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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 1.5×10^4 in tubes and 2×10^6 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).

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

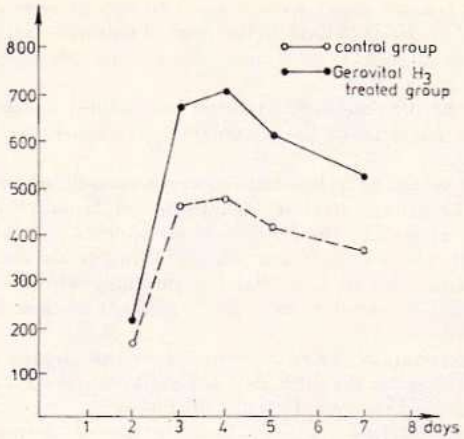

Fig. 1 – Renal cells density during the first 7 days in control and treated groups.

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 ± 0.31 as compared to 3.68 ± 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 ± 0.06	34	0.79 ± 0.08	38.3
10	1.21 ± 0.10	39.8	1.33 ± 0.31	41.0
16	1.37 ± 0.12	46.8	1.42 ± 0.13	50.0
20	1.99 ± 0.16	51.9	1.89 ± 0.37	54.0
25	2.17 ± 0.09	67.0	2.08 ± 0.15	65.9
30	2.33 ± 0.10	79.0	2.16 ± 0.08	69.9
40	2.42 ± 0.09	84.2	2.29 ± 0.10	76.4
50	3.68 ± 0.29	91.0	2.98 ± 0.31	80.0
60	4.20 ± 0.39	96.4	3.61 ± 0.28	95.0
70	—	—	4.39 ± 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 ± 0.03	16.0	0.40 ± 0.02	16.7
11	0.82 ± 0.08	19.3	0.80 ± 0.10	17.1
15	1.19 ± 0.13	26.0	1.13 ± 0.09	22.3
21	1.65 ± 0.26	30.0	1.58 ± 0.16	31.2
26	1.73 ± 0.25	34.2	1.70 ± 0.14	33.5
32	1.88 ± 0.40	35.4	1.81 ± 0.06	34.9
41	2.66 ± 0.22	60.2	2.58 ± 0.26	57.8
51	3.00 ± 0.30	74.0	2.89 ± 0.11	69.0
58	3.69 ± 0.36	88.0	3.06 ± 0.29	79.1
68	—	—	3.88 ± 0.16	89.4

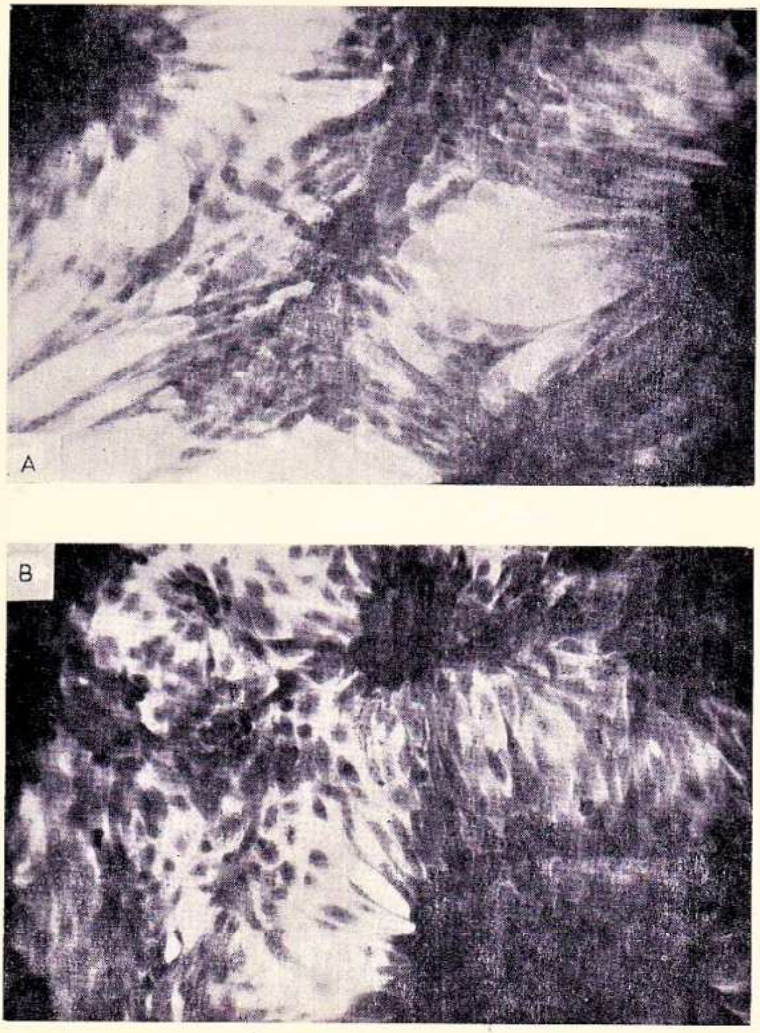

Fig. 2. — Microphotograph of a 4-day-old-cellular area: A — in the control group; B — in the group treated with Gerovital H_3. Increased cellular density in the treated culture. Staining violet crystal. 400 ×.

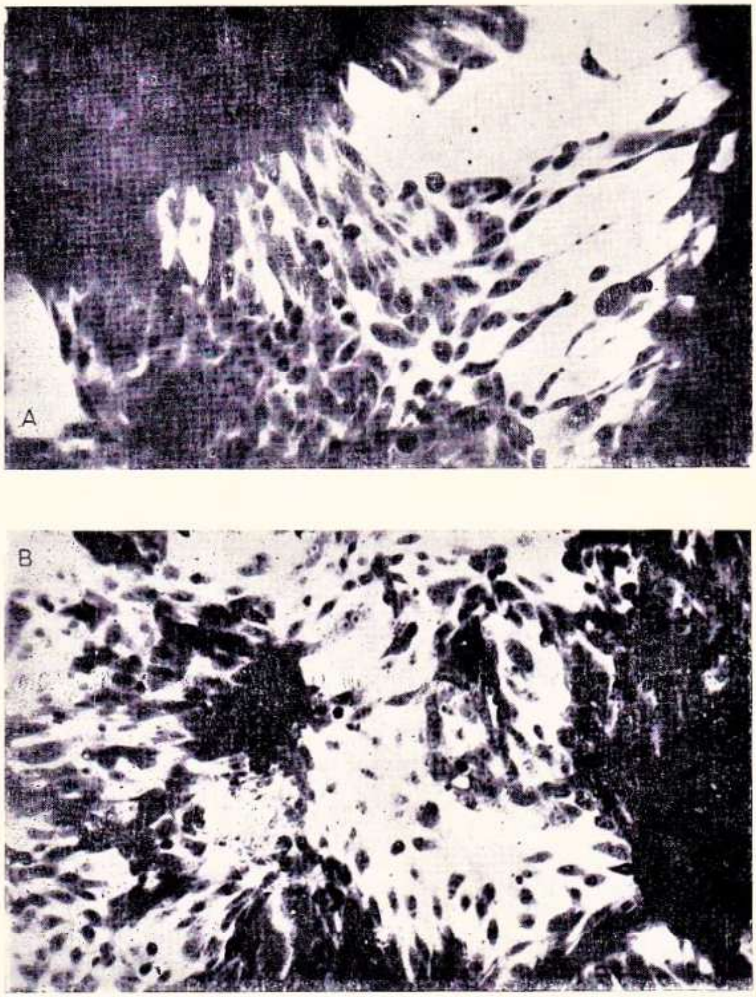

Fig. 3 — Microphotograph of 25-day-old-cellular areas; A — in the control group; B — in the group treated with Gerovital H_3. The areas are slackly populated as the cells fall from the vessel walls.
Staining violet crystal. 400 ×.

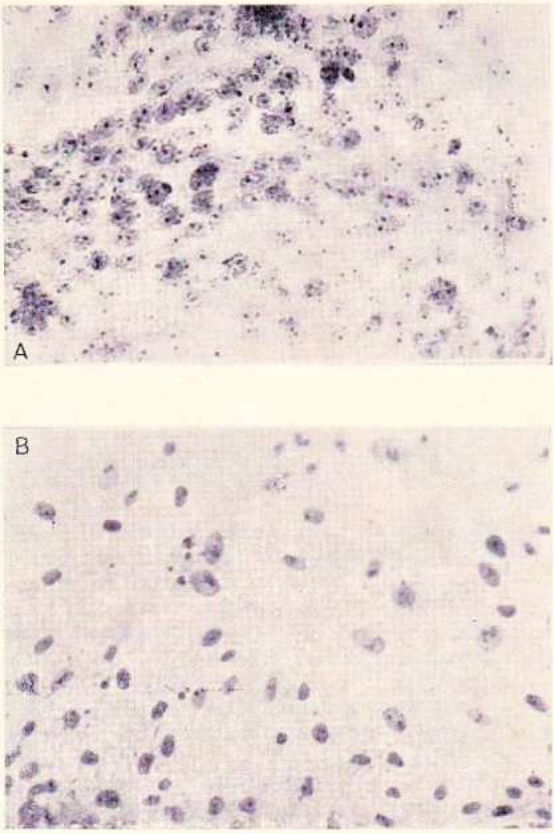

Fig. 4 — Microphotograph — 14-day-old renal cell in the control group (A) and in the group treated with Gerovital H_3 (B). Marked fat loading in the control group. Staining Nile Blue. 200 ×.

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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