Ultraweak photon emission and anther meiotic cycle in *Larix europaea*
(Experimental investigation of Nagl and Popp's electromagnetic model of differentiation)

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Summary. Synchronized microsporocytes and microspores of larch have been introduced as an excellent model system for the examination of the cell cycle dependence of biophoton emission (PE) and delayed luminescence (IPE). In agreement with the predictions of the model of Nagl and Popp for differentiation it could be experimentally confirmed that there exist: 1) sensitive dependence of PE and IPE on the cell cycle, 2) correlations to conformational states of DNA which are linked to DNase activity and 3) a hyperbolic decay of IPE.
The electromagnetic model of differentiation predicts oscillations of IPE that should depend on the wavelength of the exciting light and the cell cycle phase. In the established larch model system evidence was obtained for the first time of these oscillations which showed a dependence on both wavelength of the inducing light and the stage of the cell cycle.
Key words. Biophotons; delayed luminescence; electromagnetic model of differentiation; cell cycle dependence; microsporocytes; microspores; DNase activity.

The development and differentiation of cells and the regulation of the activity of genes have always been considered the most important problems of biology. However, despite intense research efforts, interesting results and some spectacular achievements, our knowledge and understanding of the mechanisms of differentiation are still very fragmentary and far from complete.
A new approach to these problems was presented by Nagl and Popp in their electromagnetic model of differentiation [13, 16]. The authors postulated that the biophoton field of the cell – a factor that had not previously been taken into account – plays the key role in the processes of cell development and differentiation. The model itself represents a unique combination of the physical, thermodynamic description of living systems and of the biological knowledge of the structure and function of the cell. Simultaneously, the model is the first complete theory of the origin and biological function of the ultraweak photon emission (PE) of living systems. The model seeks to explain the differentiation processes through an assumption of the existence of a feed-back loop between the conformation of DNA and the biophoton field of the cell. Such an assumption allowed the authors to propose one consistent mechanism for the basic regulation of differentiation processes at all levels of organization of living matter, from a single cell to a population of organisms.
The natural biological model of changes of DNA conformation and cell metabolism is the life cycle of the cell. However, to the best of our knowledge, no investigation of the PE phenomenon has been carried out using cells which are in the same, well-determined phase of the cell cycle, and we have started such experiments using isolated cells in successive stages of anther meiosis (fractions of microsporocytes) and in the early stages of haploid cell development (fractions of microspores) in larch.

Pure fractions of microsporocytes and microspores were acquired with the use of a specially elaborated method based on centrifugation of the plant material in a series of non-linear density gradients of sucrose (for details see Chwirot [1]). The intact male inflorescences of larch, devoid of covering hulls, were used as a reference sample. The description of the measuring apparatus can be found in Chwirot et al. [5] and a more general discussion of some methodological problems in a comprehensive review article by Popp et al. [15].
Anther meiosis or microsporogenesis is an example of a very special differentiation process of plant cells, as it results in the formation of the future pollen grains, microspores, that are well known to be able to develop in many different directions. The induction of the meiotic cycle and its course are undoubtedly controlled to a large extent by signals from the maternal plant and from the external environment, but the mechanisms of many of these interactions are still to be explained. It seems that Nagl and Popp's theory, which takes into consideration a new carrier of intercellular information – biophotons – may lead to a real progress in our understanding of the mechanisms of the control of microsporogenesis. On the other hand, the investigation of the changes in the characteristics of light emitted by the cells in the course of the meiotic cycle should be helpful in the development and clarification of the model.
Our first study concerned the developmental changes of the intensity of PE, and of the photon emission induced by irradiation of cells (IPE) with the white light including the UV component. The results of the first measurements appeared to be surprisingly clear. The patterns of the developmental variations of both PE and IPE from the cells undergoing meiosis were considerably different from those observed for the whole inflorescences (figs 1 and 2). The microsporocytes also showed a much stron-

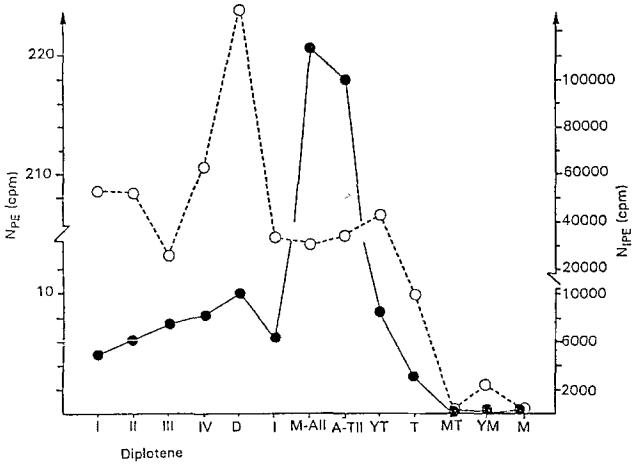

Figure 1. Ultraweak (N_{PE},●) and induced (M_{IPE},○) photon emission from microsporocytes during microsporogenesis in *L. europaea*. D, diakinesis; I, interkinesis; M-AII-metaphase-anaphase of the second meiotic division; YT, young tetrad; T, tetrad; MT, mature tetrad; YM, young microspore; M, microspore.

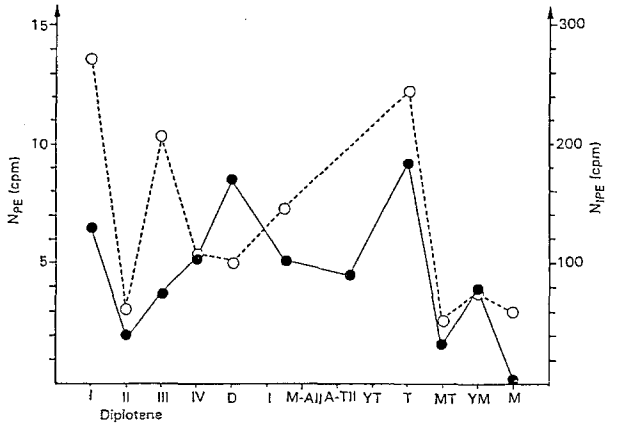

Figure 2. Ultraweak (M_{PE},●) and induced (M_{IPE},○) photon emission from inflorescences during microsporogenesis in *L. europaea*. Legend as in fig. 1.

ger response to the illumination than the inflorescences. The intensity of IPE from the microsporocytes was about 1000 times, and that from the inflorescences about 100 times, higher than the intensity of PE.

All the above-mentioned facts confirmed the developmental dissimilarity of the meiotic cells from the other cells of the inflorescence and, according to Nagl and Popp's model, the dissimilarity in the regulation of their development. The processes of reorganization and redistribution of the genetic material of the cell that take place during the first and the second meiotic division differ significantly. The unique events, characteristic for meiosis only, occur during the first division and among them are the conjugation of the homologous chromosomes and crossing-over, followed by the highly organized redistribution of the homologous chromosomes between the two sister cells. The second meiotic division, accompanied by the redistribution of the genetic material of each chromosome, the chromatids, can be considered according to many authors as being of a mitotic character. Therefore if, as is assumed by Nagl and Popp, the features of PE and IPE depend on the changes of the DNA conformation, the results obtained for the cells undergoing the first and the second meiotic division should be considerably different. Figure 1 shows that such differences were actually observed.

At present there is only a little information available about the physical nature and mechanisms of the interactions taking place at the different levels of organization of the genetic material of the cell during its divisions. The organization of the chromatin and the mutual interactions of the sister chromatids and of the whole homologous chromosomes all undergo intense and complex changes in the course of meiosis. Thus, it is extremely difficult to give a full interpretation of the data shown in figure 1. The unusually high level of the IPE intensity found for the microsporocytes in the diakinesis period could be interpreted on the basis of Nagl and Popp's theory as resulting from changes of the interaction of the homologous chromosomes. Such a conclusion, however, has to be considered a highly speculative one at the present stage of investigation and it requires further verification, for instance from measurements made using different material. The occurrence of a conspicuous increase of PE from microsporocytes at the time of the second meiotic division, however, resembles previous observations of a high level of PE from cells undergoing mitosis (Popp et al.[15] and references therein). Thus, assuming the mitotic character of the second meiotic division, one can explain this increase of the PE intensity in the frame of the electromagnetic model of differentiation.

It is of particular interest that considerable changes occur in the level of PE and IPE both from the microsporocytes and the inflorescences in the haplophase period. No PE could be observed from the haploid generative cells at this time, and the intensity of PE from inflorescence dropped to zero level at the time of the microspore stage. Such a decrease of the intensity of PE is predicted by the electromagnetic model of differentiation for cells reaching the end of their life cycle. The haploid microspores are the result of the complex meiosis process and seem to be an excellent example of such cells. On the other hand, cytological and biochemical studies[3,9] showed that intensive structural and metabolic transformations take place in cells during the period of the post-meiotic interphase in larch. There is, among other changes, an increase in activities of some respiratory and lytic enzymes and in the processes of maturation of organelles, especially mitochondria and dictyosomes. Thus, an increase rather than a drop to zero level of PE from microspores could be expected. At the same time, however, the intensity of the IPE from microspores also reaches its lowest level. The possible explanation of all these, at first sight contradictory, data may be that because of the thick sporopollenin wall of the pollen grain the future popula-

tion of the pollen cells is optically isolated from the external environment.

Popp et al. [15] and Nagl and Popp [13] suggest that the level of the IPE intensity can be considered an indicator of the sensitivity of a cell to the regulating action of biophotons. The ten times stronger response of microsporocytes, compared with inflorescences, to irradiation may be due to their localization in the anther cavity. The anther wall consists of several tissue layers and, additionally, the whole cone is covered by several layers of hulls sealed by a resin. The measurements of the external transmission of the cone hulls in conifers gave values of order of 10^{-1}–10^{-5}% [8, 17]. Thus, only a very small part of the external light can reach the center of an inflorescence. Additionally, the light is then strongly absorbed and scattered by the cells of the anther wall. It should be noted here that during the whole 6-month-long period of meiosis in larch only proplastides showing very reduced thylacoides can be seen in microsporocytes. Thus, in natural conditions the development of microsporocytes goes on in the dark, probably without the influence of the daylight. Following Popp [14] and Nagl and Popp [13] one can assume that the source of regulating biophotons may be the microsporocyte itself and/or the somatic cells of the anther. A striking, positive correlation between the variations in the intensity of PE from inflorescences and of IPE from microsporocytes seems to indicate the anther somatic cells as being the source of the regulating photons for the latter during the whole of meiosis. On the other hand, Kosinski and Giertych [10] demonstrated that the intensity and spectral composition of light reaching the bud center affected floral induction and/or flower development in spruce and pine. Our recent measurements of the external transmission spectra of scales during microsporogenesis in larch [8] showed considerably different patterns of the developmental variations observed for the scales covering the male inflorescences and the leaf buds. All the above-mentioned results might suggest that a three-step regulation could occur in inflorescences, when the anther somatic cells would respond to the daylight, previously strongly modified by the covering hulls, and then, by appropriate changes of their PE would control to some extent the development of the microsporocytes.

The commonly accepted view is that biophotons and excited molecular species in the cell originate from metabolic processes (see other parts of this review and references there). The investigation of the content and intensity of synthesis of proteins and of the activity level of some enzymes (Mg^{2+}-ATPase – E.C. 3.6.1.3, cytochrome c-oxidase – E.C. 1.9.3.2. catalase – E.C. 1.11.1.6, IDPase – E.C. 3.6.1.6, acid phosphatase – E.C. 3.1.3.2. α- and β-amylase – E.C. 3.2.1.1 and 3.2.1.2) in the microsporocytes and the anther somatic cells during microsporogenesis in larch [2, 3] showed a slight decrease in metabolic activity in cells undergoing meiosis. When comparing these results with changes of PE from microsporocytes during development a positive correlation is generally found between the latter and appropriate variations in the activity level of α- and β-amylase and catalase.

The other source of biophotons, of basic importance for the biological function of PE is, according to Popp [14] and Nagl and Popp [13], the genetic apparatus of the cell. Rattemeyer et al. [18] treated cucumber seedlings with ethidium bromide and were able to demonstrate a close correlation between changes of the degree of condensation of the cellular DNA and the PE intensity. Our investigation of the developmental variations of the activity level of DNase (E.C. 3.1.4.5) and RNase (E.C. 2.7.7.17) and of the intensity of PE and IPE from microsporocytes of larch during their development [4] revealed the close positive correlation between changes in the DNase activity and of the intensity of the IPE. These results, together with the observation that no light is emitted by the mature red cells of blood, in which the nuclear apparatus is missing [18], seem to support strongly the concept that DNA (maybe only in the form of chromatine) is one of the sources of biophotons.

As has been mentioned already, Nagl and Popp [13], seek to explain the PE phenomenon by the assumption of a mutual interaction between the genetic apparatus and the biophoton field of a cell. In particular, the coherence of the light emitted by living systems is predicted by their theory. At least partial coherence of this light is a necessary condition for considering the biophotons the intercellular carriers of information. Because of the very low intensity of the PE, direct measurements of the statistical properties of this radiation are very difficult. However, according to theoretical considerations of Popp et al. [15], Li et al. [12] and Li and Popp [11] the existence of a feed-back coupling between the biophoton field and the cell genome should result in hyperbolic decay of the IPE. Developing the model of coherent coupling between the emitted light and the biological system Li et al. [12] obtained the following law for the decay of the IPE:

$$I(t) = A^*(t + t_0)^{-\beta} \quad (1)$$

where t_0 characterizes the time of irradiation and $A(\beta)$ and β depend on the system under consideration. They also found that in most cases $1 < \beta < 3$. Such a hyperbolic function is considerably different from the exponential functions usually applied for the description of the fluorescence decays. In our study we have initially tried to fit the measured decays of the IPE by the theoretical curve given by (1). The numerical tests showed, however, that much better fits have been obtained when the following function (2) with three fitted parameters was used:

$$I(t) = A^*(t + B)^{-\beta} \quad (2)$$

A hyperbolic decay of the IPE from microsporocytes was found in eight of thirteen stages of meiosis and only in one stage was the IPE decay classified as two-exponential (examples in figs 3 and 4). It is of particular interest that

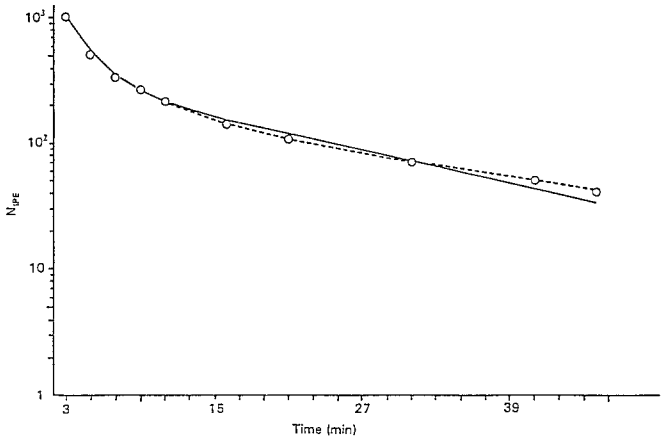

Figure 3. Decay of IPE from microsporocytes in the diplotene III stage as an example of a very good aproximation by the hyperbolic function. ---, hyperbolic; ——, two-exponential function; O, measured values.

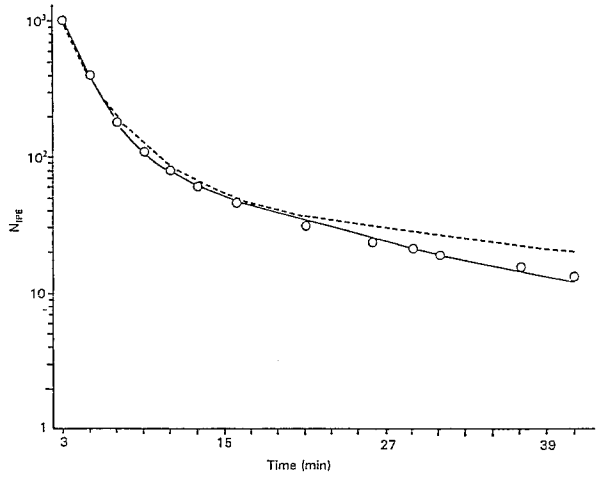

Figure 4. Decay of IPE from microsporocytes in diakinesis, which was the only one sufficiently well approximated by the two-exponential function (all captions as in fig. 3).

this two-exponential decay occurs in the diakinesis stage, in which the highest intensity of the IPE was observed. The only period of microsporogenesis for which the IPE decay could not be approximated either with the hyperbolic or with the two-exponential function was the time of the metaphase-anaphase of the second meiotic division. It should be noted that at the same time the highest level of PE intensity was measured.

For all hyperbolic decays we found $1 < \beta < 2$ and these values agree with those reported by Li et al. [12]. The long decay times of the IPE from living systems were predicted by Popp [14] as a consequence of the phenomenon of active photon storage. The first measurements of the IPE performed by Popp et al. [15] for cucumber and *Bryophyllum* have shown that living cells do indeed exhibit long-lasting (of the order of 1 h) decays of the light emission after illumination. Similar decay times of the IPE were observed in our investigation, both for the microsporocytes and for the intact male inflorescences of larch. It is also noteworthy that the occurrence of hyperbolic decay in five stages coincided with periods of almost total synchronism of microsporocyte development in the anther. This fact supports the concept of the coherent component of the PE being a carrier of intercellular information [13, 16].

The character of the decay of IPE from the whole inflorescences was quite different from that observed for the meiotic cells. No decay could be classified as hyperbolic or two-exponential. In four stages of the inflorescence development the IPE decay was approximated by both functions and for all the other decays of IPE the fit was very poor. Compared with microsporocytes, the much lower accuracy of fit obtained for the whole inflorescences may be due to their structural and cellular inhomogeneity. The IPE of an inflorescence consists mainly of photons emitted by the anther wall cells which belong to different tissues and are in different phases of development. Thus, according to the electromagnetic model of differentiation, the decay of the IPE from the whole inflorescence cannot be described by a single function but rather by a linear combination of many of them.

The occurrence of IPE decays that can be described simultaneously as hyperbolic and two-exponential may suggest the existence of two different, coherent and incoherent, components of the IPE. The linear combinations of the hyperbolic and exponential functions might be adequate to approximate such decays. However, in our opinion, more than three parameters cannot be determined by analysis of the decay curves only, without any additional information.

According to Li and Popp [11] the occurence of the coherent component of the IPE and its hyperbolic decay may be due to the process of coherent scattering of light in living systems. The effectiveness of such a scattering process depends on the light intensity and wavelength, among other things. Therefore, the aim of our next study [7] was to examine the decay of the IPE from the microsporocytes and microspores of larch after irradiation with quasi-monochromatic light (red and green) and white light without the short-wavelength UV component.

The very interesting result of these experiments was the observation of the oscillating decays of the IPE after illumination of microsporocytes with quasi-monochromatic light (examples in figs 5 and 6). Such an oscillating decay behavior was predicted by Nagl and Popp's theory (see Appendix 2 in Nagl and Popp [13]) but had in fact never been observed. Popp et al. [15] observed single local maxima appearing during the first half of the IPE decay after illumination of cucumber seedlings and *Bryophyllum* plants with quasi-monochromatic light and interpreted them as traces of the oscillations. The intensity and frequency of the oscillations we found in our study seem to depend both on the spectral composition of the exciting light and on the stage of the microsporocytes development. Such a dependence, when interpreted in the

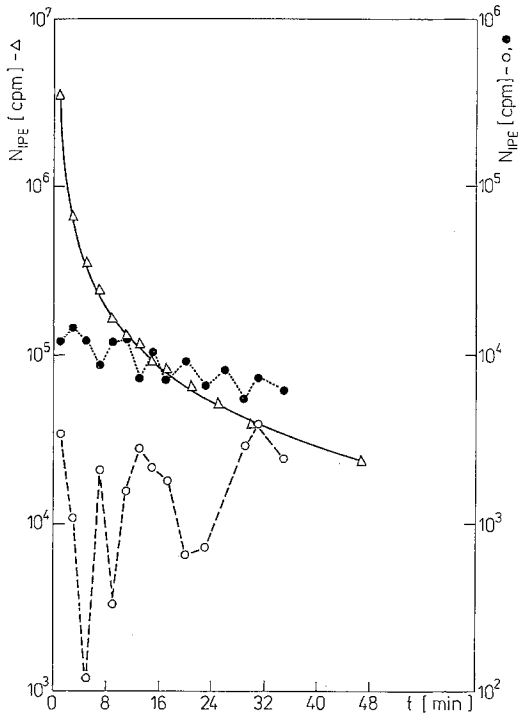

Figure 5. Monotonic and oscillating decays of the IPE observed at the time when the microsporocytes were in the period of diakinesis and metaphase of the first meiotic division (--○-- red-, ---●--- green-, —△— white ($\lambda > 330$ nm)-light excitation, the line drawn for the monotonic decay represents the fit of function (2) to experimental data).

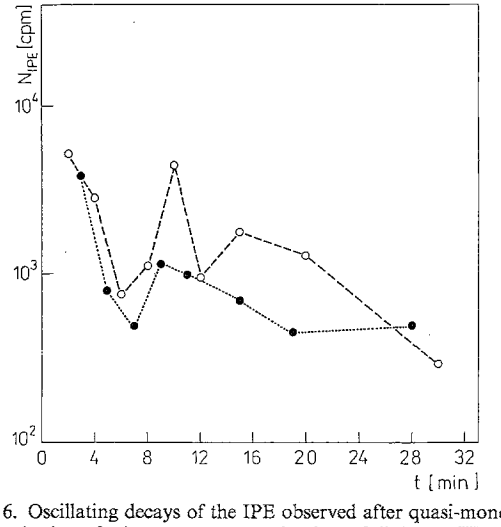

Figure 6. Oscillating decays of the IPE observed after quasi-monochromatic excitation of microsporocytes at the time of diplotene III. Legend as in fig. 5.

frame of Nagl and Popp's theory [13] may explain why the oscillations of IPE decay had not been observed in the previous investigations. Popp et al. [15] used whole plants, composed of cells belonging to many different tissues which were in different stages of differentiation, whereas in the work of Chwirot et al. [6] homogeneous fractions of cells were illuminated with non-monochromatic light. We also did not observe any oscillations of the IPE intensity after illumination of cells with non-monochromatic light of $\lambda > 330$ nm. Moreover, although the changes of the IPE level related to the development are very similar for the white-light-excitation without [7] and with [6] the UV part this is no longer true for the green- and red-light-excitation. In view of all the above-mentioned facts it seems that the necessary condition for obtaining the IPE oscillations may be the use of samples consisting of cells at the same phase of development, and of a narrow-band excitation.

The real value of any theory in science depends always on the validity of the assumptions and predictions and on its greater universality when compared with previous theories. The last feature undoubtedly characterizes the electromagnetic model of differentiation. The model seems to include in a general way all the processes of the control of the development and differentiation of organisms known up to now and it proposes a consistent explanation of their mechanisms [13, 16]. The common feature of all living organisms is their extremely high complexity. Additionally, the method of characterization of an object by studying its components, commonly used in science, cannot often be applied to the investigation of living systems; for example, a DNA molecule which is a part of a chromatine complex is very different from the pure DNA extracted from the cell. For these reasons it will take a long time until some of the very important assumptions of Nagl and Popp's theory can be verified experimentally. In this work an attempt has been made to test some of the theoretical predictions of the electromagnetic model of differentiation. Many of the results we have obtained so far strongly support the theory, but others cannot at present be interpreted in its frame. In our opinion, supported by an increasing amount of experimental data, the further development of the model, perhaps in the direction of more detailed interpretation of some well-known processes of cellular differentiation, should in the near future result in solving the problem of the functional role of PE in processes of differentiation and development of biological systems.

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Concluding remarks

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The multi-author review presented here clearly shows that biophoton research has come of age. This is demonstrated by the amount of research activity in various countries, by the organization of symposia and conferences on this topic, and by the increasing number of original articles and books recently published on bioluminescence and related fields [1–3, 6, 7, 9 10, 15, 16, 19, 20]. The stream of progress may have its source – as is often in science – in the association of two disciplines, in this case of biological questions with physical concepts, from the experimental level to the thermodynamic and quantum mechanical level. By that combination, many so far 'unlaid eggs' have become fertilized and have been enabled to develop into new theories and research strategies.

In the present concluding remarks I should like to concentrate on some new opportunities in the understanding of biological phenomena and some new perspectives. I would also like to appeal to more 'conservative' colleagues to realize that scientific progress depends on the acceptance of new results, new methods, and new theories, because 'there is simply no absolute truth, but only relative truths, theories which change with the accumulation of *new knowledge*' [5]. This is, actually, *old knowledge* for the theories of science and cognition.

There are still a large number of biological phenomena and events that cannot yet be adequately explained, or even simply described, such as cell differentiation and its regulation, morphogenesis, and evolution. Some of the unsolved, but central questions, are specified in the following. Let us first consider a phenomenon at the molecular level. The genome size varies among eukaryotic species within the range of 1 : 14.000. The minimum DNA content increases with the complexity of the organism in a given taxon, but organisms of very similar complexity often exhibit extremely different DNA amounts. It is now evident that the number of genes (as well as their function) is rather similar among organisms, and that up to 99.9 % of the genome represents non-coding, regulatory DNA [8, 11, 13]. But little is known about *how* this regulation may occur, so that some scientists neglect that mass of DNA as 'selfish' or 'junk' DNA. If we consider the level of the whole organism, the diversification of cells and organisms during ontogenesis (individual development) and phylogenesis (evolution) clearly represents a basic aspect of living matter. But how is the program of growth, differentiation, reproduction etc. controlled? And how does the ordered development come to be disturbed in some cells?

I think that not one modern scientist can believe that all of these ordered processes are just the result of random events. But all the regulators envisaged so far, such as proteins and hormones etc., require some regulator themselves to be produced or be effective. So, if we confine our studies to the physiological and biochemical levels, we shall not find an end, but only more open questions than before. The number of cell divisions that can be performed by a given cell type, and the life span of the members of a given species, are determined and limited in some way, while cancer cells are able to escape that control. What makes the difference between ordered development and chaotic growth? If we only knew this,