des collectivités des populations qui sont distribuées dans des zones bien délimitées et qui présentent des particularités en ce qui concerne le processus biologique de vieillissement au niveau populationnel.

Les recherches épidémiologiques ont été effectuées en relation avec les facteurs de risque suivants: obésité et irradiation (3).

Les recherches écologiques ont été délimitées aux facteurs particuliers de milieu d'une station balnéo-climatique à microclimat de zone sous-montagneuse, aux eaux minérales et thermales. Les éléments météorologiques et les conditions qui relèvent les caractéristiques du milieu physique de la région investiguée influencent de façon positive les processus de croissance, de développement et de vieillissement.

Dans cette région les indices démographiques montrent également une proportion plus grande des personnes longévives (85 ans et plus) par rapport à la moyenne de la Roumanie: 5,6% par rapport à 3,1%; la durée de la vie normale est de 3 ans supérieure à la moyenne. Les *indices de morbidité* par les maladies des appareils: cardio-vasculaire, digestif, respiratoire et du système neuro-endocrinien sont de façon significative plus petits par rapport à ceux du reste du pays.

*Tableau 1*

**Critères d'âge — expressions quantitatives**

| Critères par appareils et systèmes | Indices | Degré d'intensité<br>0+ \| ++ \| +++<br>Marquage numérique | | |
|---|---|---|---|---|
| I. Téguments | Elasticité | 2 | 3 | 4 |
| | Humidité | 2 | 3 | 4 |
| | Rides | 1 | 2 | 3 |
| | Taches séniles | 2 | 3 | 4 |
| II. Cheveux | Elasticité | 2 | 3 | 4 |
| | Lustre | 2 | 3 | 4 |
| | Achromotrichie | 1 | 2 | 3 |
| | Perte des cheveux (calvitie) | 1 | 2 | 3 |
| III. Ongles | Striations transversales | 1 | 2 | 3 |
| | Grossissement | 2 | 3 | 4 |
| | Friabilité | 2 | 3 | 4 |
| | Déformation | 3 | 4 | 5 |
| IV. Yeux | Acuité visuelle | 7 | 8 | 9 |
| | Eclat | 3 | 4 | 5 |
| | Gérontoxon | 8 | 9 | 10 |
| | Cataracte | 7 | 8 | 9 |
| V. Organe auditif | Acuité auditive (hypo-acousie) | 5 | 6 | 7 |
| | Acouphènes | 2 | 3 | 4 |
| VI. Tissu cellulaire sous-cutané | Turgor | 2 | 3 | 4 |
| VII. Appareil ostéo-articulaire | Arthroses | 2 | 3 | 4 |
| | Spondyloses | 3 | 4 | 5 |
| | Déformations | 4 | 5 | 6 |
| VIII. Appareil digestif | Edentation | 3 | 4 | 5 |
| | Atrophies alvéolaires | 4 | 5 | 6 |
| IX. Appareil génito-urinaire | Puissance-libido | 4 | 5 | 6 |
| | Kraurosis vulvae, atrophie de sénescence | 5 | 6 | 7 |
| | Adénome de la prostate | 6 | 7 | 8 |
| X. Appareil respiratoire | Emphysème pulmonaire | 4 | 5 | 6 |
| XI. Appareil cardio-vasculaire | Sinuosité et induration des artères | 5 | 6 | 7 |
| | Pulsations épisternales de l'aorte | 5 | 6 | 7 |
| | Tension artérielle | 3 | 4 | 5 |
| XII. Système nerveux | Signe de Noica | 5 | 6 | 7 |
| | Réflexe palmo-mentonnier | 5 | 6 | 7 |
| | Syndrome pseudo-bulbaire | 5 | 6 | 7 |
| | Démarche | 3 | 4 | 5 |
| | Attention | 4 | 5 | 6 |
| | Mémoire | 5 | 6 | 7 |
| XIII. Etat physique | Mobilité | 2 | 3 | 4 |
| | Possibilité de se servir soi-même | 5 | 6 | 7 |
| XIV. Capacité de travail | Physique | 2 | 3 | 4 |
| | Intellectuelle | 5 | 6 | 7 |

## MATÉRIEL ET MÉTHODES

Dans la période 1965—1977, dans la Section de recherches de gérontologie sociale de l'Institut National de Gérontologie et de Gériatrie de Bucarest, on a effectué des recherches *épidémiologiques* et *écologiques* concernat le *vieillissement différencié* de certains groupes d'âge: 50—59 ans, 60—69 ans et 70 et de plus, en fonction de certains facteurs de *risque* et des *conditions spécifiques de milieu.*

Les recherches ont été effectuées sur trois lots: I) personne qui habitent dans une station sous-montagneuse (Călimănești); II) personnes obèses et III) médicins radiologues. Dans les deux derniers lots sont comprises des personnes qui habitent Bucarest. Chaque lot a été constitué de 200 personnes âgées de plus de 50 ans, réparties de façon égale par décennies d'âge. Les personnes qui constituaient le lot de la zone balnéo-climatique se trouvaient dans un bon état de santé, ce qui a déterminé que ce lot soit considéré témoin, du point de vue du vieillissement.

Les sujets ont été examinés par des gérontologues et des spécialistes pour chaque appareil investigué.

Afin de connaître les particularités du vieillissement de la population sous aspect biologique chez les trois lots investigués, on a utilisé la méthode de la quantification proportionnelle des critères cliniques d'âge [1]. [2]. On a utilisé 14 groupes de critères totalisant 41 critères cliniques, au moyen desquels on a apprécié les modifications d'âge de certaines structures et fonctions des appareils et des systèmes, ainsi que les modifications intervenues au niveau de l'organisme tout entier. Chaque critère d'âge a été marqué de 1 à 10, le marquage s'effectuant en fonction de l'importance qu'on lui attribue dans l'estimation générale de l'âge biologique. Par conséquence, le marquage numérique exprime l'intensité du rythme du processus de vieillissement (Tableau 1).

On a calculé, pour chaque critère d'âge, les valeurs moyennes, en fonction du facteur investigué. Les diverses grandeurs des valeurs moyennes de chaque critère d'âge ont mis en évidence le degré de vieillissement différencié des personnes investiguées.

## RÉSULTATS ET DISCUSSIONS

Les données comparatives de l'âge biologique, exprimées par marquage numérique, pour les trois lots, sont consignées dans le tableau 2 et la figure 1.

Il ressort de ces données que le processus biologique exprimé par le marquage des critères cliniques de l'âge est différent, aussi sous l'aspect du rythme.

Ainsi, le lot de la zone balnéo-climatique présente des valeurs beaucoup plus petites par rapport au lot constitué par des personnes irradiées et à celui des personnes obèses.

L'augmentation des valeurs de l'âge biologique d'un groupe d'âge à l'autre chez le premier lot est graduelle, tandis que les deux autres lots commencent par des valeurs moyennes plus grandes et chez les personnes obèses l'augmentation est plus évidente.

Afin de connaître le degré d'atteinte de chaque tissu, appareil et système dans le processus d'involution, on a calculé la valeur moyenne du marquage de l'âge biologique pour les 14 groupes de critères d'âge chez les lots investigués.

La moyenne du marquage de l'âge biologique selon 14 groupes de critères, pour les trois lots, pour les personnes entre 50 et 59 ans, est consignée dans le tableau 2 et la structure du marquage de l'âge biologique dans la figure 2.

*Tableau 2*

**Données comparatives de l'âge biologique, exprimées par marquage numérique**

| Groupe d'âge | Lot de la zone balnéoclimatique | Lot des personnes irradiées | Lot des personnes obèses |
|---|---|---|---|
| 50—59 ans | 29,7 | 66,0 | 95 |
| 60—69 ans | 50,5 | 71,6 | 135 |
| 70 ans et plus | 71,3 | — | 152 |
| Total (pour les trois groupes) | 54,8 | 67,9 | 106 |

On observe, en examinant les données présentées, que chez le lot de la zone balnéo-climatique, les valeurs moyennes du marquage des critères d'âge sont, de façon significative, plus petites, ce qui correspond à l'avancement en âge (sous l'aspect de la structure et de la fonction), des tissus, des appareils, moins évident par comparaison aux deux autres lots.

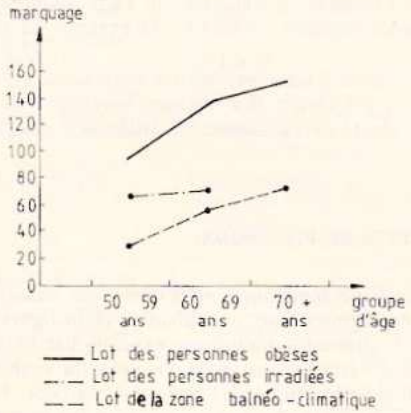


Fig. 1. — L'âge biologique exprimé par marquage, pour les trois lots.

On constate des différences significatives en comparant les valeurs moyennes de l'âge biologique pour le lot des personnes obèses à celles du lot des personnes de la zone balnéo-climatique (fig. 3).

En ce qui concerne les appareils et les systèmes, les différences des valeurs moyennes du marquage des critères d'âge apparaiissent plus manifestes:

a) *l'appareil cardio-vasculaire*, pour lequel la moyenne du marquage de l'âge biologique chez le lot de la zone balnéo-climatique est de 3,0 par comparaison à 12,8 pour les personnes obèses et 5,6 pour les personnes irradiées.

b) *Le système nerveux* présente des valeurs moyennes du marquage de l'âge biologique de 1,5 chez le premier lot, par rapport à 16,3, respectivement à 8,0 pour les deux autres lots.

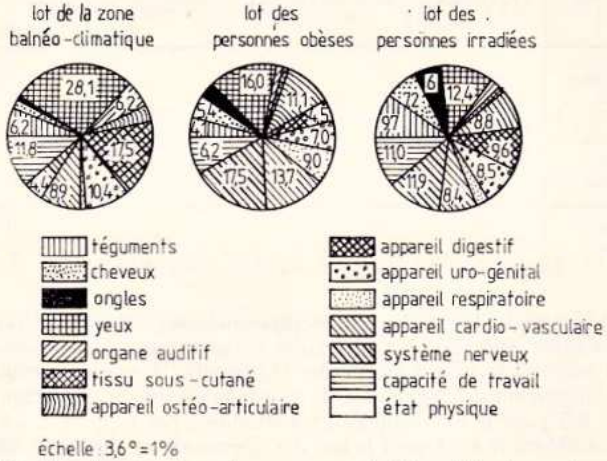


Fig. 2. — La structure du marquage de l'âge biologique selon 14 groupes de critères, chez les personnes ayant entre 50 et 59 ans.

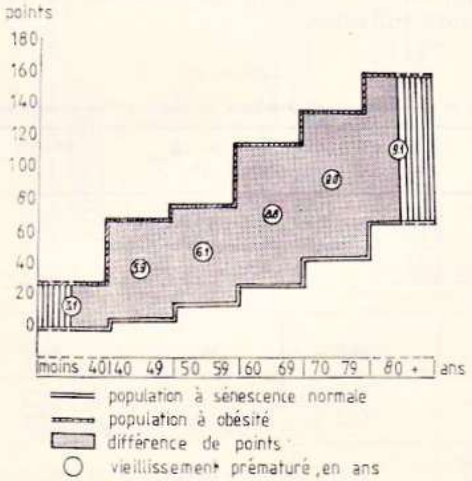


Fig. 3. — Le vieillissement prématuré des personnes obèses.

c) *Le système ostéo-articulaire* est également moins modifié, chez les personnes de la zone balnéo-climatique et beaucoup plus modifié chez les personnes obèses ou irradiées.

*Tableau*

**La moyenne du marquage de l'âge biologique selon 14**

| Lot investigué | Téguments | Cheveux | Ongles | Yeux | Organe auditif | Tissus sous-cutanés |
|---|---|---|---|---|---|---|
| Lot de la zone balnéoclimatique | 2,1 | 0,6 | 0,1 | 9,5 | 2,1 | – |
| Lot des personnes obèses | 4,4 | 5,0 | 2,7 | 14,9 | 1,3 | 0,6 |
| Lot des personnes irradiées | 6,5 | 4,8 | 4,0 | 8,3 | 1,6 | 0,9 |

d) *Les téguments et les annexes.* Ces tissus présentent une accélération du processus d'involution spécialement au lot des personnes irradiées. Ainsi, quant aux critères qui relèvent le vieillissement des téguments, la valeur moyenne du marquage, le vieillissement des téguments, la valeur moyenne du marquage des critères d'âge est de 6,5 pour le lot des personnes irradiées, par rapport à 4,4 pour le lot des personnes obèses et à 2,1 pour le lot des personnes de la zone balnéo-climatique.

e) *L'appareil respiratoire* présente un vieillissement plus retardé pour le premier lot, la valeur moyenne du marquage étant cinq fois plus petite que celle pour le lot des personnes irradiées.

*Tableau 4*

**Les valeurs moyennes du marquage de l'âge biologique par rapport à l'état de santé, par groupes d'âge**

| État de la santé (diagnostic) | Total | 50–59 ans | 60–69 ans | 70 ans + |
|---|---|---|---|---|
| Sain cliniquement | 34,1 | 18,8 | 35,8 | 54,0 |
| Maladies de l'appareil cardio-vasculaire | 75,5 | 39,0 | 73,1 | 83,1 |
| Maladies neuro-psychiques | 86,0 | 40,5 | 86,0 | 88,0 |
| Maladies de l'appareil digestif | 61,7 | 30,0 | 74,5 | 70,0 |
| Maladies de l'appareil locomoteur | 60,5 | 35,0 | 64,2 | 72,4 |

Des différences *non significatives* ont été observées aux critères d'âge qui expriment le rythme d'involution des appareils: visuel, auditif, digestif et urogénital.

3

groupes de critères, chez les personnes de 50 à 59 ans

| Appareil ostéo-articulaire | Ap. digestif | Ap. urogénital | Ap. respiratoire | Ap. cardio-vasculaire | Système nerveux | État physique | Capacité de travail |
|---|---|---|---|---|---|---|---|
| 1,3 | 5,8 | 3,5 | 0,3 | 3,0 | 1,5 | 2,4 | 4,0 |
| 10,4 | 4,2 | 6,5 | 8,4 | 12,8 | 16,3 | 6,9 | 5,8 |
| 5,9 | 6,4 | 5,7 | 1,9 | 5,6 | 8,0 | 8,1 | 7,4 |

On constate des différences *significatives* des valeurs moyennes du marquage de l'âge biologique par rapport à l'état de santé, par groupes d'âge (voir tableau 4 et figure 4).

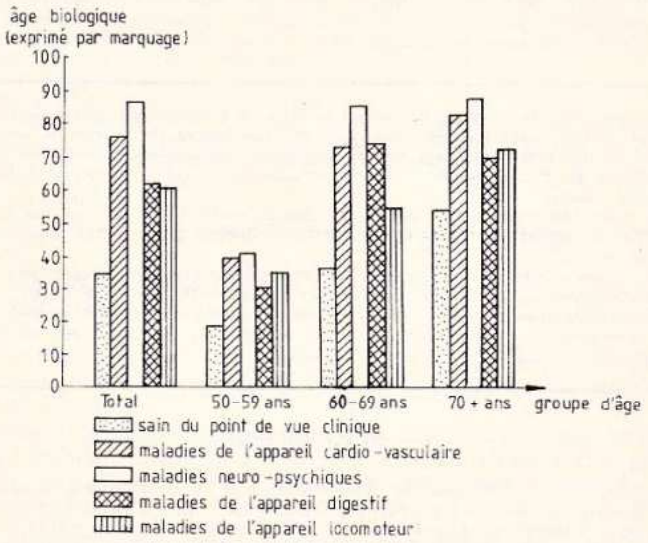


Fig. 4. — Les valeurs moyennes du marquage de l'âge biologique par rapport à l'état de santé, par groupes d'âge.

Il ressort de ces données que les maladies neuro-psychiques et les maladies de l'appareil cardio-vasculaire influencent dans une grande mesure le processus d'involution, phénomène plus manifeste après 70 ans.

## CONCLUSIONS

*1.* L'utilisation de la méthode « de la quantification proportionnelle des critères cliniques d'âge » pour apprécier le processus de vieillissement, au niveau populationnel, présentant des conditions spécifiques de milieu, a permis:

*a)* l'établissement de l'influence des divers facteurs de risque et des conditions spécifiques de milieu dans le conditionnement du vieillissement biologique;

*b)* l'analyse comparative du processus biologique aussi sous l'aspect du rythme pour les divers lots;

*c)* la détermination de certaines corrélations entre l'état de santé (prévalence par diverses maladies) et le vieillissement différencié.

*2.* Les résultats des recherches effectuées montrent que l'obésité et l'irridiation sont des facteurs de risques qui influencent dans une grande mesure surtout l'involution de l'appareil cardio-vasculaire, du système nerveux et de l'appareil ostéo-articulaire.

*3.* Les données concernant l'âge biologique montrent que les facteurs balnéoclimatiques ont une action positive de durée sur les systèmes d'intégration: neuro-endocrinien et cardio-vasculaire.

*4.* La méthode de la quantification proportionnelle des critères cliniques d'âge offre la possibilité de déterminer le *vieillissement différencié*, aussi bien au niveau de l'organisme tout entier, qu'au niveau des tissus, des organes, des appareils et des systèmes tant pour chaque individu séparément, qu'au niveau de groupe.

**Summary.** Based on the original Romanian method of proportional quantification applied to 41 clinical age indicators in epidemiologic and ecologic studies, the process of biological aging was studied on different age-groups and a comparison was made between several communities, in relation to 2 risk-factors – obesity, irradiation – and environmental factors (submountai nous resorts).

The mean biological age values of the groups under study pointed out the intensity and the different impact of the risk-factors on the biological aging of the organisms, systems and apparatuses.

The environmental factors specific to the submountainous resorts favor the orthogerous aging expressed by a mean score of 54.8 years for the biological aging, whereas the other 2 risk-factors accelerate the process of aging (particularly in cardiovascular, nervous, digestive, osteo-articular systems) by a mean score of 67.9 for the aging process in the irradiated community and 106 in the obese patients.

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# ACUTE PNEUMOPATHIES IN THE ELDERLY

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**Summary.** The study was conducted on 226 patients aged 61 to 96, suffering from acute pneumopathies; 23.4% presented normal and 76.6% accelerated aging; 2 to 4 morbide associations were noticed in the last group, with the prevalence of chronic bronchopulmonary and cardio-vascular diseases, diabetes, alcoholism, etc.

The characteristics of the clinical symptoms were cough with muco-purulent expectoration, moderate or absent fever, thoracic pains, lenkocytosis. Functionally, 35% of the patients presented severe respiratory or cardiovascular failure.

Bacteriologically, a variable uncharacteristic flora was prevalent in most cases, this pointing out the virus-bacterial mixed etiology.

The X ray pointed out that disseminated bronchopneumonias were the most frequent forms; the percentage of lobar pneumonia was lower.

A discrepancy was noticed between the dominant and persistent radiologic pattern and the clinical one.

The severe forms represented 38.1%, in 43% of the cases the evolution was slow and recurrent, pleuro-pulmonary, cardio-vascular, renal complications occurred in 59.8%, mortality reached 17% and was prevalent with the polymorbid group.

The anatomopathological examination pointed out giant cells without hepatization as a peculiarity in the pneumonic deceased patients.

The therapy administered was complex and included antibiotics, mucolytics, fluidifiants, cardiotonic agents, bronchodilators, analeptics, as well as diets with hydroelectrolytic additives and vitamins to control the deficits in the aged.

The favourable clinical evolution and the lower mortality rate in Gerovital $H_3$ treated cases were pointed out.

The analysis of the last 10 years mortality at the National Institute of Gerontology and Geriatrics revealed that acute pneumopathy was the major cause in 35% of the deaths (fig. 1).

The statistics on morbidity caused by acute diseases point out the significantly higher incidence of respiratory infections in the elderly than in adults [1, 4, 5, 6, 7, 8, 13, 17, 19, 20, 21].

The general opinion on acute pneumopathy, classified into bacterial and viral, has received a new interpretation because of the higher incidence of virus pneumonia (nearly 40%; as against 20% bacterial and 39–40% atypical mixed forms) with virus and bacterial flora and microplasms prevalence [4, 5, 6, 16, 17, 19, 20, 21].

Along epidemiological observations the modification of the clinical aspect has been noticed, particularly after the extensive use of the antibiotics with wide spectrum. Thus, the place of lobar pneumonia has been taken by the interstitial atypical pneumonia and most of the bacterial pneumonia has been noticed to follow after virus pneumonia as a result of bacteria-virus synergism [4, 5, 6, 16, 17, 19].

As shown before, the acute virus-bacterial pneumonia is more severe and lingering in the elderly, because of the factors closely correlated with advanced age, such as: the drop in functional respiratory reserves and adaptative abilities, de-

crease or lack of reactivity to infections, the diminished drug tolerance, nutritional deficiences [2, 3, 10, 11, 14, 21, 29, 30].

The pneumopathy is particularly severe when occurring in already deficient organisms suffering from different diseases such as: chronic bronchopulmonary, cardiovascular afflictions, diabetes, ethylism, etc. [12, 13, 15, 19, 20, 24, 27].

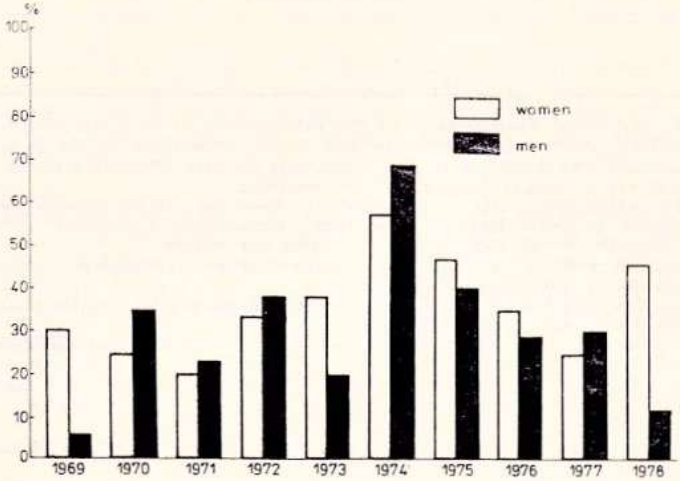


Fig. 1. — The dynamics of mortality due to acute pneumopathies (pneumonias and bronchopneumonias) by sex during the last 10 years as compared to the general mortality figures in NIGG.

From the anatomic, physiologic and immunologic aspects the local defense mechanisms involve three progressive echelons, each reacting after the aggressive factors had passed the previous one. The first is the bronchial and bronchoalveolar barrier which triggers the defense reaction of the surfactant mucus: the continuous pulmonary clearance. After the aggressive factors have passed this echelon, the lympho-reticular system starts to react setting in function the cellular and humoral immunity mechanisms, that is the leucocytes, lymphocytes, monocytes and immunoglobulins. The third echelon is the mesenchymal mechanism the reaction of which is the exudative inflammation. Under normal conditions, the clearance of the respiratory pathways is quite efficient and they are kept sterile. But if the defense mechanisms are impaired, the external aggressive factors strong and the defense ability abated, a vicious circle is created due to the onset of the pneumopathy in which the "opportunist" germs from the oropharynx are the aggressive factors [18, 19, 29]. The studies on the morpho-functional characteristics of the respiratory apparatus in the elderly, conducted at the National Institute of Gerontology and Geriatrics pointed out macro and microscopic structural changes in the alveolar wall (consisting in the thinning of the wall), bronchial mucus and local metabolic modifications [19, 22, 28]. The changes in the lung physiology with the third age and the involution of the immunologic systems favour the onset of acute diseases.

## MATERIAL AND METHOD

The study was conducted on 226 patients with acute pneumopathy aged 61 to 96, hospitalized at the National Institute of Gerontology and Geriatrics over the last 10 years.

## RESULTS AND DISCUSSION

From the gerontological standpoint, the aging was normal in only 55 subjects (23.4%); the rest presented accelerated aging and morbid associations, prior to the onset of acute pneumopathy. The morbid associations were the following:

*Table 1*

| Associated diseases | No. of cases |
|---|---|
| Chronic bronchitis and/or bronchiectasis | 61 |
| Pulmonary sclero-emphysema | 59 |
| Myocardial and vascular diseases | 57 |
| Diabetes mellitus | 15 |
| Alcoholism | 10 |
| Renal sclerosis | 9 |
| Gastro-duodenal ulcer | 6 |
| Rheumatoid polyarthritis | 4 |
| Other diseases | 4 |

The table shows that the onset of the acute pneumopathy was subsequent to a previous chronic pulmonary disease in 61% of the cases. The impairment of the bronchial stem and the deficient reliability of the pulmonary parenchyma, the defense mechanisms of which are more easily defeated because of the respiratory infection, account for the above-mentioned fact.

The second important group includes the cardiovascular diseases, diabetes mellitus and alcoholism, as deficiencies in which the natural defense mechanisms are weakened by the disease itself, dietary restrictions and metabolic disturbances thus favouring the onset of acute respiratory infections. The last part of the table mentions the less frequent associated diseases which favour the onset of respiratory diseases such as neuronal or immunologic deficiencies, etc.

Mention should be made that 2 to 4 of the diseases presented in the table were associated in 43% of the cases. The clinical symptoms were: cough, with quantitatively variable muco-purulent expectoration; fever, moderate or even absent (as a result of the poor reactivity in the elderly); thoracic pains; increased leucocytosis following closely the progress of the bronchial infection.

Our cases confirm the polymorphous aspect of the infection which is in most cases viral and bacterial, because as known, leucopenia was shown to prevail with the simple virus infections.

Functionally, 79 patients studied (35%) presented severe respiratory failure, due to the progress of suppurative bronchitis in 47% and myocardial decompen-

sation in 32% of the cases; thus, the functional pattern was that of cardio-respiratory failure.

Bacteriologically, an uncharacteristic variable flora was revealed (pneumococcus, staphylococcus, enterobacteriae).

The progress of pneumonia was quite severe in the 13 cases with Clebsiella Friedländer.

The patients with Friedländer infections were prone to necrosis with microabscess. In staphylococcal infections the giant bullae described were not noticed probably because of the fibrosis of the parenchyma displayed by the elderly, in whom the pulmonary valve phenomenon can not be achieved.

The X-ray examination revealed most frequently disseminated bronchopneumonia (85.6%); lobar pneumonia was present in only 14.4% of the cases — some peculiar forms of bronchopneumonias should be mentioned:

*1.* The uni- or bilateral disseminated lobar forms with foci ununiformly dispersed through the lungs.

*2.* Symmetric node disseminated forms with atelectasic nodes subsequent to severe chronic bronchial afflictions.

*3.* Disseminated forms with "too nice" pulmonary image revealing the quite severe acute or subacute bronchiolitis.

*4.* Systemic reticulo-nodal forms with monomorphous or polymorphous elements and progressive interstitial fibrosis.

*5.* Macronodular forms:

— confluent or partially confluent

— pseudolobar, quite severe.

Most of the cases presented peribronchovascular reaction or infiltration with increased hypertransparence due to the already existing emphysema. In the patients with virus pneumonia (14.4%) the condensation was subsegmentary, segmentary and quite seldom lobar. Mention should be made of the pseudotumoral forms, in which the pneumonic process occurring in the ventral, parahillar and paradiastinal segments raises problems for the differential diagnosis with a bronchopulmonary tumor with superadded infection [9, 23, 24].

A discordance between the clinical and radiologic patterns was frequently noticed; the radiologic pattern appeared more complex, dominant and persistent than the clinical one blurred out by therapy, particularly antibiotic.

The macroscopic anatomo-pathological investigation of the pneumonic patients revealed lungs with soft consistency, which on the microscope displayed accumulations of giant cells or pneumonia without hepatization. The more abundant lympho-poly-morphonuclear infiltration is a basic peculiarity with the aged, as against the lack of fibrin production. Areas with tissular necrosis were noticed at the level of the pneumonic or broncho-pneumonic foci. Numerous epithelial metaplasias and abundant muco-purulent exudate were noticed in the bronchia, associated with the desquamation of the epithelial cells and lymphopolynuclear detritus.

A positive diagnosis was difficult, particularly in the preexisting pulmonary afflictions, because of either an exacerbation of the chronic disease or an acute superadded process. The diagnosis was based mainly on laboratory findings and radiologic examinations.

In 44 cases (19.5%) the remission was normal with *restitutio ad integrum.*

In 109 cases (43.7%) the remission was slow, lingering from one to 4 months, recurrences occurring.

In 36 cases (16%) the disease, particularly when bronchial, became chronic. Among Friedländer patients, mortality reached 30%. The total mortality figure was 21%.

The complications which occurred were:

a — pulmonary:
  — suppuration (11 cases, 4.9%)
  — abscess

b — pleural:
  — para- or metapneumonic pleural reactions (28 cases, 12.4%)
  — encysted pleurisy (21 cases, 9.8%)

c — bronchial (19 cases, 8.3%)

d — cardiovascular (34 cases, 15%) among which:
  — acute myocardial decompensation (32 cases, 15%)
  — thromboembolism (2 cases)

e — renal (7 cases, 31%)
  — acute renal failure.

Complications occurred in 59.6% of the cases.

The associated diseases were noticed to worsen the condition of elderly patients with acute pneumopathy.

Both during and subsequent to the control of the acute pneumopathy, the patients with cardiovascular failure presented decompensation phenomena which required special steps for rehabilitation.

*Table 2*

| Medication administered | No. of cases | % |
|---|---|---|
| Antibiotics | 226 | 100% |
| Mucolytics and fluidifiants | 226 | 100% |
| Bronchodilators | 171 | 75.6% |
| Corticotherapy | 39 | 17.2% |
| Cardiotonic agents | 178 | 83.0% |
| Vasopressor, cardiac and respiratory analeptics | 108 | 48.0% |
| Heparin | 11 | 5.0% |
| Diuretics | 78 | 35.0% |
| Vitamins and reinforcing drugs | 147 | 65.0% |
| Symptomatic agents | 226 | 100% |
| Insulin, hypoglycemiant sulfonamides | 8 | 3.1% |
| Postural drenage | 87 | 38.5% |
| Diet with hydroelectrolytic additives (+Ca, ClK) (−NaCl) | 173 | 76.5% |

The therapy of choice was based mainly on antibiotics, because of the virus-bacterial pathogeny, the decreased resistance of the aged and the superadded pathology. *(Table 2).*

The therapeutic approach was changed whenever the progress of the disease was not favourable or the antibiogram pointed out a common flora demonstrating the inefficacy of the approach. The discrepancy was noticed between the antibiogram and the clinical evolution, which supports the microbial polymorphism in

the acute pneumopathy. Mention should be made that the antibiotic therapy was maintained (3 weeks on the average) even if we were tempted to discontinue the treatment because of the favourable clinical evolution. The mucolytics and fluidifiants were largely used in order to change the bronchial secretion, because of the defective expectoration in the aged and to avoid the worsening of the respiratory failure. Because of the same reason we used the bronchodilators in order to control the bronchospastic component. The corticotherapy was used with great caution, because of the diabetic and hypertensive patients included in our group and also because of poor cortisone tolerance in the aged. Cardiotonic agents and respiratory analeptics were extensively used even in patients who had not needed cardiotonic agents previously.

Intermittent oxygen therapy and bleedings were used in patients with severe cyanosis considering the polyglobulinemia of the cardio-respiratory failure. Heparin was used in a few cases in injections with 1000 u, one ampoule every 2 days, in order to control coagulability disorders.

Taking into account the specific metabolism in the polydeficient aged patients, hydro-electrolytic, lacking in sodium and rich in potassium chloride, dietary additives were included in the therapeutic schedule.

The aged institutionalized at the Home of the Institute and subjected to Gerovital $H_3$ treatment displayed a higher resistance to respiratory infections during the epidemics, the clinical progress of the disease was more favourable and mortality lower [1, 21].

*As concluding* remark, acute pneumopathy in the aged represents one of the most important chapters of clinical geriatrics, because of its high incidence, seriousness, complications and high mortality.

Therefore, prophylactic steps should be taken, such as: well-balanced lifestyle, avoidance of polluting factors, of crowds during seasonal epidemics, asiduous physical exercise, as well as increase of unspecific immunological resistance, by vaccination and biotrophic treatment.

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**Résumé.** On a étudié un nombre de 226 malades âgés entre 61 et 96 ans, avec pneumopathies aiguës; 23,4% du total avaient normalement vieilli, tandis que 76,6% présentaient un vieillissement accéléré. Ces derniers présentaient au moins 2 – 4 associations morbides, avec prédominance des maladies broncho-pulmonaires chroniques, cardio-vasculaires, diabète, éthylisme, etc.

Les signes cliniques ont été caractérisés surtout par toux à expectoration muco-purulente, fièvre modérée ou même sans fièvre, algies thoraciques et leucocitose. Du point de vue fonctionnel on a constaté chez 35% des sujets une insuffisance respiratoire ou cardio-respiratoire grave.

Du point de vue bactériologique on a mis en évidence la prédominance d'une flore variable non caractéristique, qui vient préciser l'étiologie mixte viro-bactérienne.

Du point de vue radiologique on a remarqué surtout les broncho-pneumonies disséminées, la pneumonie franche n'étant signalée qu'à un nombre réduit de sujets.

On a constaté une fréquente discordance entre la dominance et la persistance de l'aspect radiologique vis-à-vis de celui clinique.

L'incidence des aspects graves a été de 38,1%, l'évolution traînante et avec récrudescence chez 43%, avec complications pleuro-pulmonaires, cardio-vasculaires, rénales, etc. chez 59,8% des sujets.

Le pourcentage de la mortalité a été de 17%, le groupe de polymorbidité ayant une certe prédominance.

Du point de vue anatomo-pathologique, le caractère prédominant des pneumonies – pour les sujets décédés – réside dans les cellules gigantesques sans hépatisation.

La thérapie appliquée a été complexe, au premier plan se situant les antibiotiques,

les mucolytiques, les fluidifiants, les analeptiques, les cardiotoniques, les bronchodilatateurs tout en assurant une diète avec apport hydroélectrolytique et vitaminique approprié aux carences des sujets âgés.

Une évolution clinique favorable, et une mortalité diminuée ont été mises en évidence chez les sujets traités à Gerovital $H_3$.

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# THE STUDY OF CORTICAL ELECTROGENESIS IN AGED HUMANS

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**Summary.** In order to obtain data of comparative value regarding the difference between a healthy normal adult and a subject included in any of the age decades above the adult age, useful in estimating the evolution of a normal process of nervous system ageing, sixty nine subjects were grouped according to age decade into 4 lots: lot I 15 subjects as control (aged 22–44 years), lot II 22 subjects (aged 71–80 years), lot III 27 subjects (aged 81–90 years) and lot IV 5 subjects (aged 91–100 years).

EEG was carried out under the basal condition required for recording the alpha rhythm. The mean amplitude and mean frequency values of the EEG tracings were computed, noting as positive the tracings whose response to intermittent light stimulation coincided to the value of the mean frequency of the EEG tracings. Whenever the subjects' age allowed it, EEG alterations were also studied using the hyperpnea activation test for 3 minutes.

The evolution of the normal process of ageing presupposes:

1. A decrease in the mean frequency value of the EEG tracing, from 10.5 c/s to 8.5 c/s.

2. A dichotomic process of the mean amplitude evolution of the EEG tracings from 50 microV either towards hypovoltage, as in most cases, or towards slightly higher values than the adult mean (over 50 microV) as encountered in some of the cases.

3. A more or less marked decrease of the response to intermittent light stimulus and to the hyperpnea test.

In earlier works carried out on a relatively large number of subjects we reported the fact that the alpha rhythm in the healthy adult individual shows variations in its most important parameters, such as frequency and amplitude [3]. In the same works we were also reporting that the variations in frequency come within the range of a Gauss type of curve with a maximum of 10–11 c/s and 8–9 c/s and respectively 12–13 c/s the lower limits.

From a statistical standpoint the large number of normal cases (over 60%) are situated at the top of the slope, the frequencies corroborated by the amplitude of the EEG traces showing, in most cases, the presence of normovoltage within the range of 40 to 70 microvolts (mean amplitude 50 microvolts) [3].

In order to obtain data of comparative value regarding the difference between a healthy normal adult and a subject included in any of the age decades above the adult age, useful in estimating the evolution of a normal process of senescence, we also undertook a number of studies demonstrating from a statistical view the distributions of the mean values of the alpha rhythm frequencies in relation to age. This enabled us to notice that most of the alpha rhythm frequencies at advanced ages (80–90 years) come within the range of 8–9 c/s which gives a different curve than the one plotted for the adult. This made us subdivide the alpha rhythm into several compartments: (i) slow rhythm (8–9 c/s), (ii) fast rhythm (12–13 c/s) and, (iii) in-between these two extremes a medium rhythm (around 10.5 c/s). As a

result of this subdivisioning we were able to notice that the highest incidence of slow frequencies occurs in advanced age decades, a fact that entitled us to consider it as a normal feature of the senescence process.

Using hyperpnea as a test for alpha rhythm reactivity we found that unlike the normal adult values where the amplitude increased up to 20% of its initial values, the evolution of the senescence process leads, as a rule, to the attenuation of this reactivity.

The present work attempts to explore on a group of subjects believed to undergo a normal senescence process, the cognitive value of the response to intermittent light stimulation (ILS) in order to enrich the picture of the physiological modifications due to the senescence process.

## MATERIAL AND METHOD

Sixty nine subjects were grouped according to age decades into 4 lots as follows:

Lot I 15 subjects intended as controls (aged 22—44 years)
Lot II 22 subjects (aged 71—80 years)
Lot III 27 subjects (aged 81—90 years), and
Lot IV 5 subjects (aged 91—100 years).

Electroencephalography was carried out with the aid of an 8-channelled Mingograf Junior Siemens Elema, the subjects being placed in a dim lighted chamber and under the basal conditions requested for recording the alpha rhythm. ILS was performed with the aid of a stroboscope placed at 1.5 m in front of the subject and provided with a photostimulator of the type Officine Galileo R 79 C.

The electrodes were placed on the scalp according to Jasper's method (1958) using the 10—20 international system. The electrodes were connected to two standard programs.

The mean amplitude and mean frequency values of the EEG tracings were computed according to Goldstein and Beck's method [1] as modified by Racotta [2] noting as positive the tracing whose response to ILS coincided to the value of the mean frequency of the EEG tracing. Whenever the subject's age allowed it, EEG alterations were also studied using the hyperpnea activation test for 3 minutes.

## RESULTS

The results are set forth in Fig. 1 showing the EEG mean frequencies of the 3 age-groups as against the control lot. Inspection of the panels reveal a graded decrease in values from 87% in controls having a fast alpha rhythm down to 20% reached by subjects aged 91—100 years.

As concerns the slow alpha rhythm compartment (8—9 c/s), it was noticed an ascending distribution in the aged lots with values ranging from 27% to 60% as against the 0% in the control lot.

Figure 2 displays the evolution of the mean amplitude of the EEG tracings in the age groups. The evolution of the values in the groups with mediovolted and hypovolted amplitude within each lot seems to us of significance. Thus, the group with middle voltage amplitude representing 53.4% in the controls evolves to-

wards values of only 20% in lot IV whereas the group with hypovolted amplitude representing 26% in the controls, evolves towards 60% in lot IV.

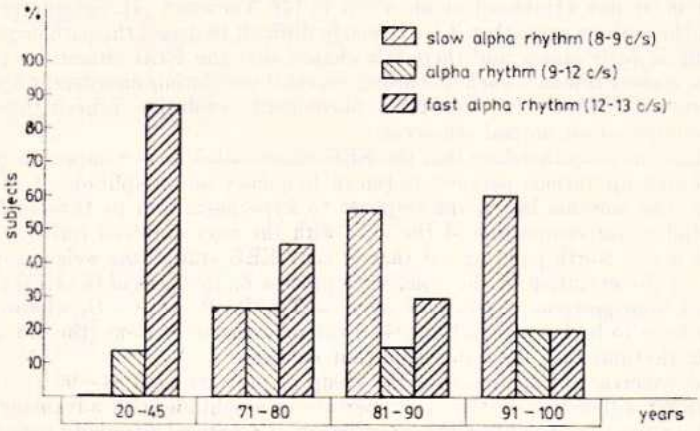


Fig. 1. — Evolution of the mean frequency of the EEG tracings in various age-groups.

At this point it is worth mentioning (see later comments) that the group of normovolted amplitude of the EEG tracings has an ascending evolution of 13.4% in lot I and reaches 22.2% in lot III.

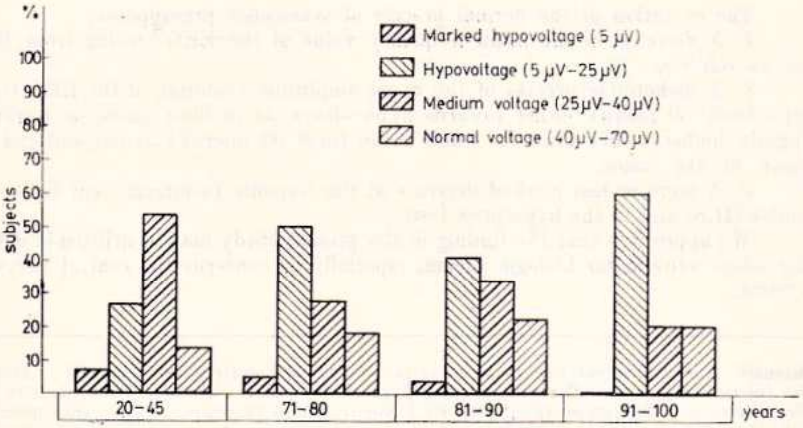


Fig. 2. — Evolution of the mean amplitude of the EEG tracings in various age-groups.

As concerns reactivity to ILS we noticed a tendency towards progressively diminished responses in relation to age, down to complete disappearance. This was objectivized both for the potential amplitude and for the response within frequency band adequate to the alpha rhythm.

## DISCUSSIONS

The normal EEG changes in the ageing process have been objectivized in a number of studies (Hubbard et al., cited in [4], Verdeaux [4], Sacerdoțeanu [3]. Most of the authors agree that it is extremely difficult to detect the pathologic event occurring in early stages and there is a chance that the EEG alterations encountered in various diseases, such as chronic cerebral circulation disorders, respiratory disorders with subsequent hypotoxic phenomena, evolutive neuropathies, etc., be superimposed on normal senescence.

It is necessary therefore that the EEG examination be as complex as possible by following up various parameters (mean frequency and amplitude of the EEG tracings, the morphic index, the response to hyperpnea, and to ILS) in longitudinal studies, for comparison of the data with the ones obtained earlier.

It is also worth pointing out that in later EEG studies the weight should be placed on the evolution of the senescence process on the basis of the EEG tracings obtained from posterior derivations, such as Tp—P; P—O; C—O, whose modifications seem to be more significant than the rolandic derivations (the Mu and the rolandic rhythm) that show nonsignificant changes.

As concerns the finding that the group of patients aged 81—90 years show a somewhat different evolution, i. e. increase in amplitude with advancing age, a possible explanation would be the intervention of a natural selection process which helped those individuals who enjoyed better health (including the nervous system) to become long-livers.

## CONCLUSIONS

The evolution of the normal process of senescence presupposes:

*1.* A decrease in the mean frequency value of the EEG tracing from 10.5 c/s to 8.5 c/s.

*2.* A dichotomic process of the mean amplitude evolution of the EEG tracings from 50 microV either towards hypovoltage, as in most cases, or towards slightly higher values than the adult mean (over 50 microV) as encountered in some of the cases.

*3.* A more or less marked decrease of the response to intermittent light stimulus (ILS) and to the hyperpnea test.

We appreciate that the finding of the present study may be utilized in defining some criteria for biologic ageing, especially as concerns the central nervous system.

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**Résumé.** Afin d'effectuer une étude à valeur comparative entre l'adulte normal et sain et les sujets encadrés en différentes décades d'âge supérieures à l'âge de l'adulte, dans le but de déceler des différences spécifiques de l'évolution d'un processus de sénescence normale, 69 sujets furent divisés en plusieurs groupes comme il suit: I^er^ groupe 15 sujets témoins (22—44 ans), II^e^ groupe 22 sujets (71—80 ans), III^e^ groupe 27 sujets (81—90 ans) et IV^e^ groupe 5 sujets (91—100 ans).

Les électro-encéphalogrammes ont été effectués en respectant les normes de base pour enregistrer le système alpha. Nous avons calculé la valeur de l'amplitude moyenne et de la fréquence moyenne des tracés électro-encéphalographiques, considérant comme positif le tracé électro-encéphalographique dont la réponse aux signaux lumineux intermittents a coïncidé avec la valeur de la fréquence moyenne du tracé. Dans le cas où l'âge du sujet nous

l'a permis, nous avons étudié aussi les modifications électro-encéphalographiques dues au test d'activation à l'hyperpnée pendant 3 minutes.

L'évolution des processus normaux de sénescence suppose:

*1.* La baisse de la valeur de la fréquence moyenne du tracé de 10,5 cycles/seconde à 8,5 cycles/seconde.

*2.* Un processus dichotomique d'évolution de l'amplitude moyenne des tracés de 50 V, soit vers un hypovoltage, situation rencontrée dans la majorité des cas, soit vers des valeurs légèrement supérieures à la moyenne de l'adulte (plus de 50 V), situation plus rarement rencontrée.

*3.* Une diminution plus ou moins accentuée de la réponse aux signaux lumineux intermittents et du test à l'hyperpnée.

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# A RELIABLE METHOD FOR FREQUENCY AND AMPLITUDE ANALYSES IN ROUTINE ELECTROENCEPHALOGRAPHY IN MAN

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**Summary.** EEG changes during human normal ageing may be studied using rugged electrodes which have a great reliability. The EEG tracings are statistically processed taking two significant EEG parameters: frequency and amplitude, and using the planimetric method which is precise, simple and economical. The data obtained are analysed in a tridimensional Cartesian reference space which allows a graphic presentation of the EEG ageing process. A "k" index of normality is used to detect possible pathologic processes overlapped on the ageing ones.

Cross-section and especially longitudinal studies of the neuraxial ageing process necessarily imply the study of cortical electrogenesis. Interpretation of the EEG tracings is, however, extremely difficult as long as there are cases that can not be framed into a clear pathology.

EEG analysis of the ageing process implies in the first place a comparative study of the tracings obtained in vigil rest state. The parameters that have to be taken into account are the frequency and amplitude of the alpha rhythm. Yet, the ageing process imposes variations of these parameters within somewhat restricted limits. In order to show these variations it is necessary to use certain recording techniques of great reliability and methods for processing the standardized data so as to allow comparable results, especially in longitudinal studies (on the same individual).

*Electrodes.* In order to reduce the artefacts that may influence considerably the EEG tracings it is necessary a close appliance of electrodes to the surface of the scalp skin. The best suited for this purpose seem to be the rugged electrodes (Fig. 1), that can be applied using either bentonite or collodium paste (Fig. 2).

The rugged electrodes are placed on the skin using the 10—20 international system (Jasper 1958) so that the resistance of each electrode should not exceed 10 kΩ. The electrodes in our study were connected to a Mingograf EEG Junior Siemens-Elema as indicated in Figs. 3 a and 3 b. As results from Fig. 3 a, the analysis of the alpha rhythm is made using the $C_3-P_3$, $P_3-O_1$ and $C_4-P_4$, $P_4-O_2$ leads, and integration of these leads may be compared to the $C_3-O_1$ and $C_4-O_2$ leads shown in Fig. 3 b.

*Statistical processing.* In our studies the EEG tracings were obtained using a 0.3 time constant, a filtration 15 and a base recorder gain of the 50 mieroV, V = 10 mm, 30 mm/s paper rolling speed.

*a.* The tracings thus obtained were statistically processed through the planimetric method (Racotta, 1968) [1] for evaluation of the mean frequency and ampli-

Fig. 1. — The rugged electrode.

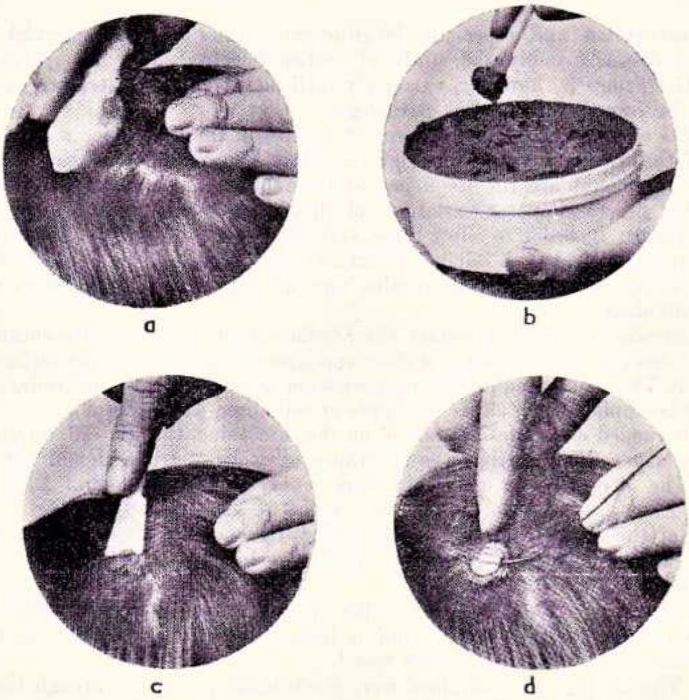


Fig. 2. — Position of the rugged electrode.

tude. This method seemed most suitable as it has an acceptable degree of accuracy and is less expensive. The procedure is to select a tracing segment (the one used in the analysis) 150 mm long which is the result of a 5 sec run (limited in Fig. 4

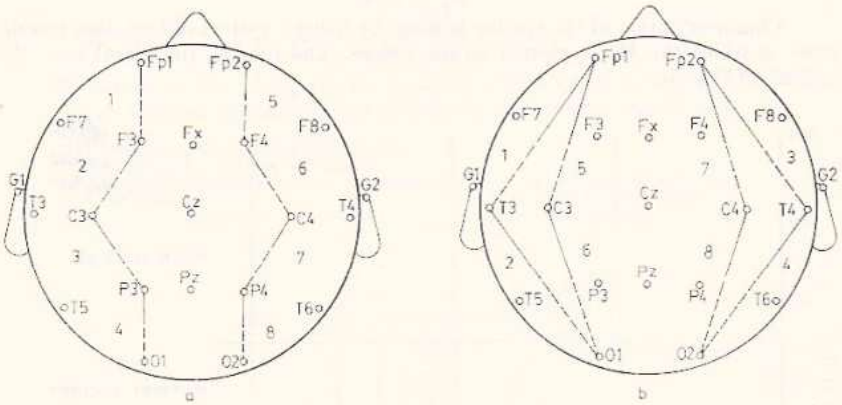


Fig. 3. — Scheme for placing the electrodes on the scalp skin.

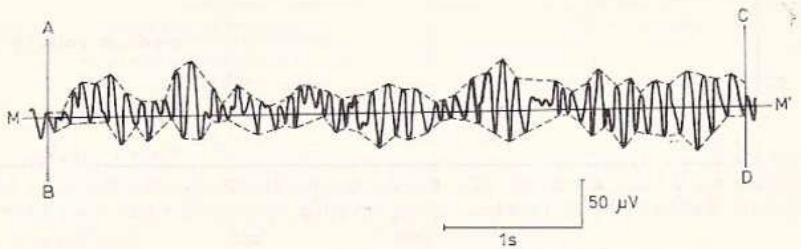


Fig. 4. — An example for computing the mean amplitude of the EEG tracings. The AB and CD segments encompass a 5-sec area. MM' = medial line; the dotted line joining the EEG waves represents the perimeter of the surface (the lined space) which is to be planimetered.

between AB and CD). This segment is divided into a superior and an inferior component by a horizontal line (MM' in Fig. 4) as near to the isoelectric line as possible. The surface covered by the EEG waves is measured in mm² using a planimeter and delineated by a contour joining the peaks of the waves (the lined surface in Fig. 4). The $S$ value (in mm²) of the surface thus obtained is divided by the length in seconds of the analyzed segment [5] multiplied by rolling speed in mm/s of the paper. This yields the mean amplitude in mm ($M_A$ mm) of the tracing under consideration according to the formula:

$$\frac{S}{5 \times 30} = M_A \text{ mm}$$

In order to obtain the mean amplitude in microV ($M_A$ microV) it is considered 10 mm equals 50 microV. This yields an equivalency relation:

$$M_A \text{ microV} = M_A \text{ mm} \cdot 5$$

In order to obtain the mean frequency $M_F$ the waves are counted either above or below the medial $MM'$ line. The number "N" of waves obtained is divided by the length (in seconds) of the segment according to the formula:

$$M_F = N/5$$

Characterization of the tracing is made by using a system of Cartesian coordinates in which the $M_F$ is plotted on the abscissa and the $M_A$ (in microV) on the ordinate (Fig. 5).

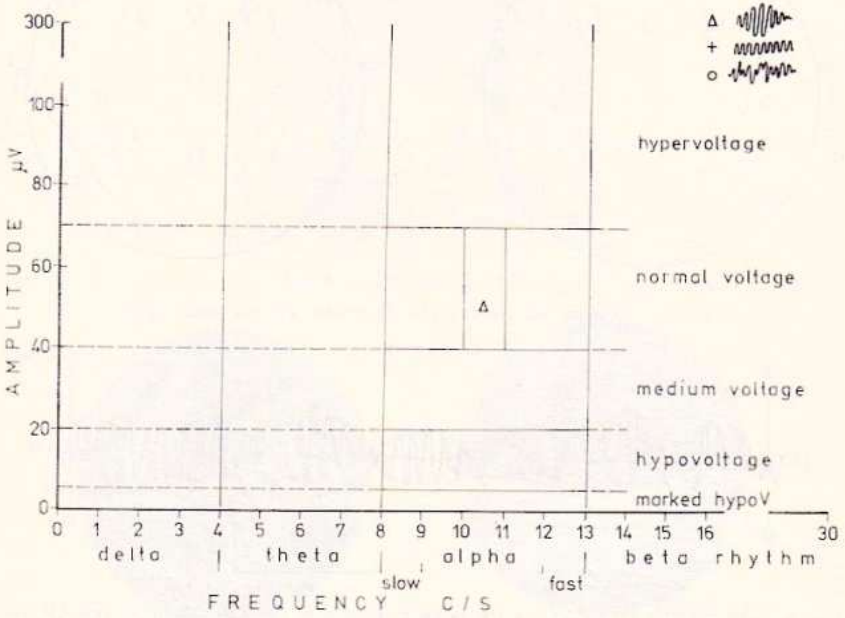


Fig. 5. — Table showing $M_A$, $M_F$ and alpha rhythm morphism in an ideal case.

In a longitudinal study, characterization of the tracing for each subject may be done using a tridimensional Cartesian system which contains in addition, the time dimension indicating the successive moments (½ yr., 1 yr., 5 yrs., etc) selected for comparative analyses of the recordings.

In this way, one may obtain curves that express in the abstract space of the coordinates the cerebral ageing process as it is reflected at the EEG level.

It is worth mentioning at this point that the healthy adult shows an alpha rhythm frequency limited between 10—10.5 c/s and a mean amplitude (in the parieto-occipital leads) around 50 microV as results from the available statistical studies.

Beginning with the age of 60 a graded decrease in frequency down to 8—8.5 c/s for the 80—90 age decade accompanied by a slight decrease in amplitude (30—40 microV) has been noticed.

Besides frequency and amplitude other elements of the EEG tracings, such as morphologic characteristics, the dynamics of the response to hyperpnea, and to

intermittent light stimulation, etc. may also be analyzed. This more complex analysis shows that with advancing age there is an increased latency.

*b.* For statistical processing of the EEG data we have also used an index of normality ($k$) defined by the relation:

$$k = \frac{M_F^2}{M_A \text{ microV}}$$

The value of this index ($k$) for healthy adults varies between 1.43—3.00. As Fig. 6 shows, any alterations of $k$ are of pathological significance.

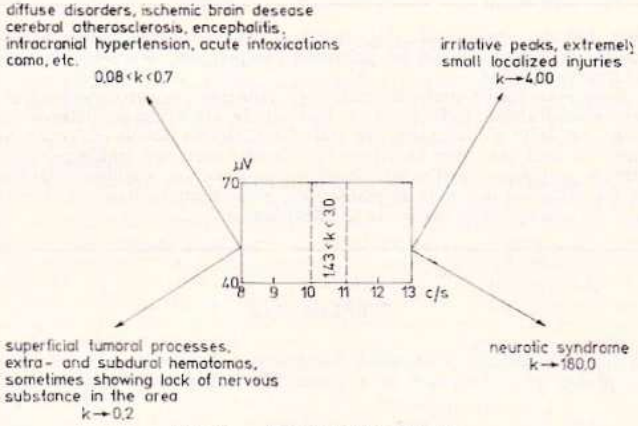


Fig. 6. — Value of the $k$ index.

In space-replacing lesions (tumors, hematomas) involving the cortical areas the $k$ index varies around 0.2. This expresses the fact that at the level of the damage the frequency (the delta rhythm) and sometimes amplitude of the EEG tracings are also reduced.

In diffuse cortical disorders (toxic, inflammatory, hypoxic, especially in cerebral atherosclerosis) the $k$ index decreases to values ranging within 0.08—0.7.

In irritative cortical processes on the contrary, the $k$ index may reach values as high as 4.00 (due to a considerable increase of the frequency, the amplitude of the waves reaches a hypervoltage value of 150—300 microV).

In neurosis, due to the flattening of the EEG tracings (decrease in amplitude) and the increase in frequency, the $k$ index rises considerably (reaching a value of 180).

In the ageing process the $k$ index does not change significantly, which means that the process takes place within normal limits. When these limits are exceeded, the $k$ values are able to point out a pathologic factor superimposed on the normal ageing.

## CONCLUSIONS

*1.* Longitudinal studies of the EEG changes during normal ageing may be successfully accomplished by using the rugged electrodes which offer a greater reliability.

2. The EEG tracings are statistically processed taking into account the two main EEG parameters, i. e. frequency and amplitude.

3. The planimetric method is used both for its greater degree of accuracy and its simplicity and inexpensiveness.

4. The data obtained are analysed in a tridimensional Cartesian reference space which allows graphic representation of the ageing process by EEG recordings.

5. Use of an additional factor, the $k$ normality index allows to pinpoint pathologic processes associated to normal ageing.

**Résumé.** Pour l'étude longitudinale des modifications électroencéphalographiques au cours du vieillissement orthogère humain on préconise l'emploi des électrodes du type « rugged », qui ont une grande fiabilité.

Les tracés sont statistiquement traités, en étudiant les deux paramètres significatifs de l'électroencéphalogramme: la fréquence et l'amplitude. On utilise la méthode planimétrique, aussi bien pour son degré d'exactitude, que pour son caractère simple et économique. Les données obtenues sont analysées dans un espace de référence cartésien tridimensionnel permettant la représentation graphique du développement du processus de vieillissement électroencéphalographique. On utilise un indice de normalité « k », permettant la détection de certains processus pathologiques superposés au processus de vieillissement.

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# SCIENTIFIC LIFE

The following national or regional scientific events of the year 1979 were held in Bucharest, under the aegis of Acad. Ana Aslan M.D., D.Sc.:

**1. The Romanian Gerontological Days** (May, 1979), organized by The National Institute of Gerontology and Geriatrics in collaboration with the Academy of Medical Sciences enjoyed the participation of more than 300 Romanian gerontologists and specialists from other related branches.

The programme included: 6 reports, 13 coreports and 77 scientific communications.

The general report was an extensive presentation of the progress achieved by Romanian gerontological and geriatric research since the founding in 1952 of the Bucharest Institute of Geriatrics, which in 1974 became The National Institute of Gerontology and Geriatrics (NIGG). Emphasis was placed on the fact that the three directions of research in this field, started in 1958, i.e. the biology of aging, clinical and social gerontology, lie at the basis of modern gerontology and geriatrics.

The results of the therapy with Gerovital $H_3$ and Aslavital, the products elaborated by Acad. Ana Aslan, have promoted the development of Romanian and foreign gerontological and geriatric researches; the prophylaxis and therapy of old age and chronic degenerative diseases as well as the prolongation of the active life-span have been proved. Numerous researches carried out in the Institute, in this country and abroad, proved scientifically the effects of the biotrophic therapy with Gerovital $H_3$ and Aslavital. The pharmacological properties and mechanisms of action of the biotrophic substances on the aging process have been studied. At present, the treatment with these substances is applied in 144 gerontological centres from industrial enterprises in Bucharest and throughout the country, in the geriatric consulting rooms of territorial polyclinics, in the pilot station, polyclinics and clinics for foreign patients.

The Institute has clinical sections in Bucharest and in the main resorts of the country; they have 1300 beds for the geriatric treatment with Gerovital $H_3$ and Aslavital, applied to foreign patients. The original Romanian products are exported in more than 45 countries; they have been approved in another 16 countries.

The researches carried out in the Institute have also been directed towards the study of age criteria, premature aging and longevity, chronic degenerative diseases related to the aging process, mechanisms of aging and mechanisms of action of the biotrophic substances, medico-social assistance for the aged.

The report on the researches in the biology of aging presented an overview of hypotheses and already known mechanisms of molecular, cellular, tissular, organic and organismic aging in the light of the current concepts on programmed or error theory of aging as a result of the action of certain endo- or exogenous factors in nucleic acids and protein synthesis, formation of crossbindings, intensification of autoimmune reactions, decrease in the immune functions, aging of intercellular and biological membrane matrix, increase in MAO activity, etc. Emphasis was laid on the contribution of Romanian researchers to the better understanding of the aging process. All the researches are consistent with Ana Aslan's concept of aging as a dystrophic generalized process due to multiple mechanisms.

The coreports and communications dealt with the main mechanisms of aging in the light of modern interpretations. They also presented researches in geriatric pharmacology which make a valuable contribution to the understanding of the mechanisms of aging and of the mechanism of action of Gerovital $H_3$ and Aslavital.

The reports and most of the communications on clinical gerontology pointed out the efficacy of Gerovital $H_3$ and Aslavital therapy in the prophylaxis of the aging process and chronic degenerative diseases.

An analysis was made of the importance of changes occurred in the immune system, with emphasis on aggressive autoimmune phenomena.

The asynchronous aging in different components of the vascular system was underlined as well as the development of adaptative and compensatory mechanisms with stress on the clinical and therapeutic peculiarities of myocardial infarction and thromboembolic diseases.

The biochemical, morphological, psychological and clinical aspects of neuraxial aging were analysed. The peculiarities of endocrine diseases hormonal biorhythms in the aged were also pointed out, with special reference to climacterium.

The acute pulmonary pathology in geriatrics was presented in terms of clinical, radiological, functional and therapeutic particularities.

Other researches dealt with the homeostasis and thrombosis in the aged in the context of interrelationships between physiologic aging and age pathology.

The report on social gerontology defined the concept of social gerontology as an integral part of the multi- and interdisciplinary aspect of gerontology; emphasis was placed on the medico-social and psychosocial aspects of the aging process.

The coreports and communications revealed in detail the breadth and depth of the social involvement in gerontology, among which mention should be made of:

1. The need to continue and extend researches in social gerontology in two main directions: *a)* the study of the health status in the active and retired aged population at national level; *b)* the development of differentiated geriatric medical assistance (more gerontological points, geriatric consulting rooms, new geriatric departments and centres for geriatric recovery).

2. The need to continue and extend the study of the possibility to have the aged taken care of in their own families, elaboration of active life patterns for the retired population in order to facilitate their social integration, means of improving the personal and social behaviour of the aged.

Taking into account the continuous increase in the aged population of Romania adequate steps will be required for ensuring the health of the aging and the aged population.

In conclusion, the synthesis, communications and discussion pointed out the main directions in the progress of modern Romanian and foreign gerontology, with emphasis on the original contributions of Romanian gerontology, the most important of which is Acad. Ana Aslan's biotrophic treatment with Gerovital $H_3$ and Aslavital as well as the steps taken for the extended prophylaxis and treatment of premature aging and chronic degenerative diseases.

The current directions in Romanian gerontological and geriatric applied researches open the prospect of medical contributions to reaching the main objectives: the prolongation of the active life span and the improvement of the health status in the aged population.

**2. Round table on Gerontoprophylaxis in the industrial enterprises from Bucharest,** organized on Acad. Ana Aslan's initiative, with the participation of physicians from 15 industrial enterprises, representatives of the Ministry of Health, physicians and other members of the staff.

Four papers were presented, followed by discussions.

The paper entitled "Role of the prophylactic check-up in maintaining and promoting the health of the aged; the efficiency of the prophylactic therapy with Gerovital $H_3$ and Aslavital in preventing premature aging", discussed the concept of gerontological prophylactic check-up and the medico-social steps taken by the Institute in order to promote the health state of active elderly workers (aged 45—62 years) from the industrial enterprises of Bucharest.

The objectives of promoting a normal or almost normal aging rhythm can be reached by means of the gerontological prophylactic check-up. A good health implies the complete survey of the functional capacity, a number of recommendations aimed at preventing the onset of chronic degenerative diseases and prolonging the active life-span, training for retirement.

In our country, Acad. Ana Aslan has elaborated and applied an original gerontoprophylactic method for workers aged 45 and over, based on the use of the biotrophic therapy with Gerovital $H_3$.

At present, this method is applied in Romania in 144 gerontoprophylactic centres, organized in industrial enterprises throughout the country, which include over 16,000 workers.

The "energizing" effect of the biotrophic therapy in the active elderly, strengthens their biologic potential, maintains their working capacity and prolongs their active life-span. This results in decreased morbidity with temporary work incapacity, the number of sick-leave days dropping by 38% as against the year prior to the application of gerontoprophylactic method, increased resistance to diseases.

The paper entitled "Researches in the mechanisms of action of Gerovital $H_3$" reviewed researches carried out at the NIGG, in other institutes and abroad on the mechanisms of action of the biotrophic products elaborated by Acad. Ana Aslan. Gerovital $H_3$ was proved to have several mechanisms of action, concordant with the mechanisms of aging.

It was pointed out that, at cellular level, Gerovital $H_3$ activates oxidative phosphorylation, in therapeutic doses stimulates the aerobic respiration, diminishes the activity of some lysosomal enzymes, stimulates DNA synthesis, inhibits monoaminoxidase activity (Bucci, MacFarlane, Hrachovec), stimulates cellular regeneration, prolongs the life-span of culture postmitotic cells and increases the number of generations in a passage (Officer). The ultrastruc-

tural examination carried out by Ana Aslan and coll. pointed out the improvements induced by Gerovital $H_3$ in nuclei, mitochondria and the cytoplasm.

The paraclinical investigations of elderly subjects, or those with chronic degenerative diseases, pointed out that Gerovital $H_3$ induces: the increase in serum total proteins; the decrease in serum cholesterol, beta and gamma-globulins, the increase in hemoglobin and hematocrit values, improved ESR. Electroencephalographic studies pointed out the increase in amplitude and frequency of the alpha rhythm. The nervous conduction speed as well as the muscular strength increases. The ECG revealed the alleviation of coronary irrigation disturbances. The increase in oscillometric indices and significant alleviation of peripheral circulatory disturbances was pointed out in the limbs, particularly in arteriosclerosis.

From the immune standpoint, the treatment with Gerovital $H_3$ and Aslavital stimulates the defense mechanisms of the organism, among which phagocytosis, coloidopexia, lymphocyte functions.

Mention was made of the researches in Aslavital mechanisms of action in atherosclerosis, carried out on subjects with thrombophilic disturbances in whom the treatment induced a decrease in the number of cases with plasma hypercoagulability, hypofibrinolysis, thrombocytic hyperfunction and increased thrombocytic thrombodynamic activity.

The authors emphasized that the use of Gerovital $H_3$ and Aslavital therapy since the age of 40–45, delays the process of aging and prevents the onset of chronic diseases or complications, thus resulting in the prolongation of the active life-span.

All these data are the scientific basis of the gerontoprophylactic action started by Acad. Ana Aslan since 1955.

The paper entitled "The role of Gerovital $H_3$ and Aslavital therapy in neuropsychic diseases induced by professional overstress" emphasized certain peculiarities of aging in the nervous system, resulting from:

– the postmitotic character of neuronal senescence;

– coordinating (or determining) the role of homeostatic, ecologic and social adaptation;

– great sensitiveness of the nervous tissue to numerous risk factors, particularly in professional activity;

– special implications of the social factors, particularly in the rational organization of work.

Two aspects should be investigated in psychological aging:

1) aging at the level of material structures and energetic processes occurring in the nervous tissue, and

2) aging at the level of informational phenomena occurring in the encephalon.

The first level involves the usual prophylactic steps specific to the prophylaxis of somatic senescence, particularly professional noxae and work conditions. The hygienic and dietary steps should be taken into consideration, in order to rule out or diminish the effects of the risk factors. Starting with the age of 45, besides hygienic steps the prophylaxis is recommended with the biotrophic therapy, according to Ana Aslan's method based on Aslavital and (or) Gerovital $H_3$, therapy which has been applied since 1954, in and outside the Institute in collaboration with C. I. Parhon. It acts at the level of the nervous system: as acetylcholine precursor (it decreases with age as a result on neuronal loss); as activator of the catecholamine subsystem (which decreases with age as a result of monoaminoxidase increased activity); improves the cerebral flow; has a general biotrophic effect on the cell systems (neuronal and probably neuroglial).

Prophylactic psychosocial steps should also be taken, aimed at ruling out the overstresses and psychic traumas.

The paper entitled "Stage results regarding the organization of gerontoprophylactic actions. Suggestions and steps to be taken during the next stage", presented the results obtained in the organization, guidance and coordination of gerontological points from the industrial enterprises of Bucharest, on problems of gerontoprophylaxis.

Based on the methodology elaborated by the NIGG of organizing gerontoprophylactic actions in the dispensaries of different enterprises, the medical staff and researchers selected some groups of hard workers, workers displaying premature aging phenomena and suffering from chronic degenerative diseases. Complex gerontoprophylactic steps were recommended, as well as the biotrophic therapy with Gerovital $H_3$ and Aslavital, according to Ana Aslan's method.

Based on the experience accumulated and the results obtained between 1976–1979 in the organization of gerontoprophylaxis in the industrial enterprises from Bucharest, the authors suggested the steps to be taken during the next stage, among which mention should be made of:

— extension of gerontoprophylactic actions in heavy industry enterprises from Bucharest, particularly in the departments with hard working conditions;

— organization of a model gerontoprophylactic point which should become a pilot station of NIGG;

— organization of prophylactic gerontological consultations given by the physicians of NIGG outpatient unit. Special attention will be given to the aged workers from noxious departments, in order to detect the onset of chronic diseases;

— introduction of problems of gerontoprophylaxis as subjects of sanitary training, particularly those related to the hygiene of work, nutrition, sleep, leisure, retirement.

Enterprise physicians emphasized the peculiar interest of the workers included in the gerontoprophylactic action, due to the positive results yielded by the prophylactic gerontological consultations and the administration of Gerovital $H_3$ and Aslavital treatments. Because the biotrophic drugs were given free of charge by the Institute, a larger number of aged workers were included in the treated groups; thus, the efficacy of the gerontoprophylactic action increased. This yielded the following results:

— decrease in the number of temporary work incapacity down to 0.44 per person/year;
— drop of arterial pressure in hypertensive cases;
— fewer angina crises;
— alleviation of insomnia;
— significant alleviation of polyarthrosis pains;
— increased resistance to different infections;
— improvement of the general health condition, increase in the working capacity;
— increased work economic efficiency.

At the end of the debate, the enterprise physicians emphasized that gerontoprophylactic actions should be extended not only in the industrial enterprises of Bucharest, but throughout the country; suggestions were advanced to ensure an increased efficiency of the steps taken.

**3. "Longevity in relation to environmental factors and age pathology",** national symposium, organized by the National Institute of Gerontology and Geriatrics in collaboration with the Academy of Medical Sciences and the Gerontological Society of the Union of Societies of Medical Sciences; over 200 participants.

The symposium included: the general report, 4 coreports and 53 communications focussed on the 3 main approaches: longevity, clinico-biological, psycho-social and experimental; considerable emphasis was laid on promoting longevity by means of old age prophylaxis and therapy with Gerovital $H_3$ and Aslavital.

The report, coreports and communications analysed thoroughly the concept of longevity, underlying the role of the genetic, physical and social environmental factors, of chronic degenerative pathology and of the factors which prolong the active life and the life-span.

The clinical and functional characteristics of longevity were presented, with particular stress on the cardio-vascular, neuropsychic indicators, those of the locomotor system, respiratory and digestive apparatus, functional and morphological indicators.

Longitudinal researches were presented among which the longest study in the world, initiated by Acad. Ana Aslan, the efficacy of the biotrophic medication being illustrated by the advanced ages reached by most of the subjects despite the chronic degenerative pathology existing before starting the treatment.

Statistically, life expectancy was exceeded by 30%. These data were correlated with the results yielded by the experimental researches in the life-span, which was prolonged by 21%.

The complex clinical, psychological, functional, biochemical, hematological, immune investigations of the subjects under the longitudinal study pointed out the improvement of most of the parametres used.

From the psychic standpoint, the alleviation of depressive states and asthenia, improved memory, attention, adaptability were pointed out; the joy of life and work increased as well as psychic performances and muscular strength; extrapyramidal rigidity diminished, gait improved. The incidence of cerebro-vascular and coronary accidents decreased; the most important diseases in the longitudinally studied subjects were those of the respiratory apparatus.

At the level of the cardio-vascular apparatus, the alleviation of the functional symptoms, dyspnea, anginous pains, was noticed as well as the increase in the capacity for effort, equilibration and stabilization of pressure values, the improvement of the left ventricle cardiodynamic indices, ECG tracings, the decrease in the intermittent claudication time, alleviation of trophic disturbances, the increase in oscillometric indices in peripheral circulatory failure.

From the humoral standpoint, in most of the subjects the dyslipemic indicators, proteinemia, hypercoagulability, hypofibrinolysis, thromboeytic hyperactivity and the hematological morphofunctional indicators were corrected or normalized.

The progress of the chronic degenerative diseases, particularly atherosclerosis, localised in different clinical sites, as well as arterial hypertension, osteoarthropathies was favorably influenced thus promoting longevity in the treated subjects.

The results obtained at the Institute are applied in the gerontological points and geriatric consulting rooms throughout the country.

Morphopathological researches were also presented and correlated with the clinical data.

The papers read at the Symposium represent a valuable contribution to the study of the mechanisms of the aging process and the possibilities of prolonging the life-span. They referred particularly to the immune, endocrine and molecular changes, most of them approached on the basis of original concepts and modern techniques.

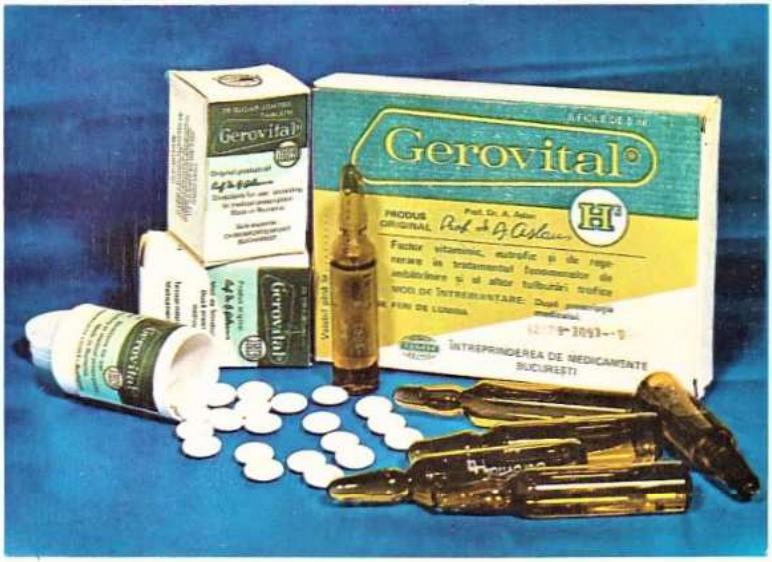
The epidemiological researches in longevity were conducted on 15,820 longevous from representative areas throughout the country.

The results of the above-mentioned researches revealed the favorable influence of the physical and social environmental factors related to the geographical environment, socio-economic status and educational level.

*Al. Vrăbiescu*

original products of Prof. Dr. A. Aslan

# GEROVITAL H3




Vitamin, eutrophic factor and regenerator used in the treatment of aging phenomena and other trophic disorders.

## VIALS AND SUGAR-COATED TABLETS

**Indications:**

- curative and prophylactic treatment of aging phenomena;
- treatment of trophic disorders;
- physical and psychic asthenia, depression, anxiety;
- neuritis, neuralgia;
- Parkinson's disease;
- degenerative rheumatism (spondylosis, arthrosis), rheumatoid polyarthritis, osteoporosis;
- dystrophies of the skin, nails and hair; neurodermitis; eczemas, alopecia, sclerodermia, psoriasis, vitiligo;
- vascular spasms;
- arteritis;
- bronchial asthma;
- gastric or duodenal ulcer

**Supply:**

- Boxes of 6, 12 and 25 vials of 5 ml
- Boxes of 100 vials of 5 ml
- Bottles of 25 sugar-coated tablets or capsules

Sole exporter: ICE "CHIMIMPORTEXPORT"

POB 525, Blvd Republicii 10, Bucharest, Romania, Telephone No. 16.06.36, Telex: 11184; 11697

Gerovital Prof. Dr. A. Aslan H3

# Gerovital

Gerovital Prof. Dr. A. Aslan H3
Gerovital Prof. Dr. A. Aslan H3


3

## Gerovital H3 hair lotion

For the care, regeneration and prevention of hair loss.

**Indications:** seborrhea, dandruff, pruritus, alopecia (baldness, partial hair loss), canities (white hair).

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Prof. Dr. A. Aslan


GEROVITAL
GEROVITAL

H3
cold
cream

Gerovital
Prof. Dr. A. Aslan H3

Prof. Dr.
# Ana Aslan

CREMĂ DE FAȚĂ
Gerovital
Prof. Dr. A. Aslan H3

CRÈME DE BEAUTÉ
Gerovital
Prof. Dr. A. Aslan H3

**H3**

Gerovital
Prof. Dr. A. Aslan H3
FACE CREAM

Ensures maintenance and nutrition of dry skin, normalizes the complexion, diminishes senile spots and acne, effaces wrinkles and other skin trophic disorders related to aging phenomena.

*Indications:*

care and regeneration of the skin; also efficient in mild burns

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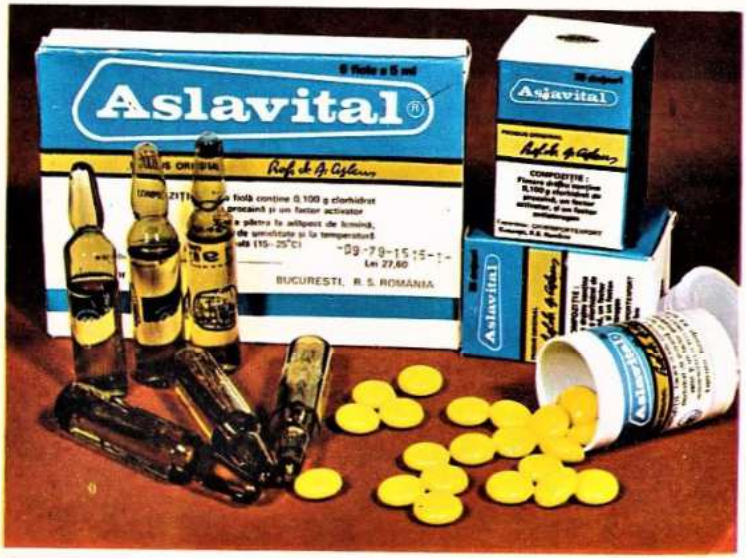
# ASLAVITAL




IMECO

Antiatherosclerotic, energising factor, active in the prophylaxis and treatment of aging phenomena of the central nervous system and cardiovascular apparatus.

## Indications:

— prophylaxis and treatment of cerebral and cardio-vascular aging ;
— troubles of memory ;
— generalized, predominantly cerebral, coronary (angina pectoris), peripheral atherosclerosis ;
— prevention and control of thrombo-embolic accidents and post-hemorrhagic sequelae.
The drug is also indicated in children with psychosomatic undergrowth and encephalomyelitic sequelae.

## Supply:

Boxes of 6, 12 and 100 vials of 5 ml.
Bottles of 25 sugar-coated tablets or capsules.

Sole exporter: ICE "CHIMIMPORTEXPORT"

POB 525, Blvd Republicii 10, Bucharest, Romania, Telephone No 16.06.36, Telex: 11184; 11697

original products of *Prof. dr. A. Aslan*


# ufrix (isobutylhydroclorothiazine)

Diuretic agent with quick and persistent action; moderate potassium depletion, decreases arterial pressure.

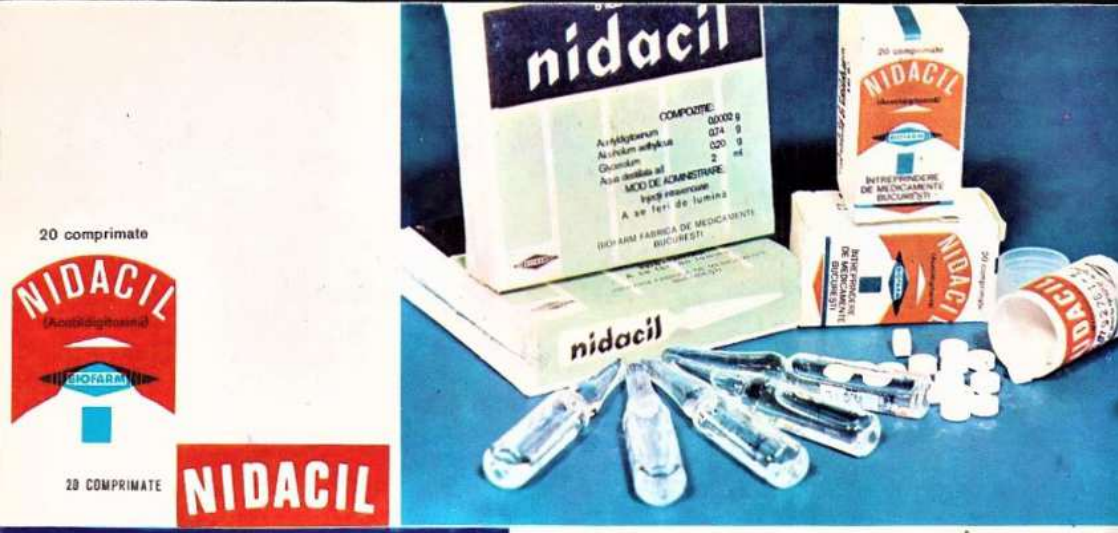
**Indications:** hydrosaline retention (without renal failure), cardiogenic oedemas, essential hypertension, cirrhosis with ascites, pregnacy with oedemas.

**Contraindications:** renal and hepatic failure; hypopotassemia.

**Supply:** tablets of 0.005 g.

UMB

Producer: *Drug Factory Bucharest, Romania.*




5 fiole de 2 ml

# nidacil

# (acetyldigitoxin)

## *Indications:*

all forms of myocardial failure with accelerated rhythm, atrial fibrillation, paroxysmal tachycardia, flutter.

**Digitaline-like cardiotonic. Positive inotropic action (increases myocardial contraction capacity), negative chronotropic action (slows down myocardial rhythm), improves coronary circulation.**

## *Contraindications:*

acute decompensated cardiopathy (myocardial failure, recent myocardial infarction), acute pulmonary oedema.
Not to be used when calcium is administered.

## *Supply:*

Vials with injectable solution containing 0.0002 g acetyldigitoxin.
Tablets containing 0.002 g acetyldigitoxin.
Solution of 0.050 g in 100 ml.
Suppositories.

*Producer:* Biofarm Factory, Bucharest, Romania

# Oxacilin


Semisynthetic penicillin; acid- and penicillinase-resistant.

**Indications:** Infections produced by staphylococci resistant to other types of penicillin; cutaneous infections; respiratory infections, urinary infections, osteomyelitis

**Contra-indications:** Intolerance to penicillin.

**Supply:** capsules containing 0.275 g sodic oxacyline monohydrate.

**Producer:** Antibiotics Factory Iaşi, Romania.

# THE DRUG, COSMETICS DYESTUFF AND VARNISH CENTRAL

Bucharest, Blvd. Ion Şulea 246

## presents


**Arginine Sorbital — perfusable solution bottles containing 12.5 g arginine chlorhydrate, 25 g sorbital and distilled water up to 250 ml.**

### Indicated in:

**Severe hyperammoniemia syndromes (hepatic coma); dystrophic states in chronic hepatic insufficiency.**

**Arginine, the amino acid which plays an important part in the urea cycle and diminishes the concentration of plasma ammonia in acute hyperammoniemic hepatic insufficiency accounts for the therapeutic effect of the drug; sorbital vehiculates arginine and is the energetic substratum required by its utilization.**

### Contraindications:

**Hyperchloremic acidosis, particularly when underlying nephropathy or anuria.**

### Producer:

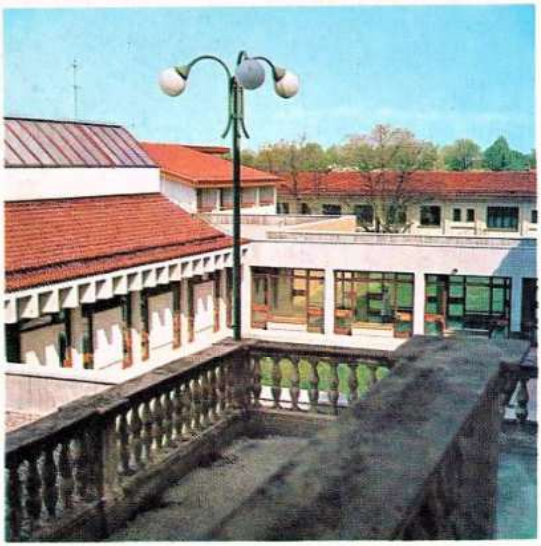
**DRUG FACTORY**

**Bucharest, Romania**

# SINAIA

At an altitude of 800 m above sea level lies the "Pearl of the Carpathians", a name by which this beautiful health-resort is also recorded.
Its microclimate is propitious to the therapy of different neuroses, depressive states, and hyperthyroidism.

**A geriatric center on the precincts of Palace Hotel provides treatment with Gerovital H 3 and Aslavital according to Prof. Dr. Ana Aslan's method.**



# Otopeni

At a distance of some 17 kms from Bucharest, on the Ploiești motorway that goes to the mountain resorts of the Southern Carpathians, stands the "Otopeni" clinical section of the National Institute of Gerontology and Geriatrics in the midst of a beautiful 70 ha park.

Prof. Dr. Ana Aslan's treatment method with *Gerovital H3* and *Aslavital* is being applied for about ten years. The excellent accommodation and services, the quiet and picturesque ambient as well as the low altitude of the place recommend it to geriatric patients.

# BĂILE HERCULANE

Herculane Spa (160 m altitude) is situated in the south-west of Romania, not far from the Iron Gate, in the picturesque valley of the Cerna river. The resort was recorded as early as the time of the Roman Empire for its curative properties and mild climate with Mediterranean influences. It is especially recommended for locomotive ailments (arthrosis, spondylosis, etc.), but also for affections of the peripheral nervous system, of the digestive tract (colitis, hypoacid gastritis, etc.), of the respiratory apparatus (chronic bronchitis), and for gynecological treatment.

A geriatric section staffed with physicians from the National Institute of Gerontology and Geriatrics provides a Gerovital H3 and Aslavital therapy according to Prof. Dr. Ana Aslan's method. Open all the year round.

# BAILE FELIX

In the north-west of Romania, close to Oradea city, lies Felix Spa.

Its microclimate and low altitude are indicated for various neurotic states, but what has made the resort famous is its treatment center against various types of rheumatism.

A geriatric section, where treatments with Gerovital H3 and Aslavital according to Prof. Dr. Ana Aslan's method are administered by physicians from the National Institute of Gerontology and Geriatrics, is open all the year round.

## COUNSEL TO AUTHORS

The Romanian Journal of Gerontology and Geriatrics publishes original papers, reports, syntheses and reviews dealing with creative research in gerontology and geriatrics. The papers should be written in one of the following languages: English, French, German, Italian.

1. The size of the manuscript (including illustrations, tables, references and summaries) should not exceed 10 pages. Reports and syntheses should not outnumber 15 pages.

The manuscript should be typed with double-spacing (31 lines per page). Two summaries containing a maximum of 15 lines, one written in a different language than the manuscript, should be included.

Illustrations will be considered as pages (one page = 150 $cm^2$).

2. The paper should be consistent with the following plan=title; authors' names; the name of the institute where the authors have conducted their research work and the respective address; summary (in the manuscript language); introduction; material and method; results; discussion; conclusions; summary (in a different language); references.

3. Figures should be drawn on tracing, white or scale paper, in India ink, preferably the size intended for publication (1/1).

Legends should be typed with double-spacing on separate sheets, as included in the manuscript.

Whenever microphotographs are used, staining and × should be mentioned. The place of figures and microphotographs will be noted in the manuscript.

4. Tables should be typed with double-spacing on separate sheets. They will be placed at the end of the text; suggested location keyed to text.

5. References should be numbered in the text with parenthetic Arabic numerals, in order of occurrence. A double-spaced bibliography at the end of the paper will include:

*a)* for papers published in periodicals: author/s; full title of paper; full title of publication; volume; number; year; first and last page;

*b)* for books, monographs, treatises: author/s; full title; publisher; town; year; page.

Romanian titles of papers will be translated into the language of the manuscript, with the indication: (in Romanian).

References should be typed on a separate sheet.

6. All manuscripts meeting these standards should be addressed to:

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## FORTHCOMING PUBLICATIONS OF THE INSTITUTE

- a volume containing the scientific communications presented at the Eighth European Congress of Clinical Gerontology of the International Association of Gerontology, Neptun, Romania, Sept. 1977;
- a volume containing scientific papers on the prophylaxis and therapy with Gerovital $H_3$, published in Romania and abroad;
- a volume containing scientific papers on the therapy with Aslavital.

ROMANIAN J. GERONT. GERIATRICS, 1980, 1 1, BUCHAREST

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