Dynamics of Growth Control in a Marine Yeast Subjected to Perturbation

By A. R. D. STEBBING,¹* J. P. NORTON² AND M. D. BRINSLEY¹

¹*Institute for Marine Environmental Research, Prospect Place, The Hoe,
Plymouth PL1 3DH, UK*

²*Department of Electronic and Electrical Engineering, The University of Birmingham,
PO Box 363, Birmingham B15 2TT, UK*

(Received 19 December 1983; revised 6 March 1984)

Normalized specific growth rates of cultures of the yeast *Rhodotorula rubra* apparently indicate the activity of a growth control mechanism, when growth is perturbed with low concentrations of a toxic inhibitor (cadmium). Data from a number of experiments, in which different cadmium concentrations were used, indicate non-linear growth control dynamics and some features of a model structure, but do not permit it to be defined. Interpretation of the oscillatory behaviour as the response of a control mechanism to perturbation suggests that inhibition of mean growth rates, indicated by a threshold on dose–response curves, is due to overloading of the control mechanism or ‘saturation’. Hormesis, the tendency of subinhibitory levels of typically toxic agents to stimulate growth, is a consequence of transient or sustained overcorrections by the control mechanism to low levels of an inhibitory stimulus.

INTRODUCTION

Biological growth is controlled at the biochemical level of organization through the rates of biosynthetic reactions (Lehninger, 1975), and at higher levels through cell number or population density, the capacity of organs and tissues to withstand physiological load or through physical size (Stebbing & Heath, 1984). Models of growth, including cell division, that depend on control theory often assume that the regulatory systems are sensitive to cell density or population size (Bard, 1979; Shymko & Glass, 1976).

We examine here the view that specific growth rate of a perturbed experimental culture with respect to a control culture represents output of a growth control system. Our proposition is that normalized specific growth is informative for interpreting growth phenomena and ultimately modelling growth control mechanisms. A price is paid for its informativeness, as careful experimental design is essential if useful specific growth rate responses are to be obtained. We shall examine in detail how experiment design constrains, and is constrained by, the information to be extracted from the specific growth rate responses.

In earlier work with the colonial hydroid *Campanularia (Laomedea) flexuosa*, we considered the growth of colonies exposed to various toxic inhibitors (Stebbing, 1976). Initially, mean growth rates over entire experiments (11 d) were used to determine concentration–response curves and threshold concentrations were derived from them. Subsequently, changes in growth rates with time were determined in order to optimize the duration of experiments, and pronounced oscillations were found in specific growth rates of organisms exposed to low levels of toxicants, with respect to organisms not exposed. The oscillations invited interpretation as the response of a feedback control mechanism to a step input (Stebbing, 1981*a*) and evidence that our data were capable of this interpretation came from work on a simulation model (Stebbing & Hiby, 1979). A non-linear model using proportional plus integral control reproduced most of the features of the data.

For a number of reasons, hydroids were unsuitable for the further development of the work.

The growth of the colonies was too slow, so a much faster-growing system was required. Estimates of biomass were based on counts of colony members and involved approximations and assumptions that reduced precision. Furthermore, believing biological growth in other taxa might be regulated by control mechanisms whose response, and thence structure, could be investigated in the same way, there was good reason to extend the work by using a species from another phylum.

The experiments described here used the marine yeast *Rhodotorula rubra*; they show the effects of a range of cadmium concentrations on the growth of the cultures, from the lowest concentration that caused significant differences in growth rate from that of the control cultures, to the highest sublethal concentration. Our objectives were first to see whether experiments of similar design to those carried out with hydroids would also provide responses showing the action of a control mechanism; second, to see the effect of progressively increasing the inhibitory load; and third, to enquire into the kind of model structure necessary to represent the control mechanism.

METHODS

Organism and its culture. *Rhodotorula rubra* (NCYC 797) was used in all experiments. The culture medium used was adapted from MYGPS (Ross & Morris, 1965). The constituents (g l^{-1}) were yeast extract (0.3), malt extract (0.3), mycological peptone (0.5), and glucose (1). The medium was made up in sea water and deionized water (3:1, v/v). This is a 1 in 10 dilution of MYGPS as normally used, in order to reduce complexation, which decreases the pool of ionic and biologically available cadmium. Growth over the short periods considered here was never limited by nutrients.

Stock cultures were maintained in Petri dishes on MYGPS made up with 1.5% (w/v) agar at room temperature. For experiments, stock suspension cultures were initiated and equilibrated in the experimental regime, clamped in a water bath at 18.5 °C under a laminar flow hood. Cells were kept as a homogeneous suspension with magnetic stirrers.

Inocula from stock suspensions were taken after 24 h or more to initiate experimental cultures which were then allowed to grow for a further 17 h before beginning an experiment. Under these conditions cell numbers doubled approximately every 2.5 h and the number used to inoculate experimental cultures was calculated to give a density of 20000 cells ml^{-1} at the beginning of an experiment.

In preliminary experiments it was found that the sensitivity of our method of measuring growth was such that specific growth rates were noticeably perturbed if the cultures were knocked, or if sampled by pouring out the required volumes. We therefore clamped flasks in the water bath and sampled with a pipette, avoiding any movement of the cultures that might perturb growth. The experiments were conducted in a laminar flow hood, not only to avoid contamination, but also so that the flasks could be left without tops to minimize disturbance when sampling.

Replication, sampling and counting. Experiments typically consisted of a single control culture and an experimental culture, each of 750 ml. Initially, experiments were carried out with three or more smaller replicate cultures for each treatment, but as we learned how critical it was to time the sampling precisely (see below), we increased the total volume of culture and limited the number of cultures per treatment to one.

Similarly, we began by taking ten subsamples of each culture for each count, but the errors introduced by the time involved in taking the subsamples were greater than the reduction in error achieved by subsampling. We therefore opted to take single large samples of 20 ml, rather than 10 subsamples.

Samples were taken at 30 min intervals, and it became clear that, with the rate of growth of *R. rubra* and our method of treating the data, the exact timing of sampling was important. A 1 min error in performing a count from a population growing exponentially, with a time constant of about 5 h, caused 0.3% error in the count. Such errors are quite significant in their effect on calculated specific rates (see later).

Although the required number of counts for each density determination took about 15 min, errors were introduced by the variability of this period or delays caused by blockages in the particle counters. We attempted to find the appropriate concentrations of several preservatives (Lugol's iodine, cupric chloride) that might arrest growth instantaneously to remove this source of error, but it proved difficult to find concentrations that did so without causing the cells to shrink or lyse to an extent that resulted in inaccurate counts.

The density of cells was determined directly by counting the suspension in plastic disposable 'Universal' bottles. Samples from control and experimental cultures were counted simultaneously using inter-calibrated Coulter counters (a model ZB and a model D). Eight counts were necessary to reduce standard errors to below 0.5%. After the appropriate coincidence corrections, population densities (cells ml^{-1}) were determined, from which specific growth rates could be calculated.

Fig. 1. Growth of *R. rubra* exposed to cadmium (3 mg l^{-1}), added at zero time, showing the derivations of normalized specific growth rates ($\bar{R}\%$) from counts (N). The data for the experimental culture are indicated by a solid line and those for the control culture by a broken line. The data are shown as numbers of cells (N) at 30 min intervals (a), mean rates (b), mean specific rates (c) and normalized mean specific rates (d) for 30 min periods.

Treatment of the raw counts. Growth of cell populations is often considered simply in terms of increases in population density of a culture with time (Fig. 1a). The effect of any inhibition is most apparent as a decrease in slope compared with the control culture. If it is assumed that rates of growth are physiologically more significant than the cumulative product of biosynthesis (Fig. 1b), the fact that rates increase as a function of density implies that specific rates (R) independent of density are more appropriate (Fig. 1c). Within the experimental regime used here, the specific rate of the unperturbed culture was approximately constant, while that of the perturbed experimental culture fluctuated above and below that of the controls. The mean specific rates of the experimental culture were expressed as percentages of $\bar{R}$ of those of the control culture (Fig. 1d).

Small and unavoidable environmental differences between the experimental and control cultures may also be expected to contribute to errors in $\bar{R}$. To assess overall reliability of the $\bar{R}$ samples, three runs were performed with no cadmium addition. The standard deviation of a total of 42 specific rates for 30 min periods was 11.1%. If this were the only evidence of accuracy, one would have to infer that the finer structural features of specific-growth-rate response cannot be detected reliably. However, we shall see that not only do several major features appear clearly in a succession of responses, but so also do some minor or short-term features which seem to vary in a systematic way as the concentration of cadmium is changed. Although it is not feasible to fit a detailed model to the responses observed so far, we conclude that some qualitative features are apparent which any candidate model should reproduce.

An important experimental constraint is the need to avoid excessive error amplification in the calculation of specific rate from measurements of cell concentration. The concentration $c(t)$ in the experimental (perturbed) culture and $c_0(t)$ in the control (unperturbed) culture are both sampled regularly at time intervals T .

The growth model relating concentration to the growth-rate 'constant' $R(t)$ is

$$c(t) = c(0) e^{R(t)t}$$

for the perturbed culture, and the same model relating c_0 and R_0 for the unperturbed culture. The specific growth rate of the perturbed culture, normalized by that of the control culture, is calculated for each sampling interval.

The mean value $\bar{R}_i$ of $R(t)/R_o(t)$ over the interval from sample $i - 1$ at time $(i - 1)T$ to sample i at iT can be calculated as:

$$\begin{aligned}\bar{R}_i &\approx \frac{\ln(c(iT)/c[(i-1)T])}{\ln(c_o(iT)/c_o[(i-1)T])} \\ &= \frac{\ln c(iT) - \ln c[(i-1)T]}{\ln c_o(iT) - \ln c_o[(i-1)T]}\end{aligned}$$

From first principles, or by using the expression for the derivative of a quotient, it is easy to see that the proportional error in the calculated $\bar{R}_i$ is the algebraic sum of the proportional errors in the numerator and denominator of this expression. This part of the calculation consequently does not have a very serious effect on total error. Serious amplification of errors may, however, occur earlier in the calculation. If $\ln c(iT) - \ln c[(i-1)T]$ is denoted by x_i , straightforward differentiation gives:

$$\frac{\partial x_i}{\partial c(iT)} = \frac{1}{c(iT)} \quad \text{and} \quad \frac{\partial x_i}{\partial c[(i-1)T]} = -\frac{1}{c[(i-1)T]}$$

so small errors $\delta c(iT)$ and $\delta c[(i-1)T]$ in the cell concentration would cause an error

$$\delta x_i = \frac{\delta c(iT)}{c(iT)} - \frac{\delta c[(i-1)T]}{c[(i-1)T]}$$

in x_i . That is to say, the *absolute* error in the numerator of the expression for $\bar{R}$ is determined by the proportional errors in two successive cell-concentration samples and would not, on average, be reduced by sampling at shorter intervals. Hence, if the interval were shortened, reducing $c(iT)/c[(i-1)T]$ by some factor, the proportional error in the numerator of $\bar{R}_i$ would increase by some factor (and similarly for the denominator). The sampling interval must therefore not be too short.

With sampling at 30 min intervals in these experiments, $\ln c(iT) - \ln c[(i-1)T]$ is around 0.1, and the measurement of $c(t)$ and $c_o(t)$ with standard deviations of about 0.25% results in the calculated $\bar{R}$ having a standard deviation of about 5%, assuming zero-mean uncorrelated measurement errors. Halving the sampling intervals would multiply the standard deviation of $\bar{R}$ by $2\sqrt{2}$.

If the responses are to yield a comprehensive picture of growth control, a wide range of response speeds is likely to be of interest. In particular, one would wish to distinguish rapid but short-lived immediate control action and much slower 'trimming' as the steady state is approached. To think for a moment in terms of signal spectra, it is theoretically necessary to sample at least twice per cycle of the highest-frequency component present in the response signal, i.e. to sample at the so-called Nyquist frequency. This is in order to avoid 'aliasing', i.e. indistinguishability of contributions from more than one frequency in the response spectrum, with consequent ambiguity in signal structure (Ziemer *et al.*, 1983). In practice, signals are not usually strongly band-limited in frequency, and noise (all variation not explicable by a model) is significant. As a result a much higher sampling rate is required. As a rule of thumb, a rate of five to ten samples per cycle of the highest-frequency component of interest is advisable. However, without knowledge of the effective signal bandwidth, the minimal acceptable sampling rate cannot be determined; since of the response signal itself is the sole source of such knowledge, the bandwidth can only be estimated from it if the sampling rate is high enough for aliasing to be ignored. Some doubt will remain about the adequacy of the sampling rate unless the response is so smooth, and the noise so small, that one can be certain that no significant behaviour is being missed or scrambled. This is seldom the case in an initial investigation.

Experimental design and constraints in relation to model structure. The first experimental aim in the selection of model structure was to check whether the responses indicated non-linear dynamics in growth control. Non-linear dynamics correspond to behaviour not describable by one or more constant-coefficient linear differential equations. The significance of non-linearity is that although a coherent body of effective technique exists for constructing and analysing models of linear dynamical systems, much less is available for non-linear systems. Also, because of the greater diversity of non-linear behaviour, those techniques that do exist for specific types of non-linear systems tend not to be very widely applicable. Identification of models for significantly non-linear dynamics tends consequently to be an *ad hoc* process. In the experiments, non-linear dynamics will be revealed by any differences between the shapes of the response to different cadmium concentrations. Entirely linear dynamics would give responses identical but for amplitudes proportional to concentration.

Another significant aspect of linear dynamical models is that powerful graphical aids to understanding their behaviour are available, largely developed in electrical and control engineering. With this in mind, the results will be discussed, when possible, in terms of linear-model behaviour; more specifically, the location and motion of

poles will be used to summarize the behaviour. To see the point of doing so, it is necessary to review briefly the meaning of poles (Ziemer *et al.*, 1983) and the idea of a root locus (D'Azzo & Houpis, 1975).

When a system with linear, time-invariant dynamics is perturbed, its response is the sum of a number of exponential components, of the form $e^{\alpha t}$, and components reflecting the nature of the perturbation. The $e^{\alpha t}$ components are related to the structure of the system through the differential equation connecting the perturbed input $u(t)$ and the observed response $y(t)$. If this equation is

$$\frac{d^n y}{dt^n} + a_1 \frac{d^{n-1} y}{dt^{n-1}} + a_2 \frac{d^{n-2} y}{dt^{n-2}} + \dots + a_n y = b_0 \frac{d^m u}{dt^m} + \dots + b_m u$$

then the values α in the exponentials are the n roots of a polynomial equation

$$s^n + a_1 s^{n-1} + a_2 s^{n-2} + \dots + a_n = 0$$

and are called the poles of the system. Normally the coefficients a_1 to a_n are real, so each pole α is either real, making $e^{\alpha t}$ an ordinary exponential, or half of a complex-conjugate pair $\alpha = \beta \pm i\gamma$. In the latter case the two exponential components have complex-conjugate amplitudes $A \pm jB$ say, and their sum is $2e^{\beta t} (A \cos \gamma t - B \sin \gamma t)$, which is an oscillation with frequency $\gamma/2\pi$ and an envelope proportional to $e^{\beta t}$. If the system is stable (in a technical rather than colloquial sense) none of the exponentials has a positive component, so all the exponents decay with time, or at worst remain constant in amplitude. Any oscillations are damped, at a rate that increases as β goes farther negative. The decay rate of such oscillations may be indicated by quoting the time constant $-1/\beta$, and similarly the time constant $-1/\alpha$ of a real exponential is often quoted.

A convenient way to keep track of n poles is to display them in the complex plane. If the dynamics of the system vary as some parameter (in these experiments the cadmium concentration) is varied, the poles will move around in the plane, generating a *root locus*. Such root loci are widely used in control-system design in engineering to see the effects on the overall behaviour of a feedback system of varying a design parameter such as a gain. In this context, there is some hope that the root locus generated as the cadmium concentration is varied will suggest the structure of the underlying feedback growth-control system, and will indicate which of its parameters depend on the concentration.

Even with these aids to interpretation, and even after taking some care in selecting a suitable cell culture and refining observational technique, experimental constraints limit considerably the information that can be extracted from the responses. The most fundamental are the overall experiment length and, as discussed above, sampling rate and accuracy (which interact because of the time taken to count a sample that is continuing to grow) and the amplification of errors in the course of calculating specific growth rate from cell counts.

The duration of the experiment is important in two respects. At the start, a period must be allowed for the culture to settle into an unperturbed steady growth state before the cadmium is added. In the series reported below, it was not always easy to do so, and the effects of environmental disturbances sometimes persisted. Secondly, the tail of the response should ideally be observed until it is clear that all significant features of the growth control action have been seen. This also was not always possible. Extension of the experiment length with the present methods is not necessarily helpful, as fatigue can increase errors, or alter the error characteristics.

Experimental procedure. After equilibration for 17 h, the growth of a pair of cultures was followed with counts at 30 min intervals to establish when specific rates had stabilized, whereupon cadmium was added (as CdCl_2 , 1 mg ml^{-1} in 1 M-HCl) to give a predetermined concentration. Sometimes, this resulted in delaying the addition of cadmium for several hours with the result that the durations of some experiments – after adding the cadmium – were rather short (e.g. at 0.3 and 1.0 mg l^{-1}). Experiments were usually terminated after 8 h. Overall experiment length was one of the chief constraints in deriving a model structure from the data, and automation of sampling and counting would be a great advantage in further experiments.

RESULTS AND DISCUSSION

Features of specific growth rate responses

Amounts of cadmium ranging from 0.3 to 8 mg l^{-1} were added to cultures of *R. rubra*, and population densities were determined at 30 min intervals for 4 to 6 h. From these data, cumulative growth curves (Fig. 1*a*), overall specific growth rates (Fig. 2) and specific rates for 30 min periods (Fig. 3) were plotted.

When presented as cumulative increase in numbers of cells with time, the data are uninformative, showing decreasing slopes with increasing cadmium concentration (Fig. 1*a*). However, when mean specific rates for entire experiments are considered in relation to cadmium concentration there is a clearly defined threshold at which the overall inhibition of

Fig. 2. Inhibition of growth of *R. rubra* by cadmium at concentrations above 2 mg l⁻¹. Data are given as normalized specific rates ($\bar{R}\%$) for entire experiments.

specific rate begins to become significant (Fig. 2). This form is typical of concentration-response curves from experiments involving a range of concentrations of a toxic inhibitor. However, analysis of the evolution of $\bar{R}\%$ throughout each experiment allows enough features to be extracted for a reason for the sharp threshold to be postulated. We now review those features.

Fig. 3 shows the responses of specific growth rate to a range of concentrations of cadmium added at zero time, after a settling period of 1 to 3 h. In every case an initial fall is followed by a recovery. Except perhaps at 8 mg l⁻¹, the recovery is not monotonic but oscillatory. To say more about the oscillations one must decide how much notice to take of the apparent short-term behaviour, in view of the standard deviation of the order of 10% for $\bar{R}$. For concentrations below 2 mg l⁻¹, the combination of very short records in some cases, the small amplitude of the oscillation and lack of simple and consistent response structure prevents any firm conclusions on the presence or characteristics of sustained rapid oscillations. Furthermore, at 1 and 1.75 mg l⁻¹ the apparent oscillation frequency is close to 0.5 cycles per sample, reason for disquiet both because (i) it is close to the two-samples-per-cycle theoretical minimum frequency (the Nyquist frequency) to avoid aliasing and (ii) any isolated errors in cell count each give rise to two successive errors in $\bar{R}$ with opposite signs. In Fig. 3 the putative oscillations are shown by a broken line, and the underlying trend by an unbroken line, where it is discernible, for concentrations up to 2 mg l⁻¹.

For the low cadmium concentrations, the recovery includes a large overcorrection, well beyond the bounds of measurement error even at 1 mg l⁻¹, retarded more and more as the concentration was increased. The final steady state, either quiescent or possibly sustained oscillations, was not reached by the end of the observations.

At 2 mg cadmium l⁻¹ there was a sharp transition to responses showing more definite oscillations about a linear recovery trend. A repeat experiment at 2 mg l⁻¹ (not shown in Fig. 3) gave a response with much more regular oscillations, like those at higher concentrations but at a higher frequency, 0.67 cycles h⁻¹. We interpreted this as corroboration of the rapid change in response character at such concentrations rather than inconsistency, as another experiment at 4 mg l⁻¹ agreed moderately well with the original response at that concentration. Physically, the sharp transition seems to indicate a change in control mode as the capability of the small perturbation control system was exceeded, and this was reflected in the marked threshold at 2 mg l⁻¹ in the concentration-response curve for overall $\bar{R}\%$ (Fig. 2).

At higher cadmium concentrations, the oscillation frequency varied only in the range 0.3 to 0.45 cycles h⁻¹, and was well below the Nyquist frequency. The phase varied very little; the first overshoot peak (in the sense of overcorrection) occurred within 0.25 h of 2.15 h for concentrations from 2.5 to 6 mg l⁻¹. Damping of the oscillations increased with concentration, although the records are too short for the damping time constant to be estimated. In terms of pole positions, the oscillatory component at concentrations above 2 mg l⁻¹ may be attributed to two complex-conjugate poles, moving leftwards because the oscillations become more rapidly

Fig. 3. Data from experiments with *R. rubra* in which various concentrations of cadmium (indicated in the figure) were added at zero time, showing the fluctuations in normalized mean specific growth rate ($\bar{R}\%$) determined for 30 min periods. The putative oscillations are shown by a broken line and the underlying trend by an unbroken line, where it is discernible.

damped. Since the oscillation frequency was near-constant, these poles move almost parallel to the real axis.

Estimation of the underlying trend, which appeared linear over the record length, is complicated by uncertainty as to whether the cell count just before the perturbation should be included in the calculation. At 2.5, 3 and probably 4 mg cadmium l⁻¹, the value immediately before perturbation is consistent with the succeeding damped-oscillation-plus-linear trend, but at 6 mg l⁻¹ the initial fall is too large for this to be so. At 8 mg l⁻¹, there is no difficulty since the oscillation, if present, is of small amplitude, and the trend is well defined by the post-perturbation points. Including the initial condition for 2.5, 3 and 4 mg l⁻¹ but not for 6 or 8 mg l⁻¹ and finding the ordinary-least-squares linear fit to the responses, rather

Fig. 4

Fig. 5

Fig. 4. Data from experiments with *R. rubra* at cadmium concentrations from 2.5 to 8 mg l⁻¹ showing fluctuations in $\bar{R}\%$ as in Fig. 3 and superimposed trend lines. Their significance with respect to possible models is discussed in the text.

Fig. 5. Data from experiments with *R. rubra* at cadmium concentrations from 4 to 8 mg l⁻¹. Superimposed upon the fluctuations in $\bar{R}\%$ is indicated a tendency, which is particularly noticeable at 4 and 6 mg l⁻¹, for points to lie on straight-line sections with similar negative slopes joined by sharp rises. The possible implications of this are considered in the text.

unconvincing trends with noticeable biases were obtained. The reason is paucity of points, two cycles coarsely sampled being too few to guarantee a representative distribution about the trend line. Trends were finally fitted by eye, and are shown in Fig. 4. The variation in trend slope and initial value with concentration may be interpreted as a component due to movement of a pole not far from the origin and on the negative real axis. The near-linearity of the trends implies that if an exponential settling transient is present, it has an extremely long time constant, i.e. it corresponds to a pole with α real, negative and small.

Subtracting the oscillatory and trend components, all that remains at cadmium concentrations above 2 mg l^{-1} is part of the initial negative excursion, i.e. the component due to a fast (large negative) real pole, in a linear-dynamics interpretation.

Further comments on model structure

As this set of responses is sufficient to suggest some features of a model structure but not to define it completely, two further remarks about possible model structures are in order. First, the response shape varies strongly with cadmium concentration, indicating non-linear dynamics, but the response to high concentrations give little evidence of time-varying dynamics within any one response. Whether this is true also at lower concentrations will not be clear until longer records, and perhaps greater experimental precision, can be obtained. Second, and speculatively, in spite of the relatively high standard deviation indicated for the specific-growth-rate samples, points on the responses at 4 and 6 mg l^{-1} show an interesting disposition to lie on straight-line sections with similar negative slopes, joined by sharp rises. Fig. 5 illustrates this, and includes a similar construction for the response at 8 mg l^{-1} , which could not be justified if that response were viewed in isolation. If such sharp rises are indeed present, they suggest abrupt transitions from one metastable state to another, by way of an unstable region. They also imply the existence of some conditioning variable, which determines the potential for rapid growth, but is not directly observed.

Hormesis

At certain subinhibitory concentrations of cadmium, cell densities become significantly greater than those in the control cultures (Fig. 2). Stimulation by low levels of typically inhibitory agents is a common occurrence in toxicological experiments (Stebbing, 1982), and is termed hormesis. The suggestion arising from our experiments with hydroids, that it may be attributed to overcorrections by a growth control mechanism in response to low levels of inhibitory load (Stebbing, 1981*b*), can be re-examined here. It is unfortunate that hormesis occurs in those experiments where the small oscillation amplitude and overall irregularity makes their interpretation less certain than at higher concentrations of cadmium (Fig. 3). Longer experiments at these concentrations would be desirable, since the responses appear not to have reached a final steady state by the end. However, even the shorter experiments cover at least one oscillation cycle, so the results provide another instance of hormesis, in a different organism, which is apparently due to a tendency to overcorrect in response to low levels of toxic inhibition.

A mechanism for inhibitory thresholds

Even a superficial examination of the results shows that the threshold concentration at which there is overall inhibition of growth by cadmium coincides with a discontinuity in the qualitative behaviour of the normalized specific-growth-rate responses and its trend as cadmium concentration is increased. At about 2 mg l^{-1} , not only do oscillations tend to become more regular and increase sharply in amplitude, but the capacity of the control mechanism to regulate adequately against disturbance begins to be exceeded (Fig. 3). The simplest explanation is that, in control engineering terms, saturation is occurring, i.e. some material or energy resource or some rate process is encountering a limit. Consequently a strong non-linearity is introduced. If this is so, and bearing in mind that above the threshold the oscillation frequency and amplitude vary little with either time or cadmium concentration, the oscillations may well be interpreted as limit cycling (Auslander *et al.*, 1974).

CONCLUSIONS

Evidence is presented from perturbation experiments with *Rhodotorula rubra* that normalized specific growth rates indicate the output of a growth control mechanism. Cadmium was used to provide the perturbation, but preliminary experiments with other agents (e.g. temperature change and copper; A. R. D. Stebbing, unpublished data) suggest that the response of the mechanism is non-specific. The dynamics are non-linear, but the experiments lack the length and the data lack the precision necessary to indicate clearly a single model (Figs. 3 and 5). Nevertheless, the data strongly indicate the output of a control mechanism that regulates specific growth rate at some preferred level, counteracting perturbations whatever their cause. This interpretation provides an explanation for the inhibitory threshold (Fig. 2) which is due to saturation or overloading of the control mechanism. Another consequence is that low concentrations of cadmium cause hormesis, the paradoxical stimulation of growth by low levels of inhibitors. This is due to large or sustained over-corrections by the growth control mechanism to low levels of inhibitory load.

We are grateful to Mrs B. Kirsop for her help in finding a suitable species of yeast with which to do this work, and to Professor D. Lloyd for reading and improving the paper.

REFERENCES

- AUSLANDER, D. M., TAKAHASHI, Y. & RABINS, M. J. (1974). *Introducing Systems and Controls*. New York: McGraw-Hill.
- 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 Botany* **79**, 121–136.
- D'AZZO, J. J. & HOUPIS, C. H. (1975). *Linear Control System Analysis and Design*. New York: McGraw-Hill.
- LEHNINGER, A. L. (1975). *Biochemistry*, 2nd edn. New York: Worth.
- ROSS, S. S. & MORRIS, E. O. (1965). An investigation of the yeast flora of marine fish from Scottish coastal waters and a fishing ground off Iceland. *Journal of Applied Bacteriology* **28**, 224–234.
- SHYMKO, R. M. & GRASS, L. (1976). Cellular and geometric control of tissue growth and mitotic instability. *Journal of Theoretical Biology* **63**, 355–374.
- STEBBING, A. R. D. (1976). The effects of low metal levels on a clonal hydroid. *Journal of the Marine Biological Association of the United Kingdom* **56**, 977–994.
- STEBBING, A. R. D. (1981*a*). 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. (1981*b*). 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. & HEATH, G. W. (1984). Is growth controlled by a hierarchical system? *Biological Journal of the Linnean Society* **80** (in the Press).
- STEBBING, A. R. D. & HIBY, A. R. (1979). Cyclical fluctuations in the growth rate of stressed hydroid colonies. In *Cyclic Phenomena in Marine Plants and Animals*, pp. 165–172. Edited by E. Naylor & R. G. Hartnoll. Oxford: Pergamon.
- ZIEMER, R. E., TRANTER, W. H. & FANNIN, D. R. (1983). *Signals and Systems*. London: Macmillan.