[5] Recently, Otsuji and Takagaki have reported the accumulation of these compounds in 6-azauracil treated bacteria, *Journal of Biochem. (Japan)*, **46**, 791 (1959).

[6] Strominger, J. L., *J. Biol. Chem.*, **224**, 509 (1957).

[7] Gray, C. H., and E. L. Tatum, these PROCEEDINGS, **30**, 404 (1944).

[8] Resumption of the DNA synthesis may have a decisive role in making the unbalanced growth of the organisms irreversible.

[9] The 60 minutes' incubation time with FU—a standard condition used in our experiments—may represent the critical time up to which the above mechanism of action of the drug predominates. On prolonged incubations inhibitions of other phases of pyrimidine metabolism may become decisive.

ACTION SPECTRA OF CHROMATIC TRANSIENTS AND THE EMERSON EFFECT IN MARINE ALGAE

BY L. R. BLINKS

HOPKINS MARINE STATION OF STANFORD UNIVERSITY, PACIFIC GROVE, CALIFORNIA

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Chromatic transients are the changes in oxygen evolution rate recorded on altering the color of light incident on a tissue, even though the intensities are adjusted to give equal steady rates of photosynthesis at both wavelengths. These were first observed[1] on exposing a red alga (*Porphyra*) alternately to red light (675 mμ) and green light (560 mμ). They have been reported[2] in a green alga (*Ulva*) on alternating illumination between (a) 490 and 540 mμ; and (b) 640 and 688 mμ. The location of these regions coincided fairly closely to the absorption *in vivo* of several pigments: chlorophyll *a* at 675–688 mμ; mixed chlorophylls at 540 mμ; chlorophyll *b* at 640–650 mμ; phycoerythrin at 560 mμ, and carotenoids at 490 mμ. (Alterations of wavelength within the absorption region of a single pigment produce little or no transient.)

The earlier studies were made by rapid change of setting on a monochromator, and the regions were limited by output energy and action spectra of the tissue to a few pairs of wavelengths. In order to obtain a more accurate delineation of the entire action spectrum for the transients, two sources of light have now been employed, one a fixed or reference wavelength, derived from a separate lamp with interference filter, the other the high-energy monochromator previously described.[3] The images of the two lamp filaments were focused carefully upon the tissue, usually an algal thallus one cell (or a few cells) thick, tightly held against a bright platinum electrode by means of a cellophane strip.[3] *Anabaena*, a blue green alga, was studied by lifting mats of filaments which had grown out very uniformly at the surface of sea water, and spreading them as smoothly as possible over the electrode.

Oxygen arriving at the electrode was reduced to H_2O_2 at an applied potential of 0.5 volt, the current being recorded by a Speedomax potentiometer connected across a fixed resistance (usually 1,000 ohms) in the circuit. Flowing or recirculated sea water, usually equilibrated against 5 per cent CO_2 in air, quickly established a base line which remained very steady in the dark, frequently at around

2 or 3 μamp, due to the passage of O_2 across the tissue. It was increased in the light to as much as 8 or 10 μamp, due to the arrival of photosynthetically produced O_2 at the electrode, the level being dependent upon light intensity. This is therefore read as a *rate* of photosynthesis and, by means of the electrochemical equivalent of H^+ ions oxidized, can be made an absolute measure of O_2 diffusing to the electrode. Furthermore, due to the short diffusion distance (a few microns only, the thickness of the cell wall), the new equilibrium value is quickly established, in a matter of one or 2 minutes (Fig. 1). This makes it possible to follow transients fairly sensitively.

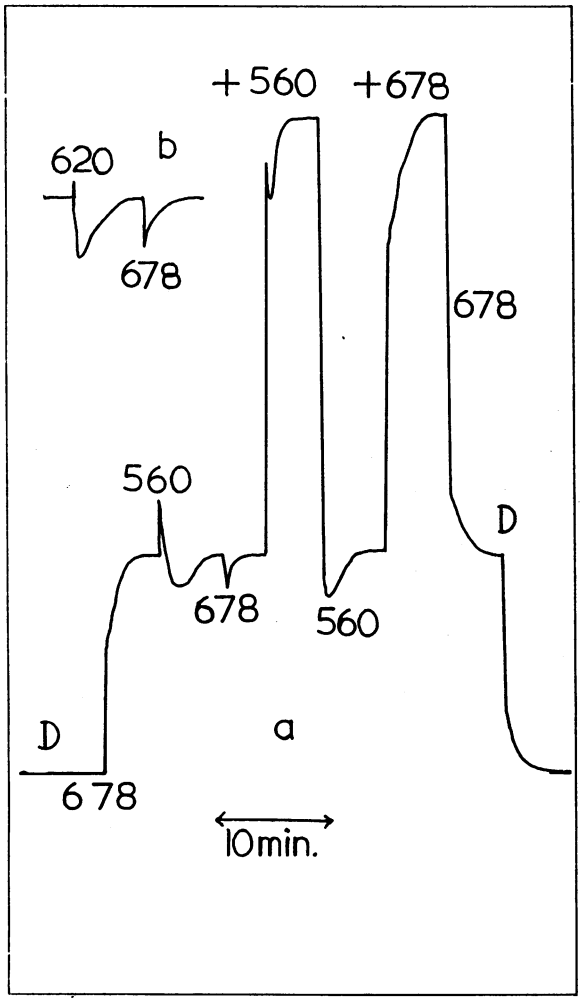


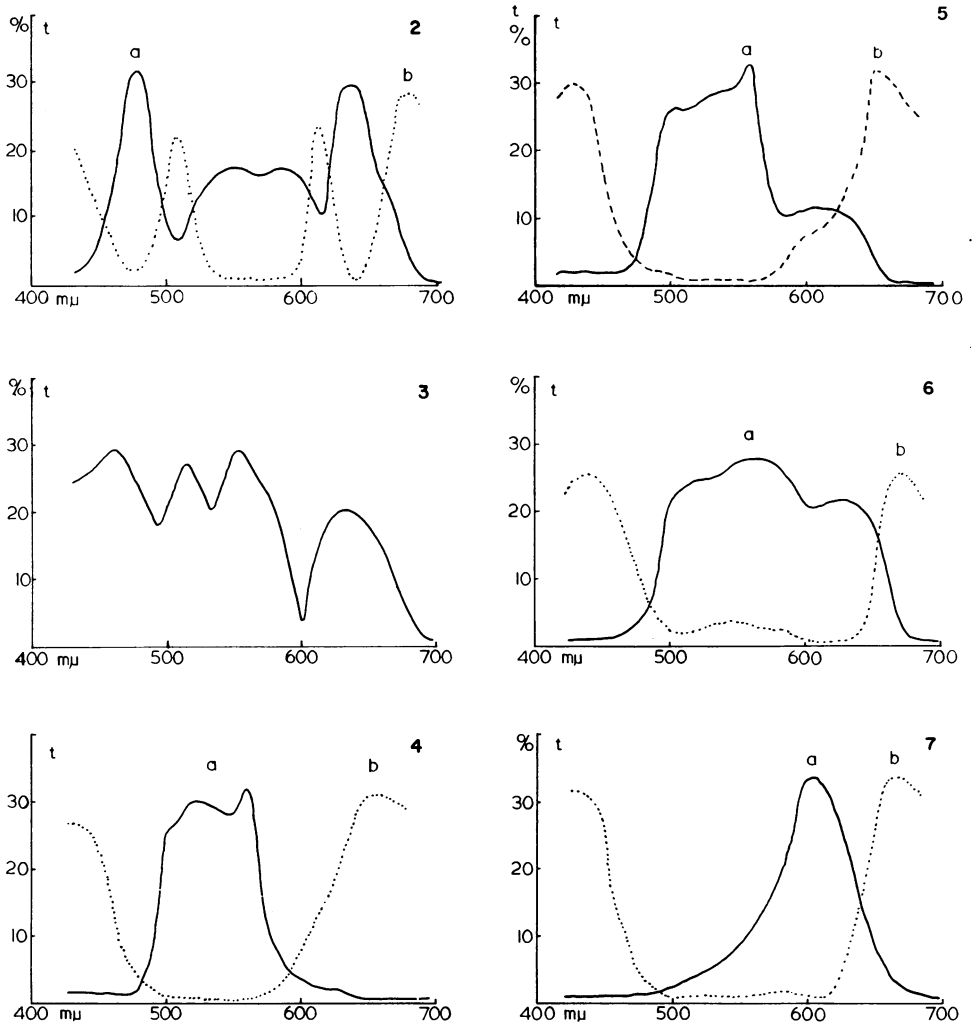
FIG. 1*a*.—Chromatic transients and the Emerson enhancement due to alternation and addition of red (678 mμ) and green light (560 mμ), in the red alga *Porphyra Nereocystis*. *b*.—Chromatic transients on alternation of 678 and 620 mμ. Explanation in text.

One precaution in recording chromatic transients is that the tissue (or the light beam) be very carefully restricted to the surface of the electrode. If the tissue projects beyond its edges, there may be a slow diffusion of O_2 from the sides to the electrode (despite good flow of sea water) which gives spurious transients if the beams of light do not exactly coincide. Careful adjustment of the beams and tissue to the electrode size or, in some cases, masking with black paper or black cellophane obviated this "edge effect" (which may be readily tested by alternating an identical wavelength from the two sources).

Another source of spurious chromatic transients is poor synchronization of the light shutters. While electrical switching partially obviated this trouble, it was found best to utilize a sliding shutter which very accurately interrupted one beam as it exposed the other. A check with photocells is desirable.

Emerson Effect.—This phenomenon, discovered by the late Robert Emerson,[4, 5] signifies the more than additive effect of two superposed wavelengths; thus the far red (700 mμ), which is usually inefficient in the photosynthesis of green plants,

becomes much more effective when shorter wavelengths are given simultaneously. This is ascribed to the low activity of chlorophyll *a*, which is markedly enhanced when light absorption by chlorophyll *b* or *c*, phycocyanin, phycoerythrin, fucoxanthin, or other carotenoids occurs simultaneously. Since two alternating light



Action spectra of chromatic transients (t):
FIG. 2.—*Enteromorpha tubulosa;* reference beam (*a*) 702 mμ; (*b*) 647 mμ. FIG. 3.—*Punctaria occidentalis;* reference beam 702 mμ. FIG. 4.—*Porphyra Thuretii;* reference beam (*a*) 702 mμ; (*b*) 566 mμ. FIG. 5.—*P. Nereocystis;* (same). FIG. 6.—*P. perforata;* reference beam (*a*) 702 mμ; (*b*) 614 mμ. FIG. 7.—*Anabaena* sp.; (same).

sources were found desirable to trace the transient action spectra, it was a simple matter to *combine* two wavelengths (ordinarily adjusted to give equal photosynthesis separately) and observe the enhancement. (Had such a double source been employed earlier, the enhancement would no doubt have been observed some time ago.)

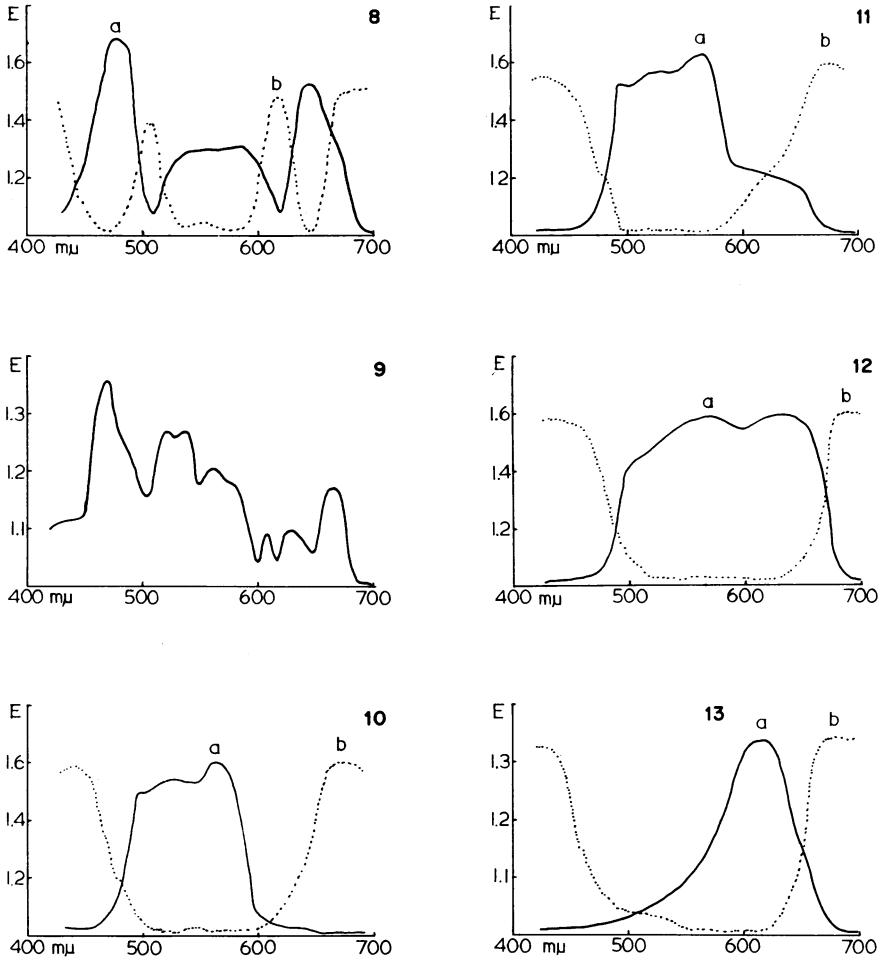
Recording.—Figure 1 shows a characteristic sequence of exposures in a red alga (*Porphyra Nereocystis*). The initial level of current (2 μamp) is due to oxygen arriving at the electrode across the tissue in the dark (D). Red light (678 mμ) near the absorption peak of chlorophyll *a* is then given; the polarographic current is increased to about twice the dark value. This represents the steady photosynthetic rate in red light; the wavelength is then changed to green (560 mμ) without an intervening dark period. A characteristic chromatic transient ensues; following the notation earlier employed[2] there is an initial cusp (a), a depression (b)—often below the steady state level—followed by a recovery (c) equal to the red value (to which it has been previously adjusted). On return to red light (678), there is a depression (d)—usually smaller than the cusp (a)—followed by a recovery to the steady state. There are somewhat different time courses found at different wavelengths; thus on alternating from chlorophyll *a* absorption to that of phycocyanin (620 mμ) the cusp (a) is often very much smaller, or almost absent, while the depression (b) is very large (Fig. 1, *b*). The next event in a typical sequence is the *addition* of the two light sources, previously *alternated.* Here green light (+560) is added to red. A very abrupt rise occurs, with a new cusp, depression and recovery, to a steady state of about 6 μamp. This is an increment of 4 μa, compared to the original red or green value of 2 μa; taken just on its additive value the enhancement is 100 per cent. But it is probably better to compare the expected response (twice the green or red value) with the observed one, giving an enhancement of 50 per cent for the combined lights, or an Emerson factor (E) of 1.5.

Decreased enhancements are found when very different relative intensities of the two light sources are added; on the whole, the best total enhancement occurs when wavelengths of approximately equal effectiveness are added. There is no evidence of a "catalytic" effect[6] at very low intensities of added light, even in the blue region.

Action Spectra.—The values for chromatic transients could be based on the height of the cusp (a). But in a few cases (as in the region of phycocyanin absorption) this was abnormally small, so it was decided to take the distance from this height to the depth of the depression (b); i.e., the greatest total variation, both upward and downward. This is plotted as percentage of the steady state photosynthesis. Figures 2 to 7 show action spectra for transients in a number of marine algae, with different accessory pigments. The majority of these were obtained by alternating a reference beam of far red light (702 mμ) with a variable beam from the monochromator. In these cases, the maxima for chromatic transients are found to coincide remarkably well with the absorption peaks of the accessory pigments. Thus in the green alga (*Enteromorpha*) the highest activity was found at 640–650 mμ (chlorophyll *b* absorption) and 480–490 mμ (carotenoid absorption). (The latter could conceivably be due partly to chlorophyll *b*, and the somewhat lower activity in the middle of the spectrum must also represent predominant absorption by chlorophyll *b*.) The peaks for the brown alga (*Punctaria*) must represent chlorophyll *c* (630 and 580 mμ), fucoxanthin (520–540 mμ), and other carotenoids (480 region); again, chlorophyll *c* might be contributing to the latter maximum.

The red algae, having no chlorophyll *b* or *c* (chlorophyll *d* seems to be nearly absent from most red algae as well), show little or no activity in the regions charac-

terizing such pigments; but they do display transients in the region 620–650 mμ depending upon the phycocyanin (and allophycocyanin) content. *Porphyra Thuretii* has the least of these pigments (it is a deep red color), and shows little or no transient activity until the phycoerythrin region is approached (wavelengths less than 600 mμ, with maximum at 560 mμ). *P. Nereocytsis* (a more purple species) has a shoulder indicating some phycocyanin activity (620 mμ), though



Action spectra for enhancement (Emerson effect, E):
FIG. 8.—*Enteromorpha.* FIG. 9.—*Punctaria.* FIG. 10.—*Porphyra Thuretii.*
FIG. 11.—*P. Nereocystis.* FIG. 12.—*P. perforata.* FIG. 13.—*Anabaena.*
(Wavelengths as in FIGS. 2–7 for the corresponding algae.)

again the major activity is at 560 mμ. Only in *P. perforata* (of a slate gray color) does the transient activity become very large in the phycocyanin region; here it extends well toward 650 mμ, corresponding to the high content of allophycocyanin in this species. It should be noted that the transient activity is very slight in the blue end of the spectrum (400–500 mμ) for all these species; quite clearly, the carotenoids are unable to generate transients against the far red (nor, obviously,

can chlorophyll *a* in this region of its absorption). However, as Figure 4 indicates, the blue end of the spectrum is perfectly capable of generating good transients when alternated with green (or orange) light absorbed by the *phycobilins*. This is clearly shown in several of the figures, where a reference beam of 566 or 614 mμ was employed. Against such a reference beam good activity is shown in *both* ends of the spectrum (the intensity of blue light has to be very high, due to its low photosynthetic efficiency in red algae).[3] It must be stressed that *either* absorption region of chlorophyll *a* is effective against the phycobilins, whether in generating transients, or in the Emerson enhancement.

The situation in *Anabaena* is particularly interesting, since (as in the photosynthetic action spectrum of a marine blue green alga[3]) it displays low chlorophyll activity. Thus good transients are generated against a far red reference beam (702 mμ) only at the phycocyanin maxim (620 mμ). None appears in the blue, or even in the green, region of the spectrum (there is little or no phycoerythrin in this blue-green alga). If, however, orange light (614 mμ) is made the reference then, as with the red algae, good transients appear in *both* the red and blue region of chlorophyll absorption.

Enhancement Spectra.—These are shown in Figures 7–12 for the same algae. The action spectrum for the Emerson effect is almost identical with that of the transients. Quite clearly the same pigments are implicated in both phenomena, and it is tempting to speculate about a common mechanism for both. What this may be is not yet clear; but it should be emphasized that enhancement occurs equally well when chlorophyll *a* is absorbing blue light as when it absorbs red. The enhancement in the former case is therefore due to absorption of a *longer* wavelength by other pigments; it is apparently not necessary that a *shorter* wave length be supplied. In a red alga, green or orange light can enhance the effect of violet or blue light.

Very similar enhancement spectra are being reported for a unicellular fresh water alga[7] and a number of other marine algae.[8]

Discussion.—It seems too early to ascribe a cause to either the chromatic transients or the Emerson enhancement. A hypothesis was proposed for the former[2] (following a suggestion by Emerson) which involved increased respiration during the absorption by various accessory pigments. There is something to commend this in the light-dark transients at the characteristic wavelengths,[1, 2] as well as in the time course of the enhancements themselves (Fig. 1). Not only is there a cusp (very like that of "a" in the transient) when green light is added to red—and missing when red is added to green; but there are nearly as marked differences when the supplementary light is removed. On turning off the red light (leaving only green), there is a marked depression (enhanced respiration?), while this is not observed when green is turned off (leaving only red). Experiments at higher and lower temperature, and with a variety of respiratory and photosynthetic inhibitors, are under way to clarify these effects.

Summary.—Action spectra are presented for chromatic transients and the Emerson effect ("enhancement") in the photosynthesis of a number of marine algae. When far red light (700 mμ) is altered or supplemented with other wavelengths, the greatest effects occur at the absorption maxima of the accessory pigments: chlorophyll *b* and carotenoids in green algae; chlorophyll *c* and fucoxan-

thin in brown algae; phycobilins in red and blue-green algae. Both effects also occur equally well when the chlorophyll *a* is absorbing in the blue end of the spectrum (435 mμ).

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[2] Blinks, L. R., *Plant Physiol.*, **34**, 200 (1959).
[3] Haxo F., and L. R. Blinks, *J. Gen. Physiol.*, **33**, 389 (1950).
[4] Emerson, R., R. Chalmers, and C. Cederstrand, these PROCEEDINGS, **43**, 133 (1957).
[5] Emerson, R., and R. Chalmers, *Phycological Soc. of America News Bulletin*, **11**, 51 (1958).
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SIGNIFICANT STRUCTURES IN LIQUIDS, III. PARTITION FUNCTION FOR FUSED SALTS

BY CHARLES M. CARLSON, HENRY EYRING, AND TAIKYUE REE

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH

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Very little has been done in the past in the way of developing partition functions to predict the thermodynamic properties of fused salts. Using the work of Walter and Eyring,[1] Wheeler[2] formulated a partition function for molten alkali halides and he, as well as Parlin and Eyring (unpublished), calculated successfully many of the thermodynamic properties of salts. The partition function used here is a further step in the application of significant structures as developed earlier.[3] For a mole of alkali halide molecules it is

$$f = \left\{ \frac{e^{\frac{E_s}{2RT}} \left(\frac{V}{V_s}\right)^{1/3}}{(1 - e^{-\theta/T})^3} \left[1 + n_h \cdot e^{\frac{-aE_s(V/V_s)^{1/3}}{2n_h RT}} \right] \right\}^{\frac{2V_s}{V} N} \times \left\{ \frac{(2\pi mkT)^{3/2} eV}{Nh^3} \frac{8\pi^2 IkT}{h^2} \frac{1}{(1 - e^{-h\nu/kT})} \right\}^{\left(1 - \frac{V_s}{V}\right) N} \quad (1)$$

$$n_h = n\left(\frac{V}{V_s} - 1\right) \quad (2)$$

The various parameters are explained as follows: E_s is the potential energy of the solid at the melting point; θ is the Einstein temperature of the solid; V_s is the volume of the solid per mole at the melting point; V is the molar volume of the liquid; n and a are parameters to be fitted to the experimental liquid data; m, I, and ν are the mass, the moment of inertia, and the ground state vibrational frequency, respectively, of the gaseous diatomic alkali halide molecule; T is the absolute temperature, R the gas constant, N Avogadro's number, k is Boltzmann's constant, and h is Planck's constant.