Mathematics and Biosciences in Interaction

FRACTALS in BIOLOGY and MEDICINE

Volume III

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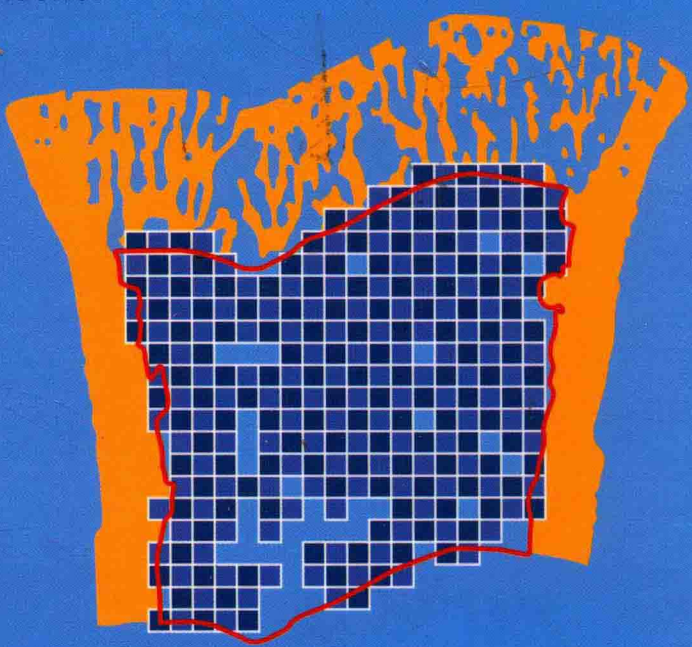

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A CIP catalogue record for this book is available form the Library of Congress, Washington D.C., USA

Deutsche Bibliothek Cataloging-in-Publication Data
Fractals in biology and medicine. - Basel ; Boston ; Berlin : Birkhäuser
Vol. 3. / Gabriele A. Losa ... (ed.). - 2002
ISBN 3-7643-6474-2

ISBN 3-7643-6474-2 Birkhäuser Verlag, Basel - Boston - Berlin

Member of the BertelsmannSpringer Publishing Group
Printed on acid-free paper produced from chlorine-free pulp. TFC ∞
Cover design: Armando Losa, graphic designer
Cover illustration: Kolmogorov fractal dimension of the trabecular network (see p. 164)
Printed in Germany
ISBN 3-7643-6474-2

9 8 7 6 5 4 3 2 1

www.birkhauser.ch

Mathematics and Biosciences in Interaction

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Mathematics and Biosciences in Interaction is devoted to the publication of advanced textbooks, monographs, and multi-authored volumes on mathematical concepts in the biological sciences. It concentrates on truly interdisciplinary research presenting currently important biological fields and relevant methods of mathematical modelling and analysis. Emphasis will be put on mathematical concepts and methods being developed and refined in close relation to problems and results relevant for experimental bioscientists.

The series aims at publishing not only monographs by individual authors presenting their own results, but welcomes, in particular, volumes arising from collaborations, joint research programs or workshops. These can feature concepts and open problems as a result of such collaborative work, possibly illustrated with computer software providing statistical analyses, simulations or visualizations.

The envisaged readership includes researchers and advanced students in applied mathematics – numerical analysis as well as statistics, genetics, cell biology, neurobiology, bioinformatics, biophysics, bio(medical) engineering, biotechnology, evolution and behavioral sciences, theoretical biology, system theory.

Foreword

This volume contains oral and poster presentations given at the Third International Symposium on *Fractals in Biology and Medicine* held in Centro Seminariale Monte Verità, Ascona, Switzerland, from March 8-11, 2000. Scientists from around the world came together again to present and discuss in an exciting atmosphere their research papers as well to exchange information on their more recent experimental findings and theoretical interpretations. Benoît Mandelbrot proposed in his book, *The Fractal Geometry of the Nature*, a way to describe irregular objects, such as clouds, the outline of a coast or the shape of a tree, by means of a new «fractal» geometry. Significant progress has been made over the last years in understanding how to analyze biological shapes and structures, favoured also by the continuing improvements in computational capabilities. Most of the participants have been focused some or all of their activities on biomedical research problems so that the potential of the fractal geometry and its practical use for describing and measuring irregular biological objects such as organs, tissues and cells as well as for understanding several complex pathogenetic processes could be explored with the adequate criticism. A special emphasis has been devoted to the complex fields of human tumours and other severe diseases, by addressing the role of fractals in the design, organization and measurement of cellular and molecular structures and functional patterns in breast and skin carcinoma, in leukemic and lymphoma cells, in bone, lung, nervous, renal and voice diseases.

In presenting the different contributions in this volume, we did not follow the chronological sequence of sessions, rather we arranged the proceedings as to grouping similar topics together.

We are especially grateful to Professor Benoît Mandelbrot for his public presentation on, *Il viaggio frattale dall'arte all'arte attraverso la matematica e la scienza,* held in the Palazzo della Società Elettrica Sopracenerina, Locarno and for his critical contribution in the saturday morning session.

We are particularly indepted to the following renowned institutions: International Society for Stereology, International Society for DiagnosticQuantitative Pathology, Swiss National Science Foundation, Swiss Academy of Sciences, Accademia di Architettura, Università della Svizzera Italiana, Institute for Scientific Interdisciplinary Studies, Research Center for Mathematics and Physics, who accepted to confer their scientific patronage and also to the sponsors, Dipartimento dell'Istruzione e della Cultura del Canton Ticino, Swiss National Science Foundation, Maurice E. Müller Foundation, Swiss Academy of Sciences, Banca del Gottardo Lugano, Banca della Svizzera Italiana Lugano, Banco di Lugano, Pharma Consulting Marion Senn GMBH Burgdorf, and Becton Dickinson BD Basel.

Our thanks are also due to Prof. Mauro Martinoni, head of the Ufficio Studi Universitari del Canton Ticino for his precious support, to our collaborators and to M. Luca Albertini, managing director of the Centro Seminariale Monte Verità, who made the conference run «fractally».

MonteVerità, Ascona 2000 — The Editors

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Summary This communication addresses why fractals are of interest in biology and medicine and presents two examples of the application of fractals concepts in biomedical research. Firstly, the analysis and simulation of the development of mosaic patterns in liver and adrenal gland of chimaeric animals and secondly the quantification of the complexity of epithelial profiles in the ventral surface of the tongue of rats treated with the carcinogen 4-nitroquinoline 1-oxide in drinking water.

1 Introduction

There are several reasons why fractals seem to have attracted interest in biomedical research. For example, there have been suggestions that natural structures with variable degrees of self-similarity arise as a consequence of deterministic growth rules, while in others cases "fractality" seems to follow as a side effect of non-equilibrium or randomness during morphogenesis. It is perhaps not surprising that numerous links have been demonstrated in physics, biology and mathematics using fractal geometry. From the perspective of physical processes, it is well documented that a wide range of complex and disordered systems exhibit some fractal properties [1]. Interestingly, from a biological standpoint, fractal geometry has been called a "design principle" in biology [2] because self similar structures have a number of attractive features such as algorithmic maximisation of area per unit of volume or the ability of coding the micro and the macro scales of objects efficiently. In developmental terms, it also seems that iterative procedures provide further benefits: as little information required for coding optimised structures, the transmission error rates are reduced and consequently there are less control requirements. In addition, fractal geometry provides methods for morphometrical characterisation and quantification [3] which has ample applications in shape and texture characterisation and consequently, in diagnosis.

Two interesting biological problems, namely, parenchyma growth during organogenesis and complexity of tissue profiles in an experimental cancer model

have been investigated using the principles of fractal geometry. The following sections show the power of fractals in generating further understanding and characterisation of such complex patterns.

2 Organogenesis

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The developmental process by which a number of cells (primordium) becomes a complete functional organ is termed organogenesis. This involves a precise and timed sequence of events which allows the successful generation of a functional organ. Initially organogenesis consists of a rapid expansion of immature parenchyma which is exponential at first and linear later, followed by differentiation into functional mature tissues. Parenchyma expansion is the result of regulated cell proliferation and spatial allocation which is *specific* to the organ (each organ has its own mechanism), and *conserved* across individuals (the mechanism applies to the same organ in different individuals of the same species). It is believed that the whole process is genetically specified [4] but discrepancies arise in terms of storage capacity in DNA given the amount information that multicellular organisms would require and the possible way that information could be stored. In other words, how can the positioning and fate of one hundred trillion cells in a human be coded in about 100,000 genes? Spatio-temporal information on a cell-per-cell basis seems not only extremely inefficient and unstable (prone to errors and sensitive to mutations) but also impossible given the limited size of the genome. It can be argued that during organogenesis not all cells may require control, or that control only applies at certain stages of development. These restrictions suggest that organogenesis may be directed by procedural mechanisms or algorithms. It is, obviously, of great interest to find out how organogenesis takes place and what those mechanisms may be.

2.1 Chimaeric Organisms

The allocation of organ primordia and their growth can be studied using experimental chimeras. These are multizygotic animals with four (or more) parents produced by amalgamation of embryonic tissues of genetically distinct strains [5, 6]. When cells from one of the parental lineages are visualised (for example, using immunostains) the "mosaic" pattern reveals the two lineages. Similarly, mosaic patterns can be produce using transgenic techniques (i.e. injecting exogenous DNA coding for a specific marker molecule absent in the species into an embryo cell nucleus and later visualising the cells that express the marker). Obviously, the mosaic patterns arising in chimaeric tissues give indications of how the parenchyma was generated. These patterns are characteristically fragmented "islands" or "patches" of one cell type embedded in the other in variable proportions. However, mosaic patterns in chimaeric rat liver and adrenal gland appear very different. In the liver, there is no characteristic patch size or anisotropy (Figure 1, left); sections in

different orientations show similar degrees of complexity. Liver patch outlines have characteristically a fractal dimension ~1.3 across locations within the same organ, across animals, and independent of the proportion of marked cells [7].

The mosaic adrenal cortex, however, has radial cords of cells in both chimaeric rat (Figure 1, right) and transgenic mice [8,9]. Moreover, the patch outlines are less irregular than those in the liver, confirmed by a fractal dimension of ~1.2 [10] (Figure 2).

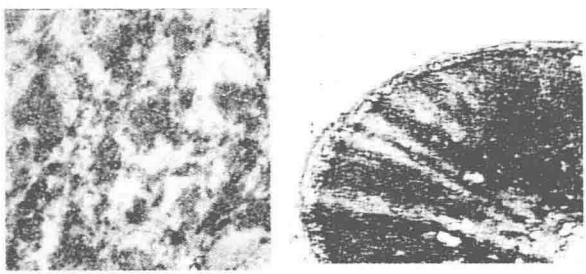

Figure 1. Greyscale digitised autoradiograms of rat chimaeric liver and adrenal gland showing the characteristic mosaic patterns. Left: fragmented or "geographic" pattern in liver (field width 2.2 mm). Right: "radial" pattern in adrenal gland (field width 1.05 mm).

Because most algorithms for constructing fractals rely on iteration and recursion involving a small number of specific rules, it has been suggested that the self-similarity of mosaic patches could also involve iterative mechanisms with simple rules [7,10]. After all, tissue growth is an iterative and recursive process driven by mitotic activity and regulated by cell adhesion, cell motility, contact inhibition and cell death. Here we have investigated a computer model of parenchyma expansion.

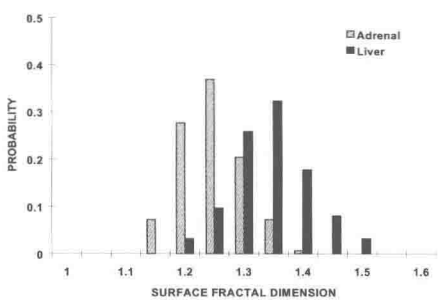

Figure 2. Distribution of fractal dimension of patches outlines in liver (n=152) and adrenal (n=62). The average values of the complexity are D = 1.32 (±0.06) and 1.22 (±0.05) for the liver and adrenal respectively.

2.2 A Model of Parenchyma Growth

The tissue expansion model presented here is the generalisation of the model of clonal growth described originally by Ransom [12]. Several variations of the model have been used to simulate cell patterning [13,14], chimera patterns [15] and fragmentation [16]. The model consists of a non-periodic square lattice, where a *primordium* (a cluster of cells containing two lineages) divides randomly and

following a random choice for the division direction. Other than the colour, the two lineages behaved identically, using the same rules and they were unaware of each other. Daughter cell allocation space is created by "pushing" the neighbouring cells. We introduced a new parameter f which represents the maximal force (in number of cells to be pushed) that a dividing cell could exert on the rest of the tissue to allocate a daughter cell. Therefore for each division cell candidate, the number of cells to be pushed in a particular direction to allocate space to a daughter cells, needs to be smaller or equal than f. The shuffling of the "tissue" produces a mixing effect which is limited to a cortical zone of width f. The parameter f may have a number of counterparts in nature, such as cell adhesion forces. We call this parameter "contact inhibition" although similar results are produced using other principles such as nutrient gradients across the tissue, cell signalling other mechanisms which operate in function of the distance between cells and an external source. The original model by Ransom [12], corresponds to the special case when $f = \infty$ and the diagram representation of the algorithm is shown in Figure 3.

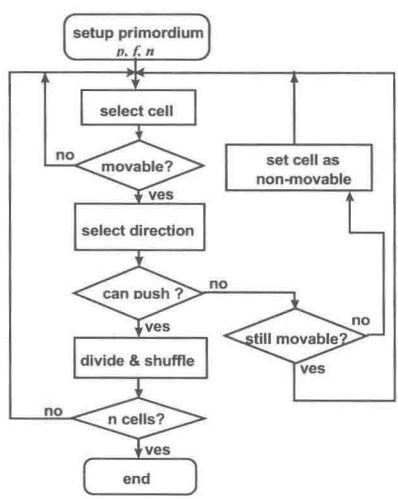

Figure 3. Flow-diagram of the parenchyma expansion model. The introduction of "movable" and "not movable" cell labelling avoided unnecessary calculations on cells that could not replicate. In simulations with low f where proliferation takes place only on a thin cortical part of the tissue, the computation was speeded up dramatically.

2.3 Simulations

One appealing characteristic of our generalised pushing model is its ability to produce radically different patterns depending on the choice of f. For large f the patterns generated with 2 lineages look fragmented and isotropic, while for small f they have a radial pattern (Figure 4). These synthetic patterns resembled those observed in sections of chimaeric liver and adrenal respectively [10,11]. Simulations were performed with f values of 2, 4, 8,16, 32, 64, 128 and 256 (208 simulations in total) and the fractal dimensions of the resulting patches outlines were estimated.

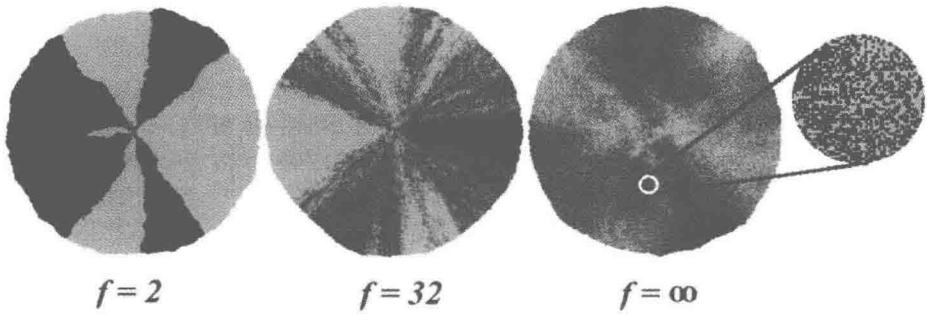

Figure 4. Simulated patterns with different values of f. Note the similarity of the radial pattern for low f and the fragmented nature of the pattern for large f. In all cases the original primordium was formed by 100 cells and a probability of 0.25 of being "marked" cells (in black in the image).

Simulations were started with 100 cell primordia seeded randomly with a proportion of marked cells of p=0.25 and continued until they reached n=4x10^5 cells. Figure 5 shows the average fractal dimension of the simulated patch outlines estimated using the area-perimeter relation [3].

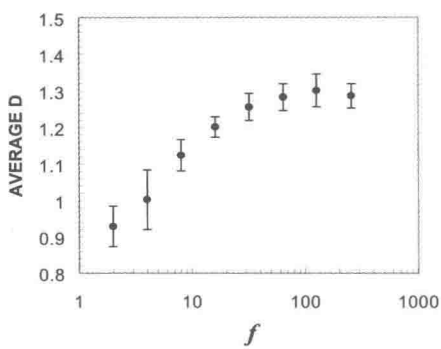

Figure 5. Average fractal dimension of the outlines estimated using the area-perimeter relation. Note that increasing values of f produce more irregular patterns. This tendency seems to stabilise around a maximum complexity value of D=1.3. The vertical bars indicate one standard deviation from the mean.

While simulations with small f exhibit outlines with little irregularity (low D), it increases with f reaching a plateau at about D=1.3 for f >64. It seems therefore possible that dissimilar patterns as observed in chimaeric liver and adrenal can be produced through the same basic mechanism, but depending only on replication restriction. Furthermore, both real and simulated adrenal mosaics showed little or no fragmentation and a radial pattern that extends through most of the cortex [8]. In 1916, Graham [17] suggested that adrenal cortex was generated by centripetal migration of cells; more recently, Morley et al. [9] working with transgenic mice also suggested that centripetal migration was responsible for such radial patterns. We suggest that comparable results can be obtained, not by active migration, but by iterative cell division and pushing for daughter cell positioning biased by contact inhibition. This is a new interpretation of the mosaic pattern: a developmental

mechanism requiring no guidance, positioning information or active cell migration during parenchyma growth, which still results in the same type of mosaic pattern [10, 18].

In conclusion, the two dissimilar mosaic patterns that develop in chimaeric liver and adrenal gland may be closely related in algorithmic terms and the observed differences may be due to a cell division restriction parameter. Despite the simplicity of our model, there is emerging evidence for genetic control of the probability of cell division [29] and daughter cell positioning following division [20, 21]. Further mathematical interpretation of the mosaic patterns and model simulations will undoubtedly contribute to a better understanding of the mechanisms of regulation and rapid expansion of parenchyma compartments during organogenesis.

3 Carcinogenesis

G. Landini, Y. Hirayama [1)], T. J. Li [2)], M. Kitano [1)]
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The irregularity of the epithelial-connective tissue interface (ECTI) is frequently used by histopathologists as a diagnostic feature (among many others) in the diagnosis of oral lichen planus, epithelial dysplasia and squamous cell carcinoma. The assessment of the ECTI complexity is, however, subjective rather than quantitative. In oral premalignancy (epithelial dysplasia) and malignancy (squamous cell carcinoma) there is a typical variability in the spatial arrangement of the epithelial cells mainly due to the loss of control in cell proliferation and loss of expression of adhesion molecules. This results in cell crowding and irregular boundaries between the epithelium and stroma. To standardise and quantify those changes, unbiased numerical procedures have been proposed and applied: generalised fractal dimension, local and local-connected fractal dimensions of the ECTI [22, 23]. Fractal dimension parameters subjected to discriminant analysis provided information about the irregularity of the ECTI in epithelial dysplasia (from human oral biopsies) which was about three times more accurate than the subjective perception of the same profiles by expert or non-expert observers [24]. Those results were encouraging, considering they were based only on the quantification of the profiles and excluded any cytological features. However, such quantitative methodology left about 25% of epithelial dysplasia cases misclassified as "normal" [23]. This could be due to limited methodological reliability or because "dysplasia" may include a number of different conditions where only a proportion exhibit detectable architectural changes. Unfortunately, there is no consensus amongst pathologists about the precise histomorphological markers for oral epithelial dysplasia [25] so it seems important to investigate the changes in the ECTI that take place in controlled conditions such as experimental models of carcinogenesis. These may help to identify the level of morphological changes that occurs in the model before full malignant transformation and stroma invasion take place. Such information will hopefully provide act as diagnostic markers to detect early changes in a reliable and quantitative way. We investigated the ECTI irregularity in the ventral surface of the tongue in two strains of rats treated with the carcinogen 4-nitroquinoline 1-oxide (4NQO) but which have *not* yet developed carcinomas.