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Is growth controlled by a hierarchical system?

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A functional view of growth inevitably leads one to consider how it is controlled. There is much information on control mechanisms that regulate growth at different levels of organization within an organism. Some evidence is presented to establish that different types of control exist. However, our interest is in how the operation of these different control mechanisms might be co-ordinated, as it is clear that they can not operate autonomously. We consider a hierarchical arrangement of control systems, some of the implications for understanding normal growth processes, how it might have evolved and an interpretation of system failure.

KEY WORDS:—Control systems – growth – hydroids.

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INTRODUCTION

Growth is the product of numerous physiological processes whose activities are controlled and linked in such a way that an organism's development follows a precisely predictable course. For a long time those who studied growth were

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concerned almost exclusively with the cumulative outcome of these processes. This can be termed the 'extrinsic' view of growth, in that it is the study of growth from without, dealing chiefly with the lifelong pattern of cumulative increase in biomass with time. The major features of this approach were embodied in D'Arcy Thompson's *On Growth and Form* and later were summarized by Medawar (1941, 1945).

Although this accumulation may be the most obvious manifestation of growth, synthesis of new tissue does not necessarily lead to increase in mass, because processes involving breakdown and loss of mass occur simultaneously and continuously. The view of growth as the integration of these two processes is partly due to Weiss & Kavenau (1957), to whom growth was "the net balance of mass produced and retained over mass destroyed and otherwise lost". Bertalanffy (1960) similarly saw growth as "the result of a counteraction of processes of anabolism and catabolism". An understanding of this turnover of materials and the consideration of organisms as hierarchical, open systems, sustained by the flux of materials within and through them (Bertalanffy, 1952) led to an interpretation of growth in a physiological sense, rather than as simply a changing state. From this functional viewpoint, and the belief that the principle of homeostasis must also apply to growth processes, came the first dynamic model incorporating some of the principles of control theory and negative feedback (Weiss & Kavenau, 1957; Kavenau, 1960).

Evidently the control organisms have over their physiological processes enables them to survive and exploit variable environments. Regulation of processes being disturbed by the variation of exogenous factors is achieved by control mechanisms incorporating the principle of feedback. In its simplest form such a system consists of a few basic components. The process or condition of the system is monitored by a sensor, and information about the output is continuously related to some preferred condition by way of feedback loops. The difference between them constitutes an error which the control elements work to minimize (Foerster 1958; Milsum, 1966). Thus the operation of the control mechanism counteracts the effect of perturbations that tend to deviate the process from its 'preferred' course (for a discussion of the teleological implication see Stebbing, 1981a). These principles have been incorporated in numerous models designed to simulate the growth of whole organisms (Hubbel, 1971; Calow, 1973, 1976; Stebbing & Hiby, 1979). In reality, the regulation of growth processes is much more complex and is the outcome of a multiplicity of control systems, regulating different processes in response to various preferenda at a number of organizational levels within organisms. Furthermore, it is evident that within metazoa the various control mechanisms involved in regulating growth must be linked in such a way that their activities are co-ordinated for morphogenesis to proceed as it does; none operate autonomously, except in a disease condition. Although there is much uncertainty, we will nonetheless consider in a conjectural manner the various forms of growth regulating mechanism and how they might be arranged to account for their observed behaviour.

Such an approach does not appear to have been attempted for two main reasons. First, because investigation of the different kinds of growth regulatory mechanisms occur in different fields of research; they are different 'paradigms' (Kuhn, 1970) with their own individual techniques, concepts and models and

therefore tend to be isolated from one another. Second, because any hypothetical framework that might be proposed must be speculative, as the mechanisms involved are unknown. Nevertheless, a holistic view is not only justifiable, but is in some senses essential. If the different kinds of control systems, at present seen largely in isolation, are considered as subsystems of a larger much more complex system, we can consider the likelihood that the complete system has attributes that cannot be anticipated from a knowledge of the constituent subsystems. It seems probable that some of an organism's properties—in health and in disease—are likely to be due to the arrangement and links between subsystems, rather than properties of the subsystems themselves.

We first consider briefly some of the evidence that growth is regulated by feedback mechanisms at different levels in response to different kinds of preferenda, before discussing their integration within a hierarchical system and how they might have evolved.

Models that purport to simulate growth processes are of a number of kinds, but we concentrate on those incorporating negative feedback, as this is the principle that underlies all self-stabilizing systems that operate to optimize some state or rate. While all systems where the output effects input have feedback, we are concerned primarily with those that operate within an organism or a species, involve active control and incorporate real feedback loops; rather than those which Calow (1976; pers comm.) terms "fictitious feedback" loops of the kind that often exist between species.

TYPES OF GROWTH REGULATION

Control of rate

All growth is attributable to the biochemical events resulting in the synthesis of new molecules, and control of these processes is therefore the most fundamental kind of growth regulation. A rate of biosynthesis is determined by a complex of hundreds of closed loop control systems (Mesarovic, 1968) that regulate the rates of reactions. Some are simple single loop systems, where the product of a sequence of reactions controls the rate at which it is produced by inhibiting the activity of an allosteric enzyme catalysing an earlier reaction (Fig. 1). Thus the intracellular concentration of a metabolite governs its rate of synthesis. The first examples of "end product inhibition" were described by Yates & Pardee (1956) and Umbarger (1956); many more complex closed loop biochemical control mechanisms have been described since.

Although most of the elements of these control mechanisms have long been known, analytical techniques sufficiently rapid and sensitive to observe the output of the mechanisms directly have only become available in recent years. Changes in concentrations can now be measured continuously against a time scale marked in minutes, and the temporal behaviour of some biochemical systems is now precisely defined (Chance *et al.*, 1973; Lloyd, Poole & Edwards, 1982).

The most appropriate technique for investigating control system behaviour is to perturb the controlled process in order to elicit a regulatory response from the control mechanism (Milsum, 1966). This method has recently been applied to the study of growth control in hydroid colonies (Stebbing, 1981a), where growth can be perturbed by low levels of metals and other toxic agents. When

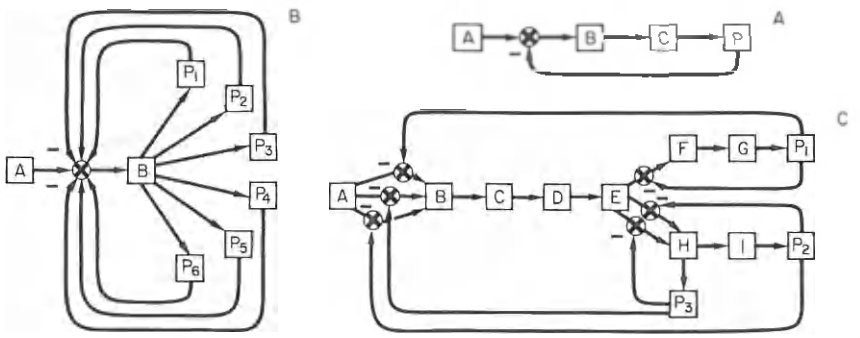

Figure 1. Examples of closed loop control mechanisms involved in the regulation of biosynthetic pathways. Letters indicate intermediate metabolites and Ps represent the various end-products of each sequence of reactions, which are identified for each pathway as follows: (A) P L-isoleucine; (B) P_1 carbamyl phosphate, P_2 glucosamine 6-phosphate, P_3 adenosine monophosphate, P_4 cytidine triphosphate, P_5 histidine, P_6 tryptophan; (C) P_1 tryptophan, P_2 phenylalanine, P_3 tyrosine (after Lehninger, 1975).

changes in specific growth rates (increase in biomass per unit time per unit biomass) attributable to the perturbation are filtered out, the output of a control mechanism is apparent with its characteristic oscillations of decaying amplitude following low levels of disturbance or a step input (Fig. 2). 'Relaxation' oscillations follow the removal of a load to which the control mechanism has equilibrated (Stebbing, 1981a). Similar effects are seen in the specific growth rate of perturbed populations of *Hydra* (Stebbing & Pomroy, 1978). Simulation models confirm that our experimental data are probably the output of a rate-sensitive feedback mechanism, in that its major features can be reproduced by a non-linear system with proportional-integral control (Stebbing & Hiby, 1979). However, some acclimation experiments and other data suggest that a more

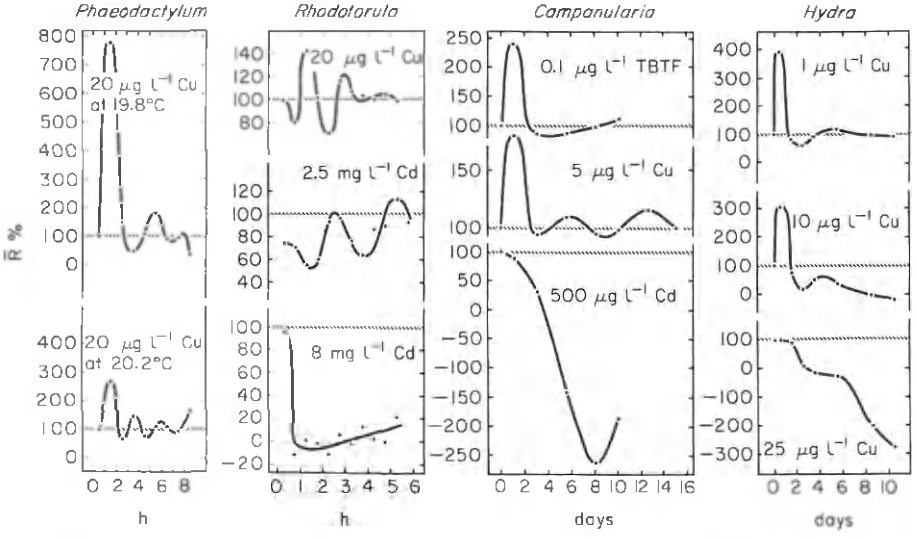

Figure 2. Examples of the oscillatory behaviour of specific growth rates (R%) in perturbation experiments with toxicants that are characteristic of the output of closed-loop control mechanisms. Data are from experiments with cell suspensions of *Phaeodactylum* (microalga) and *Rhodotorula* (yeast), colonies of *Campanularia* and budding populations of *Hydra*.

complex system is necessary to reproduce all the features of our data (Stebbing, 1981a).

The experiments do not indicate at what level of organisation within the organism such control mechanisms might operate (Fig. 2). If the data indicate the activity of biosynthetic control systems it must be assumed that the different systems involved are synchronized by the experimental perturbation, or that the action of one loop or subsystem predominates, obscuring the activity of others.

Similar perturbation experiments with suspension cultures of a marine yeast (*Rhodotorula rubra*) and the microalga (*Phaeodactylum tricornutum*) show similar behaviour following addition of small amounts of a toxicant (Fig. 2). If the same type of control mechanism is being observed in each case, its occurrence in experiments with cell suspensions suggests that a cellular or subcellular control mechanism is responsible, rather than some system peculiar to the hydroids, or to the yeast or the microalga. However, there is no corroborative evidence yet that biosynthetic control mechanisms could be responsible, or that the same hypothesis might apply to the different species investigated. Nevertheless, recent experiments with young *Mytilus* (Almada-Villela, Davenport & Gruffydd, 1982) and *Rhithropanopeus* larvae (Sanders & Costlow, 1982) exposed to temperature perturbation provide similar evidence of autoregulation, as do our own preliminary experiments on the effects of temperature change on *Rhodotorula* (Stebbing & Brinsley, unpubl.).

Whatever biochemical or other mechanism is responsible for regulating growth in this way, it must have a capacity to resist perturbation that can be related to the range of levels of a disturbing factor against which growth can be maintained at approximately its preferred rate. Thus for *Campanularia* 1 $\mu g\ l^{-1}$ is the lowest level of copper that initiates a counteractive response, while above 10 $\mu g\ l^{-1}$ growth is inhibited, indicating that there is overload. The counteractive capacity for copper is therefore $1-10\ \mu g\ l^{-1}$ and provides a measure of the homeorhetic capability of growth control mechanisms (Stebbing, 1981a). This is similar to the concept of "growth energy" (Medawar, 1940) which is measured in terms of the amount of an inhibitor required to suppress growth completely.

It may be asked why growth processes should be controlled in terms of rates of change, rather than some measure of state. Control systems incorporate sensory mechanisms which provide information continuously about the condition of the controlled process. One reason for rate-control being more suitable is that sense organs tend to be more sensitive to relative levels of what is detected over short periods of time, than to absolute levels. Thus greater precision is possible in maintaining constancy if the control mechanism is responding to rates rather than states. Furthermore, quicker response times and more rapid equilibration are possible because an alteration in a rate of change is detectable before there is a significant change in state. It seems appropriate therefore that rate-sensitive control mechanisms regulate growth processes at lower levels of organization where processes are more rapid than at higher levels. There is evolutionary advantage in achieving a rapid response, since it enables organisms to exploit changing or variable environments more effectively.

It is usual to assume that the growth of an organism is likely to be as great as the nutrient uptake and other significant variables will allow, and that

maximum growth rates indicate optimum conditions. However, control mechanisms typically regulate at preferred levels which are submaximal; homeostasis is achieved by mechanisms that stabilize and optimize, rather than maximize. Following perturbation of a controlled process oscillations of stabilizing control mechanisms often exceed their preferred levels; this can be seen in the examples given in Fig. 2 which seem to be the output of rate-sensitive mechanisms controlling growth. Sometimes such behaviour results in significant cumulative increases in the size of organisms or cultures (Stebbing, 1981b, 1982).

This raises the question considered by Calow (1982) that while the maximization principle is a reasonable initial assumption, it becomes necessary to ask how could homeostatic mechanisms have evolved that regulate processes at submaximal levels? It seems that the advantage that homeostasis confers on organisms is a capacity to resist the impact of environmental variations upon their physiology, since this makes it possible to survive in environments where significant factors have great ranges of variation. That capacity can be expressed in terms of the ability of a control mechanism to resist the impact of some factor which tends to deviate controlled processes from their preferred levels, and can be measured in terms of a range of levels over which effective control is maintained before overload becomes apparent (as at 8 mg Cd l^{-1} for *Rhodotorula*, 500 µg Cd l^{-1} for *Campanularia* or 25 µg Cu l^{-1} for *Hydra* in Fig. 2). The survival value of such a capability is clearly seen in the capacity of an organism like *Campanularia* living in estuaries to counteract the physiological impact of reduced salinity (Stebbing, 1981a), because its homeostatic capacity determines its upstream distribution.

Why then do homeostatic mechanisms regulate at submaximal rates? This is because the capacity to counteract perturbations that impose an inhibitory load, for example, is due to a stimulatory counter-response which depends upon the residual capacity for growth. Thus organisms that might grow at their maximum rates would have no residual capacity, or margin, between actual and potential growth rates with which to counter inhibitory load. Conversely, those organisms that grow at submaximal rates have a capacity to counteract inhibition or sustain load that is related to the difference between actual and potential growth rates.

Control of cell density

The growth curves of cell suspensions of various taxa—bacteria, microalgae, yeasts and protozoans—follow similar sigmoid growth curves (Pirt, 1975). As these curves become asymptotic some limitation must be imposed upon the growth potential of the system, since sub-cultured cells soon resume exponential growth. Limitation undoubtedly occurs, but whether this is intrinsic and self-imposed or due to the lack of requirements for further growth remains unclear.

Mammalian cells in culture, whether in monolayer or suspension culture, eventually stop dividing, yet remain healthy and quiescent (Holley, 1975). With cell suspensions this usually happens at what is called 'saturation density' and in monolayer culture when a contiguous sheet covers the available substratum. This is referred to as 'contact growth inhibition' and is effected by some signal passing between cells in contact. Such auto-inhibitory behaviour is analogous to

that observed in communities of sessile organisms (Stebbing, 1973) and may have evolved similarly as an adaptation to minimize intraspecific competition for space.

Reviews dealing with the control of division of mammalian cells (Holley, 1975; Leffert & Koch, 1977) have not discriminated clearly between what are described as growth regulators, or factors, that are essential for growth, or those agents that are primarily information carriers and have evolved specifically to control cell division.

One essential pre-requisite of the growth model of Weiss & Kavenau (1957) and many others designed to simulate control of cell division is a specific inhibitory regulator (Rose, 1958; Iversen, 1969; Good, 1972, 1973; Shymko & Glass, 1976; Toivonen & Rytomaa, 1978; Bard, 1978, 1979; Onda, 1979; Britton, Wright & Murray, 1982), which controls cell multiplication by limiting division. The most likely agents for this role appear to be the chalones, which correspond to the "anti-templates" of the Weiss & Kavenau model. Chalones are unstable and short-lived compounds, known mainly by their activity rather than from their chemical nature. They are tissue-specific inhibitors of mitosis, acting within the tissue that produces them as part of a closed loop control mechanism (Iversen, 1976). Their action is inhibitory, but not toxic, in that their effect is short-lived and reversible. Originally their activity was demonstrated in epidermis (Bullough & Laurence, 1960) and later in muscle, liver, kidney and other tissues and organs. There may be other growth regulatory agents than chalones, but it seems likely that they are the most important group controlling cell division. Unfortunately, definitive identification of the chemical structure of a chalone is lacking, but absence of such evidence cannot deny their existence, and their activity is now known from numerous studies (Attallah & Houck, 1976). It is possible that difficulties in analysis may be due in part to the relative instability of chalones. However, the ability to break down, or be broken down rapidly, is an important attribute of an inhibitory regulator because it determines the time delay in any response to perturbation. Bard (1978, 1979) found it necessary to assume a half-life for a chalone of less than 12 h.

How to identify the process that is regulated remains a difficulty. While it may be clear that cell division is controlled, it is not certain whether the control mechanism of which chalones are a part, is regulating the rate of cell division, their density or number, or all of these. It is only when the controlled process is identified that experiments can be designed to observe directly the output of the control mechanism. Furthermore, it is not until then that the role of chalones in regeneration, wound repair and tissue hyperplasia can be clarified (Houck, 1976).

Control in relation to size

A sustained growth capability in organisms is illustrated by their capacity for regenerative growth, which is greatest in the lower invertebrates (Berrill, 1952) and decreases markedly in the vertebrate phyla (Goss, 1969). Clearly such growth must have some kind of feedback mechanism that operates by relating actual size to a preferred or 'goal' size. Much work on regenerative growth in the lower invertebrates has been done, particularly on the polychaete worm *Nereis*,

where regenerative growth is controlled by a neurohormone from the supra-oesophageal ganglion. Its removal arrests growth, whilst implantation of ganglia from other worms restarts it (Clarke & Scully, 1964). Likewise, removal of the hypophysis or pituitary in fish arrests their growth, whilst growth hormone induces growth in hypophysectomized fish (Ball, 1969). Similar experiments have been performed on rats where the administration of pituitary extract ends all symptoms associated with its removal. In man the effects of growth hormone deficiency are likewise eliminated by hormone administration (Tanner, 1975).

Growth hormone may have effects other than causing growth (Paladini, Pena & Retegui, 1979) and the systems that mediate its release and action are still unclear in higher organisms (Wallis, 1979). Tanner (1963) suggested a size-sensitive growth control mechanism in man. He thought that actual size is related to preferred size for a given age, and that the mechanism operates to minimize the difference between them. Evidence for this is shown by 'catch-up growth', seen when growth tends to resume its original trajectory on the growth curve after removal of the causes of growth inhibition such as malnutrition or disease (Prader, Tanner & Harnack, 1963). Work by Williams & Hughes (1975) does not support Tanner's hypothesis. Our work on catch-up growth in hydroids suggests that the data showing catch-up growth could equally well be explained as a consequence of the transient stimulations that a rate-sensitive control system follows as it 'relaxes' after removal of an inhibitory load (Stebbing & Hiby, 1979; Stebbing, 1981a). Nevertheless, work on pigs, cattle and other farm animals has shown that rates of growth and the asymptotic sizes are to some extent inherited traits, so growth regulatory mechanisms must have some genetic control. It is to be expected therefore that models to simulate growth should have 'goal' or preferred sizes (Tanner, 1963; Hubbell, 1971; Calow, 1973, 1976, 1977). However, it also seems likely that the controls which operate at the highest level not only control size in the absolute sense, or size in relation to age in Tanner's sense, but they must also be responsible for co-ordinating the harmonious growth of numerous tissues and organs. Control may be imposed partly in allometric terms to maintain the relative proportions of tissues and organs.

Control in relation to physiological load

That growth is related to functional demands has long been established; Morgan (1901) considered that an organ's activity promoted its growth and Boycott (1929) that hypertrophy and atrophy followed use and disuse. Goss (1964) proposed a theory of growth regulation by functional demand and thought that since organisms have form to serve their function, growth must be controlled by those functions. Furthermore, he suggested that growth regulation independent of functions could not have sufficient selective advantage to be genetically retained. He also postulated the existence of negative feedback mechanisms that regulate and adapt physiological performance of organisms—and thus their growth—in relation to extrinsic factors.

Evidence to support this is found in the literature on compensatory growth following tissue loss and occurs for example in the liver, kidney, some endocrine glands, lymphoid and haemopoietic tissues (Goss, 1964). Increase in physiological load, leading to functional overload, is not only found where tissue

is lost: for example, high protein intake increases liver and kidney growth, hypoxia stimulates erythropoiesis and hypertension enlarges the heart.

If functional overload—whatever the cause—stimulates growth, reduction in size of an organ or tissue with a proportionate reduction in physiological load should not. There is evidence for this, but because not all tissue is functional, 100% regeneration of tissue-mass is uncommon, while complete restoration of functional capacity is usual. Where functional demand lapses, organs may atrophy, so that organisms reared under aseptic conditions have poorly-developed lymphatic systems, just as salamander larvae reared in a hyperoxic atmosphere have diminutive gills and there is decalcification of bone tissue in the absence of gravitational load. This is an example of adaptive growth in bone, called Wolff's Law, which describes how bone is continuously being broken down and reformed in a form determined by the stresses and strains imposed upon it.

Goss concluded that tissues and organs are autoregulatory and that the feedback mechanisms probably employ inhibitory control. However, details of the mechanisms are sparse, although Goss thought that the mechanism responsible for the activation of a tissue or organ might also account for its adaptive growth following overload.

There are few tissues that do not enlarge in response to increase in physiological demand; many organs have been shown to be capable of much more growth than is typically manifest, for example, chronic functional overload of some endocrine glands can result in excessive enlargement. Nevertheless, however strong the evidence for the involvement of functional demand in the control of growth other factors must be involved, for example, in controlling growth through the prefunctional stages of embryological development.

Control of population density

It is self-evident that population growth is limited, but we ask to what extent have organisms evolved means to regulate or limit their own density? Wynne-Edwards (1962, 1963) proposed that organisms are adapted to control population densities in relation to fluctuating levels of resources by means of control mechanisms operating 'homeostatically'. He considered that, in the absence of such controls, the population would grow and increase in density to the extent that all the resources available would be rapidly utilized, resulting in the depletion of food organisms, for example, and in time a reduction in the carrying capacity of the system by its over exploitation. Instead, it seems that population densities in stable ecosystems are typically regulated by control mechanisms, in such a way as to prevent the population increasing to densities that would exceed the carrying capacity of the system. This rationale would seem capable of general application, but most of the evidence in support of the theory comes from work on the social behaviour and population dynamics of higher organisms; Wynne-Edwards dealt only briefly with lower and aquatic organisms (but see Chapter 23.1 in Wynne-Edwards, 1962).

The essential component of a density controlling mechanism is a link that provides information related to present density that is fed back, and has an influence on, future density in such a way that density is maintained at a level appropriate to the available resources. Those who adhere to the thinking of

Wynne-Edwards, hold that information about population size or density from social behaviour at the breeding site, and epideictic displays, impinges directly on reproductive behaviour, fecundity and thereby on recruitment. However, difficulties with altruism and group selection have resulted in Wynne-Edwards's thesis attracting much criticism. An alternative explanation, but with essentially the same end result is that a population density that is too high will cause a shortage of necessary resources, and the reproductive output of the individual is thereby reduced. In this way information which relates to population density reaches the breeding population indirectly, but without invoking the unselfish behaviour of individuals (Dawkins, 1976). Either of these explanations could apply to mechanisms of population regulation where social stress is thought to account for population limitation in mammals (Munday, 1961; Christian, 1963), but selection for specific adaptations is more difficult to account for.

Nevertheless, it does seem that specific adaptations may be involved in some organisms and provide the appropriate information about density for population control mechanisms. In higher organisms, particularly those inhabiting terrestrial environments, light and sound are good information carriers; they can be modulated in both amplitude and frequency and information can be transmitted directionally, often over great distances. In lower organisms, particularly those inhabiting aquatic environments, pheromones appear to have a role in the limitation of growth or reproduction by inhibiting these processes at concentrations above some threshold level, often

Table 1. Some examples of autoinhibition of growth or reproduction thought to involve specific inhibitors acting exogenously

Protozoa		
Keronopsis	Density-dependent regulation of fission	Walker, 1975.
Paramecium	Inhibitor of fission	Woodruff, 1913.
Tetrahymena	Two regulators of fission	Kidder, 1941.
Coelenterata		
Hydra	Inhibitor of budding	Davis, 1966.
Platyhelminthes		
Dugesia	Inhibitor of fission	Best *et al.*, 1969.
Nematoda		
Diplenteron	Inhibitor of hatching and development	Clark, 1978.
Crustacea		
Amphiascoides	Inhibitor of sexual reproduction	Walker, 1979.
Daphnia	Inhibitor of sexual reproduction	Warren, 1900.
Insecta		
Culex	Growth inhibitor of first instar larvae	Ikeshoji & Mulla, 1970.
Gastropoda		
Biomphalaria	Inhibitor of growth and maturation	Gazzinella *et al.*, 1970. Berrie & Visser, 1963.
Bulinus	Inhibitor of growth and reproduction	Wright, 1960.
Fossaria	Growth and egg deposition	Levy *et al.*, 1973.
Pisces		
Ameiurus, *Carassius*	Growth inhibition	Allee *et al.*, 1934.
Brachydanio, *Trichogaster*	Growth inhibition	Yu & Perlmutter, 1970.
Lebistes	Reduced fecundity	Rose, 1959b.
Amphibia		
Rana	Inhibition of tadpole growth and maturation	Akin, 1966; Licht, 1967; Richards, 1958; Rose, 1960. (but see Steinwascher, 1979).

exerting the greatest effect upon the smaller members of the population (Rose, 1959a). Such signals tend to be relatively inefficient, they carry little information, have to be produced continuously, are energetically costly, and can not be directed. But for the student of population regulation, these primitive systems seem to have the advantage that the passage of information by some water-borne pheromone can be investigated experimentally to establish its stability, its effect upon growth and reproduction and its species specificity. A number of examples indicating the breadth of evidence from different taxonomic groups are listed in Table 1. The phenomenon was reviewed briefly by Rose (1960) and used by Wynne-Edwards (1962) in support of his theories. However, Thomas (1973) has discussed critically the literature on specific inhibitors in aquatic species and concludes that there is no evidence that any population is regulated by their action. Nevertheless, many authors have published accounts of experiments involving inhibitory effects (Table 1), but evidence is lacking on the isolation and chemical analysis of the inhibitory agents, so that their action cannot be rigorously interpreted. Some authors have made attempts to do so, but the low concentrations and their inherent instability seem to deter their study. Nevertheless, they could provide much more suitable model systems for the study of density regulating mechanisms than those that depend upon behavioural studies of terrestrial species.

Specific adaptations with a role in population density control, whether they are behavioural or involve specific inhibitors, do require altruism for their selection. However, as Dawkins (1976) has reasoned, reciprocal altruism is selfish in the broader context. One might in this way account for the evolution of inhibitory regulators that are apparently to the disadvantage of the individual in the short-term, but to the advantage of the species in the long-term.

HIERARCHICAL CO-ORDINATION

Hierarchical control of growth

Biological systems are hierarchically arranged in various ways according to Woodger (1930), although here we are concerned initially with his "spatial hierarchy" of multicellular organisms. He distinguished cell components, cells and components of organisms consisting of cells, at three levels of organization below that of the organism itself. There are difficulties when one elaborates such schemes to include more levels, in that some components may then fall outside the hierarchy (see Bertalanffy, 1952). It is with this in mind that the hierarchical arrangement given here is not drawn as a branching structure—as in Woodger (1930)—indicating that while all elements at one level constitute the level above, an element does not consist exclusively of elements from the level below it (Fig. 3A).

It is evident, when considering the different kinds of growth regulatory mechanism, that some are peculiar to the levels of organization at which they operate. This suggests that it should be possible to propose a hypothetical system that relates not only to the spatial or structural hierarchy, but which also satisfies the functional properties of control of different kinds at different levels.

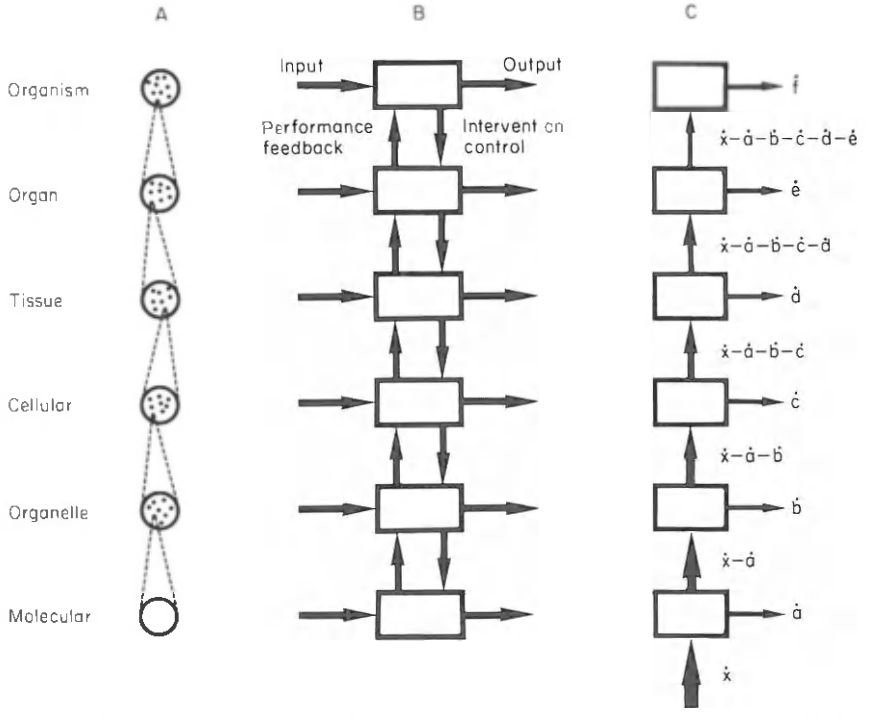

Figure 3. Hierarchical interpretations of the organization of metazoans. A, The "spatial" or structural hierarchy of Woodger (1930); B, a generalized functional hierarchy of Mesarovic *et al.* (1970) in which the subsystems are related to levels of organization; C, a similar diagram to indicate how the effect of a toxicant, for example, might cascade through a hierarchical system, its effect being dissipated as it does so by neutralizing responses of subsystems at each level acting homeostatically.

Growth control mechanisms at different levels of organization can be seen as subsystems of a larger system and must also have a hierarchical arrangement because of the spatial or structural hierarchy. For example, components of an organ clearly must be subject to control by whatever mechanism controls the organ itself, even though the components may to some extent control themselves. Thus we must consider a hierarchical system where the subsystems are semi-autonomous, they are subject to control by higher subsystems and exact control upon lower subsystems. Mesarovic, Macko & Takahara (1970) depicted the basic elements of such hierarchical systems (Fig. 3B) indicating the routes by which information and signals pass through it. Here we have related the hierarchy of control to the structural hierarchy (Fig. 3A) by assuming that control subsystems incorporate all control mechanisms operating at each level of organization. Each subsystem has its own input and output and is capable of responding to stimuli or load insofar as they relate to events localized to that level. Doubtless each is quite different, varying in complexity and the nature of its control over growth processes.

Mesarovic *et al.* (1970) have been responsible for defining and developing many of the theoretical principles of hierarchical systems in a way that seems appropriate and we have adopted their nomenclature here. Higher level systems

are supremal units and lower level systems are infimal units. In any two-level hierarchy, information about the performance of the infimal unit is passed upwards, while the supremal unit may impose what is termed intervention control, downwards. Each unit also acts independently of those above or below it, but the structure of the system does not permit complete autonomy. The occurrence of autonomy, as in neoplastic growth, indicates system failure.

Generally the rates and response times of the subsystems at higher levels are more rapid than those at lower levels. This property is perhaps related to the size of the system being controlled and the inertia, which tends to resist change and increases lag time. There are also good functional reasons for different control subsystems to have different lag times and frequencies, otherwise they would tend to interfere with each other's behaviour. At the biochemical level, oscillatory behaviour of glycolytic systems in yeast have cycles measured in seconds or minutes (Pye, 1973). At the cellular level we show, with perturbed suspensions of yeast and algae, oscillations with a cycle time measured in hours (Fig. 2). In the organs of the rat, oscillatory regenerative processes following partial hepatectomy have a cycle time of about a day (see Goss, 1964), while at the level of the whole organism data on 'catch-up' growth in man suggests a subsystem whose operation requires many months per cycle (Tanner, 1963). Thus it seems necessary for adjacent subsystems to operate at different frequencies to prevent interference with each other. A corollary is that, for such behaviour to be observed, quite different time bases are required for different subsystems.

Auto-regulation as manifest in the homeostatic or homeorhetic control of physiological states and rates, is the most universal property of life. Biologists tend to think of homeostasis in the sense that Claude Bernard expressed it: as the capacity to maintain the organism's internal environment more or less constant. It would be easy to assume that constancy itself had some adaptive advantage. More important is the advantage that homeostatic control mechanisms confer in counteracting the effect of those exogenous factors tending to disturb, or deviate, a process or condition from those states or rates best serving the requirements of the organism. This reasoning further suggests that the range of environmental conditions an organism might tolerate, and the habitats it might exploit, depends on the capacity of its homeostatic control mechanisms to maintain physiological processes within tolerable limits when under load. Each subsystem has a capacity to resist perturbations of the processes it controls. Physiological disturbances by low levels of toxic metals, for example, impinge first upon processes at the lowest level and they are counteracted by control mechanisms operating at that level. Whatever residual perturbation was not neutralized would pass upwards to the next level, where further counteraction would occur. It is clear that in the simplest case the disruption of biochemical events is likely to precede malfunction of the cell itself, just as the malfunction of numerous cells occurs before tissue function is impaired. Thus one can envisage perturbation cascading through such a hierarchical system, its impact being progressively dissipated by control subsystems operating at different levels of organization (Fig. 3C). Depending upon the severity of the perturbation, it may be completely dissipated at the lowest levels, or it may reach the highest level, affecting the organism as a whole. In principle it follows that the more levels, or the more stratified the organization of the organism, the greater its capacity to

neutralize exogenous perturbation and thus tolerate greater ranges of environmental variation.

Hydroid analogue of a two-level hierarchy

The hydroids have long been considered good model organisms for studies of growth processes. Braverman (see review 1974) at an early stage developed computer-driven models to simulate the astogeny of *Podocoryne*, conducted growth experiments that suggested control by a feedback mechanism and speculated on the nature of regulatory substances. Others have used the asexual reproduction of hydroids as models for neoplastic growth (Shostak, 1977, 1981).

A useful analogue of the simplest kind of hierarchical growth control system is afforded by comparing the control of specific growth rates and population density in hydroids. Earlier evidence was considered for the control of specific growth rates of colonies of *Campanularia* and populations of *Hydra* using toxic agents to perturb growth. Specific growth rates of perturbed colonies or populations as percentages apparently indicate the output of growth regulatory mechanism(s) whose behaviour as the system equilibrates can be followed.

Superimposed upon this system is another which regulates the density of members of a colony or the density of a population of *Hydra* (Davis, 1966). Work on colonial hydroids (Steinberg, 1954; Rose, 1957a, 1966; Tardent, 1963; Rose & Powers 1966) has led to the development of the concept of 'homologous inhibition' whereby chemical agents are produced by hydranths which inhibit the development of others nearby. A gradient of decreasing concentration away from the source permits the development of a homologous structure, once the level of inhibitor falls below some threshold concentration. Thus the spacing of members of a colony and their density on the substratum is regulated by specific inhibitors, which are produced and act internally within the system of stolons and uprights that constitute the colony. Although Fulton (1959) did not find active inhibitor acting endogenously, the evidence for control of colony formation in this manner remains strong.

Populations of *Hydra* in culture grow by budding at a more or less exponential rate, until population growth gradually slows and then stops. The culture remains healthy and reproductively quiescent until the density is reduced, or a few individuals are transferred to a new vessel, whereupon budding continues after a lag period (Stebbing, unpubl.). It has been shown that asexual reproduction in dense cultures is controlled by an inhibitor, whose activity has been demonstrated by exposing cultures to water from crowded cultures (Davis, 1966). The inhibitor was not identified, although it was established that it is a protein with a molecular weight between 5000–100 000 whose activity is destroyed by trypsin.

The elements and links of the system that is responsible for controlling specific growth rate and density in *Hydra* is indicated in the diagram (Fig. 4A) and can be considered the simplest type of "nested control hierarchy" (Bennett & Chorley, 1978). The idea of a hierarchical arrangement of complex control systems with more than two levels is best seen in a simpler representation (Fig. 4B) of the kind used by Mesarovic *et al.* (1970). Here the total system comprises two subsystems: a low-level subsystem with its own input and output, controlling specific growth rate (R) and a high-level system which limits

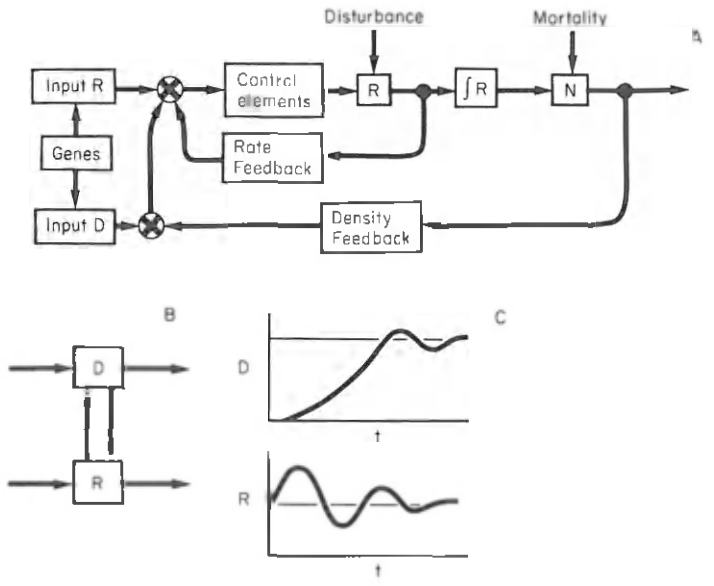

Figure 4. The kind of system that could account for the regulation of both specific rate (R) and density (D) in asexually reproducing populations of *Hydra*, seen both as a nested control mechanism (A) and as a two level hierarchy (B), together examples of the output of the two subsystems (C).

maximum density (D). Typical output of each subsystem is indicated. This simplified representation conveys the essential elements of any hierarchical arrangement of control mechanisms involved in regulating growth processes.

Evolution of hierarchical systems

Whatever the precise phylogenetic relationships between the major groups of invertebrates, the indisputable evolutionary trend has been the development of steadily increasing complexity. This has occurred through the addition of new levels in the hierarchical organization of organisms. It does not appear to have occurred by inserting new levels between existing ones, but rather by superimposing new supremal levels above existing ones in the hierarchy. We can extrapolate horizontally at the same level of organization between taxa as we do, because evolution has come about in this way (Fig. 5).

The constancy of biochemical processes, for example, in different phyla is remarkable, considering their morphological differences and, while there are inevitably some differences between phyla, biochemists extrapolate from bacteria and other micro-organisms to the mammals with confidence. Often the same molecule (e.g. that which carries genetic information) always has the same role throughout the animal kingdom and there are many other such examples (Kerkut, 1960). At higher levels of organization we see a constancy in subcellular ultrastructure and typically far greater differences exist within organisms than between them. Similarly the processes of meiosis and mitosis occur universally with little variation. Diversity has evolved with great economy throughout the living world by replication at different levels of the same basic units.

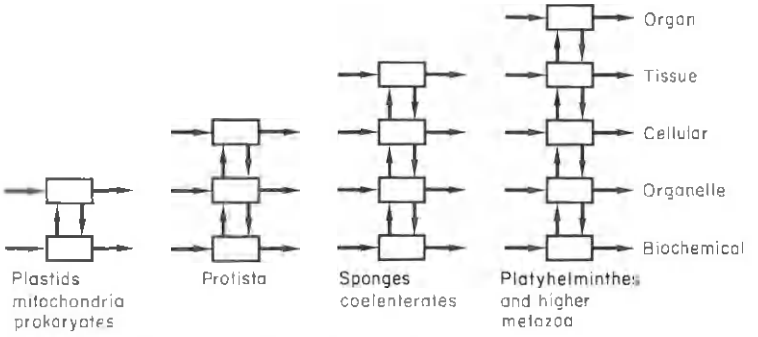

Figure 5. A scheme for the evolution of hierarchically-arranged levels of organization within organisms; the point being to suggest that new levels as they evolved were superimposed upon existing systems at the highest level, leaving those below relatively unchanged and intact.

We have already considered the idea that the role of autoregulatory subsystems responsible for homeostasis is to enable organisms to tolerate a range of environmental variations that would otherwise disrupt physiological processes. In multi-level systems, perturbation is likely to pass upwards from one subsystem to the next, until its effect is dissipated. Bronowski (1970) in his concept of "stratified stability" suggested a related hypothesis to explain the evolutionary trend toward increasing complexity. He postulated that stratification of biological systems into levels of organization (Fig. 4) provides stability against disturbance, and that the capacity to maintain stability increases with greater stratification. Thus, he argues that evolution consistently moves towards increased complexity because there is the adaptive advantage in having several levels of resistance to perturbation. Consequently a greater range of environmental variables influencing physiological processes may then be tolerated.

It is remarkable that in considering growth-regulatory mechanisms of different kinds, the most plausible mechanisms should involve specific inhibitors which control growth by limiting it (Rose, 1957b). For example, those that limit population size of single-celled species, the chalones and those involved in limiting growth or reproduction of some aquatic organisms (Table 1). We suggest therefore the possibility they had a common phyletic origin.

It is probable that each control subsystem evolved in association with the level of organization that it regulates, but it is possible that the elements of such systems are derived from earlier, more primitive systems. For example, it is likely that in early prokaryotes or protists, maximum density of any population was determined by the concentration of some excretory product or metabolite. From such precursors it is possible that pheromones evolved with the specific and exclusive effect of inhibiting mitosis in single-celled systems. Pheronomes that limit growth and/or reproduction in metazoa could readily be derived from such systems.

Another factor is involved here because, if it is to the organism's advantage to limit the maximum ratio of biomass to the volume of its aquatic habitat, the same end can be reached by limiting either growth or reproduction. A natural step with the evolution of metazoa is the possibility that inhibitory regulators,

that operated exogenously to limit density in single-celled populations, gave rise to the endogenous tissue specific inhibitor or chalone (Houck, 1976).

Evidence of the chemistry of the specific chemicals involved to support these proposed relationships is scarce. A number have been extracted, their stability under various treatments has been tested, in some an approximate molecular weight is given and in more than one case an inhibitory regulator has been identified as a protein. In no case does it seem that the molecular structure, or the actual identity of a regulatory inhibitor, is well enough known to indicate the specificity of its role, or its phylogenetic origins.

Much more effort has been devoted to the problem of identifying the chemical nature of chalones, because for a long time their existence was doubted in the absence of positive chemical characterization. However, there is now much more known about them (see Forscher & Houck, 1973, Houck, 1976) although major difficulties such as the contamination of chalone extracts by micro-organisms and non-specific inhibitors remain.

In metazoans, higher control subsystems tend to overlie and obscure the operation of subsystems operating at lower levels. Since evolution has resulted in additional levels of organization, superimposed at the highest level (Fig. 5); extrapolation laterally to more primitive organisms where the subsystem of interest is the supremal system is the usual course. The same end is often achieved by studying metazoa in cell or organ culture.

Failure of growth control mechanisms

Feedback mechanisms of the kind that are responsible for the control of growth processes can fail in various ways. The most obvious kind of breakdown might occur where the effector of control does not act on the target cells because the effector is not produced, does not reach the target cells or the target cells do not respond to the effector. The most plausible effectors considered here are inhibitors, whose specific effect is to control growth that in their absence would tend to be maximal. Clearly, failure of this kind, where a regulator does not reach its target, would result in autonomous growth, limited only by the energetic constraints of the system.

This can be demonstrated in cultures of mammalian cells which normally grow as a monolayer, cover the available substratum and then stop dividing. Neoplastic cells, on the other hand, continue to grow, forming a multilayered mass of cells, which rapidly exhausts the resources for further growth and then die. Intercellular communication in such cells is impaired by greatly increased membrane resistance, suggesting that division is normally limited by some inhibitory signal passing between the cells (Loewenstein & Kanno, 1966).

An apparently related example is provided by membrane-induced neoplasia. Subcutaneous implants of membranes, irrespective of their chemical composition, will produce sarcomas in some mammals (Bischoff & Bryson, 1964). Experiments with membrane filter implants has shown that the incidence of fibrosarcomas around the implant was inversely related to pore size (Goldhaber, 1961), highest frequencies occurring when filters without pores were implanted (Goldhaber, 1962), The implication is that such membranes have this effect because they act as a barrier, preventing the inhibitory regulator or chalone from reaching its target tissue, so that unlimited growth in the form of a

tumour follows. However, those observations also imply that a chalone is not produced by the tissue upon which it acts. This is incompatible with present thinking, unless perhaps the chalone for each tissue is produced by its epidermal layer. It is noteworthy that a number of post-mitotic tissues do not have an epidermis.

Neoplastic induction is due to the exposure of cells to some physical, chemical or biological agent that damages the genetic material in a heritable manner that impairs its own mitotic control mechanisms or its ability to respond to growth regulatory signals. Difficulties in interpreting neoplastic growth are due in part to the wide range of agents that may cause it, and the association of damaging or inhibitory agents with the stimulation of growth processes in the form of tumours.

The work we describe on the use of perturbation techniques to study the behaviour of control mechanisms (Stebbing, 1981a) provides an interpretation of growth regulation with which some of these features of neoplastic growth are entirely compatible. For example, the response of the control mechanisms we have studied (Fig. 2) is generalized, in that it is similar, whatever the nature of the inhibitor used to perturb their growth. The promotion of growth in neoplastic cells is to be expected from a control mechanism that limits growth by inhibition, because its failure to act, for whatever reason, must result in the stimulation of growth. Furthermore, the counter-response by the control mechanism to any inhibitory change is stimulatory, so failure to limit the response may once again produce the unexpected effect. Transient overcorrections by growth control mechanisms as part of the oscillatory behaviour, may result in hormesis or the stimulation of growth following exposure to low levels of toxic inhibitors (Stebbing 1981b, 1982).

It would be instructive to investigate failure in hierarchical control systems because 'intervention' control at any level is imposed from above. Neoplastic growth not only implies failure of some control subsystem, but it also suggests failure of its superior subsystem to impose intervention control. Quite separate from the question as to how such systems fail is the possibility that features of the behaviour of the hierarchy of subsystems are attributable, not to the subsystems themselves, but the way in which they are interfaced.

Burch (1976) proposed a theory to account for neoplastic growth, believing that such a theory must also explain normal growth and organization of control elements. It has a number of features we have considered, as well as some interesting differences. Briefly, the theory incorporates the principle of control by negative feedback (Fig. 6A), proposing that control is imposed by effectors which he calls mitotic control proteins (MCPs), and information about the size of the target tissue is transmitted to a central control element by the rate of release of affector signals called tissue coding factors (TCFs). Control over a target tissue, Burch proposes, is achieved by a series of such feedback loops of the kind depicted in Fig. 6B. It is clear that in each case control elements are removed from their targets, so careful consideration has been given to the problem of recognition of MCPs by target tissues and TCFs by control elements. Burch appears to assume that the control mechanisms are size-sensitive, responding to size, or size for a given age. Our belief is that size *per se* is unlikely to be the only factor to which growth control mechanisms are sensitive, and the hypertrophic and hyperplastic responses of tissues to changes in physiological load supports

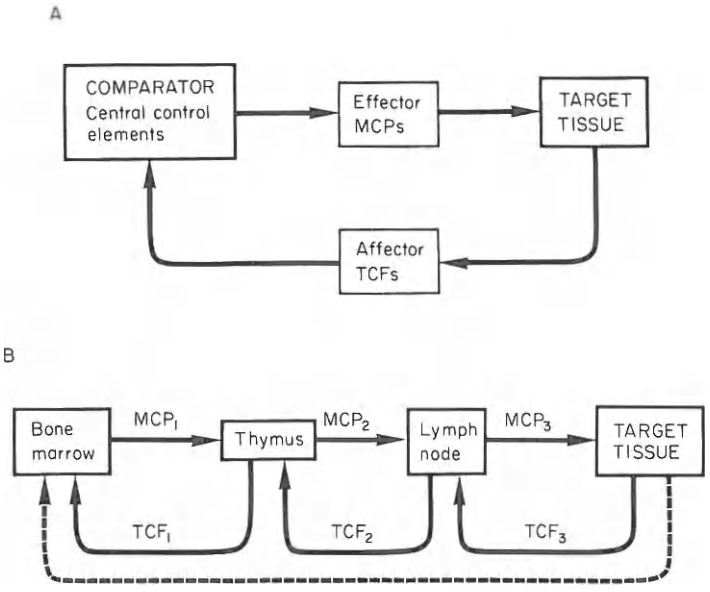

Figure 6. Hypothetical control mechanisms for the homeostatic control of growth proposed by Burch, showing the essential elements (A) and a series of proposed feedback loops (B). The diagrams have been adapted from Burch (1976).

this. Clearly the identification of factors to which control mechanisms are sensitive is crucial, as only then can attempts be made to measure their output. Also important is the belief, emphasized by Burch, that neoplastic growth should be regarded as a breakdown in biological organization, rather than a property of the cancer cell.

DISCUSSION AND CONCLUSIONS

One inevitable consequence of the sustained pursuit of a problem: the detailed investigation of processes and specialization, is an increasingly reductionist approach. Those who investigate biological systems tend to investigate processes at one level, concerning themselves, for example, at the infimal level with biosynthetic pathways, or at the supremal level with the hormonal control of growth. Each field of investigation has its own methodologies, terminology and concepts and constitutes a paradigm in the sense that Kuhn (1970) used the term. The flow of information within a paradigm is therefore inevitably far greater than that between them. The point is not that reductionism itself is limiting understanding, but that a hierarchical system, consisting of a number of subsystems associated with different levels of biological organization, is responsible for controlling growth (Fig. 4). Control of growth of the organism as a whole is a function of the co-ordinated operation of numerous sybsystems. One cannot expect that system behaviour should be the sum of the behaviour of the constituent subsystems, since many of its properties must be related to the way subsystems are interfaced.

The view of growth control, and the limitation of reproduction, put forward

here is that both are regulated by feedback mechanisms at levels that are less than maximal.

It now seems that the maximization principle may not be the most appropriate explanation (Calow, 1982) as it is not compatible with the behaviour of homeostatic systems that operate by control mechanisms which optimize rather than maximize. The capacity of such control systems to counteract perturbation, or sustain load, is related to the residual margin between actual and possible growth rates, suggesting why systems that do not maximize have survival value.

A number of conclusions can be drawn from this consideration of the control of growth. It is evident that growth is controlled at different levels of biological organization in response to various genetic, physiological and ecological preferenda. Like other autoregulatory processes, this requires mechanisms incorporating the principle of negative feedback, whose output is characteristically oscillatory when investigated using disturbance techniques. Their role is to counteract the effect of exogenous perturbations that would otherwise disrupt and derange growth processes, rather than the maintenance of physiological constancy *per se*. We believe that the numerous control mechanisms operating within an organism are hierarchically arranged and co-ordinated, each group of control mechanisms operating at a level of organization constituting a subsystem (Fig. 4). Each subsystem is semi-autonomous, but is subject to intervention control from above and imposes control upon subordinate subsystems. It seems that to some extent the course of evolution is reflected in the present arrangement of subsystems as a hierarchy, in that new subsystems have apparently been added by superimposing them at the highest level, rather than modifying existing subsystems or inserting them at a lower level (Fig. 5). We believe it is necessary that research on the control of growth uses a holistic approach, as not only will this lead to better understanding of how growth control systems work, but also how and why they fail.

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REFERENCES

AKIN, G. C., 1966. Self-inhibition of growth in *Rana pipiens* tadpoles. *Physiological Zoology, 39:* 341–356.

ALLEE, W. C., BOWEN, E. S., WELTY, J. C. & OESTING, R., 1934. The effect of homotypic conditioning of water on the growth of fishes, and chemical studies of the factors involved. *Journal of Experimental Zoology, 68:* 183–213.

ALMADA-VILLELA, P. C., DAVENPORT, J. & GRUFFYDD, L. D., 1982. The effects of temperature on the shell growth of young *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology, 59:* 275–288.

ATTALLAH, A. M. & HOUCK, J. C., 1976. Comprehensive bibliography of chalone literature. In J. C. Houck (Ed.), *Chalones:* 493–508. Amsterdam: North-Holland.

BALL, J. N., 1969. Prolactin and growth hormone. In W. S. Hoar & D. J. Randall (Eds), *Fish Physiology. Volume 2 The Endocrine System:* 207–240. New York: Academic Press.
BARD, J. B. L., 1978. A quantitative model of liver regeneration in the rat. *Journal of Theoretical Biology, 73:* 509–530.
BARD, J. B. L., 1979. A quantitative theory of liver regeneration in the rat II: matching an improved mitotic inhibitor model to the data. *Journal of Theoretical Biology, 79:* 121–136.
BENNETT, R. J. & CHORLEY, R. J., 1978. *Environmental Systems.* London: Methuen.
BERRIE, A. D. & VISSER, S. A., 1963. Investigations of a growth-inhibiting substance affecting a natural population of freshwater snails. *Physiological Zoology, 36:* 167–173.
BERRILL, N. J., 1952. Regeneration and budding in worms. *Biological Reviews, 27:* 401–438.
BERTALANFFY, L., 1952. *Problems of Life.* London: Watts.
BERTALANFFY, L., 1960. Principles and theory of growth. In W. W. Nowinski (Ed.), *Fundamental Aspects of Normal and Malignant Growth:* 137–259. Amsterdam: Elsevier.
BEST, J. B., GOODMAN, A. B. & PIGON, A., 1969. Fissioning in planarians: control by the brain. *Science, New York, 164:* 565–566.
BISCHOFF, F. & BRYSON, G., 1964. Carcinogenesis through solid state surfaces. *Progress in Experimental Tumour Research, 5:* 85–133.
BOYCOTT, A. E., 1929. The blood as a tissue: hypertrophy and atrophy of the red corpuscles. *Proceedings of the Royal Society of Medicine, 23:* 1–25.
BRAVERMAN, M., 1974. The cellular basis of morphogenesis and morphostasis in hydroids. *Oceanography and Marine Biology: an Annual Review, 12:* 129–221.
BRITTON, N. F., WRIGHT, N. A. & MURRAY, J. D., 1982. A mathematical model for cell population kinetics in the intestine. *Journal of Theoretical Biology, 98:* 531–541.
BRONOWSKI, J., 1970. New concepts in the evolution of complexity. *Synthese, 21:* 228–246.
BULLOUGH, W. S. & LAURENCE, E. B., 1960. The control of epidermal mitotic activity in the mouse. *Proceedings of the Royal Society, Series B, 151:* 517–536.
BURCH, P. R. J., 1976. *The Biology of Cancer: a New Approach.* Lancaster: MTP Press.
CALOW, P., 1973. On the regulatory nature of individual growth: some observations from freshwater snails. *Journal of Zoology, 170:* 415–428.
CALOW, P., 1976. *Biological Machines. A Cybernetic Approach to Life.* London: Arnold.
CALOW, P., 1977. Ecology, evolution and energetics: a study in metabolic adaptation. *Advances in Ecological Research, 10:* 1–60.
CALOW, P., 1982. Homeostasis and fitness. *American Naturalist, 120:* 416–419.
CHANCE, B., PYE, E. K., GHOSH, A. K. & HESS, B., 1973. *Biological and Biochemical Oscillators.* New York: Academic Press.
CLARK, W. C., 1978. Metabolite-mediated density-dependent sex determination in the free-living nematode, *Diplenteron potohikus. Journal of Zoology, 184:* 245–254.
CLARKE, R. B. & SCULLY, V., 1964. Hormonal control of growth in *Nereis diversicolor. General and Comparative Endocrinology, 4:* 82–90.
CHRISTIAN, J. J., 1963. Endocrine adaptive mechanisms and the physiologic regulation of population growth. In W. Mayer & R. Van Gaider (Eds), *Physiological Mammalogy, Vol. 1: Mammalian Populations.* London: Academic Press.
DAVIS, L. V., 1966. Inhibition of growth and regeneration in *Hydra* by crowded culture water. *Nature, London, 212:* 1215–1217.
DAWKINS, R., 1976. *The Selfish Gene.* Oxford: Oxford University Press.
FOERSTER, H., 1958. Basic concepts of homeostasis. *Brookhaven Symposia in Biology, 10:* 216–242.
FORSCHER, B. K. & HOUCK, J. C., 1973. *Chalones: Concepts and Current Researches.* Bethesda: National Cancer Institute.
FULTON, C., 1959. Re-examination of an inhibitor of regeneration in *Tubularia. Biological Bulletin. Marine Biological Laboratory, Woods Hole, 116:* 232–238.
GASSINELLI, G., RAMALHO-PINTO, F. J., PELLEGRINO, J. & GILBERT, B., 1970. Uptake of ^{59}Fe as a tool for the study of the crowding effect in *Biomphalaria glabrata. American Journal of Tropical Medicine and Hygiene, 19:* 1034–1037.
GOLDHABER, P., 1961. The influence of pore size on carcinogenicity of subcutaneous implanted millipore filters. *Proceedings of the American Association for Cancer Research, 3:* 228.
GOLDHABER, P., 1962. Further observations concerning the carcinogenicity of millipore filters. *Proceedings of the American Association for Cancer Research, 4:* 323.
GOOD, P. I., 1972. Homeostatic mechanisms and the loss of mitotic potential. *Journal of Theoretical Biology, 34:* 99–102.
GOOD, P. I., 1973. Alternative mechanisms for homeostatic regulation of mammalian cell populations. *Journal of Theoretical Biology, 40:* 543–552.
GOSS, R. J., 1964. *Adaptive Growth.* London: Logos Press.
GOSS, R. J., 1969. *Principles of Regeneration.* New York: Academic Press.
HOLLEY, R. W., 1975. Control of growth of mammalian cells in cell culture. *Nature, London, 258:* 487–490.
HOUCK, J. C., 1976. *Chalones.* Amsterdam: North-Holland.

HUBBELL, S. P., 1971. Of sowbugs and systems: the ecological bioenergetics of a terrestrial isopod. In B. C. Patten (Ed.), *Systems Analysis and Simulation in Ecology:* 269–324. New York: Academic Press.

IKESHOJI, T. & MULLA, M.S., 1970. Overcrowding factors of mosquito larvae. *Journal of Economic Entomology, 63:* 90–96.

IVERSEN, O. H., 1969. Chalones of the skin. In G. E. W. Wolstenholme & J. Knight (Eds), *Homeostatic Regulators:* 29–56. London: Churchill.

IVERSEN, O. H., 1976. The history of chalones. In J. C. Houck (Ed.), *Chalones:* 37–69. Amsterdam: North-Holland.

KAVENAU, J. L., 1960. A model of growth and growth control in mathematical terms. II. Compensatory organ growth in the adult. *Proceedings of the National Academy of Science, 46:* 1658–1673.

KERKUT, G. A., 1960. *Implications of Evolution.* Oxford: Pergamon.

KIDDER, G. W., 1941. Growth studies on ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium. *Physiological Zoology, 14:* 209–226.

KUHN, T. S., 1970. *The Structure of Scientific Revolutions,* 2nd edition. Chicago: Chicago University Press.

LEFFERT, H. L. & KOCH, K. S., 1977. Control of animal cell proliferation. In G. H. Rothblat & V. J. Cristafalo (Eds), *Growth, Nutrition and Metabolism of Cells in Culture* Vol. 3: 225–294. New York: Academic Press.

LEHNINGER, A. L., 1975. *Biochemistry,* 2nd edition. New York: Worth Publishers.

LEVY, M. G., TUNIS, M. & ISSEROFF, H., 1973. Population control in snails by natural inhibitors. *Nature, London, 241:* 65–66.

LICHT, L. E., 1967. Growth inhibition in crowded tadpoles: intraspecific and interspecific effects. *Ecology, 48:* 736–745.

LLOYD, D., POOLE, R. D. & EDWARDS, S. W., 1982. *The Cell Division Cycle: Temporal Organisation and Control of Cellular Growth and Reproduction.* London: Academic Press.

LOEWENSTEIN, W. R. & KANNO, Y., 1966. Intercellular communication and the control of tissue growth: lack of communication between cancer cells. *Nature, London, 209:* 1248–1249.

MEDAWAR, P. B., 1940. The growth, growth energy, and ageing of the chicken's heart. *Proceedings of the Royal Society, Series B, 129:* 332–355.

MEDAWAR, P. B., 1941. The 'Laws' of biological growth. *Nature, London, 148:* 772–774.

MEDAWAR, P. B., 1945. Size, shape and age. In W. E. le Gros Clark & P. B. Medawar (Eds), *Essays on Growth and Form:* 157–187. Oxford: Clarendon Press.

MESAROVIC, M. D., 1968. Systems theory and biology—view of a theoretician. In M. D. Mesarovic (Ed.), *Systems Theory and Biology:* 59–87. Berlin: Springer-Verlag.

MESAROVIC, M. D., MACKO, D., TAKAHARA, Y., 1970. *Theory of Hierarchical, Multilevel, Systems.* New York: Academic Press.

MILSUM, J. H., 1966. *Biological Control Systems Analysis.* New York: McGraw-Hill.

MORGAN, T. H., 1901. *Regeneration.* New York: Macmillan.

MUNDAY, K. A., 1961. Aspects of stress phenomena. *Symposia of the Society for Experimental Biology, 15:* 168–189.

ONDA, H., 1979. A new hypothesis on mitotic controlling mechanism in eukaryotic cells: cell-specific mitosis-inhibiting protein excretion hypothesis. *Journal of Theoretical Biology, 77:* 367–377.

PALADINI, A. C., PENA, C. & RETEGUI, L. A., 1979. The intriguing nature of the multiple actions of growth hormone. *Trends in Biochemical Sciences, 4:* 256–260.

PIRT, S. J., 1975. *Principles of Microbe and Cell Cultivation.* Oxford: Blackwell.

PRADER, A., TANNER, J. M. & HARNACK, G. A., 1963. Catch-up growth following illness or starvation. *Journal of Pediatrics, 62:* 646–659.

PYE, E. K., 1973. Glycolytic oscillations in cells and extracts of yeast—some unsolved problems. In B. Chance, E. K. Pye, A. K. Ghosh & B. Hess (Eds), *Biological and Biochemical Oscillators:* 269–284. New York: Academic Press.

RICHARDS, C. M., 1958. The inhibition of growth in crowded *Rana pipiens* tadpoles. *Physiological Zoology, 31:* 138–151.

ROSE, S. M., 1957a. Polarised inhibitory effects during regeneration in *Tubularia. Journal of Morphology, 100:* 187–205.

ROSE, S. M., 1957b. Cellular interaction during differentiation. *Biological Reviews, 32:* 351–382.

ROSE, S. M., 1958. Failure of self-inhibition in tumours. *Journal of the National Cancer Institute, 20:* 653–664.

ROSE, S. M., 1959a. Failure of survival of slowly growing members of a population. *Science, New York, 129:* 1026.

ROSE, S. M., 1959b. Population control in guppies. *American Midland Naturalist, 62:* 474–481.

ROSE, S. M., 1960. A feedback mechanism of growth control in tadpoles. *Ecology, 41:* 188–199.

ROSE, S. M., 1966. Polarised inhibitory control of regional differentiation during regeneration in *Tubularia:* II Separation of active materials by electrophoresis. *Growth, 30:* 429–447.

ROSE, S. M. & POWERS, J. A., 1966. Polarised inhibitory control of regional differentiation during regeneration in *Tubularia:* I. The effects of extracts from distal and proximal regions. *Growth, 30:* 419–427.

SANDERS, B. M. & COSTLOW, J. D., 1981. Regulation of growth in larvae of the crab *Rhithropanopeus harrisii:* the effect of cyclic temperatures. *Journal of Thermal Biology, 6:* 357–363.

SHOSTAK, S., 1977. Vegetative reproduction by budding in *Hydra*: a perspective on tumours. *Perspectives in Biology and Medicine, 20:* 545–568.
SHOSTAK, S., 1981. *Hydra* and cancer: immortality and budding. In C. J. Dawes (Ed.), *Phyletic Approaches to Cancer:* 275–286. Tokyo: Japan Scientific Society Press.
SHYMKO, R. M. & GLASS, L., 1976. Cellular and geometric control of tissue growth and mitotic instability. *Journal of Theoretical Biology, 63:* 355–374.
STEBBING, A. R. D., 1973. Competition for space between the epiphytes of *Fucus serratus* L. *Journal of the Marine Biological Association of the United Kingdom, 53:* 247–261.
STEBBING, A. R. D., 1981a. The kinetics of growth control in a colonial hydroid. *Journal of the Marine Biological Association of the United Kingdom, 61:* 35–63.
STEBBING, A. R. D., 1981b. Hormesis—stimulation of colony growth in *Campanularia flexuosa* (Hydrozoa) by copper, cadmium and other toxicants. *Aquatic Toxicology, 1:* 227–238.
STEBBING, A. R. D., 1982. Hormesis—the stimulation of growth by low levels of inhibitors. *Science of the Total Environment, 22:* 213–234.
STEBBING, A. R. D. & HIBY, A. R., 1979. Cyclical fluctuations in the growth rate of stressed hydroid colonies. In E. Naylor & R. G. Hartnoll (Eds), *Cyclic Phenomena in Marine Plants and Animals:* 165–172. Oxford: Pergamon.
STEBBING, A. R. D. & POMROY, A. J., 1978. A sublethal technique for assessing the effects of contaminants using *Hydra littoralis*. *Water Research, 12:* 631–635.
STEINBERG, M. S., 1954. Studies on the mechanism of physiological dominance in *Tubularia*. *Journal of Experimental Zoology, 127:* 1–26.
STEINWASCHER, K., 1979. Host-parasite interaction as a potential population regulating mechanism. *Ecology, 60:* 884–890.
TANNER, J. M., 1963. Regulation of growth in size in mammals. *Nature, London, 199:* 845–850.
TANNER, J. M., 1975. Towards complete success in the treatment of growth hormone deficiency. A plea for earlier ascertainment. *Health Trends, 7:* 61–65.
TARDENT, P., 1963. Regeneration in the Hydrozoa. *Biological Reviews, 38:* 293–333.
THOMAS, J. D., 1973. Schistosomiasis and the control of molluscan hosts of human schistosomes with particular reference to possible self-regulatory mechanisms. *Advances in Parasitology, 11:* 307–394.
TOIVONEN, H. & RYTOMAA, T., 1978. Monte Carlo simulation of malignant growth. Examples of the behaviour of chloroleukemia after specific perturbations. *Journal of Theoretical Biology, 72:* 257–267.
UMBARGER, H. E., 1956. Evidence for a negative feedback mechanism in the biosynthesis of isoleucine. *Science, New York, 123:* 848.
WALKER, I., 1975. Density-dependent control of fission rates in the marine ciliate *Keronopsis rubra* Ehrenberg (Hypotricha : Oxytrichidae) *Journal of Animal Ecology, 44:* 707–717.
WALKER, I., 1979. Mechanisms of density-dependent population regulation in the marine copepod *Amphiascoides* sp. (Harpacticoida). *Marine Ecology-Progress Series, 1:* 209–221.
WALLIS, M., 1979. Pituitary growth hormone. *Nature, London, 281:* 633–634.
WARREN, E., 1900. On the reaction of *Daphnia magna* (Straus) to certain changes in its environment. *Quarterly Journal of Microscopical Science, 43:* 199–224.
WEISS, P. & KAVENAU, J. L., 1957. A model of growth and growth control in mathematical terms. *Journal of General Physiology, 41:* 1–47.
WILLIAMS, J. P. G. & HUGHES, P. C. R., 1975. Catch-up growth in rats undernourished for different periods during the suckling period. *Growth, 39:* 179–193.
WOODGER, J. H., 1930. The "concept of organism" and the relation between embryology and genetics Part II. *Quarterly Review of Biology, 5:* 438–463.
WOODRUFF, L. L., 1913. The effect of excretion products of Infusoria on the same and on different species, with special reference to the protozoan sequence in fusions. *Journal of Experimental Zoology, 14:* 575–578.
WRIGHT, C. A., 1960. The crowing phenomenon in laboratory colonies of freshwater snails. *Annals of Tropical Medicine and Parasitology, 54:* 224–232.
WYNNE-EDWARDS, V. C., 1962. *Animal Dispersion in Relation to Social Behaviour*. Edinburgh: Oliver and Boyd.
WYNNE-EDWARDS, V. C., 1963. Intergroup selection in the evolution of social systems. *Nature, London, 200:* 623–626.
YATES, R. A. & PARDEE, A. B., 1956. Control of pyrimidine biosynthesis in *Escherichia coli* by a feedback mechanism. *Journal of Biological Chemistry, 221:* 757–770.
YU, M. L. & PERLMUTTER, A., 1970. Growth inhibiting factors in the zebrafish, *Brachydanio rerio* and the blue gourami, *Trichogaster trichopterus*. *Growth, 34:* 153–175.