Of the 109 postmenopausal women questioned, only 60 were certain of their age and the lapse since their menopause; five of these had had an artificially induced menopause. The present findings thus relate to 55 women. In view of the absence of published studies of the menopausal age of Zulu women, the findings are presented in spite of the small size of the sample.

The median menopausal age of these 55 women was 48.1 years, and their mean menopausal age 47.7 years (standard deviation, 5.80). More reliable figures are those based on the responses of the 33 women whose menopause had occurred within the previous 5 years; the median menopausal age of these women was 48.6 years, and the mean age was 49.2 years (S.D., 4.15).

These average values are high by comparison with those for most other groups of women. Of 22 groups cited by Pearl (*1*), only four had a later mean menopausal age, and six a later median menopausal age, than the 55 Zulu women questioned. Only one of these 22 groups had a later mean menopausal age, and only four a later median menopausal age, than the 33 Zulu women questioned within 5 years of their menopause. The findings thus suggest that the menopause tends to occur relatively late among urban Zulu women.

There is a high prevalence of malnutrition in this community (*2*). As there may be considerable involvement of the reproductive system in states of malnutrition (*3*), it is possible that malnutrition is a contributory factor to their late menopause. It is known that adolescence may be delayed in malnourished children (*4*), and it may be that the menopause is similarly delayed. Although it is commonly stated that a late menarche tends to be associated with an early menopause, there is apparently no statistical evidence for this assertion (*5*); a recent retrospective study of South African white women revealed no evidence of such an association (*6*). While ethnic, climatic, and other factors may play a role, it is noteworthy that it has been stated, in respect of maturation at an earlier phase of life, that "so far as can be ascertained from present data neither climate nor race influence the time of adolescence as greatly as nutrition, at least where the differences in nutritional status are wide" (*4*). It is possible that the late menopause of these Zulu women may reflect a slow tempo of development, partly related to malnutrition in early life or throughout life. This suggestion is in conformity with the impression (*7*) that in the United States the menopause now occurs earlier than formerly, and that business and professional women tend to have an early menopause.

Because, however, a recent study of white women in South Africa, who have considerably less malnutrition, has also revealed a late menopausal age (*6*), the importance of climatic factors cannot be excluded.

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Two c-Type Cytochromes from Light- and Dark-Grown Euglena

Abstract. A pigment-protein complex can be extracted, in aqueous 2-percent digitonin, from *Euglena* grown in the light. When further fractionated by acetone and ammonium sulfate this flagellate yields a *c*-type cytochrome. By similar extraction of dark-grown, nonphotosynthetic *Euglena*, another *c*-type cytochrome can be isolated. The cytochrome from the light-grown Euglena is like that of cytochrome *c* isolated from a photosynthetic bacterium. The cytochrome from the dark-grown *Euglena* is like cytochrome *f* found in the chloroplasts of higher plants.

It has been postulated that a photosynthetic enzyme, a cytochrome, is intimately linked with the oxidation-reduction within the chloroplast and plays a part in the primary events of photosynthesis. Such cytochromes have been isolated from higher plants, algae, and photosynthetic bacteria and have been referred to as cytochrome *f*, cytochrome b_6, and modified cytochrome *c* (*1–3*). A cytochrome with an α-band absorption maximum at 552 mμ has recently been isolated by acid extraction from the light-grown photosynthetic algal flagellate *Euglena* but not from the dark-grown *Euglena* (*4*). Although mixed porphyrins from dark-grown *Euglena* had been previously shown, no known cytochrome *c* absorption peaks were identifiable (*5*).

In previous studies the chlorophyll-protein complex, chloroplastin, obtained by digitonin extraction of chloroplasts from *Euglena*, exhibited photochemical activity, such as the photoreduction of a dye and the evolution of oxygen (*6*). The photo-oxidation of cytochrome *c* has also been demonstrated with digitonin-extracted spinach chloroplasts (*7*).

The question, then, is whether digitonin extracts a cytochrome (as well as a chlorophyll complex) which may be responsible for the photochemical activity, and if it does, whether a similar cytochrome is present in the nonphotosynthetic, dark-grown *Euglena*. We have now isolated from digitonin extracts of *Euglena* two *c*-type cytochromes: one from the photosynthetic, light-grown flagellate and another from the nonphotosynthetic, dark-grown flagellate.

Cells were collected for extraction from *Euglena gracilis* (*Z*) cultures grown in a chemically defined medium (*p*H 3.0) at 25°C under continuous illumination (300 ft-ca) and in darkness for 10 to 14 days. *Euglena* grown in the light carries on photosynthesis and synthesizes chlorophyll, and the cultures become a deep green. *Euglena* grown in the dark is nonphotosynthetic and does not synthesize chlorophyll, and the cultures are yellow to orange. The dark-grown *Euglena* cultures, which were initiated from a light-grown culture, were maintained in the dark for more than 8 months. These cultures are still capable of synthesizing chlorophyll when placed in the light. Ten grams of packed *Euglena*, after being washed twice in physiological saline, were ground with glass homogenizing beads at a salt-ice temperature (−10°C) in 8 to 10 ml of 2-percent digitonin in a Waring blender for 1 to 2 minutes. This technique gave good cell breakage and assured efficient extraction. The homogenate was further extracted at room temperature in the dark for 6 to 12 hours and was then centrifuged at 20,000*g* for 15 minutes. To separate the proteins from the pigments and lipids, the supernatant was precipitated in the cold in 80-percent acetone for several hours. This precipitate was washed in acetone, air-dried, taken up in distilled water, and brought to *p*H 9 to 10 with alkali. After standing 1 to 2 hours at room temperature, the insoluble proteins were centrifuged out, and the water-soluble fraction was neutralized with dilute sulfuric acid. It was then fractionated in the cold with ammonium sulfate at 45 percent of saturation, and the precipitate was removed by centrifugation. The brown supernatant was again fractionated with am-

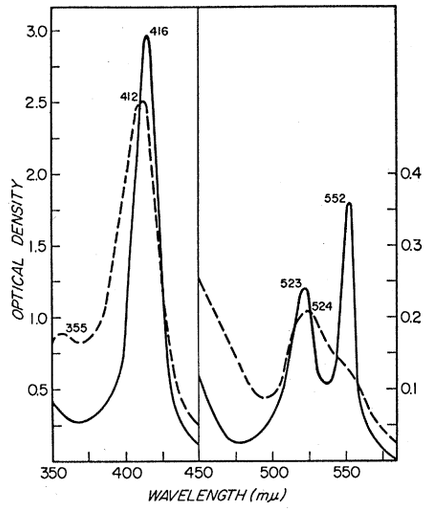

Fig. 1. Absorption spectra of the cytochrome isolated from the digitonin extract of photosynthetic, light-grown *Euglena*. Solid line, spectrum of the reduced cytochrome; broken line, spectrum of the oxidized cytochrome.

monium sulfate at 90 percent of saturation. The precipitate, now pink in color, was redissolved in 10 ml of distilled water and reprecipitated with 90-percent ammonium sulfate. This precipitate was then dissolved in 3 to 5 ml of water and dialyzed for at least 6 hours in the cold against two or three changes of 1-lit. volumes of distilled water. The nondialyzable material was cleared by centrifugation, and the absorption spectrum of the supernatant was immediately measured in the Beckman DK-1 spectrophotometer. Absorption maxima were checked in the Beckman DU spectrophotometer. The cytochrome concentration was calculated from the molar extinction coefficient of cytochrome *c* (2.8×10^4 cm^2/mole) as described by Davenport and Hill (*1*), and chlorophyll concentration was determined from the absorption spectrum of the acetone-soluble pigment fraction (*8*).

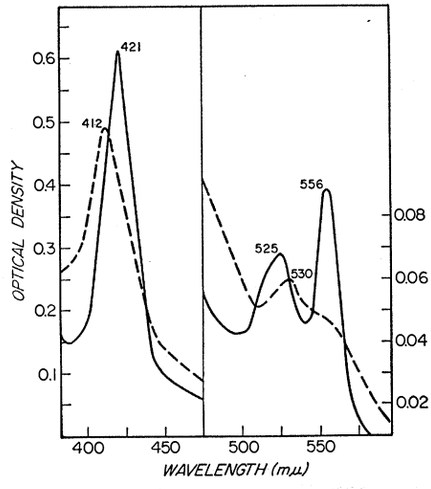

Fig. 2. Absorption spectra of the cytochrome isolated from the digitonin extract of nonphotosynthetic, dark-grown Euglena. Solid line, spectrum of the reduced cytochrome; broken line, spectrum of the oxidized cytochrome.

The absorption spectrum showed that the oxidized form of cytochrome had been isolated. A reduced absorption spectrum was obtained by adding a few crystals of sodium dithionite. The cytochrome was then reoxidized by the addition of $5 \times 10^{-3}M$ potassium ferricyanide. The initial absorption spectrum of the oxidized cytochrome and that obtained after ferricyanide treatment were identical. The absorption spectra of the reduced and oxidized cytochrome from the light-grown *Euglena* are shown in Fig. 1. Absorption peaks for the reduced cytochrome are at 552, 523, 416, and 315 mμ; for cytochrome in the oxidized state the absorption peaks are at 524, 412, and 355 mμ. These absorption maxima for the reduced and oxidized cytochrome closely resemble those for the cytochrome *c* of *Chromatium*, a photosynthetic bacterium (*3*). They differ from those for the recently reported *Euglena* cytochrome-552 (*4*) and from those for cytochrome *f* (*1*, *2*) in that the typical cytochrome *c* shift of the β-band fails to occur. That our *Euglena* cytochrome has a *c*-type heme group was determined from the spectra of the alkaline pyridine and of the dicyanide hemochromogens.

The cytochrome fraction from light-grown *Euglena* was then further purified by centrifugation in the ultracentrifuge for 2 hours at 40,000 rev/min. The supernatant containing the cytochrome was dried by lyophilization. The resulting powder was dissolved in water, and paper electrophoresis was carried out in citrate-phosphate buffer at *p*H 6.1. The electrophoretic pattern revealed three anode-migrating components: a major slow-moving band containing the cytochrome and two rapidly migrating bands. Cytochrome *f* also moves toward the anode, while the *c*-type cytochromes from photosynthetic bacteria and the *Euglena* cytochrome-552 move toward the cathode. The rate of migration of the cytochrome under study was relatively slow, being only two-fifths that of bovine albumin.

According to our calculations light-grown *Euglena* contains 6.6×10^{-7} mole of chlorophyll to every 2.3×10^{-9} mole of cytochrome. This corresponds to a molar ratio of 300:1, which is in agreement with the chlorophyll-to-cytochrome-*f* ratio obtained by others (*1*).

From dark-grown *Euglena* the cytochrome is similarly isolated in the oxidized state and has absorption peaks at 530 and 421 mμ; for the reduced cytochrome, the absorption peaks occur at 556, 525, and 412 mμ. These data are shown in Fig. 2. Although the typical cytochrome *c* shift is present, the spectrum is not that of typical cytochrome *c*. It is closer to the spectrum of cytochrome *f* (a modified cytochrome *c*), which is peculiar to photosynthetic cells. However, dark-grown *Euglena* are not photosynthetic but, as already indicated, resume photosynthesis when brought back into the light.

It was also determined that there is five times as much cytochrome in the light-grown as in the dark-grown *Euglena*. Since *Euglena* grown in the dark are nonphotosynthetic, this cytochrome may be part of the respiratory electron transport mechanism. If so, this cytochrome should also be present in the photosynthetic organisms, but its spectrum would be masked due to its low concentration. However, the similarity between the absorption spectra of this cytochrome and of the photosynthetic cytochrome *f* indicates that further studies are needed to elucidate the functional role that these enzymes play in the mechanism of photosynthesis (*9*).

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