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A historical review of the problem of mitogenetic radiation

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Summary. The 'miracle of caryokinesis' was the starting point that stimulated Alexander G. Gurwitsch to carry out his famous 'mitogenetic' experiments in 1923. The results obtained confirmed his hypothesis of a weak radiation from cells, which is able to trigger the growth of other cells. Extensive experimental work within the first two decades after this discovery indicated that the problem of mitogenetic radiation is generally related to the biological significance of UV-radiation. Both 'energetic' and 'informational' aspects have to be considered, namely radiation effective in activating molecules, and that involved in arranging them into larger units.

The molecular organization of biological structures is evidently governed by nonequilibrium conditions needing the uptake or emission of radiation. These concepts of A. G. Gurwitsch can be linked with modern approaches based on hypotheses of coherence in biology, 'synergetics' and 'dissipative structures'. However, the question of causal interrelationships between this part of non-equilibrium radiation and biological matter on different levels of evolution has to be solved now.

Key words. Mitogenetic radiation; caryokinesis; mitosis; molecular organization structure; degradation radiation; resonance coherence; synergetics; dissipative structures.

The main direction of biological suggestions

'A never-weakened interest in the miracle of caryokinesis was the basis of all my further attempts', wrote A. G. Gurwitsch. 'The most mysterious of all was for me the sporadic arising of mitoses in meristems. The question posed itself whether cell division in general might be only a causal event in cell life. The purely statistical character of the spatial distribution of mitoses demonstrated in several objects (and particularly in onion roots) supported the concept of causalness and, hence, the conclusion that mitosis as a causal event should be the result of interaction of at least two mutually independent factors'[7–10, 12, 14, 15]. However, as the author mentioned[18], these formally correct conclusions were not sufficiently substantiated because similar statistical results could be the consequence of gradually accumulated fluctuations in the rhythms of cell divisions.

On the other hand, they led to the following important suggestion. A theory of mitosis should be based upon a dual principle. That is, one of the factors which make a cell capable of division is endogeneous (a 'possibility factor') while a second one ('realization factor') is exogeneous although it may arise in the same organism[15, 18].

It was demonstrated that: 1) there is a reverse linear relationship between the surface areas of the meristematic cells and their division frequencies; 2) along the whole onion root meristem, cell surface areas increase according to the exponential law. The latter relationship may be interpreted in the following way: there exists a surface 'substance K' which remains constant during cell growth, and also a 'substance A' which increases in an assimilatorial manner, retaining its constant properties. According to this assumption, the surfaces of cells of different dimensions would be distinguished from one another only by a constantly altering relation between K and A.

The main problem was to elucidate the nature of the exogeneous factor. Initially it seemed natural to look upon it as a chemical substance, similar to Haberlandt growth hormones. Correspondingly, it was suggested that the increase in the amount of the A substance, by moving apart and thus relatively diminishing the surface sites occupied by the K substance, decreases the hormone penetration inside a cell. In this case, however, cell division frequencies should be proportional to the relation of K to a constantly increasing A, which contradicts the established fact of a reverse linear relationship between cell division frequency and cell surface area.

This contradiction led to the following suggestion: K and A sites are arranged on a cell surface as a permanently changing spatial mosaic. K sites are favorable to an exogeneous cell division factor, whereas A sites are unfavorable. If it is a mosaic-like configuration which plays a decisive role, the perception of an exogeneous impulse by a cell surface may be considered as a resonance event. This led to the hypothesis that the exogeneous division stimulating factor is an oscillatorial process which may be a radiation.

Analyzing his trend of thought later, A. G. Gurwitsch wrote in his very interesting paper devoted to the first twenty years of mitogenetic radiation studies[18]: 'It is difficult to imagine how this chain of arbitrary and physically not very literate conclusions could lead to a correct result, that is to the discovery of radiation. In our opinion, the reasons why it acutally did so are the following ones. Firstly, the very concept of the decisive role of the cell surface in mitogenetic radiation perception was substantiated, even before the nature of the cell division factor had been elucidated, since it could be derived from the synchronism of cell divisions in different syncitia, as opposed to the irregular distribution of division in a cell population.

Secondly, even in the idea of a resonance there was a grain of truth which could not be realized at that time. Indeed, as now seems obvious, one or several photons can affect the entire cell only if the photon absorbtion gives rise to a chain reaction considerably depending upon the spatial distribution of the molecules involved, that is, upon the configuration of a molecular order'.

The experimental verification of the hypothesis that the exogeneous factor is a form of radiation has evolved from the suggestion that at least some radiation should emanate from a root into the surrounding space and that it would be most probable that detectable radiation would arise from the cone-shaped tip of an onion root. An adequate detector should consist of cells ready to divide and really dividing with a certain average frequency, but at the same time capable of increasing their division frequency. The necessity for comparison with control cells was also obvious. For this purpose as well, the most suitable objects were onion roots characterized by a radial symmetrical arrangement. Therefore, the revealing of a difference in mitotic numbers between the 'irradiated' and the 'shadowed' sides of the meristem after a unilateral local stimulation seemed to be fairly possible. Already the first experiments, in which a horizontally oriented inductor-root was brought to a distance of 1.5–2 mm from the medial surface of the meristem of the vertically oriented detector root during 1–2 h, gave distinct positive results: the number of mitoses at the medial zone of the 'illuminated' side was 20–25% higher that on the other parts of the meristem (Table).

These first 'mitogenetic' experiments, conducted in 1923, were later supported by several hundred experiments[14, 15]. The first tentative experiments pointed to the UV range of radiation as the most probable one. The insertion of a glass plate no more than several dozens of µm thick between the roots did not inhibit the increase of mitoses on the illuminated side. This effect disappeared, however, when the objects were separated by a glass plate of 0.2–0.3 mm thickness. Later on, the radiation spectrum was studied in great detail[6, 20].

Radiation of an onion root causing an increase in mitosis in the meristem of another onion root. Number of mitoses in successive and corresponding microscopic fields of cross sections (thickness 10 µm) of the exposed side and of the opposite side of the root (see description in the text)

	I Exposed side	II Opposite side	III Difference
Outside of the range of exposure	36	32	4
	30	33	−3
	38	34	4
	35	31	4
	29	34	−5
	34	34	0
	51	53	−2
	54	57	−3
	66	64	2
	72	78	−6
	75	81	−6
	75	74	1
	88	82	6
	57	50	7
	55	52	3
	58	60	−2
	64	65	−1
	66	61	5
	50	52	−2
	52	53	−1
Mean value:	54.25	54	0.25
Mean deviation:	16.77	16.99	3.97
Directly exposed part of the root	64	54	10
	70	57	13
	80	50	30
	63	45	17
	58	38	20
	58	41	17
Mean value:	65.50	47.50	17.83
Mean deviation:	8.38	7.45	6.91
Outside of the range of exposure	44	40	4
	42	42	0
	43	43	0
	37	35	2
	45	43	2
	46	50	−4
	46	43	3
	42	46	−4
	54	55	−1
	64	62	2
	40	44	−4
	55	53	2
	49	53	−4
	41	40	1
	44	43	1
	36	33	3
	34	34	0
	46	47	−1
	45	49	−4
	31	37	−6
	23	25	−2
	36	30	6
Mean value:	42.86	43.05	−0.18
Mean deviation:	8.60	8.75	3.16

The direction of further investigations

The first years of intensive study were characterized by a broad range of investigations. The universality of the radiation among animal and plant objects was studied, and the most suitable method of biological detection elaborated. This method was the stimulation of yeast budding. Due to its high sensitivity it permitted the use of spectral analysis. The possibility of using Geiger-Muller photon counters was also investigated, as well as the possibility of stimulating cell division by weak physical ultraviolet radiation[6, 20, 22, 23, 27].

A certain scattering of the problems studied, inevitable in a new research area contiguous to quite different problems of natural history, did not prevent studies being made of more general and profound aspects. The important task was to reveal one of the basic mechanisms of radiation which is connected with the highly exothermic recombination of free radicals[3, 20].

It is worth mentioning that around the mid-thirties the formation and recombination of short-lived free radicals during enzymatic reactions of low caloricity was considered to be hardly probable, or even impossible. Meanwhile, it was not taken into account that these highly energy-producing events may be quite rare and hence do not influence the overall energetic reaction output but, on the other hand, they may be sufficient to generate UV radiation of a very low intensity. It is worth mentioning that the presence of atmospheric oxygen and, in some cases, illumination by visible light is necessary for the radiation of enzymatic processes [20].

With the accumulation of new data, the problems requiring further investigation have been outlined. These were: A) a study of the biological significance of UV radiation, that is, the use of the photon energy in biological processes, and B) the use of UV radiation as a subtle indicator of the molecular processes related to the different functional states of living matter.

Such a division is arbitrary to some extent, as there is now more and more evidence that photons of different energetic levels participate in practically all biological processes. However, depending on the interests of the investigator, one of these approaches may dominate in different periods of work. For example, the spectral analysis of the mitogenetic radiation of neural and muscular systems can without doubt be considered as a sensitive indicator for the structural and energetic dynamics of neuro- and myoplasms in different functional states. In this case the problem of photon participation in molecular reconstruction may not arise for some time [6].

On the other hand, already in the first years of investigation it became evident that all the events connected with mitogenetic radiation were based upon chain reactions in which light and dark links were combined. It is to this kind of process that one should relate the widespread phenomenon of secondary radiation. This phenomenon permits us to understand the very fact of the maintaining and spreading of weak UV radiation inside a biological substrate strongly absorbing UV [6, 20, 23]. We are dealing here with the 'relay' character of the transmission of excited states, both dark and light ones.

It is therefore evident that the concept of chain processes includes in itself the spreading of steric, i.e. conformational reconstructions with different degrees of reversibility. The chain processes underlying secondary radiation are of a branching character and it is breaking of the separate chains which leads to the release of energy in the form of photons.

On the other hand, the absorption of one or several UV photons may impart a chain character to chemical processes. This was demonstrated, for example, with peptide polycondensation, a process of special interest since it is necessary for the preparation of cell division and is stimulated by mitogenetic radiation [6, 20, 22, 23].

Therefore, under certain conditions, mitogenetic radiation may play the role of a creative factor. We would like to stress that such a stimulating role is played only by very weak biological UV radiation, whereas much more intensive physical UV radiation inhibits biological processes as a rule.

The primary action of the absorbed photons should obviously be considered as a very important trigger mechanism activating and participating in the chain processes which are, on the other hand, conditioned by the specific conditions constantly maintained in biological systems. In other words, the elucidation of the conditions promoting the specific 'organization' of the cellular processes which arose as secondary, tertiary etc. links in response to the absorption of single photons should be considered as the main biological problem. This general concept is apparently close to some view developed within the last 10–15 years [4, 39, 48].

Ascribing a decisive role to the chain processes excited by photon absorption, A. G. Gurwitsch suggested that the 'organization' of a substrate should create some conditions restricting their variability, that is, regulating the use of the absorbed energy.

This leads to the idea which was the basis of one of the main directions in the study of mitogenetic radiation.

The necessary condition for the development of chain processes is a certain degree of molecular 'orderliness' in a substrate. Within the classical biological concepts the latter term is no more than an unnecessary synonym for a strict concept of 'structure', but mitogenetic radiation leads to quite other concepts of cell organization.

In these concepts the notion of molecular orderliness acquires an independent content and reflects the most important features of cell organization and, obviously, cell energetics [11, 13, 15, 16, 19, 20, 21, 23].

The decisive point in the development of these concepts was the discovery of the so-called degradational radiation appearing during reversible inhibition of the metabolic level, for example during the local cooling of a system under study, narcosis of an animal, or during some other reversible interventions [6, 19–24].

The revelation of this specific and obviously universal phenomenon demonstrates that at least a part of the energy released during exothermic metabolic processes is accumulated up to the high potentials equivalent to the far and middle range UV and seems to be used normally by a stepwise degradation. The energy-requiring molecular complexes act as devices which accumulate the high energetic potentials using separate portions of metabolic energy probably not exceeding 20 kcal/mol each.

In other words, the molecular complexes or, better, the molecular 'constellations' are associations of molecules brought together, to some extent ordered and joined only by very weak interactions, permitting the energy circulation along the constellation as an entire system, with the probable cumulation of energy in one of the molecules. The migration of energy creates the conditions for a metastability of the constellations, although, on the other hand, the constellations are non-equilibrial, i.e., dynamic and labile.

The creation of non-equilibrial molecular orderliness is therefore directed against the disordered thermic molecular movements whose degrees of freedom in living systems are thus restricted. For each excited molecule, the parameters of the restriction are to some extent at least, functions of its position inside a cell. This formulation, which stresses the influence of cell parameters upon the spatial distribution of molecules, is a brief expression of the concept of cellular fields elaborated by A. G. Gurwitsch in the course of many years and related to his basic idea about the constant regulation of the spatial parameters of a living system by a coupling interaction of supracellular, cellular and molecular levels [19, 21, 24].

Some experimental data point out the changes of the steric parameters, i.e. the configurations of constellations. That gives a potential possibility for the enormous diversity of the living processes, which may be designated as 'structuralized processes', without neglecting the general systemic regulation principle [6, 20, 22, 23].

In spite of the great functional importance ascribed to the concept of non-equilibrial molecular constellations these can in no way substitute for the concept of more stable structures; there must obviously be an interaction between the different categories of ordered molecular states. In other words, the constellations may be considered as a regulatory component of the general molecular continuum, by which to some extent the character of the chemical kinetics of the system is conditioned.

Limiting ourselves here to this short description of an experimentally based statement of the problem, let us now consider the question of the attitude of some scientific circles to the problem of mitogenetic radiation.

The evolution of various views on the problem of mitogenetic radiation

'The external fate of the problem has been characterized by sharp perturbations, at least within the first decade of its existence' wrote A. G. Gurwitsch [18].

'The very wide interest in the discovery of the radiation, which arose after the first publications and continued for about 10 years, was unfortunately not based upon a solid scientific ground. The success mostly seemed to be an unhealthy sensation, insofar as most of those, who were attracted by the phenomena discovered and studied in our laboratory, were a certain kind of light-minded biologists. Some studies which seemed at the first glance to give positive results were based upon a few experiments only; therefore they could not stand up to serious criticism and even compromized the real data'.

On the other hand, many investigations negating the very fact of the radiation were also based upon a small number of experiments, whose authors not only ignored but often acted against the recommendations of the original laboratory. This is true both as far as the biological detection method is concerned as well as for the use of photon counters [26, 28, 30].

Meanwhile, despite the fact that there was so little satisfactory background, in several laboratories in different countries serious and systematic work has been performed, not only using biological methods but also by employing critically and successfully a physical method of registration (photon counters) [1, 2, 5, 31–33, 41–47].

We shall not concentrate here on individual pieces of work because, in our opinion, it would be more important and interesting to recount some general ideas suggested by A. G. Gurwitsch in 1943 [18].

'... In the course of many years, biology developed as a wide and fairly calm stream, its great successes being the confirmation of well substantiated hypotheses derived from observation, rather than unexpected novel ideas. That gives it both its greatness and its narrowness. Gradually the conviction arose that discoveries, i.e. observations which have no direct relation with the totality of our knowledge and the dominating views are actually impossible. Therefore many authors, and even those who were personally convinced of the reality of the radiation phenomenon, did not know what to do further, that is, they did not see the real physical, chemical and, most of all, biological implications of UV radiation in living systems'.

'... Along with that, demands have been made for evidence for mitogenetic radiation which are in principle both wrong and unrealizable. Particularly, only that part of the communicated facts has been recognized which could be confirmed by other, so-called classical methods. Certainly one cannot neglect the desirability of such confirmation, but to consider it as a necessary precondition means to destroy scientific logic. If a new method is more sensitive than the previously existing ones, it is only natural that it will eventually reveal events of low intensity which have escaped observation by the previous less sensitive methods. Moreover, the unrealistic character of the demands, which have been advanced mostly by biochemists, is explained by their dominating interest in substances as opposed to processes. Many important results obtained by observing mitogenetic radiation are related to the study of processes, both in models and in vivo'.

The correctness in principle and the mutual relatedness of these concepts has recently become more evident, now that the main problems of biology are increasingly concentrated around the study of energetic processes and non-equilibrial states, that is on the *dynamic* characteristics of living matter. The interest of physicists, attempting to express biological specificity in physical terms, plays an important role in the development of such an approach. Therefore, in our opinion, the problem of mitogenetic radiation has become less isolated and naturally approaches the recently formulated questions.

In conclusion, let us discuss in more detail some related ideas suggested by physicists. The main principle of the modern approaches elaborated by H. Fröhlich [4], F. A. Popp [39], H. Haken [25], I. Prigogine and G. Nicolis [35] and Z. W. Wolkowsky [48] is as follows:

Biological systems, owing to their metabolism, are far from thermodynamic equilibrium, that is, they are excited and non-equilibrial. Biological effects may be induced by the action of radiation of very low intensities and particularly by waves in the mm range [4]. The effects are related to the resonance properties of a system as a whole, depending upon its activity. The inducing actions are considered as triggers of processes prepared inside the system itself. The concept of active biological systems requires a high degree of organization of living matter, that is, cooperative collective behavior of its elements. The collective modes of excitation are related to the high dielectrical polarization of biological substances which may be coupled with coherent electrical vibrations [4].

The concepts put forward by Popp's group are in general near to these of Fröhlich. This group studies the electromagnetic radiation of biological systems in the UV and, mainly, in the visible range [29, 34, 36, 39]. The authors ascribe great importance to the energetic and informational role of radiation. They are studying the coherence of biological radiation and stress the specificity of the 'biophoton' concept. In this respect it is very interesting that according to their data DNA is one of the main sources of radiation. DNA excitation is related mainly to the approach and interaction of two or more molecules which form so-called exciplexes and gradually discharge and release their energy in the form of coherent radiation [29]. Therefore, these studies give the study of radiation a wide biological significance. Somewhat similar concepts are elaborated by M. Janbastiev [49].

One of the important approaches in modern physical and biophysical studies of the energetic and structural states of biological systems is exemplified by 'synergetics', which studies the coupled interaction of subsystems of different orders, creating a unified functional system. Obviously, the central problems here are the organization of a molecular substrate, the dynamics of spatial parameters, and energetic states.

Monographs discussing the physical premises, model observations and the conclusions derived have been published by Haken [25], Nicolis and Prigogine [35] and Prigogine [40]. Elaborating the concepts of non-equilibrial thermodynamics the authors [35] stress: 'It is important that outside the area which can be described by steady-state thermodynamics, a new type of organization may arise relating the coherent spatio-temporal behavior of a system to its inherent dynamic processes.... The new structures thus arising – 'dissipative structures' – are completely different from 'equilibrial structures'. Dissipative structures may exist far from equilibrium only at the expense of a fairly large supply of energy and substance. They are a striking example demonstrating the capacity of non-equilibricity to be a source of order, namely, order through fluctuations'.

There are certain analogies between the concept of dissipative structures and non-equilibrial molecular constellations, although the former are considered as macroscopic formations, while the latter are microscopic molecular associations, giving the theoretical possibility of considering the 'behavior' of separate molecules. One may also mention the similarity between the concept of coherence, as a regular spatio-temporal aspect of the behavior of a system, and that of non-equilibrial molecular constellations, considering the latter as a dynamic regulatory principle of cell processes.

On the other hand, the two concepts show important differences. When developing the non-equilibrial constellation concept, A. G. Gurwitsch suggested that the constellations should be the necessary link in the constantly maintained and spatially specified relations between the different biological levels – organismic, cellular and molecular [19, 21, 24].

In the work cited above [25, 35, 40] the arising of the non-equilibrial organization of matter is considered as being under certain conditions a self-organized process. It seems possible that further analysis may lead to a more profound understanding of the conditions required for the arising of spatio-temporal orderliness of biological processes and one cannot ignore the usefulness in this respect of the data related to mitogenetic radiation.

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Super-high sensitivity systems for detection and spectral analysis of ultraweak photon emission from biological cells and tissues

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Summary. In this paper we summarize and discuss the modern technology and systems, studied and established by our research group, for performing the detection and special analysis incorporated with the super-high sensitivity photon counting method for the study of ultraweak photon emission; for example, extra-weak bioluminescence and chemiluminescence from living cells and tissues, closely related to biochemical and biophysical processes and activities. An excellent sensitivity of the basic photon counting system, making it possible to achieve count rates in the very low range of one photoelectron per second to one per minute, allowed us to carry out in vivo as well as in vitro measurements, and analyses of ultraweak bioluminescence and chemiluminescence. Recent results concerning ultraweak photon emission from blood samples and organ homogenates of rats are presented and reviewed as one of the interesting and valuable applications of our modern technology for studying ultraweak cell and tissue radiation.
Key words. Ultraweak photon emission; bioluminescence; chemiluminescence; super-high sensitivity photon counting technique; photon counting spectral analyzer system; biological cells and tissues; rat blood; rat organ homogenates.