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Nonlinear Dynamics, Chaos-theory, and the "Sciences of Complexity": Their Relevance to the Study of the Interaction between Host and Microflora

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Summary

Theoretical and experimental studies of various biomedical systems, including heart, brain, immune system, and many ecosystems, have shown that many of these systems may be described in terms of nonlinear dynamics. Two important consequences of this nonlinear dynamical behaviour are irreversibility and unpredictability, due to the chaotic behaviour this type of system may exhibit. Another trend in modern science (often named "the sciences of complexity") deals with the often complex or chaotic collective behaviour of systems made up of large numbers of relatively simple entities. Very often such systems exhibit nonlinear dynamical behaviour. Many such complex and nonlinear systems have been studied successfully using computer simulation techniques.

It is proposed that, as has been demonstrated for the immune system, the intestinal microbial ecosystem may be viewed as such a complex system governed by nonlinear dynamical equations. A discussion of techniques available for the study of such systems is given, with a special emphasis on computer simulation. Finally, the results of a pilot study using computer simulation of the interaction between the anaerobic and aerobic compartments of the microflora within a simple geometric model of the small and large intestine are presented.

Introduction

In recent years there has been a great deal of interest (and indeed a great deal of hype) concerning three catch-phrases: nonlinear dynamics, chaos, and complexity. This interest (and hype) has led to a large number of popular-science articles decorated with very fancy graphics (fractals and the like). Naturally, a sceptical backlash from certain serious scientists (Horgan, 1995) has occurred. Some scepticism is of course always in place when a group of scientists claims to have opened up a new field of study which will (a) revolutionize science, and (b) explain virtually anything under the sun and beyond. Some scientists working in the fields of nonlinear dynamics and complexity have indeed made such claims. Such claims abound throughout the history of modern science from Newton down to the present day (see Prirogine and Stengers (1984)). Each

time some breakthrough was reached, far-fetched claims about the general applicability of the new theory or model cropped up. Similarly, objections by serious scientists against such claims have been heard as often as the claims themselves. Even the critics must however concede that nonlinear dynamics, chaos theory and studies of complex systems have been making solid contributions to fields of physics (e.g. Ott *et al.* 1994), meteorology (e.g. Lorenz, 1963), and ecology (Bulmer 1994, Lindgren and Nordahl, 1994) to name but a few.

Leaving aside both the exaggerated claims and the often acrimonious responses, the aim of this paper is to explore the possible implications which techniques and insights gleaned from nonlinear dynamics, chaos theory and studies of complex systems may have for the study of the intestinal microbial ecosystem and its interaction with the host. To achieve this, the meaning of the phrases "nonlinear", "chaos" and "complex" within this context will be defined. The discussion of these topics is presented without any attempt at mathematical rigour. Those interested in a more rigorous discussion are referred to Ott *et al.* (1994), or for the more philosophically minded Prirogine and Stengers (1984) and Kauffman (1995). It will then be shown that both the microbial ecosystem and the host's immune and digestive system all meet the necessary conditions to be called complex nonlinear dynamical systems. The types of behaviour which such systems may exhibit and the means to study them are explored. Two approaches to study the intestinal microflora and its interaction with the host follow naturally from this discussion: (i) computer simulation of the system, and (ii) time-series analysis of series of measurements to measure degrees of chaos and (un)predictability. There have been some attempts at the first approach already, notably by Freter *et al.* (1983), who made a mathematical model of the competition for food substrate and binding sites in a continuous flow model of the intestine. Many other types of interactions (both antagonistic and mutualistic) exist within the intestinal microflora, and it should be possible to model many of these. In this paper a pilot study, using computer simulation of the interaction between the aerobic and anaerobic compartments of the microflora, is presented. This simulation lends further support to the idea that a qualitative and quantitative theoretical understanding of a number of features of the intestinal microflora can be obtained through computer simulation. Finally, an outline of a research programme to explore the interaction between microflora and host with techniques from nonlinear dynamics and complexity studies is sketched.

Theory

What are nonlinear dynamical systems?

Probably the most important contribution of Newton and Leibnitz to science is the introduction of the concept of dynamical systems. In physics almost any system under study, whether planetary orbits, semiconductor electronics, or the Earth's atmosphere, may be considered a dynamical system. A dynamical system is a simply system which can be characterized by (a) a set of parameters the values of which define its *state* at a given point in time, and (b) a set of mathematically specified rules defining the change of state of the system in time. These rules are generally specified as differential equations, defining the rate of change of each of the parameters describing the system, as a function of the current state of the system.

This definition is very broad indeed, and many systems in biology, medicine, economics and the social sciences may be described and studied as dynamical systems (e.g. Prirogine and Stengers 1984, Kauffman 1994). A well-known example of this is the Lotka-Volterra predator-prey model ecosystem. The set of numbers describing this systems consists of (i) the number of predators, and (ii) the number of prey. The rules specify that the number of prey increase at a rate proportional to the number present (exponential growth) in the absence of predators. When predators are present the number of prey caught, which is proportional to both the number of predators and

the number of prey, must be subtracted. The predators starve in the absence of prey (exponential decay) and grow proportionally to the number of prey caught (again proportional to the product of prey and predator numbers). This system may show damped, undamped and increasing predator-prey oscillations. By specifying the initial conditions (e.g. from observation) and solving the differential equations involved, it is in principle possible to model or predict the future behaviour of the ecosystem.

The sequence of states the system passes through in time is called its *orbit*. If the system is dissipative, i.e. it loses energy in some way (and most systems do), the orbits converge to one of a small subset of all possible states called an *attractor*. The simplest kind of attractor is a single point: the system becomes stationary. The system is said to be at rest or in dynamical equilibrium. Another type of attractor is called a *limit cycle*: the system oscillates at a stable frequency and amplitude. A system may have numerous attractors, and the initial conditions determine to which attractor the system will converge. The set of initial states for which the system converges to a particular attractor is called the *basin of attraction* of that attractor. The Lotka-Volterra type ecosystem may have either a point attractor, i.e. the populations become stable, or a limit cycle attractor, i.e. predator-prey oscillations remain stable (e.g. Bulmer 1994, pp.39-45).

Depending on the kind of rules specified, dynamical systems are either linear or nonlinear. In a linear dynamical system the differential equations are linear, which means that the effects of changes in the state of the system are additive and proportional to the magnitude of the changes. The result of changing multiple parameters simultaneously is simply a superposition of the change in each individual parameter. The additive nature of changes to the system means that different parameters of the system may each be studied separately. Furthermore, the linear nature of the of the equations ensures that, given an initial condition, the orbit of the system is uniquely defined. This means the system is time-reversible and predictable: past and future may be deduced with arbitrary precision from the present state. Furthermore, the attractors are guaranteed to be simple, and the equations can be solved quite readily (even with paper and pencil in small systems).

Because of all these features, linear systems have been studied most. Before the advent of electronic computers, mathematical simplicity was one overriding reason to study linear systems, but a more subtle reason may have been equally important (Prirogine and Stengers, 1984). The uniqueness of the orbit lent credibility to the idea of a Cartesian, clockwork universe. All conditions were set at the time of creation, and the clockwork mechanism of Newtonian mechanics would automatically see to the rest. Unique orbits also provide complete determinism, which is not guaranteed to exist for nonlinear dynamical systems. Besides, it was (and is) argued that many nonlinear systems (such as the simple pendulum) can be approximated by linear systems to such a degree that there is no need to solve the more complicated nonlinear form.

By contrast, in nonlinear systems, changes in multiple parameter need neither be additive, nor proportional to the magnitude of the changes. The effects of changing individual parameters cannot in general be studied separately as in the linear case. Furthermore, the orbit of a system need not be unique for a given initial condition. In such cases bifurcations occur: places in the orbit where two possible future paths are open to the system, and no deterministic means exists to choose between the two paths. An element of randomness creeps back into the mechanics (Prirogine and Stengers, 1984).

In many nonlinear systems with more than three parameters which can be set freely (or *degrees of freedom*), an effect called *chaos* may occur. Probably the most famous example of chaos has been found in meteorology, where Lorenz (1963) has shown that deterministic, but highly irregular flow patterns exist within weather systems. When chaos occurs the attractor cannot be described by simple forms such as limit cycles, straight lines or points; the attractor has a *fractal*

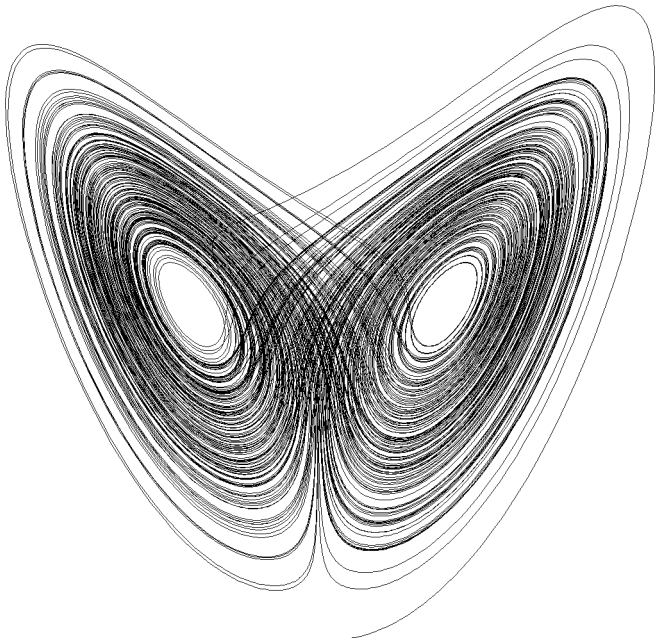

Figure 1. An example of the fractal shape of a strange attractor: the Lorenz-attractor, which may (roughly) be considered as consisting of an infinite set of different oscillations which the system may go through.

shape (Figure 1). A fractal shape shows detail at every possible magnification. Attractors with this peculiar property are usually called *strange attractors*. These attractors can be thought of as (roughly) the union of an infinite collection of limit cycles, with the system switching very rapidly between them. The corresponding motion (or orbit) may appear to be random and look something like Figure 2. Figure 2 shows the motion of a simple spring and magnet system (Moon and Holmes, 1979). The base of the spring is forced to oscillate at some frequency ω. The displacement of the end of the spring as a result of all forces is highly irregular, and yet it is not noise. The system is still deterministic. In fact, figure 2b is not a series of measurements, but the result of a computer simulation using the set differential equations describing the system, so it cannot contain truly random noise. This type of seemingly random, yet fully deterministic behaviour is one of the hallmarks of chaos.

Another hallmark is the so called “butterfly effect”: change the initial conditions of wind speed in the global weather by an amount corresponding to the beat of a wing of a butterfly in Peking, and the path of a Caribbean hurricane is altered, because the change introduced increases exponentially. In chaotic systems, infinitesimal changes in initial conditions propagate exponentially in time, resulting in drastically different outcomes from infinitesimally different initial conditions. This means that future and past cannot be deduced with arbitrary precision or for arbitrary periods in time from measured data, which always contain some finite error. It is possible to determine a degree of chaos: the Lyapunov exponent. This is a number which determines the doubling rate of the error in the prediction. If it is low the system is not very chaotic, and medium to long term predictions remain accurate over considerable periods of time. If it is high, errors increase rapidly, and only very short term prediction is possible. Other measures of degree of chaos exist, most notably the *fractal dimension* of the attractor, which is a measure of the complexity of the shape of the attractor. The more complicated the attractor, the higher the degree of chaos.

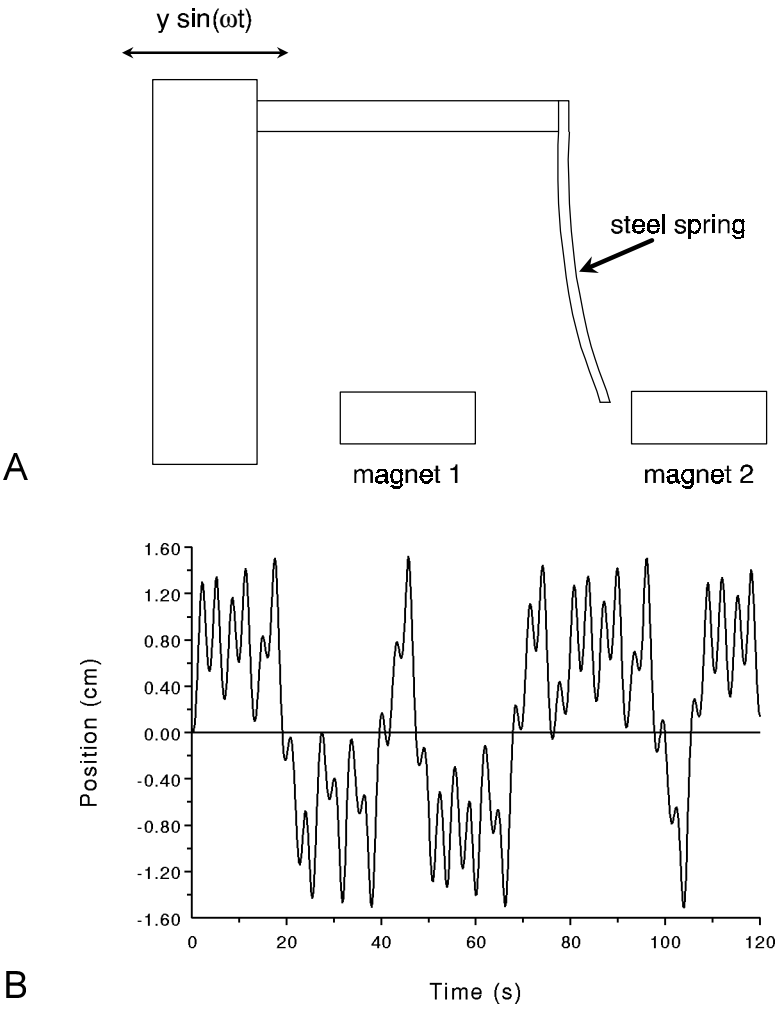

Figure 2. A simple system showing chaos (after Moon and Holmes, 1979): (a) diagram of apparatus, showing a steel spring suspended between two magnets; the top of the spring is forced to oscillate sinusoidally, (b) the graph shows the chaotic motion of the lower end of the spring.

Can microbial ecosystems be described as nonlinear dynamical systems?

Growth of bacteria, either single species (Monod, 1950), mixed cultures (Gerritse *et al.* 1992), or complete ecosystems (De Wit *et al.* 1995) can be described in terms of dynamical systems. The key feature of the dynamics of these systems is that they show autocatalytic or inhibitory loops: the presence of a bacterium is needed to make more of that kind bacterium (obviously). Furthermore, species A may inhibit species B by secretion of toxins. Species A might also enhance growth by production of metabolites which serve as food for B, or may remove substances toxic to B form the ecosystem. In systems which are far from thermodynamical equilibrium, such autocatalytic and inhibitory loops produce just the type of nonlinear dynamics which can produce highly complicated and chaotic behaviour (Prirogine and Stengers, 1984). In practice, all ecosystems are far from thermodynamical equilibrium, since large fluxes of energy or food pass through them; only death (a point attractor of any ecosystem) corresponds to thermodynamical equilibrium.

For these reasons it may be assumed that techniques for analysis and modelling of nonlinear dynamical systems in general are appropriate tools for the study of bacterial ecosystems, including the gut microflora.

Chaos and control systems

As odd as it may seem, the presence of chaos may be an advantage in control systems, if rapid responses are required. Chaotic systems would seem to be utterly unreliable, given their extreme sensitivity to initial conditions. Yet, as, e.g., Ott *et al*. (1990) have noted, that same sensitivity allows a control mechanism to control the system with very small corrective signals, provided the developing chaos can be analysed rapidly, i.e. proper feedback is available. Very small adjustments have large effects.

This may be of particular importance to biological control systems. Changing the mode of operation of, e.g. heart, nervous system or immune system rapidly, and without the expenditure of large amounts of energy is literally of vital importance to practically any organism. The constant feedback and small corrective steps to keep a such systems in the correct mode are probably not such a drawback, since the expenditure of energy can be small for chaotic systems. Chaotic dynamics have indeed been observed in, e.g., heart rate variations (Goldberger *et al.* 1984), though there is still some debate about the significance and meaning of these findings (Kaplan and Talajic, 1991). It has been observed that a *reduction* in variability (and possibly chaos) of heart rate may indicate heart disease (Kaplan *et al*. 1991, Skinner *et al.* 1991). On the other hand, fibrillations seem to be highly chaotic in nature, with high fractal dimension (Garfinkel *et al.* 1992). Too much chaos is uncontrollable.

It has also been claimed that chaos is present in electroencephalograms (EEG). Here too, there is quite a lively debate about the reality and relevance of chaos (Bullock *et al*. 1995, Pritchard *et al.* 1995). Nonetheless, the fractal dimension of the attractor has been used as a measure of complexity of the EEG patterns. Stam *et al.* (1994) found that normal controls had significantly ($p<0.001$) more complex EEG patterns than patients with Parkinson's disease. Theirs in turn was significantly ($p<0.001$) more complex than EEGs of patients with Alzheimer's disease.

What are complex systems?

The "Sciences of Complexity" deal with systems which may show complicated behaviour, stemming from the behaviour of a large number of entities which themselves show a simple behaviour. The complexity does not stem from complex rules, but rather from the large number of entities or subsystems the system is made of. An objection which has been raised is that the term complexity has not been defined particularly strictly (Horgan, 1995). Indeed a number of (more or less conflicting) definitions have been given, yet these definitions are mainly aimed at measurement of complexity, i.e. assigning a number to it. Whatever the conflict about how to measure complexity, the basic premise that complex systems are systems which are made up of large numbers of simpler objects is agreed on by all those working in the field. Any ecosystem can of course be considered as such a system, being built up of large numbers of individual organisms, each of which may show a a far simpler behaviour than the whole system. Similarly, the immune system may also be considered to be a complex system in this sense, since it is comprised of many cells which themselves exhibit rather simpler behaviour than the whole.

Having said this, what can actually be gained by calling ecosystems or the immune system "complex"? Do complex systems share certain properties which may be exploited to give extra insight into the behaviour of, e.g., the gut microflora and its interaction with the immune system? Several studies indicate that such common properties do exist (Langton 1989, 1992, Kauffman 1995). The most important feature is probably that such systems show global, coordinated behaviour, without the presence of any distinct "global controller": self-organization. Though an ecosystem might show Lotka-Volterra type predator-prey oscillations, there is no external driving force which creates this; no "invisible hand". Similarly, the immune system has no "chief lymphocyte" which directs an immune response, neither has the brain a "chief neuron"

in which central control of all behaviour is located. The behaviour of all such systems is collective, but not under any "Stalinist" rule, nor need any of the entities involved be aware of the nature of the collective behaviour. Secondly almost all such systems are nonlinear system: given the large number of interactions in such systems, some are bound to nonlinear. Given that, and the large number of entities (and therefore degrees of freedom), such systems are almost certain to show chaotic behaviour under a wide range of conditions.

Complex systems may show roughly four types of behaviour (Langton 1989, 1992): (i) steady state, (ii) periodic, (iii) "complex", and (iv) highly chaotic. Steady state is the simplest: the system is frozen into a particular state. Though there may be some initial oscillations, these die out and the system settles down into its final state. There is a gradual transition into the periodic regime: initial oscillations persisting for longer and longer times until they become effectively infinite. Even though the system is oscillating, the spatiotemporal structure may be thought of as fixed, non-adaptive. Both the steady state and periodic classes of behaviour may be thought of as solid. Conversely, in the highly chaotic regime, no oscillations persist, and no structure is apparent at all. Though determinism might be present, the degree of chaos is so high it is indistinguishable from stochastic behaviour. The system might be thought of as being in a gaseous phases. As such, the system is not adaptive either, it is just a constant mess.

The most interesting behaviour is seen at the borderline between order and chaos, which might be thought of as a phase-transition between the solid and the gaseous phase. At this borderline, periodic oscillations may persist for long periods of time, or may vanish almost instantly. Definite structures may propagate through space and time, and produce complex interactions where they meet. It has also been shown that such systems, balanced on the "edge of chaos" can perform computing tasks: manipulation, storage and transmission of data. On the edge of chaos they are neither so rigid that manipulation or transmission is impossible, nor so chaotic that stored and transmitted data are scrambled. The systems can become truly adaptive. It is an attractive, but as yet unproven conjecture of many workers in this field that all living systems (single organisms and ecosystems alike) are balanced on the edge between order and chaos, since it is only on this edge that sufficient order is present for homeostasis, along with sufficient chaos for adaptive behaviour (Langton, 1992). There is a number of theoretical studies which suggest that evolution indeed drives the evolving entities to this edge (Kauffman and Johnsen 1992, Kaneko and Suzuki 1994).

Self-organized criticality and power-law spectra in complex systems.

It has been claimed that complex systems may show what has been called "self-organized criticality": a situation in which the slightest disturbance may cause either large or small cascades of events. The classical example of this is a large pile of sand, each grain on the surface of which is *just* held in place. Toss an extra grain of sand on the pile and you may see anything may happen from just a trickle to a huge avalanche (Bak *et al.* 1988). Similarly, in an ecosystem, the introduction of a new species (or a mutation in an existing one) may cause mass extinction or no effect whatsoever. In fact, if many species are present in the ecosystem, it becomes very hard to introduce new species. Usually, they fail to colonize. Occasionally however an intruder may wipe out practically all others.

According to Bak *et al.*, self-organized, critical systems may show shifts in behaviour at all scales, but not all magnitudes of shifts are equally likely. Small changes (small trickles) are more likely than large ones (avalanches). The likelihood (p) that a shift of a given magnitude (A) occurs is given by a power law:

$$p(A) \propto A^{-\nu} \qquad (1)$$

This equation implies two things: (i) catastrophes cannot be prevented in such a system, and (ii) the rate of occurrence of catastrophes at given magnitudes can be predicted from small scale events. This power law is seen by some as a hallmark of self-organized criticality (Bak *et al.* 1988), others claim such systems do not actually show a power law (Horgan, 1995), but that very large scale events occur at lower rates than predicted by equation (1). Whatever the final outcome of that discussion, if some law may be postulated which, like equation (1), can predict the frequency of occurrence of large magnitude shifts in a dynamic system from the rate of occurrence of small magnitude events, this may become a diagnostic tool. If certain large scale shifts in the microbial ecology of the intestine are associated with disease, their rate of occurrence might be predictable from the normal, nonpathological population dynamics. If this is the case, modulating the dynamical behaviour of the flora, rather than its mean composition might become a goal of therapy. At this point in time, this idea is still very much speculation, yet there are ways to verify it. If we can determine the short to medium term (and therefore small to medium scale) fluctuations in the gut microflora of healthy volunteers, and we find a power law distribution, we can then try to predict the rate of occurrence of large scale shifts relating to certain well defined pathological situations, for which good epidemiological data are available, and in which the gut microflora is assumed to be involved in its aetiology. A good agreement between predicted and measured data would lend support to the thesis that the population dynamics of the gut microflora are involved causally.

Which techniques have been developed to study complex, nonlinear dynamical systems?

A number of different tools to study complex, nonlinear dynamical systems has been developed in the last decades. All rely on the availability of moderate to large amounts of computing power. The methods can be divided into two categories: (i) (time-series) analysis of observations, and (ii) computer simulations: science on the edge between theory and experiment.

The first set of techniques attempts to detect the "fingerprint" of nonlinear, deterministic behaviour in measured time-series. If the data are of sufficient quality, it is possible to distinguish chaotic from stochastic behaviour (Theiler *et al.* 1992). The degree of chaos may be determined be measuring Lyapunov exponents (Eckmann *et al.* 1986, Parlitz 1992), or fractal dimensions of the attractor (Grassberger and Procaccia, 1983, Brandstater and Swinney 1987). With lower grade data, spectral analysis, to measure the frequencies of shifts of different magnitudes can be performed, to see whether power law relationships are evident (e.g. Bracewell, 1986). All kinds of time series analysis described here do need larger numbers of points than are usually obtained in e.g. patient studies of the microbiology of the intestinal microflora. Some tens of sample points should be available per patient. This precludes the use of classical culturing for these types of analysis, for all but the wealthiest researchers.

The other set of tools consists of computer simulation techniques or "experimentation *in silico*". Computer simulations allow theorists to visualize what should happen if their theories concerning complex systems are correct, or which parameter settings have the most profound influence on the system's behaviour. Computer simulations by themselves do not tell us anything about the real system, they tell us something about our theories concerning the system. Without computer simulations theories of all but the simplest systems are hard to interpret in a quantitative way. Especially in the case of complex, nonlinear systems, it is virtually impossible to say how the system will behave, given a set of experimental conditions. However, if quantitative models of each of the system's components are available, it is possible to create a computer program which could mimic the behaviour of the real system. By running such programs many times with many different settings of experimental parameters, it is possible to gain a great deal of insight into the behaviour of the system. Comparison with *in vivo* and *in vitro* experimental data

must of course be performed to see whether the behaviour of the model system is anything like the real system.

Computer models come in two different basic types: *tactical* and *strategic* (Levins 1968). A tactical model strives to explain as much detail as possible of a specific system for prediction or control purposes. The results of simulations of such a model can be highly accurate, but are not widely applicable. By contrast, strategic models are more or less qualitative. They cannot predict the behaviour of a specific system in detail, but they can explain the kinds of behaviour a class of systems sharing certain features may show. Insight, rather than prediction and control is the ultimate goal of such models. The results of these kinds of simulations are not at all numerically accurate, but they are widely applicable. Most modelling in theoretical biology is of the strategic type (Bulmer 1994). A number of tactical models have been used within the field of microbiology (Jahnke *et al.* 1982, Gerritse *et al.* 1992, De Wit *et al.* 1995), usually applying to ecosystems of limited complexity. An example of more complex modelling is Cybermouse, a model murine immune system (Sieburg 1990, 1993).

The "spatial vs. chemical detail" trade-off

When modelling an ecosystem it is of course impossible to capture all detail. Tracing every single cell's interaction with every chemical is beyond the power of any computer on earth. Some intelligent simplifications are needed. When designing such a simplified model, the most important trade-off is that between the spatial resolution required and the number of (chemical or microbial) species in the model. Models can in fact be classified based on the spatial/species resolution ratio.

At one end of the spectrum are those models which model "chemistry" in high detail, but do not show any spatial detail. Usually these models are *connectionist* models, using complicated graphs (food-webs) to define the interactions between various species within the system. Such models may be used for well mixed chemostats (e.g. Gerritse *et al.* 1993), and can be used to model complex chemistry (Bagley and Farmer, 1992) or food webs (Lindgren and Nordahl, 1994). Leaving out spatial detail may be safe enough if the ecosystem is fairly homogenous, yet there is one caveat. In a study of gypsy moth population dynamics, Wilder *et al.* (1995) found that chaos occurred when spatial detail was omitted. If spatial detail (and consequently diffusion) was included, highly regular travelling waves were seen instead. Diffusion was capable of damping out chaos, and changing it to regular behaviour.

On the other side of the scale are cellular automata: (usually rectangular) grids of simple "chemostats" of limited complexity, each interacting only with its nearest neighbours through simple rules. Such systems can show high spatial detail, at the expense of biochemical realism. Nonetheless, as extremely abstract systems they lend themselves to strategic modelling of spatial self-organization processes, such as can occur in reaction diffusion systems (Markus and Hess 1990). As microbiological systems can often be seen as reaction diffusion systems (e.g. Blackburn and Blackburn 1993), it is reasonable to assume that some spatial detail must be included.

Many biological models are somewhere in between the two extremes, e.g. showing one (vertical) spatial dimension for microbial mat communities yet show a great deal of biochemical realism (De Wit *et al.* 1995). De Wit *et al.* could predict the vertical spatial distributions and diurnal cycles of coexisting cyanobacteria, purple sulphur bacteria and chemotrophic sulphur bacteria in a microbial mat community, based on detailed knowledge of metabolisms, light absorption, division rates, etc. The computations could be carried out on a simple personal computer. The success of such work strongly suggests that at least a strategic model could be made for the intestinal microflora. With considerably more computing power, and considerable input from *in vitro* measurements of microbial physiology, a tactical model could possibly be made.

An experiment *in silico*

A computer simulation has been run, using a program developed as a pilot study within the ISGNAS research program. A full description of the computer program, its capabilities and the simulations run on it is in preparation. The model intestine consists of a 6 m long axisymmetric tube of varying diameter. The first 4.98 m are the small intestine, with a radius of 1 cm; the next 18 cm are the "caecum" (radius 5 cm), followed by a "colon" of 84 cm long and 3 cm radius. The lengths and radii may be varied at will. The intestine is subdivided axially into 100 sections and radially into 10 concentric shells. Each of the 1000 volume elements may be considered a separate "chemostat" coupled to its neighbours by transport mechanisms. Continuous laminar flow and diffusion are the transport mechanisms modelled to date. Extensions for peristaltic motion may be included later. Apart from up to 6 "species of bacteria", 2 "chemical substances" are included in the model: food and oxygen. Though I will use the phrase species, each type of bacterium represents a whole category of bacteria, all of which share an aerobic or anaerobic metabolism. This means that each "species" can metabolize a far wider set of food substrates (lumped together as one substance "food"), than a single species in reality. Within each category, mutualisms, such as the use of metabolites of the one species as substrate by others, means that the effective yield of biomass per unit of substrate should be higher than in a true single species. The metabolism of each species was modelled using Monod equations with modifications for (i)

Table 1. Parameters describing a bacterial metabolism and numerical values for the three "species" used (derived from Gerritse *et al.* [1992]).

Symbol	Meaning	strict anaerobe	facultative anaerobe	strict aerobe	Units
μ_O	max. aerobic growth rate	$-1.0\cdot10^{-4}$	$4\cdot10^{-4}$	$6\cdot10^{-4}$	/s
μ_{an}	max. anaerobic growth rate	$1.0\cdot10^{-4}$	$0.75\cdot10^{-4}$	0	/s
μ_{basal}	basal metabolic rate	$1\cdot10^{-5}$	$1\cdot10^{-5}$	$1\cdot10^{-5}$	/s
K_F	aerobic food saturation uptake rate constant	$2\cdot10^{-2}$	$2\cdot10^{-2}$	$2\cdot10^{-2}$	mol/l
$K_{R,O}$	anaerobic food saturation uptake rate constant	$1\cdot10^{-6}$	$1\cdot10^{-5}$	$1\cdot10^{-5}$	mol/l
$K_{T,O}$	oxygen kill rate saturation constant[1]	$1\cdot10^{-6}$	$1\cdot10^{-5}$	$1\cdot10^{-5}$	mol/l
κ_O	max. oxygen kill rate	$1\cdot10^{-6}$	0	0	/s
α_O	efficiency of aerobic metabolism	1	1	1	
α_{an}	efficiency of anaerobic metabolism	1	1	1	
α_κ	fraction of killed bacteria returned as food[1]	0.5	1	1	
β_μ	max. respiration oxygen uptake rate	$1\cdot10^{-9}$-$1\cdot10^{-7}$	$1.5\cdot10^{-4}$	$1.5\cdot10^{-4}$	/s
β_κ	max. oxygen uptake rate as toxin	$1\cdot10^{-9}$-$1\cdot10^{-7}$	0.0	0.0	/s

[1] Value has no influence on outcome if oxygen kill rate and uptake rate are zero, but causes divide by zero errors if set to zero itself.

a basal metabolism, and (ii) mutual hindrance at high population densities. The model metabolism of each species is determined by 12 parameters, the meanings and values of which are summarized in table 1. The model metabolism is a slight variation of that used by Gerritse *et al.* (1990). All concentrations are given in mol/l: food and all bacteria in moles of organic carbon, oxygen simply in moles of molecular oxygen (O_2). To convert to numbers of bacteria, it was assumed that the volume of a single bacterium was 10^{-15} l (i.e. a maximum of 10^{12}/g), and that they contained roughly 10% w/w of organic C. This yields a conversion factor from mol/l to bacteria/g of about $1.2 \cdot 10^{11}$.

Using the above model, experiments were done to simulate colonization in a sterile intestine. One or two species of bacteria, selected from three available types (strict aerobe, facultative anaerobe and strict anaerobe), were introduced into a sterile intestine, in which the oxygen concentration of the lumen was in equilibrium with the walls (0.1 mmol/l). The input of food, oxygen, and bacteria was in block waves with a 40% duty cycle. Food concentration at maximum was 7 mol/l, oxygen concentration 0.1 mmol/l, and in most experiments the food inflow contained a maximum of $1.2 \cdot 10^3$ bacteria/g of each species. Though this may be a bit high, runs with only 12 bacteria/g showed virtually identical results, so evidently this parameter is relatively unimportant in the initial colonization phase.

Figure 3 and Table 2 summarize the results of the simulations. When strict anaerobes were introduced simultaneously with either facultative anaerobes or aerobes, the latter colonized within 1 day, reaching a maximum at day 4. After this, they were replaced by the anaerobes, which only appeared in any numbers at day 3. After 5 to 6 days a stable equilibrium was reached with strict anaerobes outnumbering facultatives or aerobes by 2.4-2.7 ^{10}log steps. Small oscillations caused by the periodic input of food remained visible. Once stabilized, the population did not change if the influx of bacteria from the “stomach” reduced to zero, thus they had colonized the lumen.

Facultative anaerobes by themselves could colonize in high numbers in the absence of strict anaerobes (2.2×10^{11}/g). Strict aerobes could colonize by themselves, but only in modest numbers compared to facultatives (1.1×10^9/g). By contrast, none of the strict anaerobes tested could colonize in the absence of bacteria with an aerobic metabolic ability.

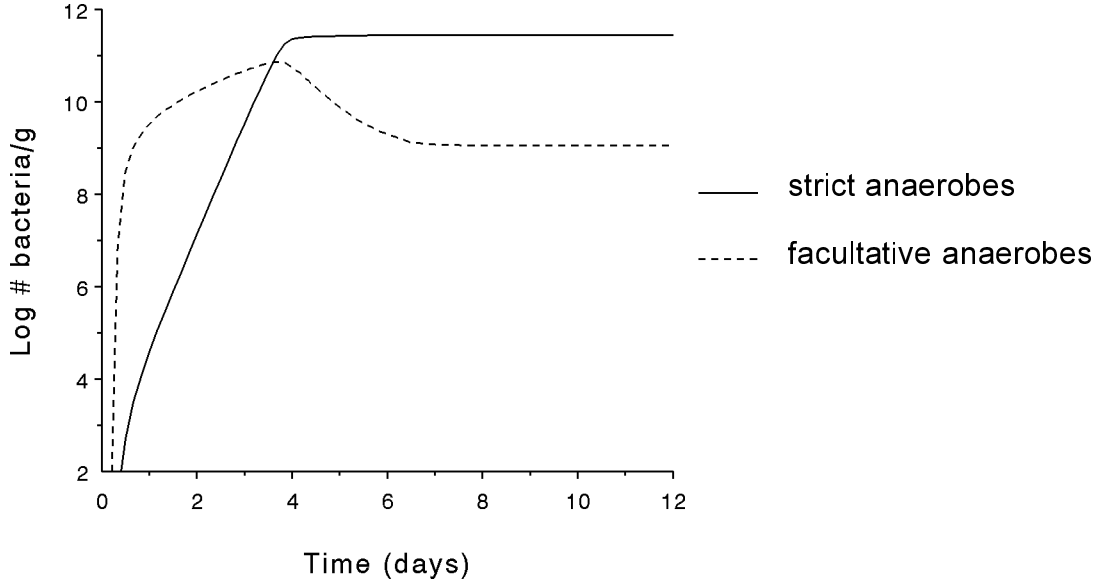

Figure 3. Colonization process in a di-associated sterile intestine modelled by computer simulation. Equal numbers of two species of bacteria (one strict and one facultative anaerobe) are fed into the sterile intestine, which contains an initial oxygen concentration of 0.1 mmol/l. Initially, the facultatives colonize, later, as oxygen levels drop, the strict anaerobes outcompete the facultatives.

Table 2. Mean numbers of bacteria per gram at equilibrium, 12 days after colonization, for a mono- or di-associated model intestine.

Mono-associated with:	Small intestine (^{10}log)	Large intestine (^{10}log)
strict anaerobe[1]	$4.42 \cdot 10^2$ (2.65)	$6.77 \cdot 10^2$ (2.83)
strict aerobe	$1.04 \cdot 10^8$ (8.02)	$1.10 \cdot 10^9$ (9.04)
facultative anaerobe	$7.73 \cdot 10^8$ (8.86)	$2.22 \cdot 10^{11}$ (11.35)
Di-associated		
strict anaerobe +	$1.65 \cdot 10^9$ (9.22)	$2.8 \cdot 10^{11}$ (11.45)
strict aerobe	$1.02 \cdot 10^8$ (8.01)	$4.42 \cdot 10^8$ (8.65)
strict anaerobe +	$1.66 \cdot 10^9$ (9.22)	$2.8 \cdot 10^{11}$ (11.45)
facultative aerobe	$9.871 \cdot 10^7$ (7.99)	$9.89 \cdot 10^8$ (8.99)

[1] Does not represent colonization, as the maximum input density of bacteria was $1.2 \cdot 10^3$/g (mean $4.8 \cdot 10^2$/g), and when the input density was reduced to zero, all anaerobes were washed out of the intestine with 3-4 days.

A second experiment started with the stable mixed populations at t=12 days found with the first experiment. At that point, the aerobic fraction of the microflora was eliminated and the influx of aerobes halted, as a (crude) simulation of selective decontamination of the digestive tract. Depending on the oxygen uptake rate of the anaerobes (both β-parameters in Table 1), the populations could remain stable, even in the total absence of aerobes. Only if the inhibition of growth and destruction of bacteria required less than 1.2×10^{-8} mol O_2 per mol C bacterial biomass did the population become unstable and die out due to the increased oxygen concentration. The extreme sensitivity to the value of both β-parameters (Table 1) is shown in figure 4. When both are set at 1.2×10^{-8}, the flora remains stable, but at 1.1×10^{-8} a steady decline does set in after 4 or 5 days, and at 1.0×10^{-8} the decline starts 2 days earlier.

To test the stability of the ecosystem to perturbations around this critical point, the supply of food was altered in two ways: (i) above the stability threshold the period was increased while

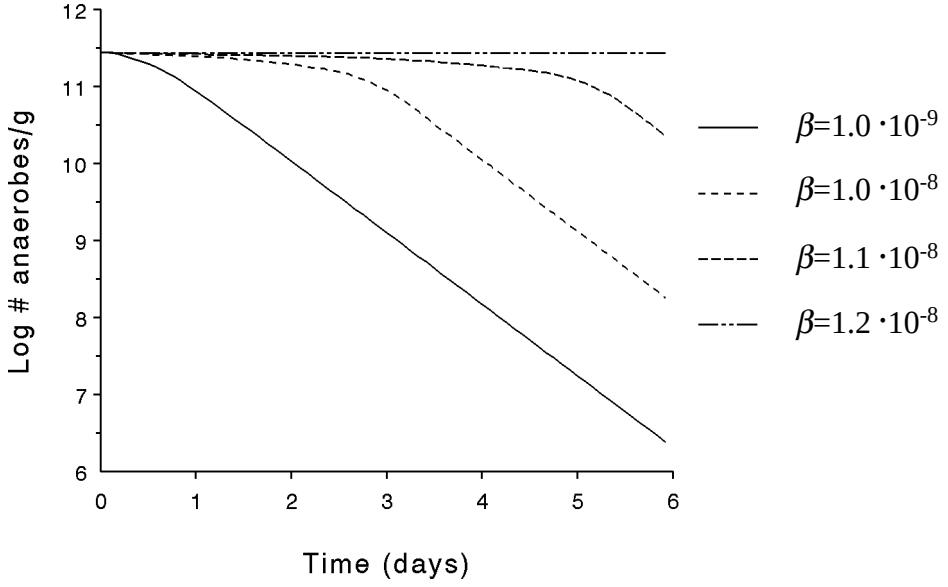

Figure 4. The survival of strict anaerobes when all facultative anaerobes and aerobes have been removed: depending on the oxygen uptake parameters $\beta=\beta_\mu=\beta_\kappa$ (Table 1) the bacteria survive or die out.

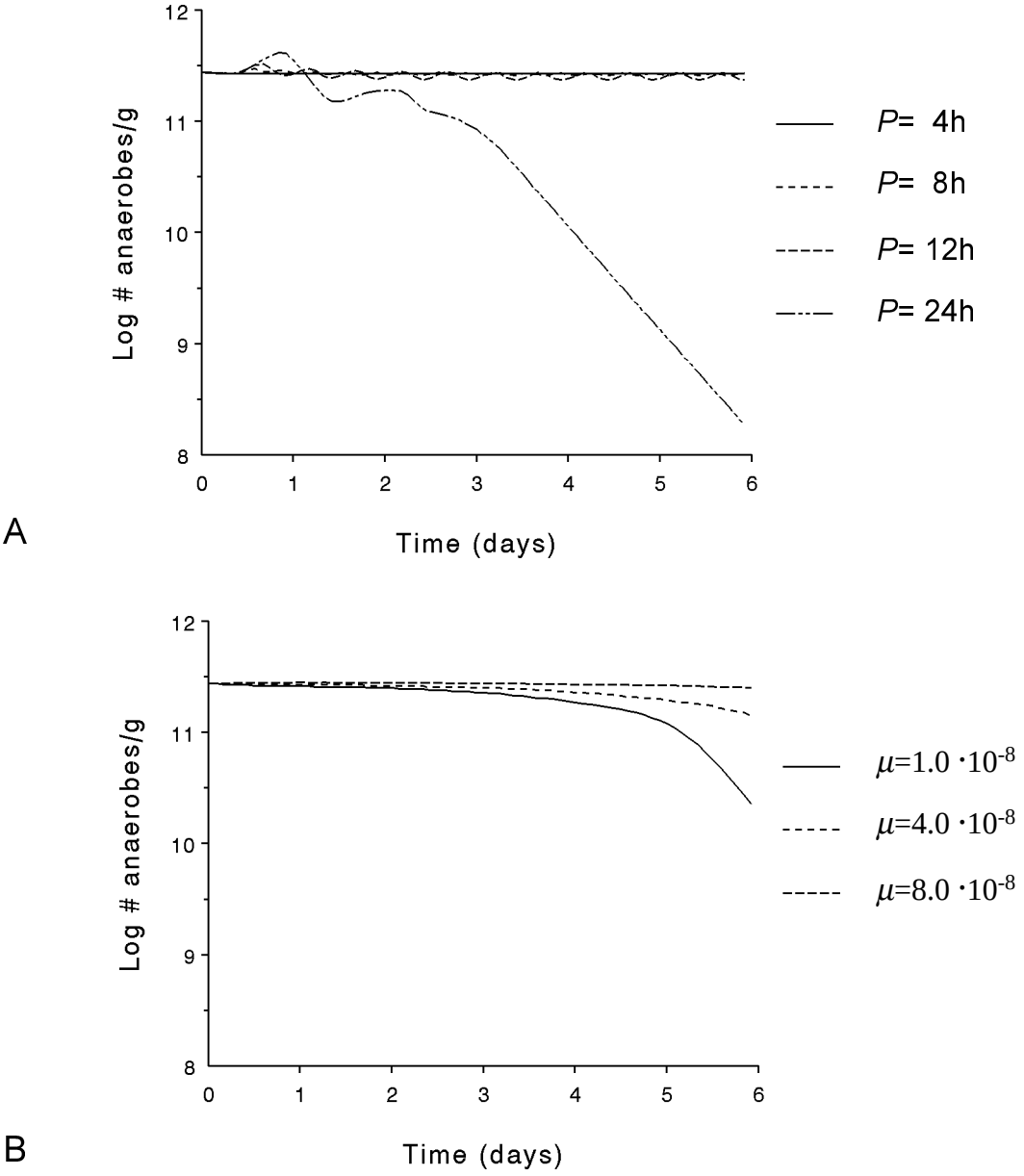

Figure 5. Modulating the survival of strict anaerobes when all facultative anaerobes and aerobes have been removed: (a) increasing the period P of the food supply cycle for the survivors in figure 4 (β=1.2 x 10^8) causes increasing oscillations which destabilize the population; (b) increasing food supply through mucus production (μ) increases survival for bacteria with β=1.1 x 10^8.

retaining the same total food supply (i.e. a few large amounts of food in stead of many small amounts), and (ii) below the stability threshold increasing the production of mucus.
Figure 5a shows the results of the first perturbation. As the period between meals increases, the oscillations in the population density increase, which is expected in many types of damping systems. When food is supplied only once a day, the oscillations become so large that the population becomes unstable and dies out. Figure 5b shows the results of the second experiment. With increasing food supply, the survival increases, though in this experiment no permanent survival was observed.

Discussion

In my view, no "leap of faith" is needed to describe the intestinal microflora and the immune system as complex, nonlinear dynamical systems. In fact, it is merely a generalization of the modelling work of, e.g. Freter *et al.* (1983). Once this is accepted, it is a logical step to use nonlinear time series analysis techniques and computer simulation as tools to study these systems. Computer simulation is probably the only way to verify that certain models work, i.e. explain observed data, in any system with more than 3 interacting objects when it is not in an equilibrium state. Computer simulation can distinguish the essential from the accidental parameters. Used properly nonlinear dynamics may tell us both how to interpret our data within the framework of a complex model (i.e. a lot of simple interacting objects), and which parameters should be observed to distinguish between competing models.

What might we learn from nonlinear dynamics in the intestinal microflora?

Here we enter the realm of speculation. Leaving aside a number of "ifs", computer simulation and time-series analysis might give us insight into the following issues:

- Under which conditions does the microflora become more or less self-regulating?
- If we extrapolate the power law spectrum of the population dynamics (if it exists), could we explain the occurrence of certain intestinal disorders as a consequence of this power law? If so, this could lead to preventive therapy: can we modulate the flora to change the power of the power law?
- What is the link between the power or fractal dimension of the time series and the number of species in the flora? Does this conform to the conventional notion of some 400 species?
- how does all this influence colonization resistance, i.e. can we predict colonization resistances from population dynamics?
- What role do bacteriophages play?
- What attributes does a bacterium need to survive in the intestines?
- How do the mechanics (intestinal motility, lumen viscosity, etc.) influence the spatial and species distribution?
- What role does the immune system (e.g., modelled using CYBERMOUSE) play in modulating the flora?

These issues (and probably a lot more) can of course not be resolved by computer modelling work alone, but should be addressed by a concerted effort, incorporating the development of new theories and more accurate methods of observation. High quality data will be essential for the nonlinear analysis approaches to work. The problems with cultural counts can however be surmounted with a number of techniques, such as measurement of microflora associated characteristics (MACs) (Midtvedt, 1985) and digital image analysis (Meijer *et al.* 1991, Wilkinson *et al.* 1995) especially in combination with 16S rRNA targeted fluorescence *in situ* hybridization (Langendijk *et al*. 1995) and measurements of metabolic activity (Nwoguh *et al.* 1995, Gribbon and Barer, 1994). Such techniques promise to deliver both the data quality and achievable sampling rates needed for the kind of analysis envisaged.

What has been learned from the pilot study?

First of all it should be stated that no true chaos was observed in any of the simulations. Secondly, a number of things may be learned from the omissions in the model. Adherence sites on the epithelium were not modelled, yet in the absence of a true mucosal flora attached to the wall, a luminal flora could become perfectly stable. Evidently, bacteria *can* colonize the lumen without colonizing the mucosa. Without an immune system reasonable ratios of aerobes to anaerobes were found. Thus, it is reasonable to assume that the immune system does not in fact regulate

this ratio, but that the reduction of oxygen by aerobes creates an anoxic environment, in which they are outcompeted for food by strict anaerobes. Far from being a new idea, this has already been suggested by (e.g.) Meynell (1963), Schaedler *et al*. (1965) and Schaedler (1973) on the basis of experimental data. However, none of these authors could give a estimate of the magnitude of the effect on theoretical grounds.

Apart from the final numbers and ratios, sequence of the colonization in figure 3 is very reminiscent of the colonization of the gut of germ free and new-born mice (Schaedler *et al*., 1965, Schaedler 1973), where the "normal" flora (fusiforms, *Bacteroides*, etc.) are preceded by the coliform facultatives. For about 2 days, the facultatives dominate the strict anaerobes, after which the anaerobes outcompete the coliforms. The difference between these observations and the simulation lies in the lactobacilli and lactococci, which are the first to appear in new-born mice. However, many lactobacilli grow readily at high oxygen levels (even in air (Schut, personal communication)), and do not lower redox potential (Eh) (Meynell, 1963). Furthermore, they are not thought to enter into direct substrate competition with the coliforms, fusiforms, *Bacteroides*, etc. (Schaedler *et al*., 1965). Thus, they may not interfere with the type of interaction modelled in this experiment.

Selective decontamination could lead to a destruction of highly oxygen sensitive anaerobes, even when totally resistant to the antibiotics used. This effect should be larger in patients with reduced mucus production due to epithelial damage than in healthy volunteers. On the other hand, the oxygen uptake by anaerobes need not be unrealistically high (Gerritse *et al*. 1992) to retain a perfectly stable anaerobic flora when all aerobes have been removed. Even in these cases it is likely that the anaerobes become more sensitive to extra stress factors, such as a residual antibiotic resistance.

Since the main supply of oxygen in the large intestine is the diffusion through the mucosa, oxygen availability should not change with food supply, to a first order approximation. Therefore, if the host is starved or if little or no fibre is contained in the diet, a shift in ecological balance towards a more aerobic flora is expected. This may suggest that an increased risk of intestinal overgrowth by aerobic pathogens during malnutrition can exist, even before the immune system is affected. Similarly, if the mucosa is damaged the diffusion of oxygen may increase, causing an increase in the numbers of aerobes, which in turn may result in more damage. This type of vicious circle may be considered an attractor in dynamical terms. Viewed in this way, an aerobic infection may contain a form of self-organisation, the bacteria creating the conditions for their own success. Furthermore, if the epithelium is damaged by irradiation or chemotherapy, both the production of mucus and the oxygen uptake by the epithelium may be impaired. Both effects should contribute to an increase in aerobic bacteria. Both in man and in mice such an increase has been observed after irradiation (Van der Waaij, 1978).

Concluding remarks

More work, *in vivo*, *in vitro* and *in silico*, is needed to show whether the tentative conclusions drawn from this pilot study hold up. A more complicated model, taking more microbial and chemical species into account, and the inclusion of receptors on the intestinal epithelium, an immune system, etc., are needed for the *in silico* part of the work. Simultaneously, the data analysis techniques reviewed here should be used to examine data from *in vivo* measurements.

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