Reports

Regulation of Mitosis in Stentor coeruleus

Abstract. When *Stentor coeruleus* was cut into anterior and posterior halves, the micronuclei in the posterior half underwent mitosis about 5 to 6 hours later, as shown in stained preparations. It is suggested that the division of the micronuclei was initiated by metabolic changes which resulted from the lack of adoral membranelles.

Evidence is accumulating that a minimum amount of chemically bound energy is required for the initiation of mitosis. This is borne out by the investigations of Swann (*1*) and Bullough (*2*), who have demonstrated the need for a minimum energy supply for the initiation of mitosis in sea urchin eggs and mouse epidermis, respectively.

The role of energy supply in the rhythmic alternation between cell growth and cell division can be studied in the ciliate *Stentor coeruleus,* since its anterior end has a conspicuous energy-consuming ciliary apparatus, and because it contains a highly polyploid macronucleus, surrounded by a varying number of micronuclei, which extends throughout most of the cell. The organism, therefore, can be cut into halves in such a way that the two halves receive approximately equal shares of the macronucleus and the micronuclei, while only the anterior half receives the ciliary apparatus. The behavior of the nuclei in such halves can then be correlated with the energy consumption, which should be relatively increased in the anterior fragment but decreased in the posterior fragment, as compared with the energy consumption of the whole cell.

Schwartz found previously (*3*) that removal of the adoral apparatus in *S. coeruleus* results in a temporary contraction of the macronucleus similar to that seen during normal cell division. After regeneration of the organism, he found an unexpectedly large number of micronuclei—a finding which indicated that the micronuclei had undergone mitosis. Since this experiment has direct bearing upon the problem outlined above, we have repeated it in order to establish, in stained preparations, whether or not the loss of the adoral organelles is inevitably followed by mitosis of the micronuclei (*4*).

About 1 hour after division, a large number of cells were cut with a glass needle into anterior and posterior fragments, which received approximately equal parts of the macronucleus, together with micronuclei. At this stage, according to Weisz (*5*), the anterior and the posterior portions of the macronucleus are functionally equal. The living pieces were examined from time to time with the aid of a stereomicroscope. When left alone, both parts regenerated and, after more than 40 hours, divided. About 5 to 6 hours after the cutting, the macronuclei in the posterior fragments became contracted and remained so for more than 1 hour, while no contraction occurred in the anterior pieces. Those fragments whose macronuclei were contracted were fixed on albuminized cover slides (mercuric chloride-ethanol-glacial acetic acid), stained with alum carmine, and examined under a phase microscope. In 17 out of 125 cases the micronuclei were found to be in mitosis in the posterior fragments, whereas no mitosis and no increase in the number of micronuclei was observed in the anterior fragments, regardless of the respective sizes of macronuclei and fragments. In controls, the micronuclei did not undergo mitosis before the next cell division, which occurred about 24 to 30 hours after the preceding one.

Since the main difference between the anterior and posterior fragments consisted in the presence or absence of the ciliary apparatus, it would appear that the advance in onset of mitosis in the posterior halves was a consequence of the diversion of energy and precursors from processes concerned with the operation of the ciliary apparatus to mitosis. We suggest that the loss of the ciliary apparatus causes a considerable decrease in the utilization of adenosine triphosphate, and that this results in a reduced level of adenosine diphosphate available for further phosphorylation. An inadequate amount of adenosine diphosphate as phosphate acceptor would result in a shift toward a reduced state of the respiratory chain, as demonstrated in in vitro experiments by Chance (*6*) and by Lardy (*7*). An increase in both phosphorylation pressure and reducing power would indeed satisfy some of the known requirements for mitosis, such as the need for deoxyribose and triphosphates (*8*) as specific building blocks for the chromosomes and the need for a disulfide reductase activity (*9*) to initiate the spindle mechanism, as suggested by Mazia (*10*).

Weisz' observation (*11*) that the fusion of enucleated fragments of *S. coeruleus* with nucleated individuals in early interphase advanced the onset of mitosis considerably is also pertinent here, since Brachet has shown (*12*) that aerobic adenosine triphosphate production in enucleated cell fragments continues undiminished. The fusion of an enucleated fragment with a nucleated cell therefore brings about a relative increase in the production of chemically bound energy, while such energy-requiring processes as ribonucleic acid and protein synthesis (*13*), which are largely nucleus-dependent (*14*), probably do not show a proportional acceleration. Plesner's recent finding (*15*) that nucleoside triphosphates accumulate in synchronized cultures of *Tetrahymena* just prior to mitosis substantiates the above interpretation and suggests that an imbalance similar to that produced in the experiments with *Stentor* is responsible for the initiation of mitosis towards the end of interphase during normal growth.

The role that competition for a supply of energy for various cellular processes plays in the regulation of mitosis is now being subjected to further scrutiny in the myxomycete *Physarum polycephalum,* which provides synchronized mitoses (*16*) in a mass of tissue large enough for chemical analysis.

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References and Notes

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

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Pepsin-like Enzyme in Larvae of Stable Flies

Abstract. A pepsin-like enzyme has been found in larvae of the stable fly. The activity is detectable in homogenates of whole larvae and in homogenates of isolated midguts, the latter of which possess a *p*H environment low enough to support a peptic enzyme. The *p*H optimum of the activity was determined to be 2.4.

Prior to the work of Greenberg and Paretsky (*1*), it was assumed that the enzyme pepsin was absent from insects, and indeed, was restricted to vertebrates. However, these workers showed that a pepsin-like activity is present in all three larval stages of the house fly. Although Champlain and Fisk (*2*) were unable to detect pepsin in homogenates of whole, adult, blood-fed stable flies, we felt that the results of Greenberg and Paretsky warranted a closer investigation in other stages of the stable fly, and also in the expected site of protein digestion, the midgut. The present report (*3*) summarizes the results of a study of a pepsin-like enzyme in the stable fly, *Stomoxys calcitrans* (L.), and the house fly, *Musca domestica* (L.).

Fully mature, third instar larvae, reared on CSMA medium after the method of Champlain *et al.* (*4*) were used throughout. Homogenates of whole larvae (final concentration 10 per milliliter) or dissected midguts (final concentration 50 per milliliter) were prepared with a Teflon-Pyrex tissue grinder in glycine-NaOH buffer (*p*H 2.5) in the cold, and the cuticular debris was removed by centrifugation. Homogenates were stored frozen before use. Estimation of pepsin-like activity was made essentially by the method of Fisk and Shambaugh (*5*) by incubating 0.1 ml of homogenate with 0.9 ml of glycine-NaOH buffer (*p*H 2.5) and 0.25 ml of sulfanilic acid azoalbumin substrate (*6*) at 37°C for 2 hours. Analysis of the diazotized, colored amino acid or peptide fragments, released in direct proportion to enzyme activity (*5*), was accomplished with a Klett-Summerson photoelectric colorimeter with the 420-mμ filter. Controls consisted of reaction mixtures containing homogenate which had been placed in a boiling water bath for 5 minutes prior to assay, and also of reaction mixtures in which the homogenate was replaced by an equal volume of buffer to determine autolysis and residual color of the substrate. Although the investigation was primarily qualitative, the same wet weight of tissue, or number of midguts or whole larvae, was used for comparable assays.

Table 1 is a summary of the results, which confirm the presence of a pepsin-like enzyme in both whole larvae and midguts of *Musca domestica* and *Stomoxys calcitrans*. There is a small but consistent increase in Klett-Summerson colorimeter units in the unaltered mixtures compared with the values obtained for boiled homogenate mixtures or with the residual color of the substrate alone. Variations occurred from preparation to preparation. An additional series of experiments was carried out in which the Folin-Ciocalteau technique employed by Greenberg and Paretsky was followed. By this technique we showed that larvae of both species possessed nearly equal pepsin-like activities. A later comparison of the activities of homogenates from third instar larvae, pupae, and adult stable flies showed that the larvae had the most activity, followed closely by the pupae, but with the adults quite low.

To insure that the enzyme activity was not due to microorganisms, tests were repeated with axenic larvae (*7*). Essentially the same results were obtained (see Table 1). Sterility checks on representative samples of media and larvae showed them to be negative for bacteria, yeasts, and molds.

It was also necessary to determine whether the larval stable fly possessed an alimentary *p*H acid enough to support peptic activity. An experimental procedure was followed (*8*) in which young third instar larvae were reared on enriched artificial medium plus *p*H indicator dyes. The larvae fed for 2 days and were then dissected to determine *p*H. The *p*H of the medium itself was also noted.

The results of the *p*H tests of the digestive tract clearly indicated that the anterior portion of the midgut, and part of the mid portion of the midgut actually could provide a suitable *p*H environment for peptic activity. The following *p*H ranges were recorded: anterior midgut, 2.8 to 3.0; middle midgut, 2.8 to 4.8; posterior midgut, 6.8 to 7.9; hindgut, 7.9 to 9.6. These conditions prevailed although the food itself was above *p*H 6.8 before ingestion.

The *p*H optimum was determined for the pepsin-like activity by measuring both *p*H and increase in Klett reading after 2 hours' incubation of reaction mixtures buffered at 0.3 *p*H intervals between *p*H 1.0 to 3.1. The highest activity was found at *p*H 2.4, but the activity was only slightly less at *p*H 2.8. Activity was lowest below *p*H 1.3. These results are similar to those of Greenberg and Paretsky (*1*) who found the optimum to be *p*H 2.5 in both whole homogenates and dissected guts of the house fly.

At present it is not known whether the enzyme activity is similar to true gastric pepsin of higher animals or is a cathepsin acting at low *p*H. It is also unknown

Table 1. Results of tests confirming presence of pepsin-like activity in *Musca domestica* (L.) and showing similar activity in third instar larvae of *Stomoxys calcitrans* (L). Except as noted, activities are averages of seven replicates, while boiled homogenate or homogenateless mixtures represent two replicates.

Enzyme source	No. of whole insects or guts per replicate	Reading		Net increase due to active homogenate	Reading of homogenateless mixture
		Active mixture*	Boiled homogenate		
		Normal larvae			
Musca					
Whole larvae	1	44.3	41.5	2.8	
Midguts	5	37.2	20.9	16.3	
Stomoxys					
Whole larvae	1	43.9	36.5	7.4	21.0
Midguts	5	32.8	21.0	11.8	19.9
Midguts	5	23.7	13.4	10.3	8.5
Midguts	5	18.4	12.0	6.4	8.5
		Axenic larvae			
Stomoxys					
Whole larvae	4	16.5†	0‡	16.5	
Fore and Midguts	4	18.3†§	0	18.3	

* Klett units (Klett-Summerson photoelectric colorimeter with 420-mμ filter).
† Klett units derived from optical density readings with Spectronic 20 colorimeter at 440 mμ.
‡ Readings made with boiled homogenate tubes arbitrarily set at 0.
§ Activity readings here represent just six replicates.