*Biochemistry (Moscow), Vol. 68, No. 2, 2003, pp. 242-246. Translated from Biokhimiya, Vol. 68, No. 2, 2003, pp. 292-298.*


**DISCUSSIONS**

# Games with Glass-Beads for Biologists or Science for the Day After Tomorrow?

## (Review of paper by A. M. Olovnikov "The Redusome Hypothesis of Aging and the Control of Biological Time during Individual Development")

**V. N. Anisimov**

*Petrov Research Institute for Oncology, Ministry of Public Health, Pesochnyi 2, St. Petersburg, 197758 Russia; E-mail: aging@mail.ru*

The whole history of ideas and concepts in biology of aging may be shortly characterized as the history of a search for the biological "clock" of aging. Selection of the "clock" determined approaches for treatment of a mechanism of this clock that could increase longevity and operative effectiveness of this "fragile" mechanism. The existence of a large number of theories and hypotheses explaining mechanisms of aging reflects the disappointing fact of the absence of a generally established universal theory [1]. L. Hayflick was right stating [2] that any theory of aging should answer at least three questions.

1. Why are living organisms subjected to progressive and irreversible reduction of physiological functions at the last (declining) part of their life?

2. Why do intra- and interspecies differences in expected duration of life or aging rate exist?

3. Why do such experimental treatments as nutritional calorie limitation attenuate the onset of numerous physiological and pathological changes and increase average and maximal duration of life of experimental animals?

Since genetic and environmental factors may influence aging processes several additional questions are quite reasonable.

1. Do the factors which influence aging and age-related diseases operate independently from each other?

2. Do age-related changes in the organism increase susceptibility for diseases or do diseases develop independently and "create" a certain background for acceleration of aging?

The latter question is especially important because a correct answer would link cell immortalization (i.e., factors of external and internal media leading to cancer development) and aging of the organism.

Finally, there is the other principal question: Can we influence the aging process in man?

The recent decades are characterized by the introduction of molecular biology and gene engineering methods into life sciences. This resulted in cardinal changes in our knowledge on the nature of aging and the appearance of theories of aging based on late and the latest experimental data. In 1971 using new data on principles of DNA synthesis in cells A. M. Olovnikov proposed a hypothesis of marginotomy [3], which represented a basis for the telomere theory of aging. Olovnikov suggested that during template synthesis of polynucleotides DNA polymerase is unable to reproduce the whole stretch of the linear template and the resultant replica is always shorter in its marginal part. Thus, each cell division is accompanied by shortening of cellular DNA and this phenomenon limits proliferative potential of the cell and acts as the "counter" of number of cell divisions which determines life span of the cultivated cells. In 1985 telomerase, the enzyme adding nucleotides to shortened telomeres in infusoria, sex cells, and tumor cells (and imparting immortalization to them) was discovered [4]. This discovery gave a "new life" to Olovnikov's hypothesis and stimulated numerous studies. These studies revealed many fundamental facts of the biology of a cell and its individual life span. These studies also stimulated hot discussions on the role of telomeres and telomerase in basic processes underlying the fate of cells and whole organisms in terms of aging and death and also malignization (immortalization). Some authors even question the correctness of extrapolation of *in vitro* data to *in vivo* processes and extrapolation of data obtained on experimental animals (e.g., mice) to processes occurring in man [5, 6].

Now we are offered a new hypothesis of aging which appears to answer all six key questions required to be answered by universal theory of aging. The set-point of Olovnikov's new hypothesis [7] consists in a reasonable notion that some control function for biological time

0006-2979/03/6802-0242$25.00 ©2003 MAIK "Nauka/Interperiodica"

coupled to aging process still remains *terra incognita*, because structures underlying biological time of living organisms still remain unclear (we will consider this point later). The paper begins with well-written principles of molecular and cell biology suitable for students of the School of Biology. However, soon the reader desperately wants to look for literature references in Internet on the unknown structures which are so brightly described by the author. I was very surprised to learn complete absence of any reference for any unknown structure. Finally, I realized that Olovnikov developed some model of genetic structure which contains "unreal personages". Being involved into collaboration with serious mathematicians working on mathematical imitation modeling of processes involved in aging of various organisms (from yeast and nematodes to man and population of whole countries) I have become adapted to their language, which is too formalized for me, medical doctor by specialty and biologist by occupation. So I decided to continue this fascinating reading and made a break only at the list of references.

Moving among fantastic description of unreal (or it is better to say undiscovered) organelles and mechanisms of their work within the cell, I gradually distinguished some contours of slightly recognizable structures. During subsequent reading I found some familiar "guides" and became more confident in that different fantastic World opened by the author without any warning.

What does this new work by Olovnikov represent? It represents well formulated description of hypothetical, i.e., completely unknown in modern science microstructures of the cell nucleus. These structures have bright and well-sounding names: redusome, printomere, protoprintomere, chronosome and chronomere with protochronomere, acromere, and acrosome. Olovnikov proposes clear description of some hypothetical mechanisms responsible for interaction of these structures. This results in a logic system containing contours of known or possible principles. The introduction ends by clear definition of biological time, which (according to Olovnikov) represents span of sequentially changed events of individual development and this span is measured and controlled in higher animals by the organism itself on the basis of changes of genetic composition of shortened chronomeres. At this moment introduction of a new organelle (chronomere) does not cause deep intrinsic protest. Two other postulates do not cause any objections, because the referee completely agrees with them: "Biological time is characterized by stepwise rather than gradual run. Biological time and astronomical time run independently."

In the next part Olovnikov introduces a course of this new science. He describes in detail his hypothetical structures and their properties and activities. I call this course the "glass-beads game for beginners" which was brightly described by Herman Hesse in his novel. The key role in this new structural and functional organization of a living cell belongs to a "printosome"–"chronomere" couple. According to Olovnikov's hypothesis they are involved in control of biological time and aging, but printosome and chronomere play different roles and their (printomere and chronomere) DNAs undergo different time-dependent shortening. At this moment a reader not very well familiar with Hayflick's limit problem might conclude that Olovnikov begins to exploit again his good old marginotomy theory in attempts to omit accumulated contradictions in this theory. However, as one character of Russian history said the reader won't be right again, because at this moment Olovnikov begins an advanced course for the "glass-bead game". He introduces certain principles for the interaction of structures which should be formulated even if these structures do not exist, because these principles are related not only to the hypothetical structures, they involve into the sphere of interaction known structures and principles, which still exist as "beliefs" and require better understanding and interpretation. (Here I use the term "belief" to describe simple lack of knowledge rather than the religious attitude to some fact. Known Russian biologist Aleksandrov describing translation process indicated that "messenger RNA, enters cytoplasm" and he indicated that the word "enters" hides "the abyss of our lack of knowledge" [8].)

So, Olovnikov explains to his readers what printosomes and chronosomes are doing and how they function. For example, the "mission of printomeres consists in solution of problems of interpretation and memory of position information during morphological differentiations". Dealing with the mission of printomeres the reader naturally comes to idea that it cannot be otherwise, because the telomere system of the cell (like the Ptolemaic system) is not entirely logical (harmonic).

In the next part Olovnikov deals with basically brilliant idea of chronomere, which measures time of the individual development by a "chronomeric ruler" localized in neuroendocrine cells. According to Olovnikov the chronomere is responsible for directed flow of biological time during eternal repeat of individual development of organisms. He describes further principles of chronomere organization, its functioning and why (at least at the level of the neuroendocrine cells) biological time is characterized by a stepwise run (because of stepwise loss of chronomeric genes). Olovnikov suggests that the rate of chronomere shortening may be reduced in a senile period. This is consistent with modern notions on slow down of aging processes in the senile period [9].

According to Olovnikov viewpoint the central nervous system (CNS) is the initial substrate for aging and within CNS cells DNA is the primary substrate for aging. This is a principally important viewpoint which is supported by experimental results. For example, Akifiev and Potapenko [10] demonstrated that incorporation of 5-bromo-2′-deoxyuridine (BrdUrd), a synthetic analog of thymidine, into DNA caused mutations. Aging related to

BrdUrd-induced damage of hypothalamic structures was demonstrated in rats and mice [11, 12]. Addition of BrdUrd to a cell culture also accelerated cell aging [13]. Data on tissue-specific differences in age-related accumulation of mutations do not contradict this hypothesis [14, 15].

Olovnikov believes that age-related changes in energy homeostasis are ultimately important for life span. In the seventies of the last century V. M. Dilman [16] suggested that gradual increase in the threshold of sensitivity of the hypothalamus–pituitary system to homeostatic signals from peripheral endocrine glands is a basis for aging. Olovnikov draws our attention to recent and very impressive studies in modern gerontology. Experiments on *C. elegans* revealed that mutations in genes encoding proteins involved in insulin signaling in neurons significantly increased life span of these nematodes [17, 18]. Olovnikov suggests that this effect cannot be attributed to unfavorable changes in free radical formation because their production in wild-type cells should be maximally equilibrated by the functioning of antioxidant defense system(s). Nevertheless, he leaves unanswered the question on mechanism(s) underlying the positive effect of mutations in neurons on life span of *C. elegans*. In other words, the key question why changes in energy metabolism of *C. elegans* reduce the aging rate still remains. Recently, it was shown that addition of 1-butyl-biguanide increased life span of *C. elegans* by 23.4% [19]. Earlier it was found that anti-diabetic biguanides increased tissue sensitivity to insulin and decreased threshold for sensitivity of hypothalamus to homeostatic signals in rats and mice [20, 21]. Anisimov et al. studied age-associated allele distribution for the apolipoprotein C-III gene T-455C polymorphism [22]. The latter (T-455C) is located in the apoC-III 5′-untranslated region within a functional insulin-response (IRE) element. These authors found a correlation between frequencies of the mutational event in IRE of human *APO CIII* gene and life span [22]. This suggests that the mutation in insulin signal transduction system located below *daf-16* gene is directly linked to longevity [22]. In this part of the paper, using proposed mechanisms regulating new (hypothetical) structures Olovnikov gives plausible explanation of possible reasons for interspecies differences in life span.

A large section of his paper Olovnikov reserved for analysis of the role of biological rhythms in measurement of biological time. He suggested so-called T-rhythms, and infradian biorhythms of neuroendocrine system activity are the best candidates for the role of these rhythms. Emphasizing the special role of changes in energy metabolism for the rate of aging, Olovnikov suggests that T-rhythms should be based on rhythmic hormonal activity of the GH/IGF-I axis and the existence of such rhythmic secretion of growth hormone and IGF-I has been already recognized [23]. The authors suggest that oscillation of calcium ions, second messengers in signal transduction from hormone-activated receptors of plasma membrane inside cell, may regulate gene transcription. Certain frequency characteristics of hormonal bursts operating in T-rhythms and acting via intracellular oscillation system may be involved into arrangement of superintensive transcription of certain genes. Olovnikov illustrates this idea by convincing evidences that demonstrate the existence of such (or similar) mechanisms.

Olovnikov's idea on chronomere localization in CNS regions does not cause objections and dogmas on the role of hypothalamic structures and their age-related mechanisms of aging are rather correct and consistent with the framework of the whole hypothesis. Olovnikov reserves an especially important role to the pineal gland which is generally considered as "aging clock" and to its hormone, melatonin, the main hormone regulating circadian rhythms in the body [24, 25]. To support his hypothesis Olovnikov cites data on the involvement of calcium ions in mediating melatonin action and its oscillatory synthesis and secretion [26] and melatonin-dependent expression of genes regulating calcium release in cells [27]. Subsequent development and deepening of Olovnikov's hypothesis should include data on circadian and other oscillations and their genetic control in all cells of an organism which function even *in vitro* [28-30] and reevaluation of the key section in the hypothesis on the regulation of T-rhythm. In this connection it is relevant to mention that one chapter of a recently published review on coordination of circadian rhythms in mammals was dedicated to so-called nuclear timesome [31] and protein complexes mCRY–mPER–CKIε/CKIδ associated with transcriptional factors CLOCK and BMAL1, regulating transcription of circadian rhythm genes seem to play the role of this timesome [32]. This might represent a reasonable appendix to Olovnikov's chronosomes!

Within the framework of his hypothesis Olovnikov considers further such important problems of modern gerontology as mechanisms of *in vitro* aging of nonproliferating cells, Werner's syndrome, and the role of telomeres which may serve as a witness rather than direct participant of cell immortalization. The author gives very interesting interpretation of data on reduction of life span in transgenic mice with excess of overexpressed telomerase [33]. (This paper induced very vivid and still continuing discussion in the literature.)

The chapter entitled "How can the notion of redusomes help to overcome the main current difficulties in the biology of aging?" is very interesting, because readers meet a whole firework of suggestions. Olovnikov suggests that a principal feature of his "program of aging" operating on the basis of interaction between T-rhythm and chronomere DNA consists in dynamic changes of material carrier of this program and lack of any special genes responsible for realization of the aging program. This suggestion does not contradict modern concepts on candidate aging genes in man [34, 35] and the latest data on

age-related time-course of transcriptome expression in *C. elegans* [36, 37].

The new hypothesis may explain some "problematic" points of Hayflick's concept and the intriguing fact that telomere length does not correlate to species longevity. Some of Olovnikov's notions correspond to known tenets in science and therefore can be interpreted as their subsequent development. For example, Olovnikov indicates that "impairment of extracellular environment promotes impairment in effective functioning of cells during aging of the organism, and the quality of environment deteriorates in dependence on chronomere shortening in CNS and hormonal and neurotrophic supply to all target cells deviates more and more from optimum which was achieved in youth". As Olovnikov mentions there is analogy with the "law of homeostasis deviation" formulated by V. M. Dilman [16].

Within the framework of his hypothesis Olovnikov also considers the exponential age-related increase of frequencies of cancer incidences. Discussions on the role of telomeres and the role of cell senescence in cancer became especially hot in recent years [5, 38, 39]. According to Olovnikov, "Cell senescence does not represent a mode for anticancer defense (as it may be suggested here) via slowing cell divisions, apoptosis, etc., as it is often suggested in the literature. Moreover, cell senescence itself is obviously the main factor provoking (even in the absence of exogenous carcinogens) appearance and developments of neoplasms." I agree with this viewpoint and am its supporter. Skeptical position of the author on the effectiveness of treatment with antioxidants as geroprotectors is also supported [40]. At the same time I am not confident to analyze Olovnikov's explanation of one of the oldest mystery of genetics, the non-accidental gene linkage in chromosomes. However, the schemes within his speculative hypothesis seem to be rather logical.

The final section before conclusion deals with facts obtained in certain studies (particularly cytogenetic ones) which may support possible existence of redusomes. Of course examples given in the paper require independent validation and only single-minded search may confirm or refute their reality. Some aspects of this hypothesis expressed as suggestions are now well known and therefore they may be interpreted as arguments for support of this hypothesis.

Slow down of aging processes in the senile period reflecting the reduction of mortality rate is considered as one of the most intriguing discoveries of demographic studies of longevity [9, 41]. However, it remains unclear why reduction in the mortality rate observed in older ages is also observed in yeast, worms, and drosophila [41, 42]. Olovnikov suggests that the reason for this phenomenon might consists in a decrease of chronomere transcription rate and, consequently, in more economic consumption of their remnants in very old humans. During the last 160 years expected human longevity in the most economically developed countries has increased by three months per year [43]. This is related to radical changes in style of life and achievements of civilization and medicine. However, how can we explain increased life span of some laboratory animals [42]? Interestingly, a tendency for reduced mortality was noted in male mice maintaining under standard condition of the same animal house during a ten year period (from 1988 to 1998); at the same time mortality of female mice increased in spite of stable weight of male and female animals [44]. It would be interesting to know Olovnikov's interpretation of this "challenge" from demographers. Another important problem which still requires exhaustive explanation is the nature of the increase in longevity induced by mild stresses [45]. Hypothesis pretending to be a universal should also pay some attention to plant aging especially in terms of the biological time of plants.

It should be noted that the redusome theory provides a definitive answer on very old (but still unsolved) question whether a program of aging exists. According to Olovnikov, the program of organism aging not only exists but represents a part of wider species strategy directed to protection of gene pool of the whole species [46, 47] and aging of organism as a part of sequential events of its development proceeds on the basis of universal mechanism of chronomere shortening.

In conclusion, Olovnikov summarizes his hypothetical constructions of beautiful in its harmony hypothesis into simple dogmas, which do not seem to be so fantastic and do not look like the glass-bead game at the end of reading. Perhaps Olovnikov's manuscript looks like a textbook for biology which we mysteriously received from the far future. I have read this manuscript "on a single breath" as I used to read my favorite books by Asimov and Shackley. I enjoyed reading this manuscript which is excellent in deepness of analysis of material, in clear compositions and fine writing. Unquestionably this manuscript may by published under the heading "Hypothesis". I do believe that Olovnikov is one of the few distinguished scientists who has undoubted right to publish such manuscripts in the submitted version. Some comments or (more correctly) specifications and additions which I have made may be used for subsequent work on this hypothesis, which gives great stimulus to look for this wonderful and beautiful world opened for us by A. M. Olovnikov.

How long will it take to confirm this hypothesis? Which questions should Olovnikov answer for us? Which new problems appear in medicine? What can the scientific community do for accelerated verification of this hypothesis in order to avoid a quarter of century waiting which passed since the first publication of marginotomy hypothesis in 1971 up to its verification at the end of XX century? This period of waiting significantly reduced our telomeres and as we know now redusomes as well. (The latter is even more important.)

I think that limits of a paper in a scientific journal are too narrow for Olovnikov and his hypothesis should be

published as a monograph. I do believe that any scientific journal would by happy to publish this paper by Olovnikov. His work was supported by a grant from Russian Foundation for Basic Research (RFBR). This also suggests high level of this theoretical consideration made by Olovnikov, but *vice versa* this work increases the authority of RFBR by "a feedback mechanism". Astronomic time (which as we known now is independent from biological time) will show whether this hypothesis will be verified or not. If yes, we will get a key to control biological time.

## REFERENCES


1. Anisimov, V. N., and Soloviev, M. V. (1999) *Evolution of Concepts in Gerontology* [in Russian], Eskulap, St. Petersburg.
2. Hayflick, L. (2000) *Nature*, **403**, 365.
3. Olovnikov, A. M. (1971) *Dokl. Akad. Nauk SSSR*, **201**, 1496-1499.
4. Greider, C. W., and Blackburn, E. H. (1985) *Cell*, **51**, 405-413.
5. Rubin, H. (2002) *Nature Biotechnol.*, **20**, 675-681.
6. Wright, W. E., and Shay, J. W. (2002) *Nature Biotechnol.*, **20**, 682-688.
7. Olovnikov, A. M. (2003) *Biochemistry (Moscow)*, **68**, 2-33.
8. Aleksandrov, V. Ya. (1970) *Usp. Sovrem. Biol.*, **69**, 221-240.
9. Ukraintseva, S. V., and Yashin, A. I. (2001) *Mech. Aging Dev.*, **122**, 1447-1460.
10. Akifiev, A. P., and Potapenko, A. I. (2001) *Genetika*, **37**, 1445-1458.
11. Anisimov, V. N., and Osipova, G. Y. (1992) *Mutat. Res.*, **275**, 97-110.
12. Anisimov, V. N. (1994) *Ann. N. Y. Acad. Sci.*, **719**, 494-501.
13. Michishita, E., Nakabayashi, K., Suzuki, T., Kaul, S. C., Ogino, H., Fujii, M., Mitsui, Y., and Ayusawa, D. (1999) *J. Biochem.*, **125**, 1052-1059.
14. Dolle, M. E. T., Giese, H., Hopkins, C. L., Martus, H.-J., Hasudorff, J. M., and Vijg, J. (1997) *Nature Genet.*, **17**, 431-434.
15. Dolle, M. E. T., Snyder, W. K., Dunson, D. B., and Vijh, J. (2002) *Nucleic Acids Res.*, **30**, 545-549.
16. Dilman, V. M. (1994) *Development, Aging, and Disease. A New Rationale for and Intervention Strategy*, Chur: Harwood Academic Publ., Switzerland.
17. Tatar, M., Kopelman, A., Epstein, D., Tu, M. P., Yin, C. M., and Garofalo, R. S. (2001) *Science*, **292**, 107-110.
18. Wolkow, C. A., Kimura, K. D., Lee, M. S., and Ruvkun, G. (2000) *Science*, **290**, 147-150.
19. Bakaev, V. V., Bakaeva, L. M., Nikitin, Yu. P., and Shabalin, A. V. (2002) *Biogerontology*, **3**, Suppl., 23-24.
20. Dilman, V. M., and Anisimov, V. N. (1980) *Gerontology*, **26**, 241-245.
21. Anisimov, V. N. (1980) *Vopr. Onkol.*, **26**, 42-48.
22. Anisimov, S. V., Volkova, M. V., Lenskaya, L. V., Khavinson, V. Kh., Solovieva, D. V., and Schwartz, E. I. (2001) *J. Gerontol. A Biol. Sci. Med. Sci.*, **56A**, B27-B32.
23. Roelfsema, F., Biermasz, N. R., and Veldhuis, J. D. (2002) *Clin. Endocrinol.*, **56**, 79-87.
24. Pevet, P., Bothorel, B., Slotten, H., and Saboureau, M. (2002) *Cell Tissue Res.*, **309**, 183-191.
25. Vanecek, J. (1998) *Physiol. Rev.*, 7**8**, 687-721.
26. Foulkes, N. S., Cremakian, N., Whitmore, D., and Sassone-Corsi, P. (2000) *Novartis Found. Symp.*, **227**, 5-14.
27. Anisimov, S. V., Boheler, K. R., and Anisimov, V. N. (2002) *Dokl. Ros. Akad. Nauk*, **383**, 276-281.
28. Sakamoto, K., Oishi, K., Nagase, Y., Miyazaki, K., and Ishida, N. (2002) *Neuroreport*, **13**, 1239-1242.
29. Duffield, G. E., Best, J. D., Meurers, B. H., Bittner, A., Loros, J. J., and Dunlap, J. C. (2002) *Curr. Biol.*, **12**, 551-557.
30. Aktar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H., and Kyriacou, C. P. (2002) *Curr. Biol.*, **12**, 540-550.
31. Reppert, S. M., and Weaver, D. R. (2002) *Nature*, **418**, 935-941.
32. Lee, C., Etchegaray, J. P., Cagampang, F. R., Loudon, A. S., and Reppert, S. M. (2001) *Cell*, **107**, 855-867.
33. Tyner, S. D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., Lu, X., Soron, G., Cooper, B., Brayton, C., Park, S. H., Thompson, T., Karsenty, G., Bradley, A., and Donehower, L. A. (2002) *Nature*, **415**, 45-53.
34. De Benedictis, G., Tan, Q., Jeune, B., Christensen, K., Ukraintseva, S. V., Bonafe, M., Franceschi, C., Vaupel, J. W., and Yashin, A. I. (2001) *Mech. Aging Dev.*, **122**, 909-920.
35. Bonafe, M., Barbi, C., Stroci, G., Salviolo, S., Capri, M., Olivieri, F., Valensin, S., Monti, D., Gonos, E., De Benedictis, G., and Franceschi, C. (2002) *Exp. Gerontol.*, **37**, in press.
36. Lund, J., Tedesco, P., Duke, K., Wand, G., Kim, S. K., and Johnson, T. E. (2002) *Curr. Biol.*, **12**, 1566-1573.
37. Dillin, A., Crafford, D. K., and Kenyon, C. (2002) *Science*, **298**, 830-834.
38. Campisi, J. (2002) *Mech. Aging Dev.*, **123**, 567-573.
39. Anisimov, V. N. (2002) *Adv. Gerontol.*, **10**, 99-125.
40. Anisimov, V. N. (2001) *Exp. Gerontol.*, **36**, 1101-1236.
41. Vaupel, J. W., Carey, J. R., Christensen, K., Johnson, T. E., Yashin, A. I., Holm, N. V., Iashine, I. A., Kannisto, V., Khazaeli, A. A., Liedo, P., Longo, V. D., Zeng, Y., Manton, K. G., and Curtsinger, J. W. (1998) *Science*, **280**, 855-860.
42. Carey, J. R., Liedo, P., and Vaupel, J. W. (1995) *Exp. Gerontol.*, **30**, 605-629.
43. Oeppen, J., and Vaupel, J. W. (2002) *Science*, **296**, 1029-1031.
44. Eiben, R. (2001) *Exp. Toxicol. Pathol.*, **53**, 399-408.
45. Rattan, S. (2001) *Hum. Exp. Toxicol.*, **20**, 281-285.
46. Skulachev, V. P. (2001) *Exp. Gerontol.*, **36**, 995-1024.
47. Skulachev, V. P. (2002) *Ann. N. Y. Acad. Sci.*, **959**, 214-237.