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.

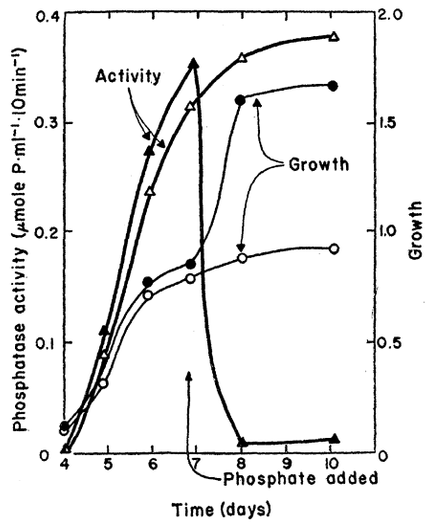

Fig. 1. Time course of phosphatase activity and growth in phosphate-deficient *Euglena.* One flask (△—○) was given 100 μmole of phosphate per liter of medium at time of inoculation and allowed to exhaust phosphate. The second flask (▲—●) was treated identically until 6.9 days, at which time (see arrow) 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*).

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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

occurred. After a hiatus of a month (in which all animals were moved from Vancouver, British Columbia, to Edmonton, Alberta) nine further menstrual cycles were recorded. The third recorded postoperative menstrual cycle was 45 days long, but the remaining eight were of normal length (28.2 ± 1.5 days).

While control animals menstruated regularly over the winter season, their first recorded menstrual cycles after removal from British Columbia to Alberta were often also irregularly long, and thus this third long postoperative menstrual cycle in animal XIX was believed in some way due to the change in environment and not due to experimental effect. As is illustrated in Fig. 1 (top), vaginal exfoliation rates varied systematically with stages in the menstrual cycle. This is typical of the data in the five control animals (*2*), in which sediment varied between 0.01 and 2.0 ml/day, and in the other unilaterally lobectomized animal in which 11 preoperative wintertime control menstrual cycles did not significantly differ in length from nine postoperative experimental menstrual cycles (31.1 ± 5.9 versus 30.8 ± 10.6 days).

In contrast to this, in the two bilaterally lesioned animals, 13 of their combined postoperative menstrual cycles averaged 40.3 ± 13.8 days standard deviation. There was a statistically significant difference in length (p between .01 and .001) when these results were compared with the lengths of their preoperative control wintertime menstrual cycles, which averaged 28.8 ± 4.1 days. A significant difference also occurred between these experimental postoperative cycles and those of the concurrently recorded control group. A brief protocol of the two bilaterally lobectomized animals summarizes the results.

Right and left temporal lobes were removed from animal VI on 6 April and 3 May, respectively. After a short (20-day) menstrual cycle, a 138-day period followed with no menstrual bleeding. Then two menstrual cycles of normal

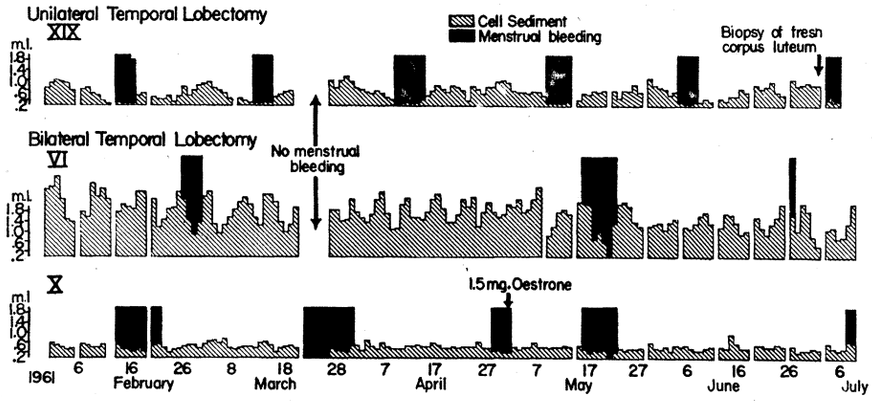

Fig. 1. Total daily vaginal exfoliate during menstrual cycles of one (XIX) unilaterally and two (VI and X) bilaterally temporal lobectomized rhesus monkeys. Lengths of menstrual bleeding are indicated by solid bars.

length (28 and 28 days) with normal vaginal exfoliation patterns occurred. Beginning in November and extending until 25 June 1961, three irregularly long menstrual cycles were recorded (56, 39, and 41 days) together with a 79-day amenorrheic period. An unfamiliar, but graded fluctuation in vaginal exfoliation rates occurred as illustrated in the second graph of Fig. 1. The five postoperative menstrual cycles and the amenorrheic period averaged 45 ± 19 days while preoperative wintertime control menstrual cycles averaged 30 ± 5.5 days.

The finding in animal X after bilateral temporal lobe removal was first a 124-day immediately postoperative period of amenorrhea followed by seven consecutive menstrual cycles, of which one was regular (26 days) and the others irregularly long (35 to 40 days).

The reduced and relatively invariant exfoliation rate that then occurred (bottom graph, Fig. 1) was the characteristic exfoliation pattern obtained in records of castrated but not intact animals.

The intramuscular injection of 1.5 mg of estrone in propylene glycol during day 4 of the fourth postoperative menstrual cycle was associated with an immediately shortened period of menses (4 days) and with a uterine bleeding 18 days later (bottom graph, Fig. 1). There was a similar finding in two out of four control animals injected at the same time in the menstrual cycle, but at twice the dosage. These phenomena in animal X were therefore interpreted to be related to estrone injection. In view of the likelihood that this was not a true menses—a point under further investigation—this 18-day period was excluded from calculation of menstrual cycle lengths.

During July 1961 the remaining temporal lobe in unilaterally lesioned animals was removed. Menstrual cycles in the recently lobectomized animals (XIX, I) are irregular, confirming the findings in animals VI and X.

Biopsies of corpora lutea taken during laparotomies in unilaterally lobectomized animals (top graph, Fig. 1) shows that this experimental group was capable of ovulation. Further experiments are necessary to determine if ovulation occurs in bilaterally operated animals.

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