## Repression of Acid Phosphatase Synthesis in Euglena gracilis

*Abstract.* Phosphate-repressible phosphatase synthesis occurs in the algae, *Euglena gracilis.* This phenomenon differs from the similar process known to occur in bacteria in that (i) the enzyme is an acid rather than an alkaline phosphatase, and (ii) enzyme activity nearly disappears after addition of phosphate to the culture.

The synthesis of phosphomonoesterase activity in *Euglena gracilis* (Klebs), z strain, is repressed by inorganic phosphate in a manner generally similar to, but different in detail from that observed earlier with *Escherichia coli* (*1, 2*). There are two differences: (i) the enzyme is an acid rather than an alkaline phosphatase, and (ii) the enzyme activity is not stable in vivo, but falls almost to zero soon after the addition of phosphate.

*Euglena* were grown heterotrophically with $10^{-4}M$ phosphate in an otherwise complete nutrient medium (*3*). The medium contained inorganic salts, thiamine, cyanocobalamin, ethanol, and glutamate. Cells were washed with water, suspended in $0.01M$ sodium malate buffer, *p*H 5, and frozen. Phosphomonoesterate activity in the thawed cells was estimated with $10^{-3}M$ nitrophenylphosphate at 30°C by a modification of the method of Bessey *et al.* (*4*). Enzyme activity was linear with time for at least 20 minutes and linear with enzyme concentration over the range employed. Optimum activity was found at *p*H 5.0 with $0.1M$ malate buffer.

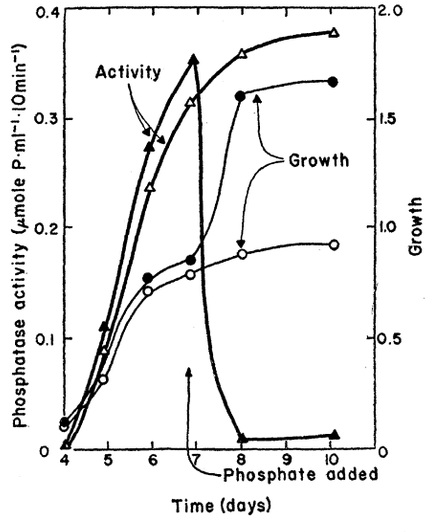


Fig. 1. Time course of phosphatase activity and growth in phosphate-deficient *Euglena.* One flask (△—○) was given 100 μmole of phosphate per liter of medium at time of inoculation and allowed to exhaust phosphate. The second flask (▲—●) was treated identically until 6.9 days, at which time (see arrow) 2000 μmole of phosphate per liter of medium was added. Growth (○—●) was measured as optical density of the culture suspension, corrected for self-absorbance. Phosphatase activity (△—▲) was measured as described in text. Final specific activity of phosphate-deficient cells was 18 times greater than initial activity and 52 times greater than activity of cells that had received additional phosphate.

The time course of growth, as measured by corrected optical density (*3*), and of total phosphatase activity is shown in Fig. 1. Two results are immediately evident: (i) activity was virtually zero until growth departed from the exponential rate, after which activity increased rapidly; and (ii) although upon addition of 2 μmole of phosphate per milliliter growth resumed, total activity fell to near the initial value.

The time course of enzyme synthesis with *Euglena* was directly comparable to that observed with *Escherichia coli* (*1, 2*) and *Bacillus subtilus* (*5*). The effect of adding phosphate, however, was markedly different. With the bacteria, addition of phosphate resulted simply in cessation of synthesis; with the algae, enzyme activity decreased as rapidly as the enzyme was formed, falling virtually to zero. The activity of phosphate-deficient and phosphate-repressed cells mixed together was 98 percent of the activities measured separately; so inhibition by a high concentration of phosphate in the reaction mixture could not account for the sharply decreased activity in repression.

Hewitt and Tatham (*6*) reported a 20-fold increase in acid phosphatase activity in phosphate-deficient tomato leaves. Although Kuo and Blumenthal (*2*) found no phosphate-repressible phosphatase activity among 20 strains of *Staphylococcus aureus*, seven out of ten strains of *Escherichia coli*, and one strain of *Neurospora crassa*, the findings of Hewitt and Tatham together with the data presented here indicate that this possible control mechanism of phosphorus metabolism may occur widely in the plant kingdom (*7*).

C. A. PRICE

*Department of Plant Physiology, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick*

### References and Notes

T. Horiuchi, S. Horiuchi, D. Mizuno, *Nature* **183**, 1529 (1955
A. Torriani, *Biochim. et Biophys. Acta* **38**, 460 (1960).
M. H. Kuo and H. J. Blumenthal, *Nature* **190**, 29 (1961).
3. C. A. Price, *Biochem. J.* **82**, in press.
4. O. A. Bessey, O. H. Lowry, M. J. Brock, *J. Biol. Chem.* **164**, 321 (1946).
5. C. Anagnostopoulos, *Federation Proc.* **19**, 48 (1960).
E. J. Hewitt and P. Tatham, *J. Exptl. Botany* **11**, 367 (1960).
7. This study was supported by a grant (No. A3767) from the U.S. Public Health Service. I am indebted to Dr. Donald Plocke, S.J., for his association in an early stage of this study. This report is a paper of the journal series of the New Jersey Agricultural Experiment Station, New Brunswick.

18 July 1961

## Menstrual Irregularities in Temporal Lobectomized Rhesus Monkeys (Macaca mulatta)

*Abstract.* In four experimental and five control monkeys, we recorded 178 menstrual cycles. Unilateral temporal lobectomy in two animals had no effect upon lengths of menstrual cycles. Bilateral lesions in two animals affected menstrual function, producing significantly lengthened menstrual cycles with unfamiliar vaginal exfoliation patterns.

The purpose of this preliminary study was to demonstrate whether or not temporal lobe removal, with ablation of the amygdaloid nucleus, which is important in reproductive function in rabbits (*1*), resulted in a demonstrable effect on menstrual cycles.

In the spring of 1960, four experimental rhesus monkeys (*Macaca mulatta*) were anesthetized with Nembutal (0.6 gr/kg), the squamous plate of the temporal bone removed, and all of the temporal lobe except the superior temporal gyrus was sucked out. In five control animals sham operations were performed.

During 12 of the following 13 months, while consecutive menstrual cycles were recorded in experimental and control groups, vaginal canals were washed to recover all possible exfoliated material daily. Twenty-two control menstrual cycles during this period averaged in length (with standard deviation) 29.3 ± 1.8 days, and did not differ significantly in length from 64 control menstrual cycles recorded prior to the experiment.

Fifteen experimental menstrual cycles recorded in unilaterally temporal lobectomized animals averaged 29.6 ± 7.2 days and did not differ significantly in length from the concurrently recorded control menstrual cycles, or preoperative cycles of this experimental group.

Animal XIX received a unilateral temporal lobectomy on 13 June 1960, after 17 control menstrual cycles which averaged 26.8 ± 2.0 days. After surgery, beginning 21 June, two consecutive menstrual cycles of 30 days' length