6. L. M. Vorobyova and A. A. Krasnovsky, *Biokhimiya* **21**, 126 (1956); **23**, 760 (1958).
7. S. S. Brody, *Science* **128**, 838 (1958).
8. E. Rabinowitch, *Photosynthesis and Related Processes* (Interscience, New York, 1951), vol. 2, pt. 1, Figs. 21-25, p. 641.
9. We are thankful to Prof. J. B. Thomas, Mrs. Rajni Govindjee, and Mr. Carl Cederstrand for their assistance in this investigation, which was carried out with the support of National Science Foundation grant G4969.

21 March 1960

Capacity Electrode for Chronic Stimulation

Abstract. An electrode is described which can be used for electrical stimulation over prolonged periods without danger of contaminating tissue with electrode products. Use of a thoroughly insulated metal surface precludes all electrode processes, although a transient current can occur to stimulate the tissue.

It has not been demonstrated conclusively that ionic and gaseous products resulting from an electrode process can produce toxic depression of excitability and, perhaps, even block of conduction. Nevertheless, the suspicion that such effects might occur has encouraged the development of an electrode wherein the possibility of electrode reaction products is completely precluded. Such an electrode has been developed and tested and found to behave perfectly in the electrical stimulation of excitable tissues.

The mechanism underlying the behavior of this electrode depends merely on the electric field which arises as the primary event in any conventional electrode when the metal wire is initially charged upon being connected to the source of current. If the metallic wire should be deliberately insulated, the migration of ions by the action of the primary field will give rise to an accumulation of ions at the outer surface of the insulation which will abolish the field in the surrounding volume. The time interval required for this event to take place—the "charging" time—is usually very brief, depending primarily upon the area of electrode surface immersed in the solution and the thickness and dielectric constant of the insulation. We assume, of course, that the impedance of the charging source is negligible. The brevity of this charging event unfortunately makes such an electric field rather useless for the stimulation of excitable tissues, since, in view of the current strength-duration relationship of most tissues, the peak currents necessary would demand prohibitive charging potentials and would immediately cause dielectric breakdown of the insulating surface layer.

What is required is to extend the duration of current flow and also to confine the current to a small area of excitable tissue. This can be done with a large area of insulated metallic surface enclosed by an insulated cavity—the cavity being filled with electrolyte, namely, Ringer's solution—which opens to the tissue via a polyethylene tube. This electrode displays the behavior of a series resistor-capacitor network, where the capacitance for a given thickness of insulation is proportional to the surface area of the wire and the resistance is proportional to the length and inversely proportional to the area of cross section of the tube leading from the cavity. Thus, when a square-wave voltage pulse is applied, a differentiated ("biphasic") response of current is obtained. The indifferent electrode need only be a conventional wire electrode in most cases.

A typical construction of the capacity electrode is shown in Fig. 1: The ¼- by ½-inch cavity is milled from a polyethylene block (¾ by ⅝ by ⅛ inch), a thin wall of material being left to serve as the floor of the cavity. In the final assembly the cavity is closed with a thin plate of polyethylene which is sealed to the cavity walls by heating with a soldering iron. The current from the cavity is conveyed to the nerve via a 1-cm length of PE 50 polyethylene tubing (*1*). A length of PE 240 tubing is used as a cuff, slit to allow entry of the nerve. The metallic surface is provided most practically by a four-layer Teflon-coated No. 30 copper or stainless steel wire (*2*). The wire is coiled to fit the cavity and is threaded through the PE 90 tubing which is sealed with a small flame. Filling is accomplished quite easily by immersing the electrode in Ringer's solution and pumping down for 30 minutes with a filter pump. In the assembly shown in Fig. 1, a length of wire approximately 2 ft long and approximately 1 cm of PE 50 tubing results in an electrode with a time constant of approximately 80 μsec. Such an electrode, when used to stimulate a frog sciatic nerve, requires about a 10-volt square pulse (0.2 msec duration) to obtain a current of sufficient magnitude and duration for excitation. The phrenic nerve of the dog required about a 20-volt pulse. Larger cavities have been used to accommodate lengths of wire of about 6 ft to provide a longer time constant for cardiac stimulation. In this case it has been found convenient to bring out the polyethylene tube from the wall of the cavity instead of the end as shown in Fig. 1. Thus, when the cavity is sutured to the ventricular surface the polyethylene tube (several millimeters long) of necessity presses into the myocardium. (In a more recent version the tube has been eliminated entirely, whereupon the hole in the wall of the cavity is flush with the ventricular surface.) Variants of this design are being explored for use in cortical stimulation. A "bipolar" electrode can be constructed with two cavities adjacent to one another with the pair of polyethylene tubes extending to the cuff in the case of nerve stimulation or into the myocardium in the case of cardiac stimulation.

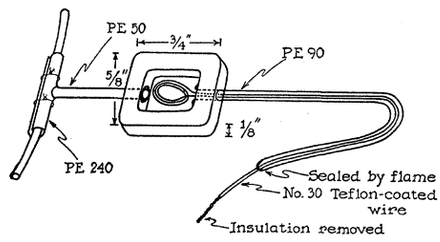

Fig. 1. Sketch of capacity electrode.

The capacity electrode should be useful in any situation where it is found necessary, for whatever reason, to completely eliminate the possibility of electrode reaction products.

ALEXANDER MAURO
Rockefeller Institute, New York

Notes

1. The polyethylene tubing was obtained from the Clay-Adams Co.
2. Wire was obtained from Hitemp Co., Mineola, N.Y. Note that ordinary single-layer Teflon-coated wire is inadequate because of the presence of occasional pin holes.

17 March 1960

Age at Menopause of Urban Zulu Women

Abstract. Interviews with a population sample of Zulu women residing in Durban, Union of South Africa, indicated a tendency for the permanent cessation of menstruation to occur late. The median menopausal age of 33 women questioned within 5 years of their menopause was 48.6 years, and their mean menopausal age was 49.2 years. It is suggested that this may be an effect of malnutrition or of climatic factors.

There is considerable variation in the average menopausal age of various groups of women (*1*). This variation has been ascribed to genetic and other factors.

Recently the opportunity arose, in the course of two investigations carried out in Durban, Union of South Africa, to inquire into the menopausal age of urban Zulu women. The female subjects in two population samples were asked whether or not their menses had ceased, and the time lapse since their menopause.

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.

J. H. ABRAMSON, B. GAMPEL, C. SLOME*, N. SCOTCH†
Department of Social, Preventive and Family Medicine, University of Natal, Durban, Union of South Africa
C. C. MAJOLA
Institute of Family and Community Health, Durban

References and Notes

1. R. Pearl, *The Natural History of Population* (Oxford Univ. Press, London, 1939).
2. J. H. Abramson, B. Gampel, C. Slome, N. Scotch. *Amer. J. Clin. Nutrition*, in press.
3. J. Gillman and T. Gillman, *Perspectives in Human Malnutrition* (Grune and Stratton, New York, 1951).
4. J. M. Tanner, *Growth at Adolescence* (Blackwell Scientific Publications, Oxford, 1955).
5. T. N. MacGregor, *Practitioner* **182**, 539 (1959).
6. F. Benjamin, *S. African Med. J.* **34**, 316 (1960).
7. E. C. Hamblen, in *Geriatric Medicine*, E. J. Stieglitz, Ed. (Saunders, Philadelphia and London, 1949).

* Present address: Hebrew University-Hadassah Medical School, Jerusalem, Israel.
† Department of Sociology and Anthropology, Washington State University, Pullman. Work done during the tenure of a research fellowship of the U.S. Public Health Service.

3 May 1960

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-