Pergamon

European Journal of Cancer Vol. 32A, No. 8, pp. 1283–1292, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

PII: S0959-8049(96)00075-5

Review

Cancer: A Challenge for Control Theory and Computer Modelling

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INITIATION AND MOTIVATION

IN ONCOLOGY, one characteristic feature of cancer is uncontrolled cell proliferation. This observation has stimulated numerous biomathematicians to construct cell proliferation models (continuous, discrete, deterministic, stochastic) based on, for example, differential equations describing growth and kinetics of abnormal cell multiplication [1–11]. During the past decade, oncogenes, suppressor and repair genes have shifted the origin of cancer to the molecular biology level, i.e. to genetic alterations [12–15]. By this, a previous proposed, attractive working hypothesis of our group [16] was revitalised to interpret cancer as structurally unstable, negative feedback control loops. The main question in this scenario is how is proliferation of normal and abnormal cells regulated and how can it be elucidated at (i) the molecular level, (ii) the cellular level, and (iii) the organ level? Currently, the experimental data on the control mechanisms at the first level are still insufficient. For this reason, we have restricted our considerations on constructing feedback control models which describe the cell division of normal and tumour cells to the cellular level. Before going into details, some general remarks of modelling and simulation shall be given. By modelling, we mean the study of a system using basic physical laws and relationships [17]. Models are only mappings of a more complex reality! So, what we call a system model is actually a reflection of the modeller's understanding of the reality, its components and their interrelations (Figure 1). Therefore, one should keep in mind which aspects have been omitted and what assumptions have been made in modelling. Thus, the computer model is an operational computer programme system implementing a system model. The output of a computer run may be the predicted time behaviour of the dynamic system. In this sense, simulation can be regarded as the art and science of experimenting with models [18–21]. There are many rationales why simulations are valuable, including: verification and optimisation of the design of a system before its construction; helping to avoid costly design errors; helping to ensure safe designs or to test hypotheses; and to vary comfortably the system's structure and parameter sets. Examples of modern areas of application include space flight simulations, migration in urban systems as well as pattern formation or spread of epidemics. In contrast to the goals of many biostatisticians, our primary aim is not data fitting in a simple manner, but stimulation of scientific questions by applying well-established methods of control theory [22] to the regulation mechanisms of the cell division process (Figure 2). One crucial question is: Under what conditions does the negative-feedback closed-loop circuit become unstable?

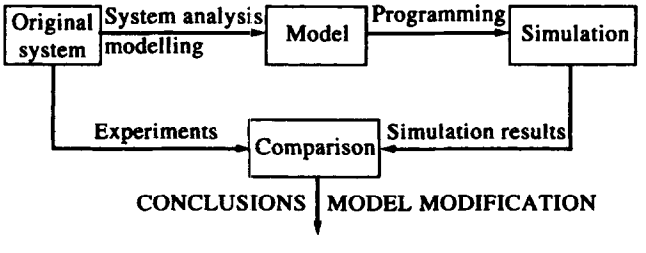

Figure 1. Steps of modelling.

MODELLING STRATEGY

For studying the process of carcinogenesis tumours are either induced in animals or developed as cell cultures, i.e. *in vivo* or *in vitro* models, respectively, are used. Our approach is concerned with computer models, which means, strictly speaking, with "models of the models".

The computer modelling of highly complex biological systems requires a breakdown of the problem and appropriate simplifications. These are dependent on the ultimate aim of modelling. If one wants to model the spatial surgical removal of a tumour *in vivo*, for instance in the skin, one has to assume a limited tissue volume, a constant volume of a cubic cell, a homogeneous tissue structure, and only horizontal and vertical correspondence to neighbouring cells mainly caused by restrictions of computer capacity. At the present stage, heterogeneity, immunological reactions, and the formation of metastases have to be neglected. The computer model is com-

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Received 29 Jan. 1996; accepted 7 Feb. 1996.

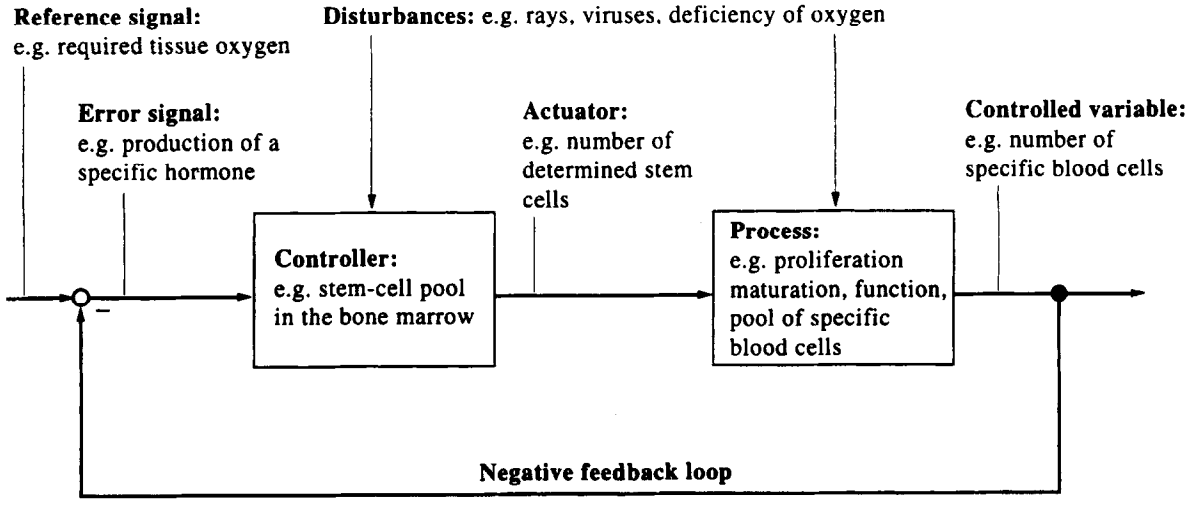


Figure 2. Schematic illustration of a disturbed closed-loop control circuit describing cell renewal processes.

posed by separate models for normal and malignant cells with different cell production rules (e.g. a tumour cell is theoretically capable of unlimited division). These models are connected by a set of cell-to-cell interaction rules which may be position and energy-dependent. That means that if the oxygen or glucose supply of a proliferating cell becomes low, the cell will enter the dormant G0-phase. The required experimental data are the initial temporal and spatial configuration of the cell system and the cell cycle times, which are created by a pseudo random number generator in the computer. For representing the results, a two-dimensional or three-dimensional spatial visualisation of normal and malignant tissue is required. If one wants to study the temporal and spatial growth of an *in vitro* tumour spheroid (a model of non-vascularised early tumour growth) with the aim of investigating various treatment schedules, we have to construct additionally a cytokinetic model of a tumour cell implementing distinct cell cycle phase durations (T_{G1}, T_S, T_{G2}, T_M). If we intend to optimise cancer treatment, one needs a treatment model, and in order to consider side-effects on normal tissue, we have to model different cell renewal processes of rapidly and slowly proliferating normal tissue. This procedure requires a set of more than 10 variables and approximately 30 parameters, which may be partially dose-dependent. Every scientist knows about the difficulty in getting or estimating these parameters by experiments. Therefore, a crucial bottleneck very often is the lack of sufficient experimental data which are the basis of all modelling activities.

For example, Figure 3 represents the growth model of a tumour spheroid [23–24]. In this case, one underlying assumption is that the internal (autocrine) cellular control loop of a cell has become unstable [16, 22] and the transformation process from a normal cell to a cancer cell is complete. It should be mentioned that in Figure 3 the time-dependence of the proliferation is controlled by the spatial status of oxygen and glucose (described by a system of differential equations related to diffusion processes) introduced from experimental data. Therefore, the simulation run on a VAX workstation with a VAX station 3200 which takes 4–6 days is interrupted every 3 h by a simulation loop which checks whether the actual simulation results (cell number in the various stages of the cell cycle) are in accordance with the experimentally

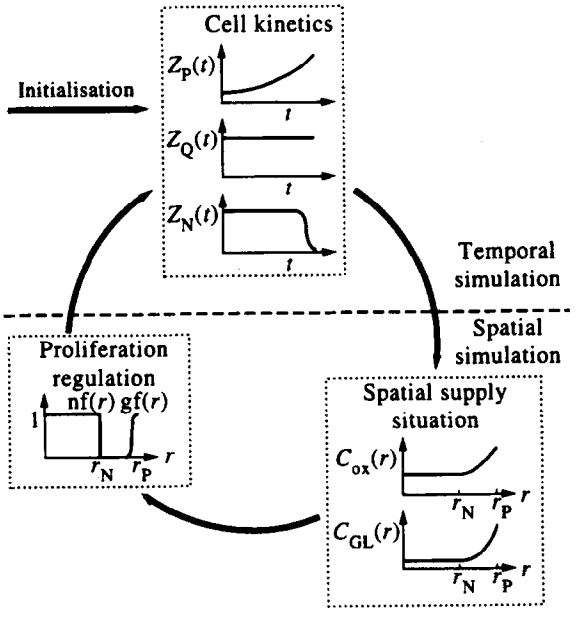


Figure 3. Growth model of a tumour spheroid. *Z*, number of cells; *C*, concentration; *r*, radius of the tumour spheroid; nf(*r*), necrotic fraction; gf(*r*), growth fraction; OX, oxygen; GL, glucose; P, proliferating; Q, quiescent; N, necrotic.

gained supply data. If not, the proliferation mechanism starts its correction work.

In Figure 4, a completely different model is presented [25]. It symbolises a cell kinetic compartment model describing the time behaviour of normal cells after apoptosis, which has disturbed the balance of cells in the separate compartments. From the viewpoint of the control theory, the modelling of the interacting signals in the communication paths between the different compartments has been performed in the way that the number of cells is tried to be kept constant in the stem-cell and transit-cell pool [26] via the interacting signals e_{ss}, e_{ts}, e_{tt}, e_{est}, m_{ss}, m_{st} (Figure 4).

The complex models described here have been transformed into computer programmes (FORTRAN IV or C++ and blockoriented simulation languages (Matrix$_x$, MATLAB) have been used). The modelling of different temporal and spatial responses of cell growth to inner and outer per-

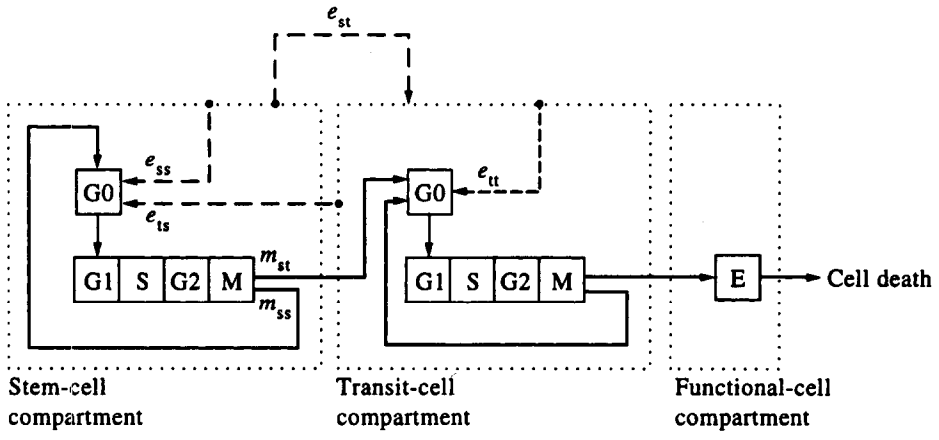


Figure 4. Cell kinetic model of rapidly proliferating normal tissue. m_{ss}, share of stem cells which derive from stem cells during mitosis; m_{st}, share of transit cells which derive from stem cells during mitosis; e_{ss}, share of lacking stem cells which stimulates stem cells to leave the dormant phase G0; e_{ts}, share of lacking transit cells which stimulates stem cells to leave the dormant phase G0; e_{tt}, share of lacking transit cells which stimulates transit cells to leave the dormant phase G0; e_{st}, share of lacking stem cells which stimulates transit cells to migrate into the stem-cell compartment.

turbances (e.g. chemical agents or radiation dose) allows a better understanding of the various cell regulation mechanisms.

TUMOUR GROWTH

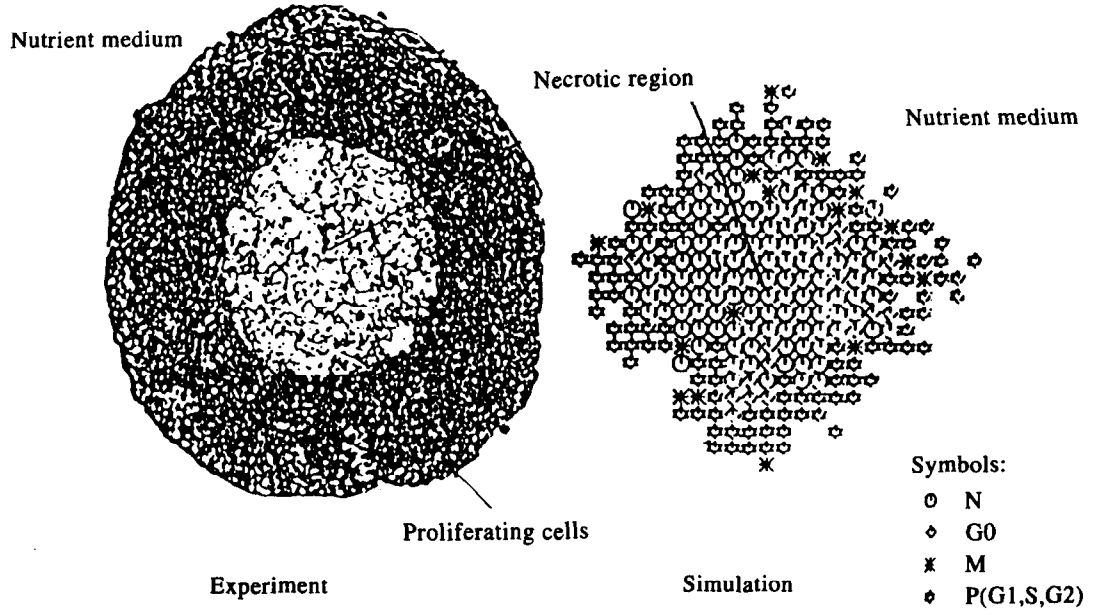
Let us now start with computer experiments simulating the *in vitro* growth of a tumour spheroid [23]. For this purpose, a single tumour cell (in mitotic phase) has been placed in the centre of a nutrient medium of a three-dimensional cell space, whose volume amounts to 1 mm^3. The model is based on cell cycle regulation mechanisms, and the input data are the cell cycle phase durations which are created by a pseudo random number generator. After growing according to the cell production and interaction rules of the computer model [27] and the spatial two-dimensional steady state is illustrated in Figure 5a. A good qualitative agreement with the experiment can be observed in the cross-section (Figure 5a) as well as via the three-dimensional plot of Figure 5b. An improved computer model [24] considering additionally the oxygen and glucose supply in detail leads to a very good quantitative fit of the simulated time course with experimentally gained data of the mamma sarcoma of the mouse (EMT6/Ro-tumour spheroid) given in Figure 5c. The transition to the simulation of *in vivo* tumour growth is much more complicated. In a very first step we have neglected the capillary system. Furthermore, in the modelling approach [28], we have assumed that one can only distinguish between normal and malignant cells. Thus, it was possible to study the growth of a tobacco leaf cancer [28] starting from a nucleus of about 20 transformed cells which have been placed in the outside rim of the tissue. The spatial two-dimensional configuration after growth of the tumour is represented in Figure 6a. Parallel to this work, we have been thinking about the introduction of the vascular system into our modelling world. In another study [29], the approach was to study the formation of the capillary network of a brain segment of a rat as a regulatory process during the oncogenesis of evolution (Figure 6b). Thus, we could simulate in an extended computer model [30] the formation of an *in vivo* micrometastasis, starting with a single transformed cell which was inoculated in the direct neighbourhood of a capillary (Figure 6c). The power of the model may be seen from the two-dimensional plot (Figure 6c) in which the possibility of visualising the neovascularisation (tumourangiogenesis effect) is included.

TUMOUR SURGERY

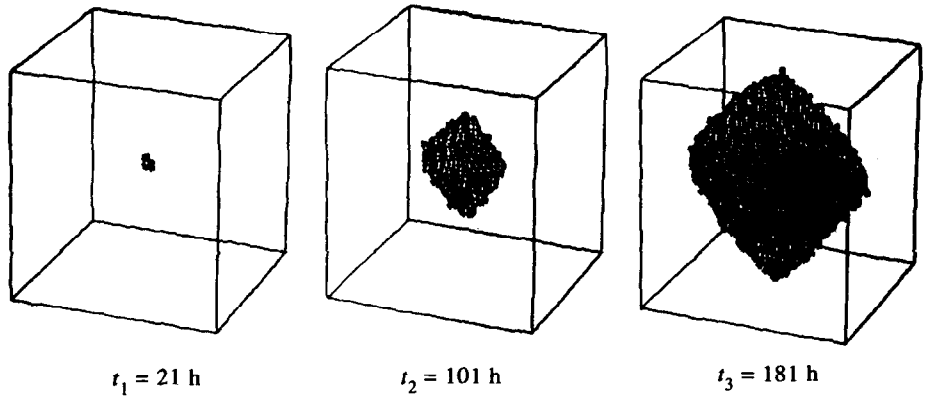
If we want to model cancer treatment, we have to extend the models describing tumour growth by developing additional programme packages considering the various treatment methods and schedules. A relatively simple case is that of a surgical removal of tumour cells [28] because in our computer model we have to eradicate the undesired cells only. This has been performed in the example of Figure 7. In this case, we have restricted the cell space symbolising a section of the skin to a two-dimensional 100×100 cell matrix consisting of normal cells coded by the symbol 1 which characterises the specific mean life-span of a cell. The growth pattern of a normal cell renewal system is generated by a set of cell production and interaction rules [28]. For some cells, the multistage transformation process of normal into cancer cells is complete. So, the existence of a tumour nucleus according to Figure 7a may be assumed.

The multiplication of a tumour cell does not obey production rules for normal cells, but has special instructions valid only for tumour cells, which dictates that division of a tumour cell can take place even if there is no vacancy for one or two daughter cells [28]. The input data of our model are the mean life-span of normal and tumour cells, the percentage of cell loss and the initial configuration of the tumour. The spatial step-by-step increase of the tumour is illustrated in Figure 7b. At $t = 50$ units of time (Figure 7c), most of the tumour cells are removed by a surgical operation. However, the remaining tumour cells rapidly proliferate to produce new tumour cells (Figure 7d). Thus, an extremely complex biological system can be simulated near to reality by means of a few and simple assumptions. The model enables the influence and variability of the mean life-span of a tumour cell, of the size and initial configuration of the tumour nucleus and of the percental tumour cell loss to be studied. One important practical application is the training of surgeons by simulating the operational procedure prior to clinical treatment; a further use is the importance of the tumour extension considered in the model for microsurgery.

(a) Two-dimensional cross-section of a tumour spheroid



(b) Three-dimensional growth of a tumour spheroid



(c) Tumour volume as a function of time

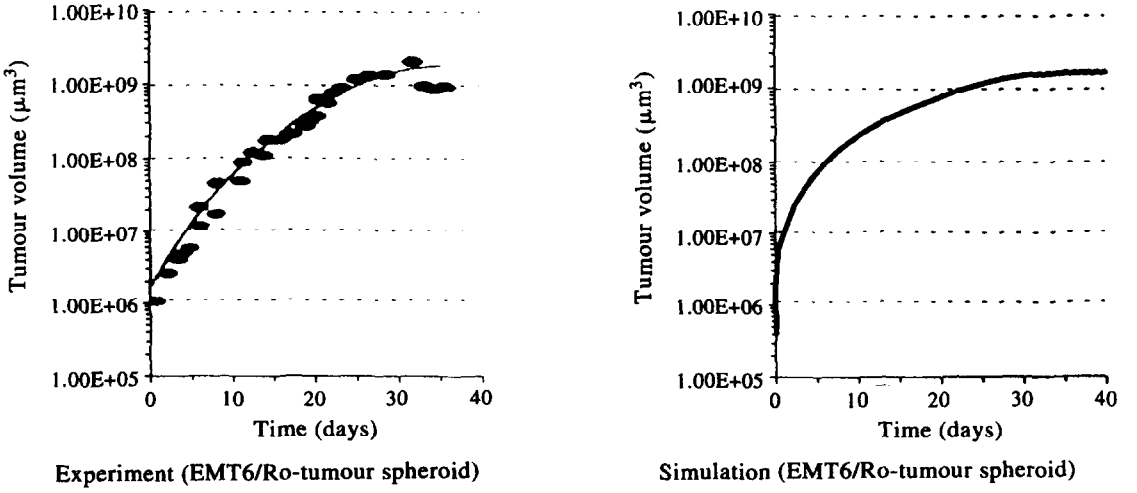
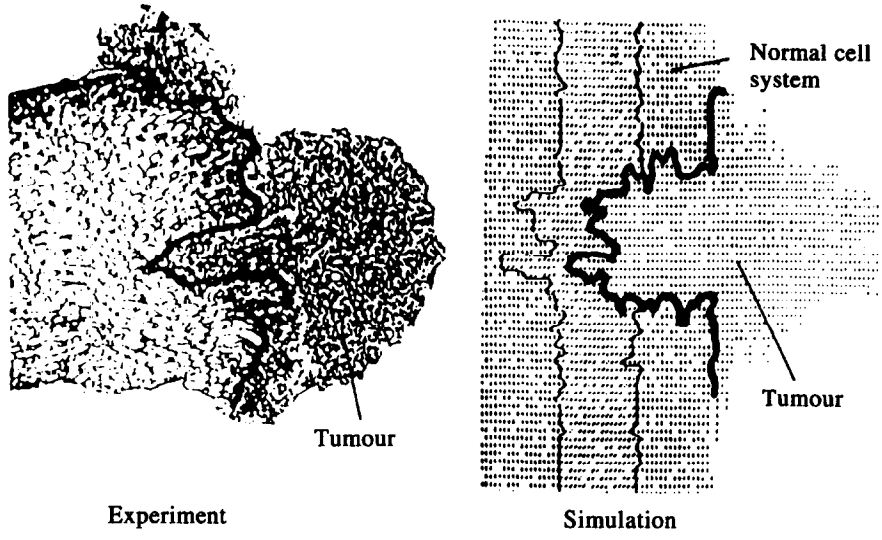


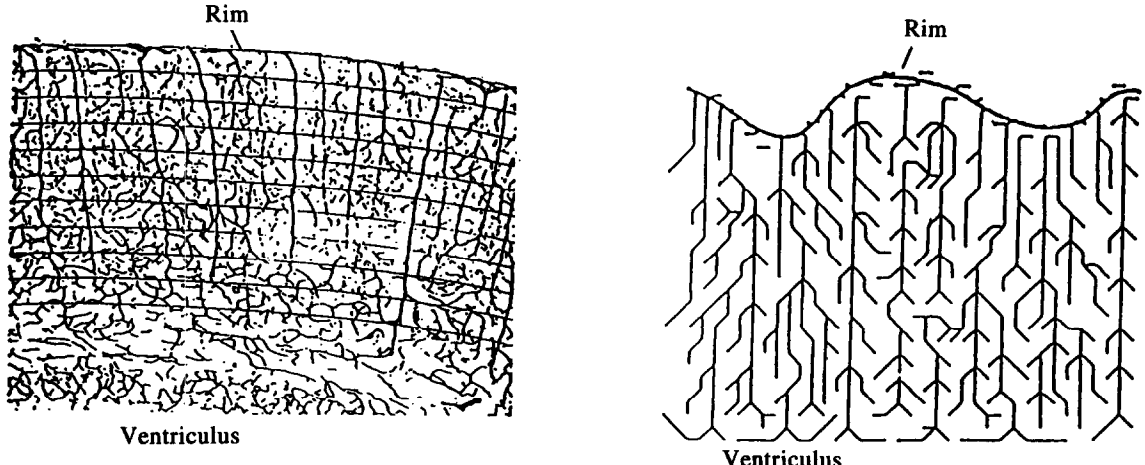
Figure 5. Tumour growth *in vitro*.

(a) Two-dimensional growth of a tumour in a tobacco leaf



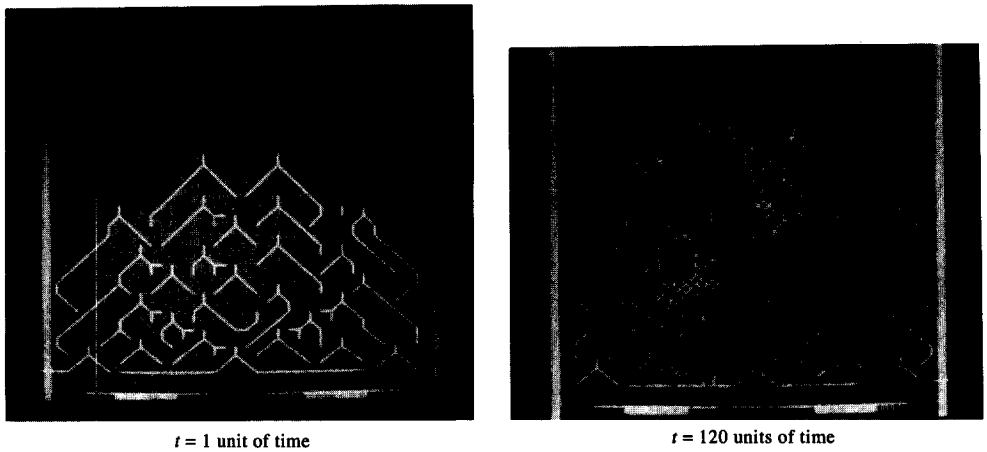
Experiment Simulation

(b) Two-dimensional capillary network of the cortex of a rat



Experiment Simulation

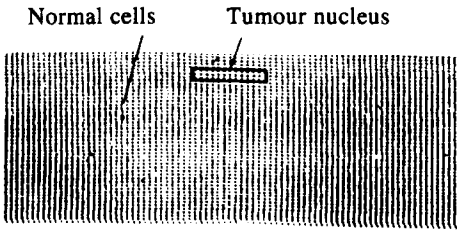
(c) Two-dimensional formation of a micrometastasis (simulation)



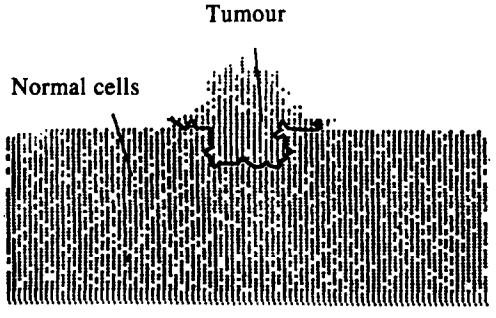
t = 1 unit of time t = 120 units of time

Figure 6. Tumour growth *in vivo*.

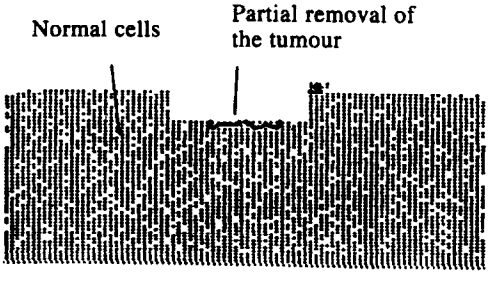
(a) Tumour nucleus (initial configuration) at $t = 1$ unit of time



(b) Tumour growth (configuration at $t = 25$ units of time)



(c) Tumour eradication



(d) Recidiv (configuration at $t = 50$ units of time)

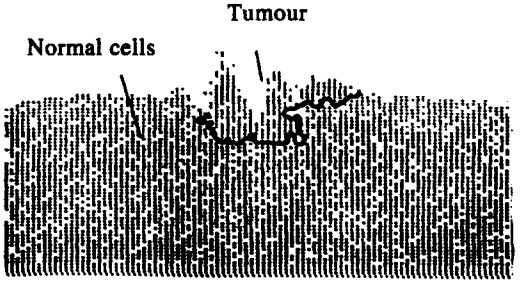


Figure 7. Surgical removal of tumour cells.

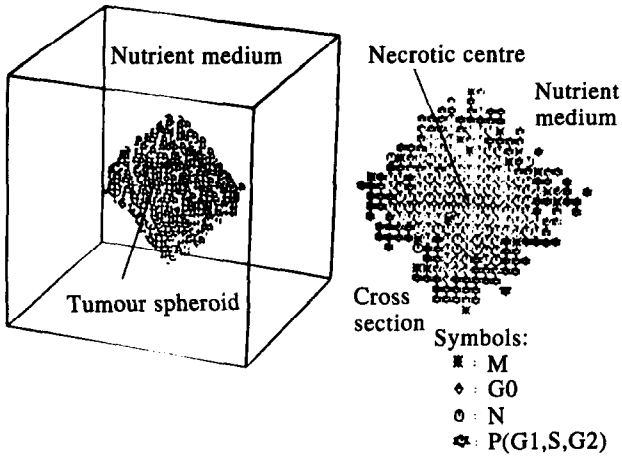
CHEMOTHERAPY

Cell cultures may be used to study not only the division of tumour cells, but also to determine the cytotoxicity of chemotherapeutic drugs. Many chemotherapeutic agents preferentially affect cells which are in a particular phase of the cell cycle, that is, they are phase-specific [31, 32]. Now, tumour cell growth in a cell culture is a very good "*in vitro* system" which can be simulated by a computer model [24, 27, 33–34]. In contrast to the oversimplified model of the previous section, in which one could only distinguish between normal and malignant cells, in this case our model is based on the cell cycle regulation mechanisms which make it possible to differ between tumour cells residing in different stages of the cell cycle. Furthermore, cell production and interaction rules allow the construction of the actual spatial (two- and three-dimensional) configuration. The input data of the model are cell kinetic data (cell cycle phase durations) and treatment schedules [27]. The effect of drug resistance has not yet been implemented in our model. However, this phenomenon can be taken into account by the dose-dependence of the diffusion of a drug through cell membranes (passive permeation) and using methods and results in [24]; work in this direction is in progress. The example in Figure 8 illustrates the application of an agent which has killed all proliferating tumour cells (Figure 8b) of the outer rim of the tumour spheroid at $t = 201$ units of time. By this procedure, the outer quiescent G0-tumour cells are recruited into the cell cycle again and tumour growth proceeds according to Figure 8c. The time at which cytotoxic treatment is given is extremely important. From the time course plotted in Figure 8d, one can see the optimal moment when the cytotoxic drug has to be administered for a second time. This is not the time when the total number of tumour cells has reached its minimum (at $t = 250$ units of time in Figure 8d), but, following our model, the drug should be administered when the number of G0-cells has reached a minimum (at $t = 205$ units of time in Figure 8d), because then the maximal number of tumour cells resides in the proliferating phase and can optimally be hit by the phase-specific cytotoxic drug. It should be stressed that this approach mainly provides qualitative results which allow new ideas concerning the variation of treatment schedules to be tested. Simulation results have to be confirmed by experiments (*in vitro*, *in vivo*) and clinical evidence. A noteworthy improvement of the computer simulation model is possible using investigations [35–37 and references therein] on the cytotoxicity of some well known anticancer drugs (e.g. doxorubicin, epirubicin, cyclophosphamide, isofosfamide, cisplatinum, carboplatinum), relating to dose–effect relationships, mechanisms of DNA–interstrand crosslinkings and DNA–protein crosslinking monitored in some cell lines (monolayers, spheroids). The status of the simulation model [24], presented below, on radiotherapy alone could be extended, using the results of these investigations, to include cancer chemotherapy, and the integrated modality of chemotherapy and radiotherapy. Work in this direction is in progress.

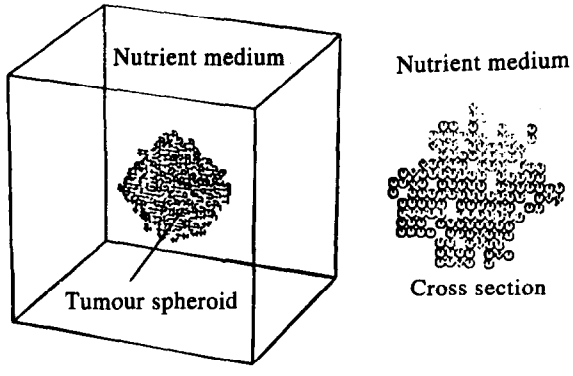
RADIATION THERAPY

The tumour spheroid model used in this case is based on the cell cycle model described in preceding sections. It has been extended and improved by considering metabolic factors such as oxygen and glucose supply [24]. The simulation of radiation therapy is much more complicated than that of surgical removal of a tumour. Firstly, an appropriate radiation model is required. In our case, we have chosen the linear-quadratic survival function [38–39] as the dose–response relationship of cell cultures (monolayers and spheroids). The aim of radiation treatment is the maximum kill of tumour cells and the minimum damage of normal cells [40–42]. Thus, in a first step, we have applied five different clinical treatment

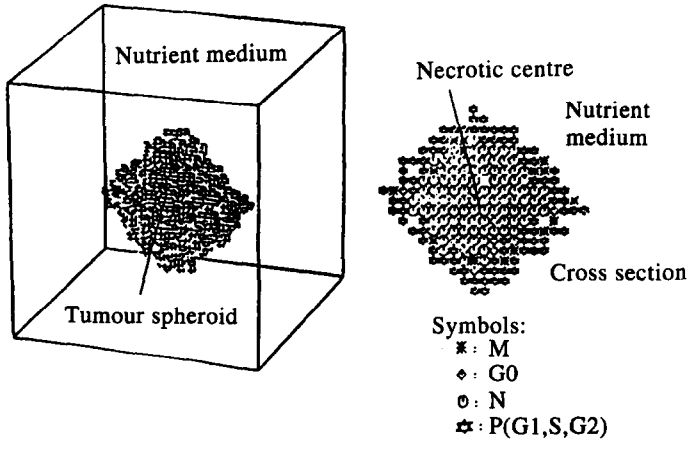
(a) Configuration at $t = 200$ units of time



(b) Chemotherapy a $t = 201$ units of time



(c) Tumour configuration at 100 units of time after chemotherapeutic treatment



(d) Number of tumour cells as a function of time

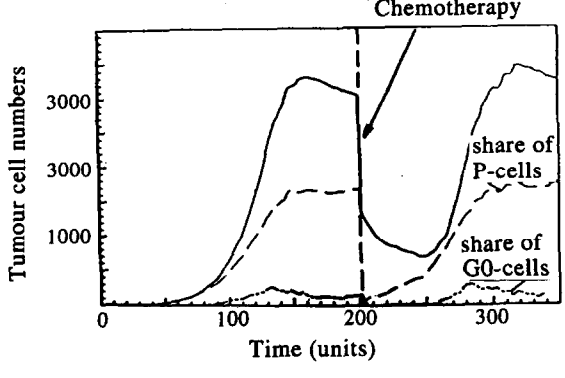


Figure 8. Chemotherapy applied to a tumour spheroid.

Table 1. Fractionation schemes

Scheme	Dose
Standard fractionation	1 × 2 Gy per day; 5 days per week; 60 Gy total
Hyperfractionation*	2 × 1.2 Gy per day; 5 days per week; 72 Gy total
Accelerated fractionation*	2 × 2 Gy per day; 5 days per week; 60 Gy total
Accelerated hyperfractionation*	3 × 1.5 Gy per day; 5 days per week; 54 Gy total
Hypofractionation	1 × 6 Gy per week; 60 Gy total

*Interval between treatments per day: 6 h.

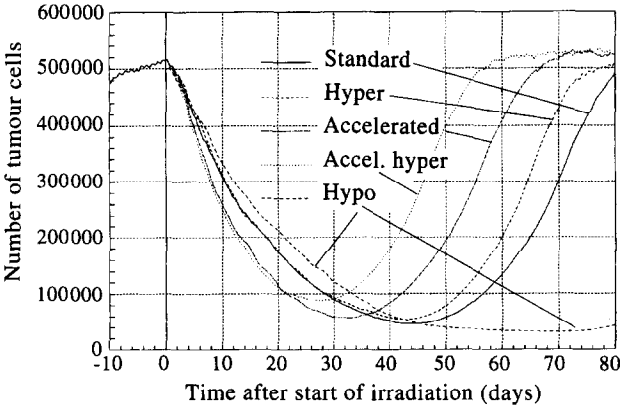


Figure 9. Irradiation of a EMT6/Ro-tumour spheroid (of the mamma sarcoma of the mouse) with five different fractionation schemes (Table 1).

schemes [43](Table 1) to the computer tumour spheroid model of the mamma sarcoma of the mouse [24]. Standard fractionation (5 × 2 Gy per week) is the usual schedule and it is still preferred in most hospitals. However, clinical studies to evaluate modifications of this standard scheme with regard to an improved tumour cell kill effect and eventually reduced side-effects to normal tissue are in progress. Therefore, the essential question is: What is more favourable, a multifractionated irradiation or an irradiation with a weekly high single dose? In agreement with qualitative clinical observations [44–46], the following simulation results will show that a general answer cannot be given to this question. Indeed, only a specific solution for each case is possible because, in addition, the side-effects of radiation to normal cells have to be considered also. Examination of the results of irradiation of the *in vitro* EMT6/Ro-tumour spheroid (of the mamma sarcoma of the mouse) in Figure 9, starting when the tumour has reached its saturation state (Figure 5c) at $T = 40$ days indicates that only the hypofractionation (Table 1) leads to a substantial tumour cell kill [24], although this is certainly dependent on the cell line under consideration. The radiation effect to a rapidly regenerating normal cell renewal system has to be studied. If we apply the radiation model to the cell renewal compartment model [25, 47] of Figure 4, the influence of irradiation to fast proliferating normal cells can be visualised. As an example of a single-dose irradiation, Figure 10 illustrates the response curves of the epidermis of the mouse. The irradiation of the epidermis of the mouse with five different clinical treatment schedules according to Table 1 has been published elsewhere [24]. However, the simulation results tell us that a recovery of the cell system can be expected, unless an accelerated irradiation scheme is applied. Clinical experience is often that

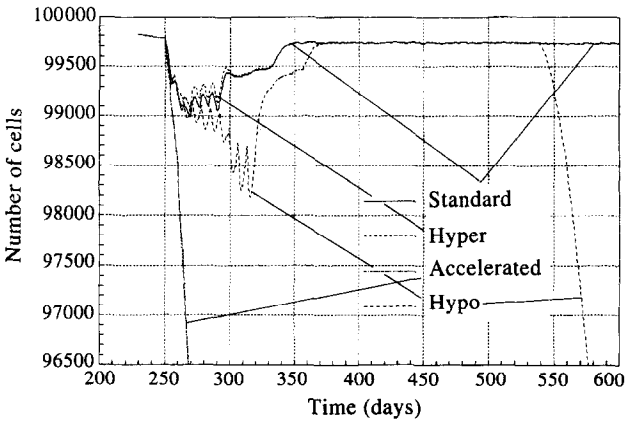


Figure 11. Irradiation of the lung parenchyma of the mouse with four different fractionation schemes (Table 1).

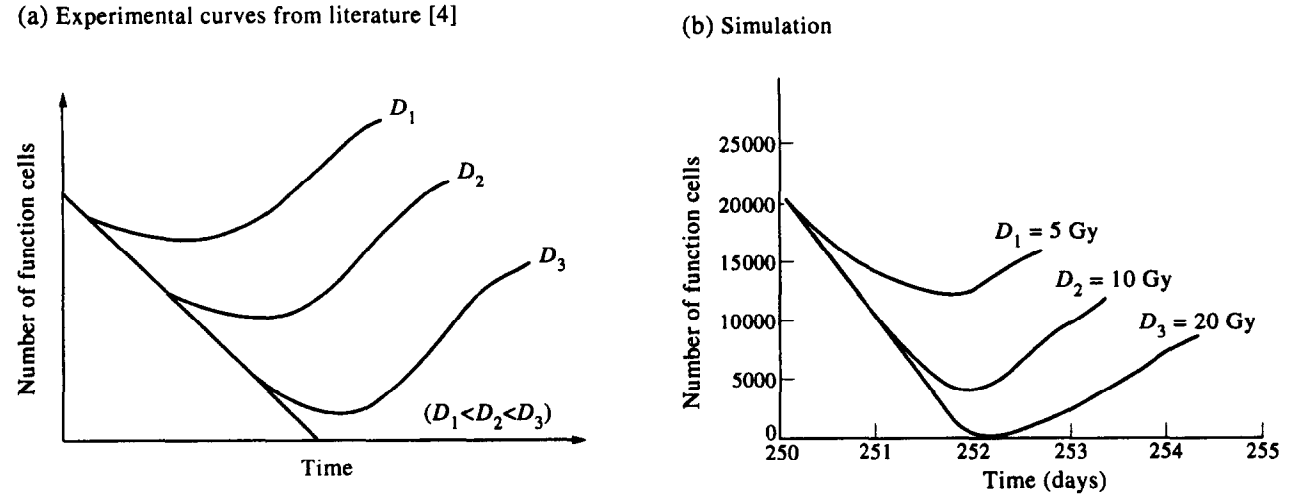


Figure 10. Cell number of function cells of the epidermis of the mouse after a single-dose irradiation at $t = 250$ days. D, dose.

the late effects of irradiation are really the dose-limiting factor [43–46]. Therefore, we had to construct a modified computer model [47–48] which considers the cell kinetic behaviour of slowly regenerating normal cell systems (lung, brain) with dominantly quiescent G0-cells. Again, applying the five different clinical fractionation schemes (Table 1) to the lung parenchyma of the mouse, Figure 11 shows that now the two schedules hyper- and hypofractionation lead to severe (late) damage of the normal cells [24]. However, the qualitative simulation results always have to be confirmed by experiments and/or clinical evidence.

Finally, an important advantage of the simulation approach shall be stressed. In the biological reality, you can only perform experiments with a tumour embedded in a mixture of rapidly and slowly regenerating normal tissue. By applying the computer modelling, it is possible to study the influences of treatment to each tissue separately and to compare the results with each other.

OUTLOOK

Here, we have shown that computer models are obviously essential simplifications of the true complexity of real life. Thus, much remains to be done. Some promising avenues of future research include:

- —Initiating new biological experiments for measuring currently unknown data and parameters.
- —Considering facts which had been neglected so far (formation of metastases, immunological reactions, drug resistance, heterogeneity).
- —Extension of the cell space which is currently limited to approximately 1 mm^3 tissue volume.
- —Considering growth factors in haematopoietic cell renewal models.
- —Generating a more realistic initial configuration of a tumour by combining image processing techniques (computer tomography, magnetic resonance, positron emission tomography) with our predictive models describing tumour growth.
- —Improving cancer treatment strategies by studying alternative schedules such as continuous irradiation with low doses, administration of chemotherapeutic drugs and/or combined therapy modalities, which are becoming increasingly important in the treatment regimen of human tumour diseases. Priority should be given to considering cell metabolism and the non-linear dependence of the metabolic variables.
- —Systematic comparison of different treatment methods and protocols.
- —Partial substitution of long and expensive biological test series by simulation experiments.
- —Construction of computer models at the gene-regulation level by introducing methods from computer science, e.g. Petri-networks (event-oriented asynchronous switch-networks).

An important precondition (*conditio sine qua non*) of all modelling activities is the stepwise reduction of antipathy against the systematic modelling approach which is created by scientists predominantly working empirically. The authors sincerely hope that this review will help to inspire more confidence in modelling.

1. Tautu P. Mathematical models in oncology: a bird's-eye view. *Z. Krebsforschung* 1978, **91**, 223–235.
2. Cherruault Y. *Mathematical Modelling in Biomedicine*. Dordrecht, D. Reidel Publishing Company, 1986.
3. Thompson JR, Brown BW (eds). *Cancer Modelling*. New York, Marcel Dekker Inc., 1987.
4. Wheldon T. *Mathematical Models in Cancer Research*. Bristol, Adam Hilger, 1988.
5. Sandblad B, Meinzer HP. Modelling and simulation of complex control structures in cell biology. *Math Inform Med* 1992, **31**, 36–43.
6. Murray JD. *Mathematical Biology*. Berlin, Springer-Verlag, 1993.
7. Drasdo D. *Monte-Carlo-Simulationen in zwei Dimensionen zur Beschreibung von Wachstumskinetik und Strukturbildungsphänomenen in Zellpopulationen*. Aachen, Verlag Shaker, 1993.
8. Levin SA (ed.). *Frontiers in Mathematical Biology*. Berlin, Springer-Verlag, 1994.
9. Rowe G. *Theoretical Models in Biology*. Oxford, Clarendon Press, 1994.
10. Mosekilde E, Mouritsen OG (eds). *Modelling the Dynamics of Biological Systems*. Berlin, Springer-Verlag, 1995.
11. Solyanik GI, Berezetskaya NM, Bulkiewicz RI, Kulik GI. Different growth patterns of a cancer cell population as a function of its starting growth characteristics: analysis by mathematical modelling. *Cell Prolif* 1995, **28**, 263–278.
12. Denekamp J. *Cellkinetics and Cancer Therapy*. Springfield, Illinois, C.C. Thomas, 1982.
13. Bannasch P (ed.). *Cancer Therapy, New Trends*. Berlin, Springer-Verlag, 1989.
14. Horwich A (ed.). *Oncology—A Multidisciplinary Textbook*. London, Chapman & Hall, 1995.
15. DeVita VT, Hellman S, Rosenberg SA. *Important Advances in Oncology 1995*. Philadelphia, J.B. Lippincott Company, 1995.
16. Düchting W. Krebs, ein instabiler Regelkreis, Versuch einer Systemanalyse. *Kybernetik* 1968, **5**, 70–77.
17. Bossel H. *Modellbildung und Simulation*. Braunschweig, Vieweg Verlag, 1992.
18. Hogeweg P. Simulating the growth of cellular forms. *Simulation* 1978, September, 90–96.
19. Meinhard H. *Models of Biological Pattern Formation*. London, Academic Press, 1982.
20. Keen RE, Spain JD. *Computer Simulation in Biology*. New York, Wiley-Liss, 1992.
21. Anderson JG, Katzper M (eds). *Simulation in the Health Sciences*. The Society for Computer Simulation, San Diego, 1994.
22. Düchting W. Tumor growth simulation. *Computer & Graphics* 1990, **3/4**, 505–508.
23. Durand RE, Multicell spheroids as a model for cell kinetics studies. *Cell Tissue Kin* 1990, **23**, 141–159.
24. Ginsberg T. *Modellierung und Simulation der Proliferationsregulation und Strahlentherapie normaler und maligner Gewebe*. Siegen, Dissertation, 1995.
25. Düchting W, Ulmer W, Ginsberg T, Saile C. Radiogenic responses of normal tissue induced by fractionated irradiation—a simulation study. I. Acute effects. *Strahlenther Onkol* 1995, **171**, 460–467.
26. Potten CS. *Stem Cells*. Edinburgh, Churchill Livingstone, 1983.
27. Düchting W, Vogelsaenger T. Three-dimensional pattern generation applied to spheroidal tumor growth in a nutrient medium. *Int J Bio-Medical Computing* 1981, **12**, 377–392.
28. Düchting W, Dehl G. Spatial structure of tumor growth: a simulation study. *IEEE Transactions on Systems, Man and Cybernetics* 1980, **10**, 292–296.
29. Vogelsaenger T. *Modellbildung und Simulation von Regelungsmechanismen wachsender Blutgefäßstrukturen in normalen Geweben und malignen Tumoren*. Siegen, Dissertation, 1986.
30. Düchting W. Computer models applied to cancer research. In Blaquiere A, ed. *Modeling and Control of Systems*. Berlin, Springer-Verlag, 1988, 397–411.
31. Chabner BA, Collins JM. *Cancer Chemotherapy*. Philadelphia, J.B. Lippincott Company, 1990.
32. Armitage JO, Antman KH (eds). *High-Dose Cancer Therapy*. Baltimore, Williams & Wilkins, 1992.
33. Eisen M. *Mathematical Models in Cell Biology and Cancer Chemotherapy*. Berlin, Springer-Verlag, 1979.

34. Knolle H. *Cell Kinetic Modelling and the Chemotherapy of Cancer*. Berlin, Springer-Verlag, 1989.
35. Kohn KW, Ewig RA. DNA cross-linkings and cytotoxicity of normal and transformed cells. *Cancer Res* 1978, **38**, 3197–3204.
36. Ulmer W. Dose-effect relationships and 31P-NMR-spectroscopy of L1210 cells (monolayers) and 9L glioma cells (monolayers and tumor spheroids) treated with activated isophosphamide, adriamycin, epirubicin and 6MeV electrons. *Strahlenther Onkol* 1991, **167**, 484–493.
37. Ulmer W. In vivo investigations on murine C3H mammary adenocarcinoma treated with cis-platinum, adriamyacin, epirubicin, activated cyclophosphamide, activated isophosphamide and irradiation with 10 MeV electrons—verification of the therapeutic results with 31P-NMR-spectroscopy. *Strahlenther Onkol* 1991, **167**, 553–560.
38. Thames HD, Hendry JH. *Fractionation in Radiotherapy*. London, Taylor & Francis, 1987.
39. Düchting W, Ulmer W, Lehrig R, Ginsberg T, Dedeleit E. Computer simulation and modelling of tumor spheroid growth and their relevance for optimization of fractioned radiotherapy. *Strahlenther Onkol* 1992, **168**, 354–360.
40. Swan GM. *Optimization of Human Cancer Radiotherapy*. Berlin, Springer-Verlag, 1981.
41. Perez CA, Brady LW. *Principles of Radiation Oncology*. Philadelphia, J.B. Lippincott Company, 1989.
42. Steel GG, Adams GE, Horwich A (eds.). *The Biological Basis of Radiotherapy*. Amsterdam, Elsevier, 1989.
43. Withers HR. Some changes in concepts of dose fractionation over 20 years. *Front Radiat Ther Oncol* 1988, **22**, 1–13.
44. Freyer JP. Spheroids in radiobiology research. In Bjerkvig R, ed *Spheroid Culture in Cancer Research*. Boca Raton, Florida, CRC Press, 1992, 217–275.
45. Beck-Bornholdt HP (ed.). *Current Topics in Clinical Radiobiology of Tumors*. Berlin, Springer-Verlag, 1993.
46. Kocher M, Treuer H. Reoxygenation of hypoxic cells by tumor shrinkage during irradiation. *Strahlenther Onkol* 1995, **171**, 219–230.
47. Düchting W, Ginsberg T, Ulmer W. Modelling of radiogenic responses induced by fractionated irradiation in malignant and normal tissue. *Stem Cells* 1995, **13**, (Suppl I), 301–306.
48. Düchting W, Ulmer W, Ginsberg T, Kikhounga-N'Got O, Saile C. Radiogenic responses of normal cells induced by fractionated irradiation—a simulation study, Part II. Late responses. *Strahlenther Onkol* 1995, **171**, 525–533.