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A 2-D MODEL FOR THE GROWTH OF ARTERIES

Konrad Sandau[1], Haymo Kurz[2]

[1]FB Mathematik der Fachhochschule, Schöfferstr. 3, D-64295 Darmstadt;
[2]Anatomisches Institut II der Universität, Albertstr. 17, D-79001 Freiburg

ABSTRACT

This study is based on observations of the growth of arteries and capillary networks in the chorioallantoic membrane (CAM) of incubated chicken eggs. The structure of the capillary layer can be described as a planar, area-filling hexagonal grid, whereas the arteries are seen in a plane underneath the capillary plexus, resembling a bifurcating vessel tree. Further biological observations strongly suggest that the larger vessels do not form sprouts but that they exclusively originate from capillary proliferation and enlargement. A model for this mechanism of blood vessel formation has been developed and compared with anatomical and physiological data.

In a computer simulation, growth of the network is driven by a stochastic process starting in a point source. The probabilities for the formation of new capillary elements are derived from the flow theorem of Hagen-Poiseuille and the diameter exponent. The hexagonal grid is visualized as being supported by a flat limited area, a cylinder, and a sphere. The time course of growth and of blood pressure is obtained. The resulting arterial tree is considered to have limited fractal properties, and the dimension of its border is compared with the biological data.

Key words: angiogenesis, blood vessel, chorioallantoic membrane, computer simulation, development, diameter exponent, fractal, Hagen-Poiseuille, stochastic process.

INTRODUCTION

The development of tree-like structures like the mammalian bronchial system or the vertebrate vessel system has been of special interest to both anatomists, biophysicists and mathematicians because of their optimality properties concerning surface-volume ratios (Weibel 1984), specific volume density (Sernetz et al. 1985), and conductance (Spatz 1991). Moreover, they seem to follow comparatively simple developmental rules (Weibel 1985). The concepts of fractal geometry, of self-similarity and of iterative functions rendered the first synthetic models of mostly regular trees (Mandelbrot 1977, Mandelbrot 1982, Bittner 1991), which already compared favorably with nature (Peitgen and Saupe 1988). In the case of trees, self-similarity of the growing branch tips, together with the diameter exponent Δ, allowed to synthesize nice regular patterns. The diameter exponent essentially describes a relation of the branch thickness before (d) and after (d1, d2) a bifurcation by

$$d^{\Delta} = d_1{}^{\Delta} + d_2{}^{\Delta} \qquad (1)$$

This relation describes the branch thickness completely except for a scaling factor, which may be given by the thickness of the trunk. If a fluid is to flow through the tree, further limiting conditions can be derived from hydrodynamics, i.e., in the ideal case of laminar flow of a Newtonian fluid, from Hagen-Poiseuille's law.

Nevertheless, the actual (cellular) mechanisms of blood vessel formation or angiogenesis, were hardly appropriately portrayed with these models, which had gained their merits mostly from their aesthetical and functional properties. We therefore based our simulation on observations of a particular vascular growth process, the 'intussusceptive' capillary growth (Burri and Tarek 1990, Patan et al. 1993), and tried to build a model which would combine the developmental and the physiological findings, and which should display an irregularity as observed in nature.

This growth process was first described in the developing mammalian lung after birth, but also has been suggested for another respiratory organ, the CAM, which is used by the avian embryo before hatching. This organ has great practical advantages, since it is easily accessible and may be inspected even in the living state, and it is of considerable clinical importance as an established growth assay of blood vessels or transplanted tumors. From the point of modelling, it is matchless, because this membrane topologically resembles a sphere, but most of all, because both its capillary layer and its conductive arterial system can be considered as contained in locally two-dimensional tissue sheets.

One major observation was that the growth processes in the capillary layer drives the growth of the underlying vessels (Kurz et al. 1993). A second observation, which will be fundamental to our model was that the capillary network is built like a hexagonal grid (cf. Zeltner and Burri 1987).

The advantages (and the shortcomings as well) of a model lie in its simplification of reality. In the case of the CAM, reality is comparatively simple so that we can hope that the odds are outweighed by the evens. We thus assume capillary elements, or net elements, growing on a regular, planar, hexagonal grid. Each element has a predecessor, whose diameter increases in proportion to blood flow as required to support its successors. This is the deterministic part of the model, which also includes the definition of neighborhood and the physical laws. The second part achieves the stochastic nature: development takes place by adding new net elements to the existing system, which is intended to parallel the intussuceptive growth (cf. Patan et al. 1993), and which is realized as a stochastic process under constraints derived from the topological and physical context. The third part finally provides the visualization of the simulation and its numerical evaluation, which are compared with the morphology and physiology of the CAM.

THE PHYSICAL FRAMEWORK

The network of blood vessels is essentially determined by the set of the appearing bifurcation points and their connection. These bifurcation points correspond to the net elements in the simulation (Fig. 1). It is assumed, that the three branches of the net elements all have the same length h and that the flow is always laminar. Further dynamical vasodilatory processes are not considered.

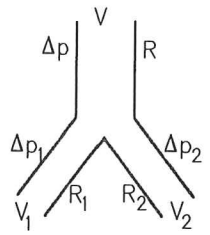

Fig. 1. A net element

Within a fixed time interval t the law of Hagen-Poiseuille is given by

$$V = \frac{\pi t}{8\eta h} \cdot \Delta p \cdot R^4 , \tag{2}$$

where η denotes the dynamical viscosity, Δp the difference of pressure along h, and R the radius of the branch. Assuming that the continuity principle holds (i.e. no fluid is lost through the capillary wall), we get the equation

$$\Delta p \cdot R^4 = \Delta p_1 \cdot R_1^4 + \Delta p_2 \cdot R_2^4 . \tag{3}$$

To complete the physical assumptions, we assume, that the flow at all branch tips is a constant volume V_e within t, that the pressure difference also is a constant, Δp_e, and that the radius is a constant, R_e. Then the flow in a net element depends on the number of successors of this net element. It makes sense to assume that the displaced V within t is a multiple of V_e because if $V_1 = k \cdot V_e$ and $V_2 = m \cdot V_e$, continuity yields $V = V_1 + V_2 = (k+m) \cdot V_e$, $k, m \in \mathbb{N}$. Let $V = l \cdot V_e$, $l \in \mathbb{N}$, then

$$V = l \cdot V_e = \frac{\pi t}{8\eta h} \cdot \Delta p \cdot R^4 = \frac{\pi t}{8\eta h} \cdot l \cdot \Delta p_e \cdot R_e^4 . \tag{4}$$

Now we introduce two functions p(l) and r(l) describing Δp and R in the considered net element in dependence of Δp_e and R_e, by

$$\Delta p = p(l) \cdot \Delta p_e, \qquad R^4 = r(l) \cdot R_e^4 . \tag{5}$$

Introducing this functions in Eq. (4) implies that

$$p(l) \cdot r(l) = l \tag{6}$$

must be fulfilled for all $l \in \mathbb{N}$. A very simple class of functions are the power functions and assuming that p and r are elements of this class we get, considering Eq. (6)

$$p(l) = l^{\alpha}, \quad r(l) = l^{1-\alpha}, \quad \alpha \in \mathbb{R}. \tag{7}$$

These functions are used in the following and a meaningful interval of α shall be derived now. It is well-known that the blood pressure decreases only little in the large vessels connected to the heart and that the precapillary and capillary vessels induce the bulk of loss in pressure. We hence assume that the pressure differences decrease with increasing number l. This implies a negative α. Further we consider the mean velocity $\bar{v}$ of flow given by

$$\bar{v} = \frac{V}{A \cdot t} = \frac{1}{8\eta h} \cdot \Delta p \cdot R^2 = \frac{1}{8\eta h} \cdot \Delta p_e \cdot R_e^2 \cdot p(l) \cdot \sqrt{r(l)} = \frac{1}{8\eta h} \cdot \Delta p_e \cdot R_e^2 \cdot l^{\left(\frac{1+\alpha}{2}\right)}, \tag{8}$$

where $A = \pi R^2$ means the area of the cross-section of the branch. This velocity decreases with decreasing radius which implies $(1+\alpha)/2 > 0$ and therefore $\alpha > -1$.

Now the deterministic part of the model is completely described and it may be noted that there is a relation between α and the diameter exponent Δ. Transforming Eq. (1) we get

$$R^{\Delta} = {R_1}^{\Delta} + {R_2}^{\Delta} \tag{9}$$

and, using Eq. (5), this additivity is fulfilled if $r(l)^{\frac{\Delta}{4}} = l$. This relation combines with Eq. (7) to

$$\alpha = 1 - \frac{4}{\Delta} \quad \text{and} \quad \Delta = \frac{4}{1-\alpha} . \tag{10}$$

Especially the result of Suwa and Takahashi (1971) of Δ=2.7, leads to $\alpha = -0.48$.

THE STOCHASTIC PART OF THE MODEL

The growth of the network is realized in the model by running through all border elements of the network at the actual stage of the network in a random sequence. A border element will receive a new successor with a given probability. This probability depends on the conditions present in the already existing network. The entire border can be very rough reaching even interior regions of the system. The conditions of a border element are restricted to local information, and the two main questions of the stochastic part are: what is local information, and how can this information influence the probability.

In view of the physiological aspects, local information at any single border element is the pressure, and the amount of flow, its direction, and its velocity. We assume that the probability of adding a new element increases with increasing pressure, with decreasing flow, and with decreasing velocity. In addition, any directional change will decrease the probability of getting a successor.

These principal relations were implemented in a variety of ways. In this study, we used weights directly proportional or reciprocally proportional to the parameters mentioned, except for the flow, where proportionality to the square root was taken.

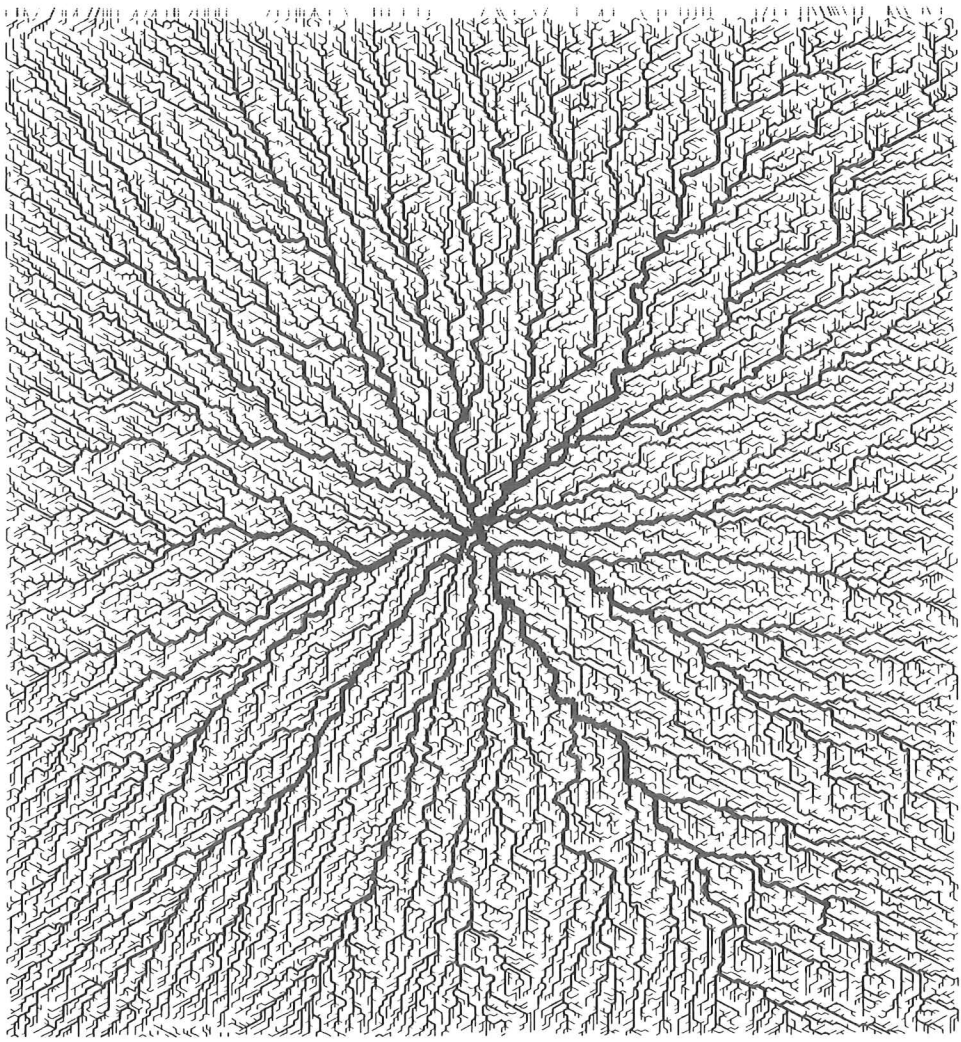

Fig. 2. A simulation was run on a cylindrical surface. The dashed lines at the upper border show the connections to the lower border. The parameters are $\alpha = -0.33$ and therefore $\Delta = 3$.

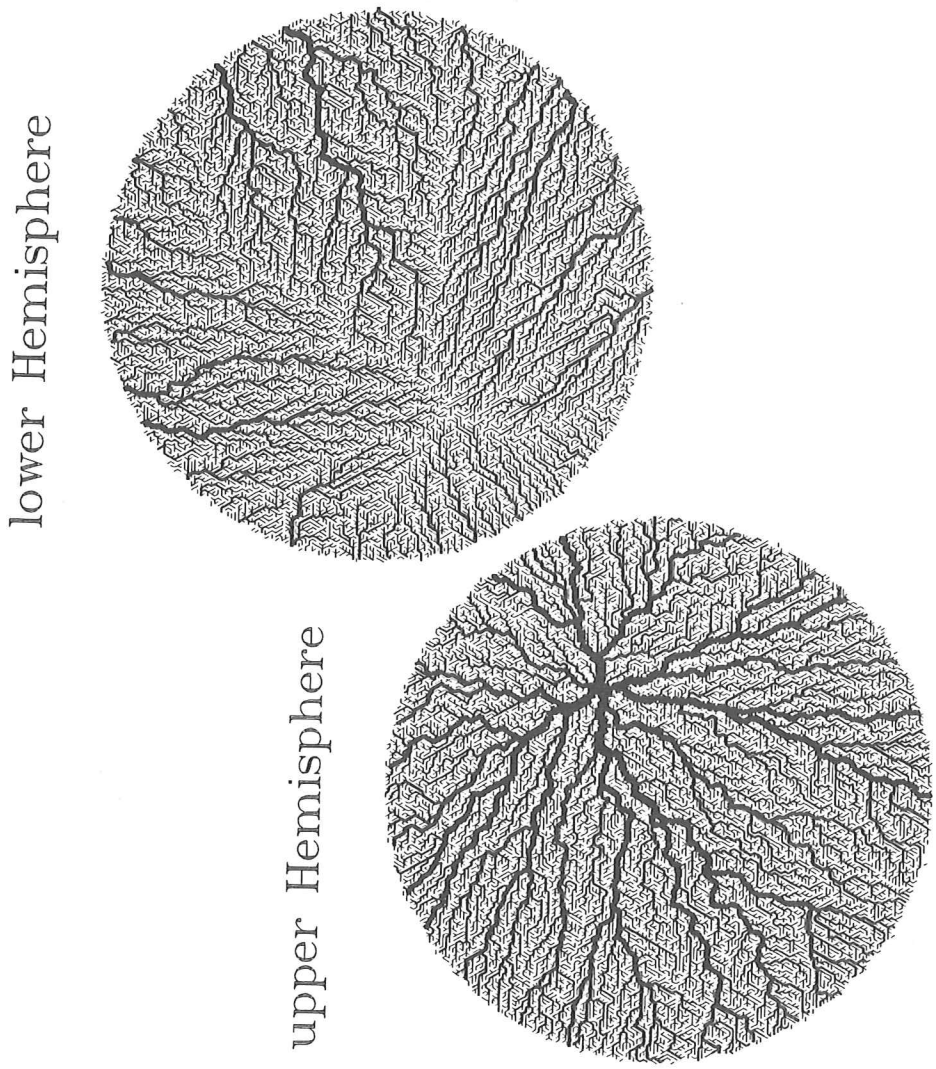

Fig. 3. A simulation was run on a spherical surface, divided into two hemispheres. The borders of the hemispheres are connected by a shift. The parameters are $\alpha = -0.48$ and therefore $\Delta = 2.7$.

THE SIMULATION

The algorithm starts at a single source element located in the middle of the area. The sequence of newly formed net elements is successively stored in a matrix representing the complete grid area. This matrix was afterwards used for extraction of numerical information and for visualization. In Figs. 2. and 3., the fully developed systems are shown on two surfaces of different topological structure, and for different α. The trees, by virtue of their stochastic irregularity, appear much more natural than most synthetical vessel patterns generated so far. The influence of the underlying regular hexagonal grid, however, remains visible at a closer look. It should be noted that an astonishing variety of local patterns may be contained in a single system, with very small branches connected to large vessels near the source point, and with the appearance of loops. One should be aware of that these patterns are self-organizing in that the differences in thickness, and consequently in flow, pressure etc. influence the growth probabilities.

THE FRACTAL DIMENSION

The complexity or roughness of a tree or a network may be characterized with its fractal dimension D. Both the CAM, and our simulated vessels grow in a plane, and hence their D is expected to be in the interval [1,2). The parameters of the model, e.g. α, may influence D. But we suppose that D is nearly independent from these parameters, because D can only be measured correctly within a limited interval of magnification. It moreover depends on the underlying grid, which in the hexagonal case implies approximative bounds to D, because a macroscopically straight line is obtained in a limited number of ways. Because of the possible hexagonal 'generators', D is expected in the interval [1.26,1.69]. Table 1 shows that this is in accordance with the estimate of D by the box counting method (BCM, cf. Mandelbrot 1982, Stoyan and Stoyan 1992) in both the model and the CAM (Kurz et al. 1993).

Table 1. The fractal D of vessel patterns was determined with the BCM for two 2-D growth models and the CAM (cf. Kurz et al. 1993) at various times of incubation. Measurement fields were positioned at the locations indicated below. The areal fraction of the binary images ranged from 25.4% to 53.4% for the simulations and from 16.3% to 35.7% for the CAM.

Location	pole of upper hemisphere/ centre field	upper equator/ middle left field	lower equator/ lower left corner	pole of lower hemisphere/ lower left corner
Simulation cf. Fig.2, α=-0.48	1.29	1.33	1.36	1.42
Simulation cf. Fig.3, α=-0.33	1.32	1.41	1.31	1.39
Incubation (d)	6	8	10	12
CAM	1.27	1.31	1.31	1.32

NUMERICAL EVALUATION

The number of net elements versus time is a sigmoidal function, whereas the plot of maximum pressure difference vs. time appears as a (rugged) root function (cf. Fig. 4). The first observation corresponds to the initially exponential and then, due to the limited surface of the egg shell, plateauing growth curve of CAM vessels (cf. Ausprunk et al. 1974, Strick et al. 1991, Kurz et al. 1993). The second phenomenon shows that the crucial pressure drop occurs in the few last generations of small 'arterioles', whereas the main stems do not contribute much resistance to the network. This shows that our networks solve the problem of supplying many vessel endpoints spread over a huge area from a single source with good efficiency and even fits the available blood pressure in the embryo (Van Mierop and Bertuch 1967).

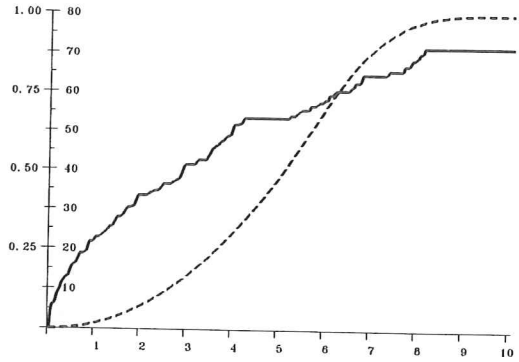

Fig. 4. An internal time is defined in the simulation and adapted to the developmental time (d) in the CAM starting in the 4th day of the embryo. The number of net elements is depicted versus this time (relative to the total number which is 65535, dashed line) and also the internal maximal pressure is depicted in units of Δp_e. The data were obtained from the run shown in Fig. 2.

DISCUSSION

The model of blood vessel patterning presented here borrows from the biological paradigm, the CAM, that the only site of angiogenesis is a capillary bed, which at any instant is connected to the circulation and is perfused. This condition is quite different from 'blind' sprouting (angiogenesis proper), or from differentiation of unconnected vessels (vasculogenesis), and was termed 'intussusceptive' vascular growth by Caduff et al. (1986). The cellular events were described in detail by Burri and Tarek (1990) and Patan et al. (1993), and it is plausible that this mechanism of extending a vascular bed is most efficient in the case of such vital organs as the mammalian lung or the avian CAM, with their gas exchanging surfaces. Their depiction of capillary meshes inspired the hexagonal grid we used, whereas we did not intend to model exactly the deformation of endothelial cells etc. described in this literature. Rather we were interested in how larger vessels can be derived from such a meshwork, because from cytokinetic studies it is known that endothelial proliferation is practically restricted to the capillary bed (Ausprunk et al. 1974, Kurz et al. 1993b). This implies that the endothelia lining the larger conducting vessels were initially formed in the gas exchanging capillaries, which later were transformed to arterioles. It does not exclude that endothelial cells may have migrated upstream and contributed to the growing vessel wall there, a well-known behavior in arteries (Wilms et al. 1991).

We did not address the question, how at the molecular level the control of endothelial proliferation and differentiation could be modeled, but our physical approach implies that endothelia are mechanosensitive or chemosensitive (cf. Ingber et al. 1987). In the CAM they obviously form new meshes only, where according to $T \sim p \cdot R$ (Laplace's law), wall tension T is low, because pressure p and radius R are low. Moreover, oxygen partial pressure (pO_2) is highest in the CAM, whereas all inner structures of the embryo are at a low pO_2. This aspect has hardly been treated appropriately in the physiological literature (Adair et al. 1990, Strick et al. 1991). It seems possible however, that this local physical information is used for influencing cell shape via modification of genetic expression, whereas the general developmental trigger may be one or more growth factors (Ingber 1991, Wilting et al. 1991, 1993). The model may be fine-tuned to include more molecular details, as soon as they will be elucidated.

At the moment, one impressive feature of the stochastically self-organizing physical model is that from a very limited set of instructions, and on an absolutely regular matrix, a complex, irregular, and in certain aspects optimal vascular system emerges. It has features in common with nature like its growth characteristics, and its fractal dimension, but fails of course with respect to completeness and generality: we modeled only a single source and assumed that the drainage through veins would be accomplished analogously. The areas spared by the arterial branches may indeed, by way of interdigitating branches, be used by the veins. On the other hand, the extension of the model to three-dimensional growth, or the inclusion of loop formation (e.g. arterial and venous anastomoses), of inversion of flow direction, of obliteration etc. is all but trivial. We believe, however, that already a 2-D model can provide considerable insight into vascular growth processes, which rather often are restricted to coplanar extension of a network.

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