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THE EFFECTS OF LOAD AND STRESS INDUCED BY CADMIUM ON THE GROWTH OF A YEAST

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ABSTRACT

Data from experiments on the growth of a marine yeast (Rhodotorula rubra) exposed to various concentrations of cadmium can be given as raw cumulative data for each concentration, or as mean specific growth rates ($\bar{R}$) presented as concentration-response curves, which show more clearly the inhibition of growth above a threshold concentration. However, it is in some ways more informative to consider these data as R% determined at frequent intervals in time. This measure of growth, in the yeast and some other organisms, apparently indicates the output of a growth regulatory mechanism, allowing one to interpret the inhibitory threshold in concentration - response curves as the consequence of overloading the capacity of the control mechanism to counteract further inhibition.

INTRODUCTION

The work described here is a development of that on the growth of hydroid colonies exposed to various inhibitory agents (Stebbing and Hiby, 1979; Stebbing, 1981b) in which we have shown that temporal oscillations in specific growth rates (R%) are likely to be the output of a control mechanism regulating growth processes. Here we extend the approach in experiments with cell suspensions of a yeast (Rhodotorula rubra) exposed to cadmium, used here as a growth inhibitor to impose a load upon the control mechanism(s) that regulate growth. The results are discussed in terms of control and counteractions, "load" and "stress" (Stebbing, 1981a).

MATERIALS AND METHODS

The data presented here are from a series of experiments on the marine yeast Rhodotorula rubra (NCYC 797). Stock cultures were for convenience maintained on agar plates, but for experiments suspension cultures were grown in 1/10 strength MYGPS (Ross and Morris, 1965). Cultures of 750 ml were grown in 1 l Erlenmeyer flasks clamped in a water bath at 18°C and continuously stirred. Typically experiments consisted of two such cultures, one to which cadmium was added and the other a control. Cell densities were determined for each culture simultaneously using two intercalibrated

Coulter Counters, by counting 20 ml of the undiluted culture in specimen tubes. The mean of seven counts was taken and corrected for coincidence and particulate background in the culture medium. Standard errors of the mean count were typically <0.5%. Densities were determined in this way at 30 minute intervals and from these data specific growth rates ($\bar{R}$) were calculated. Once $\bar{R}$ for both cultures was approximately equal and stable, experiments were begun by the addition of cadmium (as cadmium chloride in 1N HCl) to the experimental culture. Specific growth rates of the experimental culture ($\bar{R}_{exp}$) are expressed as percentages of those for the control culture ($\bar{R}_{con}$) to filter out changes in R due to the toxicant alone ($\bar{R}\%$).

RESULTS

Growth data depicted as cumulative increases in cell densities show that normal growth is log-linear and specific growth rates are therefore constant (Fig. 1 left), at least for the densities and periods involved here. The cumulative data do show progressive decreases in slope of cultures grown in higher concentrations of cadmium (Fig. 1 middle), although it is difficult to determine the lowest concentrations at which inhibition becomes significant. For this purpose it is better to plot the mean $\bar{R}\%$ for the duration of exposure to cadmium against concentration; this provides the typical concentration - response curve with a clearly defined threshold, indicating at what level cadmium begins to inhibit growth rate (Fig. 2).

When R% is calculated at frequent intervals in time, temporal fluctuations become apparent that have the characteristic form of the output of an underdamped feedback mechanism following perturbation (Fig. 1 right). At lower cadmium concentrations, oscillations have less amplitude and there is a tendency to overshoot, and $\bar{R}_{exp}$ is for much of the duration of the experiments greater than R_{con}. Above 2.0 mg/l this ceases to be the case; the amplitude of fluctuations increases and $\bar{R}_{exp}$ varies about a level that becomes less than R_{con} at successive higher levels. Nevertheless at 2.5 and 4.0 mg/l it can be seen that the level about which R_{exp} varies increases as the experiment proceeds, but at 6.0 mg/l this is not so and at 8.0 mg/l the decline is rapid and there are no oscillations at all.

When these data are interpreted in terms of the capacity of a control mechanism to counteract inhibition it appears that at the lower levels (0.3-2.0 mg/l) there is effective counteraction of the inhibition and $\bar{R}_{exp}$ would probably stabilize at the level of the controls in time. At intermediate levels (2.0-4.0 mg/l) the capacity of the control mechanism to counteract is initially exceeded, but increases in the level about which R_{exp} oscillates may indicate a recovery. At 8.0 mg/l or more there is clearly immediate overloading of the control mechanism and sustained inhibition of growth from which the culture does not recover.

These data and the behaviour of the control mechanism can be used to explain features of concentration - response curves that are not obvious from the curves themselves (Fig. 2). At lower levels of cadmium, below those that cause a lasting inhibition of growth,

oscillations in R_{exp} indicate the operation of the control mechanism, whose response is presumably necessary to maintain R_{exp} at or about the level of the controls. In fact the control mechanism tends to overcorrect and within a narrow range of concentrations, cultures may grow significantly larger than the control cultures

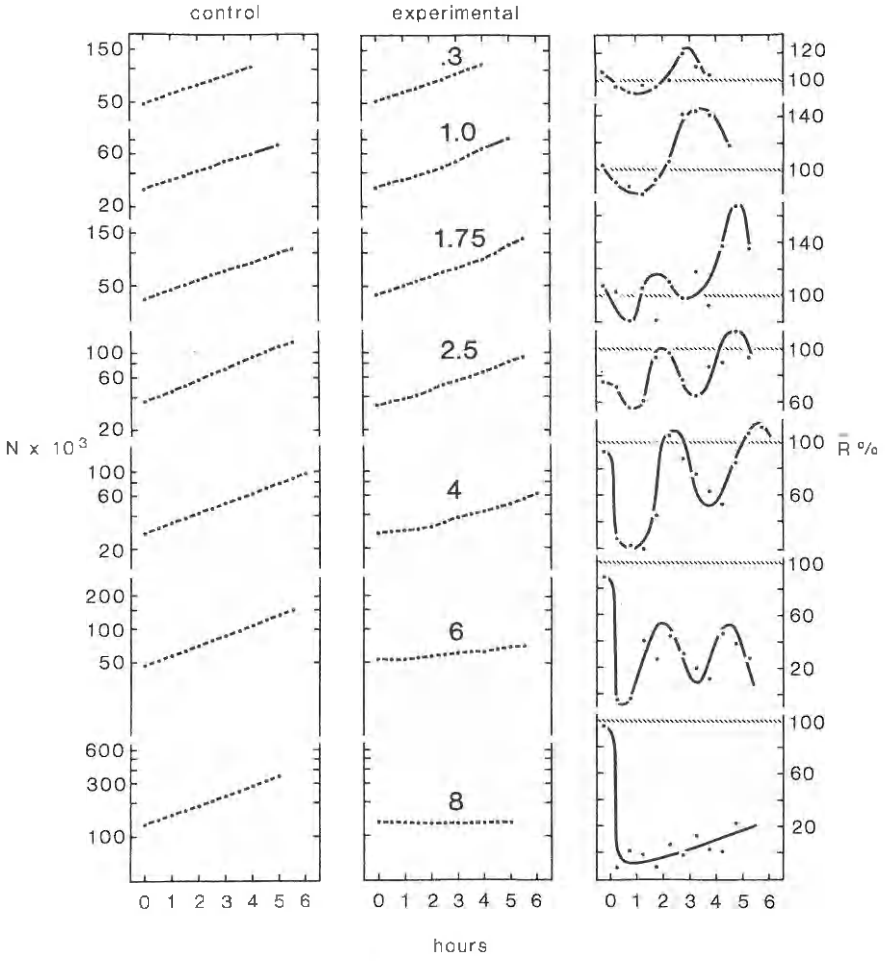

Fig. 1. Rhodotorula rubra. The growth of cultures exposed to different concentrations of cadmium. Cumulative growth curves show the increase in cell density with time of control (left) and experimental cultures (middle) to which cadmium was added. The data are also expressed as specific growth rates of the experimental cultures as a percentage of those of the control cultures ($\bar{R}$%) at 30 min intervals for each level of cadmium (right).

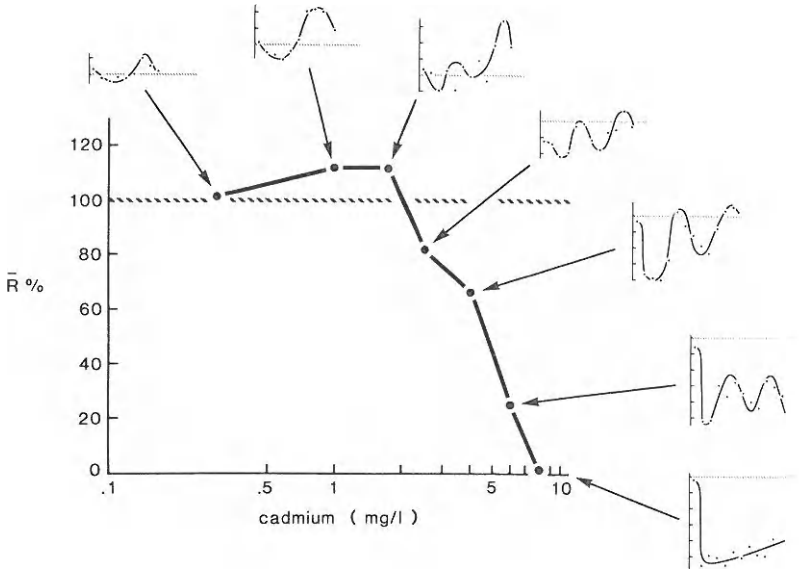

Fig. 2. Rhodotorula rubra. The growth of cultures exposed to different concentrations of cadmium. The data are presented as mean specific growth rates as percentages (R%) for the duration of each experiment. The plots showing temporal variations in R% with time from Figure 1 right are given as insets to assist interpretation.

(Fig. 2). This phenomenon is termed hormesis and is common in the toxicological literature (Stebbing, 1982). Experiments with the colonial hydroid Laomedea (Campanularia) flexuosa, showing hormesis resulting from exposure to a number of toxicants, indicated that the stimulation of growth can be explained as a consequence of the integration of adaptive overcorrections by a rate-sensitive growth control mechanism (Stebbing, 1981b).

At concentrations from 2-6 mg/l oscillations are at their greatest amplitude, but there is significant inhibition of the mean R% which increases as the degree to which the capacity of the control mechanism to counteract inhibition is exceeded. The recovery at 2.5 and 4.0 mg/l is probably due to a decrease in the level of available cadmium, although an increase in counter response could also explain the recovery. At 6 mg/l the degree of overload is even more marked and so the inhibition of mean R% becomes greater, while at 8 mg/l all cell division is quickly arrested and the mean growth rate is almost zero.

DISCUSSION

The characteristic form of our data from experiments with Rhodotorula, and the development of simulation models (Stebbing et al., 1984), indicates that the most plausible explanation is that they represent the output of a control mechanism regulating growth. A similar investigation of growth in a colonial hydroid led us to the same conclusion (Stebbing and Hiby, 1979). This hypothesis provides the most likely explanation of our data in its different forms, including the paradoxical stimulation of growth by low levels of toxicants (Stebbing, 1982).

Little importance should be attached to the threshold concentration itself (Fig. 2) as total levels of metal added do not indicate the ionic activity of cadmium to which availability and therefore toxicity are related (Engel and Fowler, 1979). Most of the metal is probably bound to organic constituents in the medium and to inorganic ligands in seawater. Ionic activity is also likely to decrease as metal ions are bound by cells with the growth of the culture. In the environment where the binding capacities are obviously less, inhibition would probably occur at concentrations an order of magnitude lower than those observed here (Fig. 2).

As toxicological data are usually presented in such a way as to demonstrate inhibitory thresholds and as the biological response measured is often one that is homeostatically controlled, it is helpful to attempt to generalise the interpretation we have used here to explain the effects of toxic inhibition on growth. Similar evidence for control, when growth data are treated in the same way, has been found in young Mytilus edulis (Almada-Villela et al., 1982) and Rhithropanopeus harrisii (Sanders and Costlow, 1981).

We also wish to link this interpretation to the related concepts of load, stress and health. In the sense that Selye (1950) first used it, stress can be considered as any external force or stimulus that elicits the generalised adaptive responses of organisms (Stebbing, 1981a). The responses of homeostatic or homeorhetic control mechanisms are a particular kind of adaptive response, although they are generalised in that the response is the same, whatever agent is used to cause an inhibitory perturbation.

The concentration-response curve (Fig. 3 top) can be considered in terms of the counter response that operates to neutralise the inhibitory effect of a toxicant upon growth (Fig. 3 bottom). At subthreshold levels nearly an order of magnitude below those that inhibit growth, the inhibitory challenge is sufficient to initiate a response of the control mechanism, as the oscillations at these levels indicate. The counter response increases due to the inhibitory load caused by higher levels of toxicant, but once the limits of the capacity of the control mechanism to counteract inhibition - or sustain the inhibitory load - are reached (Fig. 3 bottom), further increases in level result in significant growth inhibition (Fig. 3 top). The threshold concentration not only coincides with, but is explained by the onset of overloading of the control mechanism. Since this relates to one definition of stress (Shorter Oxford English Dictionary) as "the overpowering pressure of some adverse force or influence", we consider that superthreshold levels of a toxicant impose stress while subthreshold levels may impose load (Fig. 3).

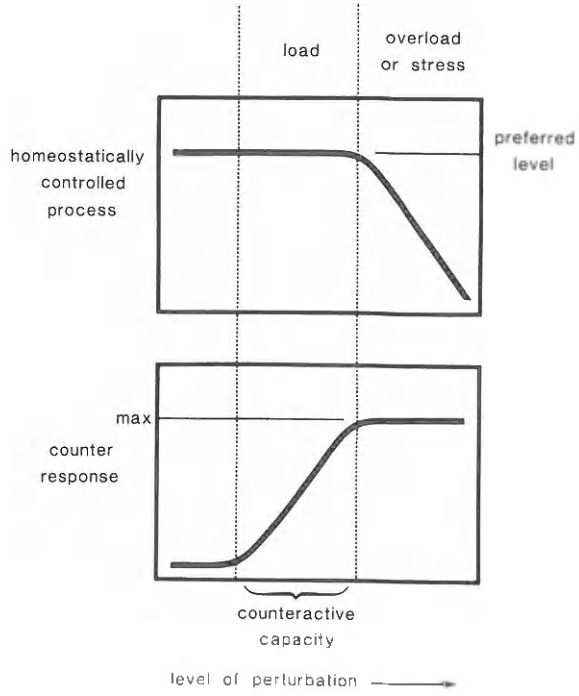

Fig. 3. The relationship between a typical concentration-response curve (top) and the counter response (bottom) due to some homeostatic or homeorhetic control mechanism.

While "stress" as a concept is useful in toxicology, of more practical utility would be ready means of quantifying and estimating the health of organisms. The use of load tolerance tests in medicine provides just such a means because health is measured in terms of the capacity of a process to withstand load (Frenster, 1962). Thus a healthier organism has a greater capacity to sustain load, and the health of an organism from the environment already under some unknown load is its residual capacity to withstand further load before overload occurs. In this way the health of an organism brought into the laboratory from the environment can be quantified in terms of the level of some load factor that can be sustained, and can be measured experimentally by determining the residual capacity to sustain imposed loads.

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