lated from animals after the repeated administration of smaller doses of the hormone.

Cortisol leads to an (approximately) threefold increase in the incorporation of tritiated orotic acid into both nuclear RNA fractions. At the same time, as shown before, it induces the formation of new species of mRNA whose binding to DNA cannot be prevented by excess nuclear RNA from hypophysectomized rats that were not treated with cortisol (Table 2). If nuclear RNA extracted at *p*H 8.3 from growth hormone-treated rats (H^3-labeled) and the corresponding RNA from rats treated with P^{32}-labeled cortisol are hybridized in the presence of increasing concentrations of unlabeled nuclear RNA from rats treated with either cortisol or growth hormone it becomes clear that unlabeled RNA from the livers of cortisol-treated animals can compete equally well with both labeled fractions, while RNA from growth hormone-treated rats competes only partially with P^{32}-labeled RNA from cortisol-treated rats. Thus, in comparison to RNA from cortisol-treated rats, RNA from animals treated with growth hormone behaves like RNA from hypophysectomized rats that did not receive any treatment at all (Fig. 1). These results (Table 2) suggest that the effect of cortisol on RNA synthesis involves regulatory mechanisms which are not influenced by growth hormone, an interpretation supported by the findings of Florini and Breuer (*5*) who studied the effects of testosterone and of growth hormone on the priming activity of chromatin from skeletal muscle and on the activity of an aggregate RNA polymerase from the same tissue. Whereas testosterone increased the priming activity of the chromatin template almost twofold, no such effect was found for growth hormone. Instead, growth hormone stimulated the activity of a preparation of DNA-dependent RNA polymerase by more than 50 percent. The additive effects of these two hormones on RNA synthesis could be explained on the basis of their different modes of action. An increase of the priming activity of chromatin, as found by Marushige and Bonner (*6*), in rat liver after cortisol administration and by Florini and Breuer after testosterone treatment in muscle correlates well with the finding that cortisol induces the formation of new types of mRNA. The fact that growth hormone, while stimulating total RNA synthesis, does not lead to the synthesis of new types of mRNA appears to be consistent with the inability of this hormone to produce changes in the template activity of muscle chromatin. Several enzymes inducible by cortisol and many of its analogs in rat liver are not induced by growth hormone (*7*). On the basis of our results as well as those of Florini and Breuer this may be due to the inability of growth hormone to alter template functions of chromatin and to initiate the synthesis of new species of mRNA.

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11 April 1967

Phycomyces Sporangiophores: Fungal Stretch Receptors

Abstract. *The application of mechanical force to the sporangiophore of* Phycomyces *elicits a transient growth response. The immediate stimulus may be the deformation of the sporangiophore under the applied force. Compression of the cell causes an interval of faster growth, and extension causes an interval of slower growth.*

The sporangiophore of *Phycomyces* is a rapidly growing single cell, capable of responding to light (*1*) and to several other stimuli. Investigation of its response to gravity and to centrifugation (*2*) showed that the cell is sensitive not only to acceleration but also to its own physical deformation resulting from the action of acceleration. We now report that the sporangiophore gives a growth response following deformation by purely mechanical means and that a change in length (positive or negative) caused by this deformation is always associated with an opposite change in growth rate: stretching the cell causes a period of slower growth, and compressing the cell causes a period of more rapid growth. Apparently the cell can function as a transducer of mechanical stimuli, but the output is altered growth rate instead of the nerve impulses of animal mechanoreceptor cells. In common with some animal mechanoreceptors, a *Phycomyces* sporangiophore can adapt to mechanical stimuli: its response to a sustained stimulus drops to zero.

To simplify the mechanical situation, we restricted the stimulus to the application or release of a tension along the axis of the cell, thereby ensuring that the deformation is symmetrical about this axis. The *Phycomyces* culture vial was held in an inverted position so that the sporangiophores hung downward. A double-pronged wire hook was hung on the sporangium of the cell selected for study, and a weight was hung from the hook by a single silk fiber (Fig. 1). We could release tension at will by raising a platform just high enough to support the weight (the weight of the hook, 0.2 mg, acts continuously). We measured the position of the sporangium with red light, using a long-focus horizontal microscope equipped with a filar micrometer eyepiece.

We investigated the overall mechanical properties of the cell by measuring its passive extension as a function of load. By spacing several weights along the silk fiber, we could apply loads of 1, 2, 4, and 8 mg in rapid succession and measure the resultant stretching of the cell. Generally the curves of extension as a function of load are nonlinear, the cell showing a greater stiffness as the load is increased. Also, the stiffness decreases during maturation of the sporangiophore, which is characterized by a darkening of the sporangium and a gradual increase in the growth rate. The total extension due to the application of an 8-mg load is 0.20 mm (mean of 105 measurements) for cells ranging from 25 to 40 mm in length. Measurements of the movement of starch grain markers on the cell wall indicated that in a cell 30 mm long about 50 percent of the extension occurs in the terminal 1.5 mm of the sporangiophore, a re-

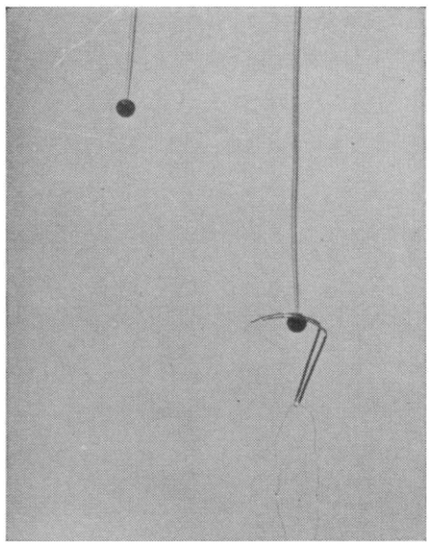


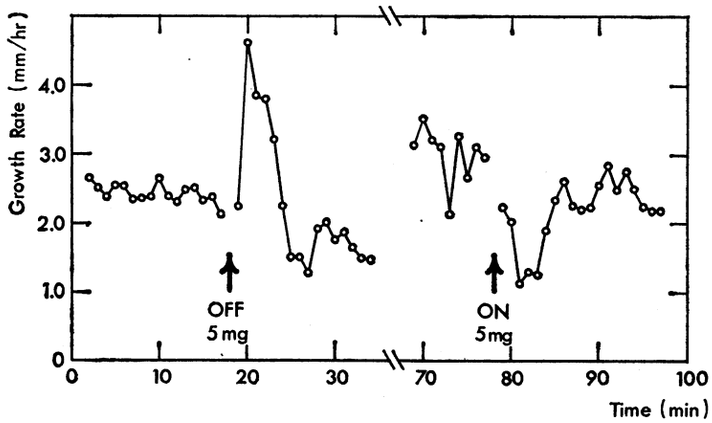
Fig. 1. A mature sporangiophore with a double-pronged wire hook resting on the spherical sporangium at the end of the cell. The diameter of the sporangium is 0.5 mm. Weights are attached to the lower end of a 5-cm silk fiber, whose upper end is threaded through the wire hook as shown. The growing region of the sporangiophore is confined to the terminal 3 mm, adjacent to the sporangium.

gion that includes the point of maximum relative elemental growth rate (*3*).

An unexpected finding is that the sporangiophore is highly elastic. To examine this, we measured in rapid succession the unloaded position, the loaded position, and the unloaded position of the sporangium (total elapsed time, 1 minute). In a perfectly elastic system the amount of contraction would equal the amount of extension. When the load is removed, the sporangium should resume its original unloaded position. If a nonelastic component is present, the sporangium will not resume its original position. We found slow growing, immature sporangiophores return to the original unloaded position. Sporangia of mature sporangiophores do not return completely to their original unloaded positions, but this can be largely explained by the amount of growth that occurs during the experiment (a growth of 0.05 mm in 1 minute is typical of a mature sporangiophore). If measurements of the final unloaded position are corrected for the growth during the experimental interval, we find that when the load is removed the sporangium returns 93 ± 3 percent of the distance from the loaded position to the original unloaded position (mean based on 70 determinations, standard error of the mean given). Thus the cell has at most only a small nonelastic component.

A sporangiophore was allowed to grow for 60 minutes while under a load of 5 mg, and by the end of this period the growth rate was relatively constant at between 2.0 and 2.5 mm/hr (Fig. 2). At the time indicated by "OFF 5 mg," the load was removed, causing a passive shortening of the cell that was complete within 30 seconds. This abrupt change in cell length is not shown in the record of the growth rate. The response to the removal of the load is evident as a transient rise in growth rate, lasting about 5 minutes and having a latency of about 1 minute. About 60 minutes later the unloaded cell again reached a relatively stable growth rate, this time about 3 mm/hr. Then the load was reapplied ("ON 5 mg"), causing a passive stretching of the cell complete within 30 seconds. The response to the application of load is a transient slowing of growth, which also lasts for about 5 minutes and has a latency of about 1 minute.

It should be emphasized that whether the response is more rapid growth or slower growth, the change in growth rate (positive or negative) is always opposite to the change in length caused by addition or removal of the load. A stretching of the cell (caused by a heavier load) elicits a slowing of the growth rate, while contraction of the cell (caused by a lighter load) brings about a more rapid growth rate. This same symmetry was noted earlier in the transient tropic response to mechanically induced bending of the cell (*2*). If a sporangiophore is bent slightly to the left by a lateral force applied to the sporangium with a fine glass fiber, the cell responds during the next 5 minutes by bending vigorously to the right, against the direction of the original deformation. This bending response is quite compatible with the growth responses presented here, if we assume that the lateral force of the glass fiber causes a compression along one flank of the cell and a stretch-



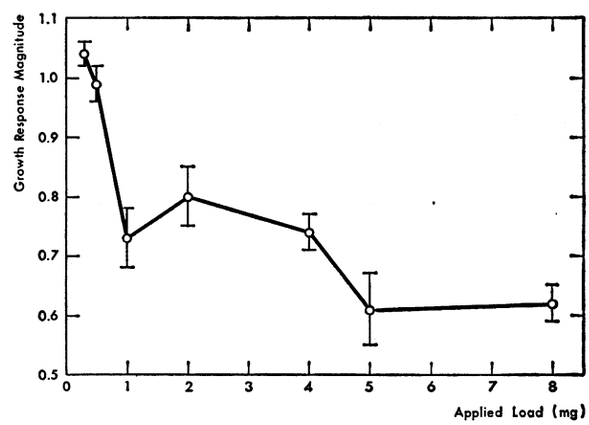


Fig. 2 (left). Growth responses to changing the load on the cell. The growth rate is obtained by subtracting successive measurements of the sporangium position made at 1-minute intervals. A mature sporangiophore typically elongates at a steady rate of 2 to 3 mm/hour for many hours. A positive growth response is seen following the removal of a 5-mg load ("OFF 5 mg"), and a negative growth response is seen following the application of a 5-mg load ("ON 5 mg"). Fig. 3 (right). Variation of the growth response with applied load. Response magnitude is defined as the ratio of growth in the 5 minutes following the stimulus to that in the 5 minutes preceding the stimulus. A response magnitude of 1.0 indicates a lack of response. Each point is an average of from 4 to 38 measurements, and the bars indicate ± standard error of the mean. The experiments were performed in red light with intervals of 60 minutes between stimuli.

ing along the opposite flank. The growth rate on the compressed side would increase, while that on the stretched side would decrease, resulting in a bend in the opposite direction, as has been observed.

Another important property of this growth response is that of adaptation; though the growth rate deviates from normal for 5 minutes after the change in load, it subsequently returns to normal even though the load remains constant. To test whether this adaptation is complete, we compared growth rates before and after the application of a load, using the following procedure. Before the hook was attached, the growth rate was measured for 10 minutes. Then the load was applied for 60 minutes, removed for 60 minutes, and finally reapplied for 60 minutes. Average growth rates were obtained for the 10-minute control period and the last 10 minutes of each of the 60-minute periods. We used only the last 10 minutes to reduce the contribution of the growth response at the beginning of each 60-minute period. Having combined the data for loads of 5, 8, 10, and 20 mg, we obtained the following average growth rates (percentage of control rate): load applied first time, 74 ± 5 percent; load removed, 80 ± 7 percent; load applied second time, 79 ± 5 percent (standard error of the mean given). Thus, no significant effect of load on growth rate was noted 50 minutes after the load was applied. This is consistent with the observation that the cell is mostly elastic, since one might expect a cell with a large plastic component to undergo continuous slow deformation when loaded and hence show a greater growth rate with the load applied than with it removed.

The ability of the sporangiophore to adapt completely to the applied load suggests that the deformation itself is the stimulus or is closely related to it. Since the sporangiophore is mostly elastic, most of the deformation occurs within a few seconds of the load's being changed. Since little deformation occurs thereafter, there will be no further stimulus acting to affect the growth rate as long as the load is constant.

We have also examined some of the factors affecting the magnitude of this growth response. We define the magnitude of the response as the ratio of the total growth during the 5-minute interval following the stimulus to the total growth during the 5-minute interval preceding the stimulus. Thus a response to an added load would be a number less than 1 (typically 0.6 to 0.8), and a response to a reduced load would be a number greater than 1 (typically 1.3 to 1.6).

Two factors affecting the response size are the length of the interval between stimuli and light. If the interval is shortened to 20 minutes, the responses are of smaller magnitude than those when there is an interval of 60 minutes between stimuli. Illumination with blue light causes a definite but highly variable reduction of the response's magnitude; this inhibition is largely removed by 60 minutes of darkness.

The weight of the load also affects the size of the response. In Fig. 3 are presented the mean response magnitudes at stimulus loads varying from 0.3 to 8 mg. Although variability is high, there is a rather sharp threshold at a load between 0.5 to 1.0 mg; at 0.5 mg and below there is no response, and at 1.0 mg and above the response is relatively large. The lack of response at 0.5 mg is not due to a lack of deformation, since at this load the total deformation of the cell is 0.13 ± 0.02 mm.

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27 March 1967

Force, Shortening, and Work in Muscular Contraction: Relative Contributions to Overall Energy Utilization

Abstract. *The amounts of energy used during muscular contraction under different loads have been compared by measurement of the total amount of disturbance of the concentration of reduced nicotinamide-adenine dinucleotide during the period of oxidative recovery. The results are in quantitative agreement with the concept that three parameters determine the energy utilization: namely the time-integral of the force development, the time-integral of the shortening process, and the mechanical work.*

When a muscle shortens against a lever bearing a load, the amount of energy expended in the process is highly dependent on the load that is moved. This was first pointed out by Fenn in his studies on the heat developed during and after twitches and short tetanuses (*1*). Until relatively recently, three processes were thought to account for this so-called Fenn effect: activation (and its maintenance), shortening, and the performance of mechanical work (*2*). We have found that two parameters, the time-integrals of the developed force ($\int P_o$) and of shortening ($\int S_o$) suffice for the quantitative description of energy used in isometric and minimally loaded isotonic contractions, respectively. In addition these parameters, together with the mechanical work performed, describe the Fenn effect quantitatively. This finding is at variance with the concept that the activation energy is a major contribution, independent of load, to the total energy utilization (*2–5*). It is also in disagreement with the concept that the energy requirement for shortening is linearly related, or very nearly so, to the distance of shortening (*2, 5*). But in contrast to other recent results (*3, 6*), we find there is a major contribution by the shortening process.

Energy utilization in various types of contractions was compared on the basis of changes in the amount of mitochondrial reduced nicotineamide-adenine dinucleotide (NAD) within the muscle at rest. During oxidative metabolism the average redox steady-state of several members of the respiratory chain varies depending on the rate of electron transport (*7*). A close correlation exists between the amount of reduced NAD at equilibrium, the rate of oxygen uptake, and the rate of oxidative phosphorylation. Thus, it is possible to monitor the metabolism during recovery in an intact muscle after contractile activity if the amount of reduced mitochondrial NAD can be measured. When the reduced form of the coenzyme (NADH) is excited with light with a wavelength of 366 mμ, the main part of the fluorescent light is emitted in the region of 400 to 500 mμ, with a broad maximum around 460 mμ. The intensity of the