Fully Deuterated Euglena gracilis

Abstract. Euglena gracilis *has been grown in 99.4 percent D_2O on fully deuterated substrates. It is the first organism with distinct animal characteristics in which at least 99 percent of the hydrogen normally present has been replaced by deuterium.*

Heretofore only algae, bacteria, and fungi have been successfully subjected to full isotopic replacement of hydrogen by deuterium. Higher plants and even the simplest animals resist full deuteration (*1*). The unicellular organism *Euglena gracilis* can be adapted to growth in greater than 99 percent D_2O on fully deuterated nutrients.

Euglena gracilis (strain Z) was obtained from the Indiana University Culture Collection. The organisms were grown in erlenmeyer flasks (125 ml) at 25°C at a light intensity of 4300 lu/m^2. Culture was heterotrophic, but access to air was maintained through a cotton plug. Isotopic exchange of D_2O with H_2O vapor of the air was very slow under our conditions, and our nominal 99.6 percent D_2O medium always was more than 99 percent D_2O by isotopic analysis (*2*). Aseptic techniques were used at all times.

A modified Hutner's nutrient medium (*3*) was used for the deuterated cultures. This medium contained the following components dissolved in 99.6 percent D_2O: ND_4NO_3, 3.0 g/liter; $MgSO_4$, 0.02 g/liter; K_3PO_4, 0.16 g/liter; $Ca(NO_3)_2$, 0.016 g/liter; $FeSO_4 \cdot 7H_2O$, 0.007 g/liter; B, 0.5 parts per million (ppm); Mn, 0.25 ppm; Zn, 0.05 ppm; Cu, 0.02 ppm; Mo, 0.05 ppm; Co, 0.01 ppm; vitamin mixture made up in D_2O (*2*); vitamin B_{12}, 10^{-7} g/liter; deuterated glucose, 1 percent; hot D_2O extract of deuterated algae (*4*), 0.3 percent; and a cold D_2O extract of whole deuterated *Scenedesmus obliquus* cells, 0.2 percent. The hot-water extract of algae can be replaced by 0.3 percent fully deuterated succinate or glutamate, but the cold water (D_2O) extract of whole deuterated algae cells is indispensable, as without it there is no growth at D_2O concentrations greater than about 80 percent.

Serial subculture into progressively higher concentrations of D_2O is necessary to obtain the fully deuterated organism. The following progression is suitable: H_2O, 50, 60, 70, 80, 85, 90, 95, 97, 99.6 percent D_2O. Sucrose was used in place of deuterated glucose in all except the final transfer step in order to conserve the supply of the deuterated sugar. The primary toxic effect seems to be associated with the deuterium of the water of the medium rather than with the nonexchangeable —C—D bond of the organic substrates. Adaptation requires about 6 months. Upon transfer to media of higher content of D_2O, the *Euglena* become spherical and motionless, and undergo a reduction in chlorophyll content. After several days the cells either lyse or slowly adapt to the new medium. It is often necessary to subculture at the same D_2O concentration to obtain vigorous growth before the next increase in deuterium content.

Fully deuterated *Euglena* are shorter and broader than normal *Euglena*. Internally, the deuterated organisms appear granular and have smaller and fewer chloroplasts. Flagella appear unaffected by deuteration. In new cultures in fully deuterated media, the eyespot is visible in fewer than 5 percent of the cells, but as growth continues more eyespots appear, though they are still only faintly visible. Upon subculture back to H_2O media the eyespots are clearly visible in all cells within 4 days. The morphological changes attendant on deuteration are illustrated in Fig. 1.

A phototactic response, while reduced, is easily observed in the fully

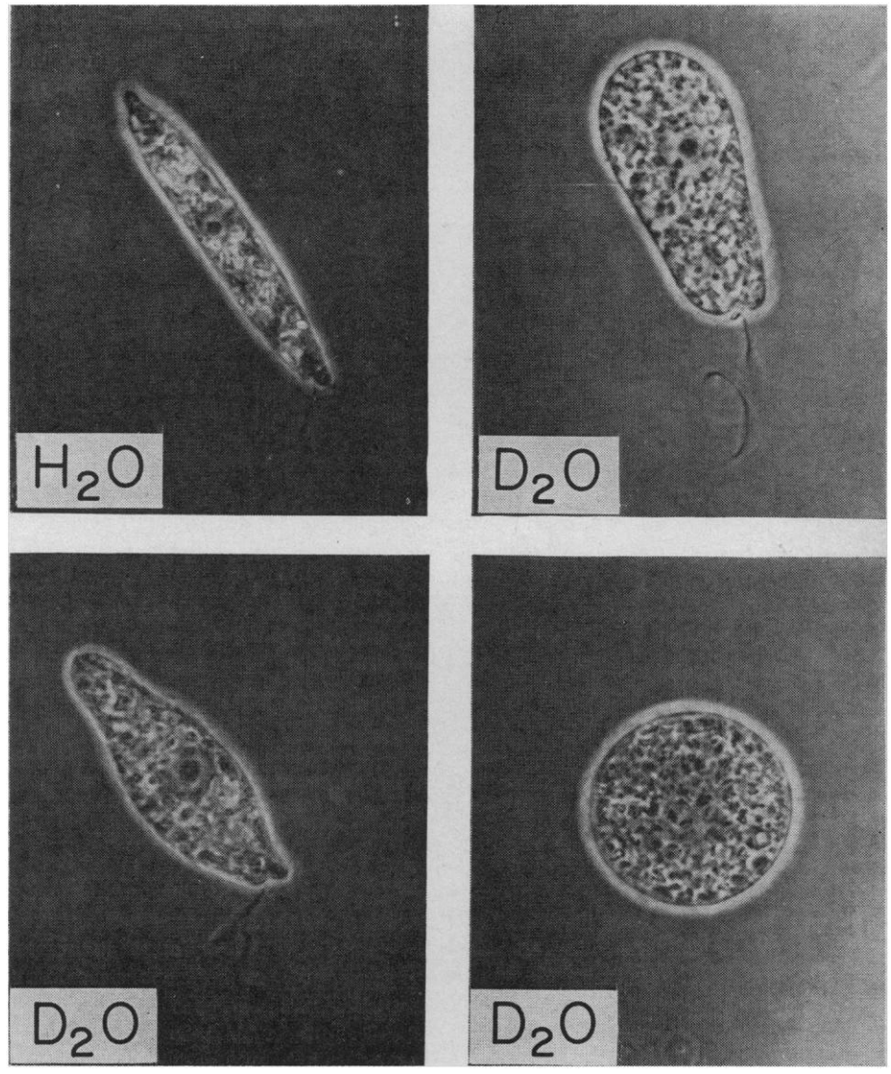

Fig. 1. Phase contrast photomicrographs of formalin-fixed *E. gracilis* grown in H_2O and D_2O. The cell labeled "H_2O" is typical of *Euglena* under our conditions in H_2O nutrient medium. The cells labeled "D_2O" are three typical cell shapes found in a culture at 99.4 percent D_2O. No single cell shape predominates. In general, the cells grown in D_2O are shorter and broader than normal, tend to "ball" much more than those grown in H_2O, and show a marked granulation. Flagella appear to be unaffected. Magnification, × 6000.

deuterated cells. The striking effect of partial deuteration upon the circadian rhythm in *Euglena* has previously been reported (*5*), and it will be interesting to explore this behavior in fully deuterated organisms.

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References and Notes

1. E. Flaumenhaft, S. Bose, H. L. Crespi, J. J. Katz, *Intern. Rev. Cytol.*, in press.
2. H. L. Crespi and J. J. Katz, *Anal. Biochem.* **2**, 274 (1961).
3. C. A. Price and B. L. Vallee, *Plant Physiol.* **37**, 1428 (1962).
4. H. L. Crespi, J. Marmur, J. J. Katz, *J. Am. Chem. Soc.* **84**, 3489 (1962).
5. V. G. Bruce and C. S. Pittendrigh, *J. Cellular Comp. Physiol.* **56**, 25 (1960).
6. Based on work performed under the auspices of the U.S. Atomic Energy Commission.

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Autonomic Mediation of the Effect of Raised Arterial Glucose upon Free Fatty Acids

Abstract. *The intravenous infusion of 600 milligrams of glucose over 30 minutes caused a 17 percent fall in the concentration of free fatty acids in arterial blood of subjects who had fasted overnight. This response to glucose was abolished in subjects treated previously with ganglionic or adrenergic blocking agents. Small amounts of insulin were secreted in response to these glucose infusions, but in insulin-dependent diabetics incapable of altering plasma insulin to any great extent, the effect of glucose upon free fatty acids could be obtained provided the subjects were primed with long-acting insulin before the infusions were begun. The response of free fatty acids to doses of glucose which elevated the concentration of glucose in arterial blood by only 3 mg percent and the blockade of this response by autonomic and adrenergic blocking agents suggest that centers in the central nervous system exist which are capable of responding to elevations of arterial glucose by inhibiting the sympathetic tone partially responsible for sustaining lipolysis in fasting.*

The role of the sympathetic nervous system in sustaining lipolysis during fasting has been amply documented (*1*, *2*). Tonic activity of the sympathetic fibers supplying the adipose tissue cells directly (*1*) and centrally mediated release of epinephrine and norepinephrine from the adrenal medulla (*3*) have been established as potential pathways for this activity. The stimuli important for initiating and sustaining this tonic sympathetic activity have received little attention. Dunér (*3*) demonstrated that raising the concentration of glucose in the carotid circulation or directly in the hypothalamus caused a prompt decrease in adrenal medullary secretion in cats. Dunér did not examine the rate of lipolysis in his experiments but rather concluded that the mechanisms under study were primarily concerned with regulating the concentration of glucose in the blood. In this report we present evidence that small increases in the concentration of glucose in the arterial blood of man inhibit lipolysis by decreasing central sympathetic tone (*4*).

Experimental subjects were studied after an overnight fast, in bed, in a quiet room. An intravenous needle for infusion was placed in a forearm vein and an indwelling needle for drawing blood samples was placed in a brachial artery. After a 30- to 45-minute rest period, two or three control samples were withdrawn at 15-minute intervals. Thereafter, solutions were delivered intravenously at a constant rate with a motor-driven syringe pump, and samples of arterial blood were collected periodically. Plasma was analyzed in duplicate for glucose (glucose oxidase) and free fatty acids (*5*).

To study the mechanism of action of glucose in decreasing lipolysis, the concentration of glucose in blood from fasting subjects was raised with small intravenous infusions of glucose. Doses of glucose were sought which would be just large enough to produce measurable changes in the free fatty acids but small enough to avoid major or prolonged alteration of arterial glucose concentration. The dose of glucose which would regularly produce a measurable decline in the free fatty acids in normal subjects proved to be 500 to 600 mg. This amount of glucose, delivered either as a single injection or at a rate of 20 mg/min for 30 minutes, was accompanied by a 20 percent decrease in free fatty acids; after the infusion, the concentration of these acids increased above the control values. To avoid the unpredictable surge of arterial glucose during the first circulation after a single injection, the short infusion technique was adopted for use in further studies. The pattern of response to infusion with 20 mg of glucose per minute for 30 minutes in eight normal subjects is illustrated in Fig. 1. The mean decrease in free fatty acids was −17 percent from the control values. At the same time, arterial glucose was raised by 2 to 3 mg percent. Increasing the rate of infusion to 60 mg of glucose per minute for 30 minutes caused an average decline of 25 percent in the concentration of free fatty acids, and after the infusion there was a more pronounced rise in the concentration (Table 1). Arterial glucose was raised 4 to 7 mg percent by this infusion rate.

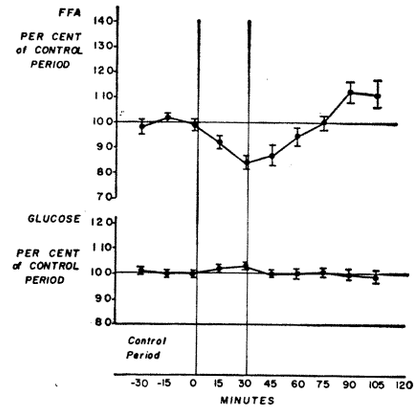

Fig. 1. The response to intravenous infusion of glucose at a rate of 20 mg per minute for 30 minutes (total dose, 600 mg) in eight normal fasting subjects. The vertical lines show two standard errors. Arterial samples were analyzed in duplicate for free fatty acids (*FFA*) and glucose.

Utilizing these small glucose infusions as stimuli, we examined the effect of treating the subjects with various autonomic blocking agents prior to and during the infusions. In normal fasting subjects under the conditions of our experiments, short-acting drugs such as hexamathonium (ganglionic blockade) or the adrenergic blocking agent, *n*-isopropylmethoxamine (*6*), produced a 30 to 35 percent fall in free fatty acids. Infusion of glucose at a rate of 60 mg/min failed to produce a further fall in free fatty acids at the height of blockade with either agent. To avoid the problem of low concentrations of free fatty acids at the time of challenge, prolonged ganglionic blockade was induced in three subjects with pentolinium. Subcutaneous injections of 5 mg were given every 4 hours during the 16 hours before study, and 10 mg was injected 30 minutes before the control period. The subjects were kept in bed through both treatment and experimental periods. The adequacy of blockade was easily demonstrated in all three subjects by