Two Forms of Chlorophyll a in vivo with Distinct Photochemical Functions

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Two Forms of Chlorophyll a in vivo with Distinct Photochemical Functions

Abstract. Action spectra of the Emerson effect in *Chlorella* and *Navicula* reveal peaks at 670 mμ, in addition to those at 650 mμ (*Chlorella*) and 630 mμ (*Navicula*) attributable to chlorophylls *b* and *c*. Thus, excitation of chlorophyll *a* form "chlorophyll *a* 670" can supplement, in these algae, the excitation of the form "chlorophyll *a* 680–700." The effect of the auxiliary pigments in these algae may be mediated by energy transfer to "chlorophyll *a* 670."

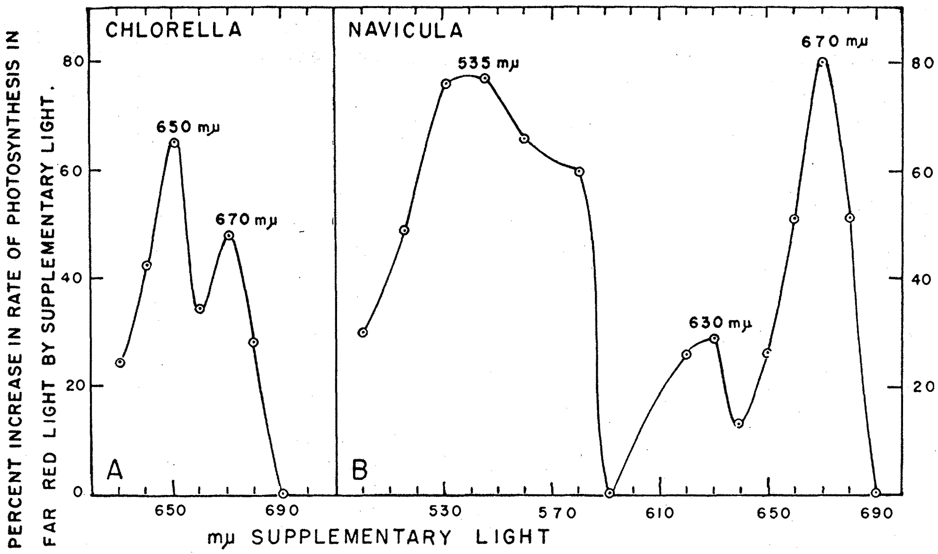

Fig. 1. Action spectra of the Emerson effect in (*A*) *Chlorella pyrenoidosa* Chick, strain 3, and (*B*) *Navicula minima* Grun. 535 mμ: at this wavelength, the largest proportion of light is absorbed by fucoxanthol; 630 mμ, by chlorophyll *c*; 650 mμ, by chlorophyll *b*; 670 mμ, by "chlorophyll *a* 670."

Emerson and his co-workers (*1, 2*) found that the low quantum yield of photosynthesis at the red end of the absorption spectrum of algae (> 680 mμ in green and brown algae, > 650 mμ in red algae) can be significantly improved by auxiliary light of shorter wavelengths. The action spectrum of this "second Emerson effect" (*3*) was found to follow the absorption spectrum of the main auxiliary pigments (chlorophyll *b* in green algae, chlorophyll *c* and fucoxanthol in brown algae, the phycobilins in red and blue-green algae).

On the basis of a hypothesis first proposed by Duysens (*4*) to explain the action spectrum of chlorophyll fluorescence in red algae it has been suggested (*2*) that the reason the excitation of the auxiliary pigments improves the efficiency of photosynthesis in the long-wave region may be that this excitation is transferred, by resonance, preferentially to a particular photochemically active form of chlorophyll *a* which is not, or not strongly enough, excited by direct absorption of light > 680 mμ in green and brown algae, and > 650 mμ in red algae. If this is so, it should be possible to produce the same enhancement effect by direct absorption of light in this form of chlorophyll *a*, at least in green and brown algae, where it must be present in relative abundance to account for the high yield of photosynthesis in the 650- to 680-mμ range.

This is in fact the case. Figures 1*A* and 1*B* show the action spectra of the second Emerson effect (enhancement of the yield of photosynthesis in a band 685 to 700 mμ by monochromatic light). In addition to the previously observed peaks at 650 mμ (due to chlorophyll *b*) in *Chlorella*, and 630 mμ and 535 mμ (due to chlorophyll *c* and fucoxanthol, respectively) in *Navicula*, these curves have a sharp peak at 670 mμ missed in earlier measurements (see Figs. 7 and 8 in Emerson and Rabinowitch, *2*) partly because of wider spacing of measured points. The presence in the absorption spectrum of these algae of a band at about 670 mμ (in addition to one at about 680 mμ) is confirmed by closer consideration of the shape of this spectrum, particularly by the method of derivative spectra (French, *5*). Only a single red band appears in this region in the spectrum of chlorophyll extracted from the algae; the 670 mμ and the 680 mμ bands, in vivo, must therefore belong to one and the same pigment, in different states of association or aggregation.

Krasnovsky and his co-workers (*6*) found evidence of preferential bleaching in leaf homogenates of a chlorophyll form with a band at 670 to 672 mμ; they considered it to be the monomeric, fluorescent, and photochemically active component; but French (*5*), while confirming the existence of two bands (at 673 and 684 mμ respectively), found no essential difference in the rate of bleaching of "chlorophyll *a* 670" and "chlorophyll *a* 680." Brody (*7*) concluded, from fluorescence and spectroscopic studies, that the absorption band of chlorophyll *a* in vivo contains a component with a peak at about 690 mμ, which he attributed to a photochemically inactive and, at room temperature, nonfluorescent, dimeric chlorophyll molecule (since a similar band can be observed also in very concentrated chlorophyll *a* solutions). French (*5*) also saw indications of a third, minor band at 695 mμ in the derivative spectrum of *Chlorella.*

Exclusive association of "chlorophyll *a* 670" with both fluorescence and photochemical activity of chlorophyll in vivo seems to be an oversimplification; but a specific photochemical function of the form of chlorophyll *a* in vivo which has a band peak near 670 mμ is clearly confirmed by our experiments.

With the accumulating evidence of three spectroscopically different forms of chlorophyll *a* in vivo, the situation in algae becomes similar to that familiar from the case of purple bacteria, where three forms of bacteriochlorophyll (with absorption bands at 800, 850 and 890 mμ) were long known to appear in vivo, and to give rise to only one band (at about 770 mμ) upon extraction. The wider separation of these bands is in agreement with the generally greater influence of solvent on the position of the absorption bands of bacteriochlorophyll compared to those of chlorophyll *a* (*8*). It remains to be seen whether distinct photochemical functions must be attributed to all these forms, in bacteria as well as in algae (*9*).

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References and Notes

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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.

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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.

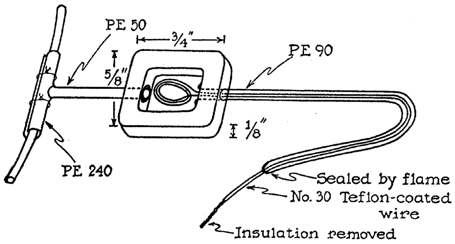

Fig. 1. Sketch of capacity electrode.

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.

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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.