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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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