UCRL-17490

University of California

Ernest O. Lawrence Radiation Laboratory

THERMODYNAMICS OF LIGHT EMISSION AND FREE-ENERGY STORAGE IN PHOTOSYNTHESIS

DISCLAIMER

Submitted to Biophysics Journal

UCRL-17490
Preprint

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

THERMODYNAMICS OF LIGHT EMISSION AND FREE-ENERGY STORAGE IN PHOTOSYNTHESIS

Robert T. Ross and Melvin Calvin

April 1967

UCRL-17490

THERMODYNAMICS OF LIGHT EMISSION AND FREE-ENERGY STORAGE IN PHOTOSYNTHESIS

ROBERT T. ROSS AND MELVIN CALVIN

From the Laboratory of Chemical Biodynamics, the Department of Chemistry and the Lawrence Radiation Laboratory, University of California, Berkeley

ABSTRACT. A Planck law relationship between absorption and emission spectra is used to compute the fluorescence spectra of some photosynthetic systems from their absorption spectra. Calculated luminescence spectra of purple bacteria agree well but not perfectly with published experimental spectra. Application of the Planck law relation to published activation spectra for Systems I and II of spinach chloroplasts permits independent calculation of the luminescence spectra of the two systems; if the luminescence yield of System I is taken to be one-third the yield of System II, then the combined luminescence spectrum closely fits published experimental measurement.

Consideration of the entropy associated with the excited state of the absorbing molecules is used to compute the oxidation-reduction potentials and maximum free-energy storage resulting from light absorption. Spinach chloroplasts under an illumination of 1 kilolux of white light can produce at most a potential difference of 1.32 eV for System I, and 1.36 eV for System II. In the absence of non-radiative losses, the maximum amount of free energy stored is 1.19 eV and 1.23 eV per photon absorbed for Systems I and II, respectively.

The bacterium Chromatium under an illumination of 1 milliwatt/cm^2 of Na D radiation can produce at most a potential difference of 0.90 eV; the maximum amount of free energy stored is 0.79 eV per photon absorbed.

The combined effect of partial thermodynamic reversibility and a finite trapping rate on the amount of luminescence is considered briefly.

I. INTRODUCTION

Photosynthesis in green plants converts radiant energy in the wavelength region from 400 nm to 700 nm into chemical free energy. A photon having a wavelength of 700 nm has an energy of 1.8 electron-volts, but measurements of oxygen evolution from green plants indicate that only about 0.6 eV per quantum absorbed is stored as free energy in the form of stable chemical products. One of the major purposes of this paper is to understand the reasons for which much of the "missing" 2/3 of the photon's energy is "lost".

A significant amount of free-energy is lost in the complex biochemical pathways between the absorption of light and the output of carbohydrate; it is possible that these losses may be considered in a general thermodynamic manner, but in this paper we shall be concerned with two "losses" which are incurred immediately upon absorption of the light.

The first of these is simply a consideration of the entropy associated with the absorbed radiation; in other words, free-energy is not the same as energy. The first worker to consider this limitation on the energy conversion process of photosynthesis was L. N. M. Duysens (1958), who did so by a general and somewhat intuitive thermodynamic approach which is strictly applicable only for systems which absorb only in a narrow frequency range. Since

then, Mortimer and Mazo (1961) and Bell (1964) have considered the thermodynamics of monochromatic radiant energy conversion in a more general context; their work has expressed Duysens' insight in more formal terms, but it has not altered the basic argument. Application of the narrow-band theory to photosynthesis requires some extensions in order to make it applicable to photochemical systems absorbing over broad bands; this has been done recently (Ross, 1966b; 1967), and we review this theory in the next section.

The second immediate loss is due to a degree of irreversibility which is necessary to cause a net flow of energy into any radiation absorber. If an absorber were in equilibrium with a radiation field, then it would reradiate at the same rate at which it received photons, meaning that the quantum yield for energy storage processes would be zero. In order to get a net retention of photons, the entropy of the absorber must be greater than the entropy of the radiation field. This and other losses have recently been considered for the general problem of narrow-band radiant energy conversion (Ross, 1966a), and this loss has more recently been considered in the broad-band context (Ross, 1966b; 1967). This theory will also be reviewed in the next section.

The evaluation of the thermodynamics of any broad-band-absorbing photochemical system rests largely on the existence of a universal Planck law relationship between the absorption and emission spectra of any photochemical system. In Section III we consider some of the available information on the absorption and fluorescence spectra of photosynthetic systems, and relate them to the theory developed. In Section IV we use these spectra and the theory, together with a little data on the intensities of the light fields in which photosynthesis operates, in order to calculate the chemical potentials which may be developed in different photosynthetic systems. These are then related to observed biochemical oxidation-reduction potentials, and the agreement is found to be rather good.

II. THEORY

The thermodynamic theory which is used in this paper can be derived in a completely general manner (Ross, 1967). However, here we shall present a derivation which has less generality, but which--hopefully--may assist the reader in getting a better physical picture.

In this particular derivation we assume that the thermodynamics and kinetics of any species considered is identical in behavior to an ideal gas; in other words, molecules are considered to be non-interacting and to obey Boltzmann statistics.

Light Emission and the Maximum Potential. Consider a dilute solution of chlorophyll in a black box which is at 295°K. Thermal processes cause transitions from the ground electronic state of the chlorophyll, Chl, to the first excited singlet state, Chl*, and from the excited state to the ground state. Some of these transitions occur with the absorption or emission of a photon, while others involve only vibrational transitions.

From the principle of detailed balance, we know that the total number of radiative transitions from Chl to Chl* equals the number of radiative transitions from Chl* to Chl. We know further that the number of Chl*-to-Chl transitions accompanied by the emission of radiation within a certain frequency interval must be equal to the number of Chl-to-Chl* transitions which are accompanied by absorption of radiation in the same band.

It is possible to calculate the wavelength distribution of these thermal radiative transitions by simply taking the product of the electronic absorption spectrum of chlorophyll with the blackbody radiation curve for 295°K. This is shown in Fig. 1.

In general, this rate is

$$8\pi\, \sigma(\nu)\, (n\nu/c)^2 \exp(-h\nu/kT), \qquad (1)$$

in units of quanta/cm^2 sec sec^{-1}, where $\sigma(\nu)$ is the absorption cross-section

of the chlorophyll and n is the refractive index of the medium. This expression has been simplified by omission of a term corresponding to induced emission.

Now let us shine an external light source into the solution. This additional light will increase the rate of excitations, and thus increase the number of Chl* molecules.

If, in the presence of the external light, thermal equilibrium is maintained among all of the vibrational levels of the Chl*, then the proportion of Chl*-to-Chl transitions which occurs by any particular mechanism will remain the same. One consequence of this is that the proportion of radiation emitted at any frequency will be the same as that computed for the rate of Chl*-to-Chl transitions in a thermal enclosure, irregardless of the frequency distribution of the impinging radiation. This means that the wavelength or frequency distribution of radiative transitions shown in Fig. 1 and given by equation (1) is always the emission spectrum of chlorophyll at 295°K. This Planck law relation between absorption and emission spectra enables one to calculate emission spectra from absorption spectra. We consider its application to photosynthetic systems in Section III.

Let us specify that the intensity of the external light is such that the population of Chl* becomes Q times what it was in the absence of the external lamp.

We can express Q as

$$Q = R'/R_o, \tag{2}$$

where R_o is the rate of Chl-Chl* thermal transitions, and R' is the rate of Chl-Chl* transitions in the presence of the external light.

The rate of thermal transitions is

$$R_o = \int I_{BB}(\nu)\ \sigma(\nu)\ d\nu + R_{n-r}, \tag{3}$$

where I_{BB} is the blackbody intensity at the ambient temperature and R_{n-r} is the rate of non-radiative transitions.

The rate in the presence of the external light is

$$R' = \int I_S(\nu)\ \sigma(\nu)\ d\nu + R_o, \tag{4}$$

where I_S is the intensity of the external light. We shall assume that R_o is negligible compared to the rate of transitions stimulated by the external light, so that

$$Q = \int I_S(\nu)\ \sigma(\nu)\ d\nu/\alpha \int I_{BB}(\nu)\ \sigma(\nu)\ d\nu, \tag{5}$$

where we have expressed the rate of non-radiative thermal transitions (R_{n-r}) as being (α-1) times the rate of radiative thermal transitions.

If our basic assumption of vibrational equilibrium within Chl* is true, then the proportion of Chl*-to-Chl transitions which occurs radiatively should be $1/\alpha$, so that α is simply the inverse of the quantum yield of luminescence.

We now have the situation that the population of the excited state is Q times the thermal population. This means that the partial molar free energy of the Chl* is increased by kT ln Q over its thermal value. We are considering light levels at which the population of the ground state is not seriously depleted by excitations into the Chl* state, so that the partial molar free-energy of the Chl remains at its thermal value. This means that the difference in the partial molar free-energies of the Chl and Chl* is kT ln Q, or

$$\mu = kT \ln[\phi_{lum} \int I_S(\lambda)\ \sigma(\lambda)\ d\lambda / \int I_{BB}(\lambda)\ \sigma(\lambda)\ d\lambda] \tag{6}$$

Note that for the evaluation of this potential difference one needs only to know a temperature (which will give I_{BB}), the wavelength distribution of the incident radiation, and an absorption spectrum (which does not need to be normalized). Using this formula with a knowledge of the absorption spectrum and typical illumination intensities, it is possible to evaluate a typical μ for various photosynthetic organisms. It should be noted that equation (6) can be derived in a completely general fashion, and is not dependent on any of the physical assumptions contained in the derivation presented here (Ross, 1967).

So long as Chl-to-Chl* excitations are caused with a quantum yield of one or less, this free-energy difference represents an upper limit on the amount of free-energy which can result from the absorption of a photon. By our approach of perturbing a perfect thermal equilibrium, it should be clear that μ is not determined by the energy of the quanta involved, and can be much less than $h\nu$.

Losses from the Irreversibility of Net Flow. At this point, let us note that the free-energy difference which we have been calculating has taken no note whatsoever of the quantum yield for the energy storing process. As we turn on an energy utilizing pathway which had not been considered in our previous discussion, the population of the excited state will be decreased to some population P* which will be less than the population in the absence of the energy storage process P^*_{max}. The quantum yield for the processes which lead to energy storage is then

$$\phi_{st} = 1 - P^*/P^*_{max}, \tag{7}$$

assuming first-order rate constants for the energy storage process and for the loss processes.

As the population of the excited state is decreased, so is the free-energy difference between the excited and ground states. One can write this potential as being

$$\mu = \mu_{max} - kT \ln (P^*/P^*_{max}). \tag{8}$$

We are interested in maximizing the product of this potential, and the quantum yield for energy storage. It is easy to solve for the condition for this maximum power storage, and it is approximately that

$$P^*/P^*_{max} \approx kT/\mu_{max}. \tag{9}$$

This means that the optimum free-energy difference between the ground and excited states is roughly

$$\mu \approx \mu_{max} - kT \ln (\mu_{max}/kT). \tag{10}$$

As with the calculation of μ_{max}, this optimal potential and its associated quantum yield may be derived without reference to any specific mechanistic assumptions about the system (Ross, 1967). We compare the results of thermodynamic calculation with the current state of experimental knowledge about photosynthesis in Section IV.

Losses from Slow Excitation Transfer. At this point we can consider a kinetic limitation on the amount of power stored which is due to the finite rate of transfer of the excitation from the absorbing pigment molecules into species which do not interact significantly with a radiation field.

Consider that we have the situation diagrammed in Fig. 2(a). Here the excited state of the pigment molecules Chl* is in thermal equilibrium with a trap state. As diagrammed, the trap might be a triplet state of the pigment molecule, or some isomerization of it, but actually the arguments which we will make apply equally to chemical reactions where the trap is a distinct chemical species.

Excitations are transferred from the excited state to the trap with what we assume to be a first-order rate constant, K_{tran}. Since Fig. 2(a) describes an equilibrium situation, the return rate must be the same, and the chemical potential of the trap will be the same as the excited state, μ_{max}. The population of the excited state is P^*_{max}, and the rate of excitations is equal to the rate of radiative and non-radiative decay, $\alpha K_{rad} P^*_{max}$.

Now consider that excitations are tapped from the trap for storage, so that the thermodynamic activity (e.g., the concentration) of the trap species drops to some fraction, δ, of the activity which would be in equilibrium with an excited state population of P^*_{max}. The resulting situation is diagrammed in Fig. 2(b).

The rate of the reverse reaction Trap → Chl* is dropped to $\delta K_{tran} P^*_{max}$, causing the population of the excited state to drop to P*. The quantum yield for storage is, as before,

$$\phi_{st} = 1 - P^*/P^*_{max}, \tag{7}$$

but our object in the current situation is to maximize the power stored as measured at the trap: in other words, to maximize the product $\phi_{st}\mu_{trap}$.

By equating the fluxes into and out of the excited state, we find the relationship

$$\alpha K_{rad}(P^* - P^*_{max}) + K_{tran}(\delta P^*_{max} - P^*) = 0, \tag{11}$$

which can be rearranged to give

$$P^*/P^*_{max} = (\alpha K_{rad} + \delta K_{tran})/(\alpha K_{rad} + K_{tran}). \tag{12}$$

Substituting equation (12) into equation (7), we find that the quantum yield for power storage is

$$\phi_{st} = [K_{tran}/(\alpha K_{rad} + K_{tran})](1 - \delta). \tag{13}$$

The expression within the brackets is the usual kinetically determined quantum yield in the absence of any reversibility in the Chl*, Trap reaction (i.e., $\delta = 0$), and the expression $(1 - \delta)$ is thermodynamically equivalent to the $(1 - P^*/P^*_{max})$ of equation (7). This means that the quantum yield for energy storage factors into two independent fractions, one of which is determined kinetically and the other of which is determined thermodynamically. Derivation of the optimal μ_{trap} and maximal power storage is equivalent to the earlier treatment where the excited state itself was considered. The only difference is that the quantum yield is lowered by the kinetic factor shown in equation (13).

When the kinetic and thermodynamic factors of equation (13) are both close to one, then the lost quantum yield is approximately

$$\phi_{loss} \approx K_{rad}/(\alpha K_{rad} + K_{tran}) + \delta. \tag{14}$$

The luminescence yield is $1/\alpha$ of this, or

$$\phi_{lum} \approx K_{rad}/(\alpha K_{rad} + K_{tran}) + \exp[(\mu - \mu_{max})/kT], \tag{15}$$

where μ_{max} is the maximum potential, corrected for the presence of non-radiative losses.

The first term in equation (15) is due to the finite rate of transfer out of the excited state, and the second is due to the reversibility of the system. The kinetic term is simple fluorescence which is independent of any chemistry, and this portion of the luminescence should decay rapidly and exponentially when illumination is terminated. On the other hand, the thermodynamic term is dependent on chemistry, so that the decay of this light emission may be expected to be considerably slower and have complex kinetics. This chemiluminescence was first observed in plants by Strehler and Arnold (1951) and is currently being studied in several laboratories (vis. Clayton, 1966).

III. LUMINESCENCE SPECTRA

Bacteria. Olson and Stanton (1966) have recently published absorption and fluorescence spectra for several species of photosynthetic bacteria. By multiplying their absorption spectra with the Planck factor for 295°K, we have calculated the luminescence spectra for these species. The results of this calculation are compared with experiment in Fig. 3.

The calculated and observed spectra have been normalized so that their peak heights match. Agreement between prediction and experiment is reasonably good, and is probably within the accuracy of the experimental data. This agreement adds confidence to our assumption of reasonably good thermal equilibrium in the excited states of photosynthetic pigment systems,[1] and provides one more evidence that there is only one photosynthetic system in bacteria.

Spinach. For the purpose of making quantum yield measurements, Sauer and Biggins (1965) made careful measurements of the absorption spectrum of the photosynthetic apparatus of spinach. Using a tabulation of their absorption data, we applied the Planck factor to calculate the luminescence spectrum which is displayed in Fig. 4. This is the luminescence spectrum which one would expect if the excited states of all of the pigment molecules in plant photosynthesis were in thermal equilibrium.

However, plant photosynthesis does not appear to be comprised of one photochemical system, but rather two. One of these, called System II, can be driven only with light having a wavelength less than about 680 nm; the other, called System I, can utilize radiation of longer wavelengths. The manner and degree of any interaction between these two systems at the level of electronic excitations is not known; the most popular current hypothesis is that there is no significant interaction, and that each system may be considered as an independent entity with its own independent absorption spectrum. We shall assume that this "separate box" hypothesis is correct (vis. Weiss, 1966).

One way of separating the two photochemical systems is to take a preparation of the photosynthetic apparatus of a plant, chloroplasts, and add to it metabolic poisons and spectroscopically observable redox agents with appropriate potentials. By use of the appropriate chemicals, one may observe the light-driven progress of only one of the two photochemical systems.

By using this technique, Sauer and Park (1965) and Kelly and Sauer (1965) have determined quantum yields for each of the two systems in spinach over a wide range of wavelengths. Their original data were distorted slightly because of the band pass of their instrument, but correction for this indicates that the quantum yield for System I plus the quantum yield for System II is within experimental error of 1.0 at all wavelengths (Kelly and Sauer, 1965).

Using the assumption of separate boxes, we have smoothed their data somewhat to obtain the quantum yield partitioning diagrammed in Fig. 5. These quantum yields may be used to calculate an activation spectrum for each of the two systems; this has been done by Kelly and Sauer, and Fig. 6 shows this on a logarithmic plot.

Separate activation spectra for the two systems permits a decomposition of the luminescence spectrum shown in Fig. 4 into a component due to System I and a component due to System II. The result is displayed in Fig. 7. The curve for System II has been magnified by a factor of 5 in order to make the area under the two curves approximately equal. If the luminescence yields for Systems I and II were about the same, then the emission spectrum of spinach should look something like the sum indicated in the figure.

Comparison of the experimental fluorescence spectrum of spinach chloroplasts (Murata, Nishimura, and Takamiya, 1966) with Fig. 7 suggests that the fluorescence yield for System I is somewhat less than the fluorescence yield for System II. By adjusting the relative magnitudes of System I luminescence

and System II luminescence to obtain the best fit with the experimental curve of Murata et al., it appears that the fluorescence yield for System I is about 1/3 that of System II. The resulting fit between the theoretically calculated luminescence spectrum and the experimental spectrum is shown in Fig. 8. Considering all the sources of error, we feel that the agreement between the two is quite good.

These calculations reinforce the notion that the fluorescence yield for System I is less than that for System II, and that the luminescence at 740 nm has a relatively greater contribution from System I than does the luminescence around 685 nm (vis. Butler, 1966).

IV. POTENTIAL DIFFERENCES AND FREE-ENERGY STORAGE

We saw in Section II that the first step in evaluating the energetics of a photochemical system is to determine the light-driven potential which is developed when the rate of luminescent emission is equal to the rate of absorption.

Recalling the equation we derived,

$$\mu = kT \ln[\phi_{lum} \int I_s(\nu)\, \sigma(\nu)\, d\nu \,/\, \int 8\pi\, \sigma(\nu)\, (n\nu/c)^2 \exp(-h\nu/kT)\, d\nu\,], \quad (6')$$

we remember that only three quantities are necessary to evaluate the maximum potential: an incident light flux, I_s, an absorption spectrum, σ, and the ambient temperature.

In order to be certain that μ_{max} is correctly evaluated, the absorption spectrum should be taken on an organism which has been grown at light intensity I_s. Otherwise there is the possibility that an organism may vary its absorption spectrum depending on the light intensity. This has actually been observed in several species of bacteria (Fuller, Conti, and Mellin, 1963), and the change in absorption spectrum is in a direction which would tend to keep the potential developed independent of light intensity. In the following discussion we will not be too careful about this point, partly because the data are not available, but chiefly because an error or a millivolt or so in the computed potential is insignificant when compared to other sources of error.

Once the maximum potential has been calculated, then the potential for maximum power storage can be obtained in the manner outlined in Section II.

Spinach. The range of light intensities for effective plant growth is limited at the lower end by the compensation point, at which the rate of photosynthesis is just adequate to balance respiration. The upper limit is set by the saturation of the various chemical reactions which make up the energy storing process. The compensation point generally occurs at a light intensity of between 20 and 500 lux[2] of white light. Photosynthesis becomes half-saturated somewhere between 1 and 10 kilolux (Rabinowitch, 1951).

The spinach whose absorption spectrum we used in Section III was grown at a light intensity of about 15 kilolux (Park, 1966). However, because of the high optical density of spinach leaves, a typical photosynthetic unit might see a light intensity of more like 1 kilolux. We shall use this figure in our calculations.

By taking the product of the spectral distribution of the quantum flux from a tungsten bulb with the absorption spectrum for spinach photosynthesis, we find that 1 kilolux of white light produces pigment excitation at the same rate as would 0.9 nanoeinsteins/cm^2sec incident at the red absorption maximum at about 680 nm. This gives us the numerator for equation (6), and we assume that this is split equally between Systems I and II.

The integral in the denominator of equation (6) is evaluated by finding the area under the curves in Fig. 7, with appropriate consideration of how the vertical scale is defined. Performing the necessary arithmetic, we find that μ_{max} for System I is 1.32 eV and that μ_{max} for System II is 1.36 eV.

These maximum potentials have been evaluated with the assumption that non-useful non-radiative decay is negligible. This is probably not true, and the potential must be corrected downwards by kT ln α, where α is the reciprocal of the quantum yield of fluorescence in the absence of the energy storage process.

The fluorescing species in plants is chlorophyll a, dilute solutions of which have an α of 3 (vis. Clayton, 1966). If the pigments of System II have an equivalent or greater amount of non-radiative losses, then the maximum potential for this system is 1.33 eV or less.

Evidence is accumulating that the species responsible for the longest wavelength absorption in plants are one or more aggregated forms of chlorophyll (Dratz, Schultz, and Sauer, 1967). Presumably these aggregated forms belong largely to System I, so that if non-radiative losses from aggregated chlorophyll should be greater than from monomers, System I would be most affected.

Recall from Section III that the observed fluorescence yield of System I of spinach appears to be only 1/3 that of System II. One cause for this could be a greater rate of non-radiative decay in System I. If this should be the sole reason, then we can guess that α for System I is 9, which would give a maximum potential of 1.26 eV for this system.

Applying the theory outlined in Section II to the assumed maximum potentials of 1.26 and 1.33 eV for Systems I and II under 1 kilolux of illumination, we find that the optimal fraction of quanta lost for thermodynamic reasons is slightly more than 2% for each system. The optimum potentials at the trap are 1.16 eV for System I and 1.23 eV for System II.

At this point we should ask how critically dependent the amount of free energy stored is on the potential at the trap. The dependence of power stored on the potential is shown in Fig. 9 for a ν_{max} of 1.30 eV. The potential for maximum power storage is 1.20 eV, but the potential can range between 1.12 eV and 1.24 eV with the amount of power stored remaining greater than 95% of this maximum.

Over this range of potential for nearly maximum power storage, the quantum yield for loss processes caused by thermodynamic reversibility ranges from 0.1% to 10%. Because power storage is so insensitive to this parameter (in the current theory at least), and because the kinetically determined losses may differ between Systems I and II, we have no assurance that ϕ_{loss} should be the same for Systems I and II. For this reason, although it seems quite plausible, the assignment of a larger proportion of non-radiative decay to System I remains speculative with the information accumulated so far.

Recent work by Bertsch, Azzi, and Davidson (1967) indicates that the delayed light emission from System I of plants is several hundred times weaker than the delayed light from System II. If this is true, and our estimate of a System I/System II luminescence yield ratio of 1/3 from the data of Murata

et al. is accurate, then the proportion of non-radiative decay from System I cannot be more than 3 times the proportion from System II.

Furthermore, such a large ratio of System II to System I delayed light would imply that the potential of System II is towards the upper end of the range which gives nearly maximal power storage, while the potential of System I is towards the lower end of the range which gives near maximal power storage. This would suggest a thermodynamically-determined lost quantum yield of roughly 10^{-3} for System I, and 0.1 for System II. It may be that System II sacrifices quantum yield in order to develop the chemical potential necessary to oxidize water to molecular oxygen.

Purple Bacteria. We do not know the light intensities used for growing the bacteria whose absorption and fluorescence spectra were discussed in Section III. Even if we did, it is unlikely that the figure would be meaningful, as typical bacterial cultures have a high optical density, so that the mean intensity incident on a bacterium is much lower than the intensity incident on the culture as a whole.

Katz, Wassink, and Dorrestein (1942) found that the rate of photosynthesis of the purple bacterium Chromatium, as they cultured it, became half-saturated at 6 to 10 kiloergs/cm^{2}sec of incident sodium lamp radiation when the optical density of the bacterial suspension was low. One kiloerg from such a lamp represents 0.49 nanoeinsteins of 589 nm light.

Bacterial photosynthesis has a somewhat S-shaped dependence on light intensity, so that the efficiency of photosynthesis drops at light intensities much below the half-saturation point. For this reason we shall calculate the potential developed for 10 kiloergs/cm^{2}sec of sodium radiation.

For the present calculation we shall use the absorption and fluorescence spectra of Chromatium obtained by Olson and Stanton which were discussed in Section III. The spectrum of the culture used by Katz et al. may have been

different because of different growth conditions, but this should not introduce a serious error in the potential calculated.

The information necessary to evaluate the denominator of equation (6) is contained in the calculations for Fig. 3(b). Combining all of the appropriate factors, we find that μ_{max} is 0.90 eV. The potential for maximum free storage is 0.81 eV and the maximum free energy storage per photon is 0.79 eV. An α of 3 would lower each of these values by 0.03 eV.

Redox Potentials of Light-Generated Biochemicals. In the previous section we found that the thermodynamic potential generated by the two systems of plant photosynthesis is about 1.2 eV, with the potential of System II being slightly higher than System I. The thermodynamic potential developed in purple bacteria is about 0.8 eV.

When the electronic excitations carrying these potentials are converted into chemical energy, it is thought--at present at least--that the most probable immediate chemical consequence is an oxidation-reduction reaction. It is possible that one might have a conformational change using at least part of the energy relatively early in the process, but an ionization seems to be the most rapid possible, and hence preferable, first step.

If the primary oxidation and reduction reactions are one electron processes, then the difference between the redox potentials of these two half-reactions should be equal to the thermodynamic potentials just calculated.

One can attempt to represent the electron transport chains of bacterial and plant photosynthesis by the potential diagrams shown in Figs. 10 and 11.[3] Here the vertical arrows represent the input of free energy in the light-driven reactions, while downward arrows indicate spontaneous, or "dark" reactions. Points at which this electron transport process is thought to be coupled to energy-storing phosphorylation are indicated with the curved dotted lines.

Chemicals which have been identified as participating in the electron transport pathway are indicated by their initials, and placed according to

our estimate of their redox potential when the organism is illuminated. If the primary molecules to be oxidized and reduced are largely in their "acceptor" oxidation states, then the actual potential will be shifted from the midpoint potential, which is indicated in parentheses.

Fd stands for ferredoxin; FP for flavoprotein; PN for pyridine nucleotide; Cyt. for cytochrome; PQ for plastoquinone; and P_{890} and P_{700} for as yet uncharacterized molecules having absorption peaks at 890 and 700 nm which can be bleached by light, and also reversibly bleached chemically with the midpoint potentials indicated.

In the case of bacteria, the available free energy appears to be adequately explained by the difference in redox potentials between the well-characterized c-type cytochromes and P_{890}, and bacterial ferredoxin. The thermodynamics is also in accord with a proposal by Loach (1966) that a two electron/photon oxidation-reduction occurs with midpoint reduction potentials of -0.02 and +0.44 V.

In System I of plants, shown as the solid vertical arrow of Fig. 11, the available energy significantly exceeds the potential difference between spinach ferredoxin, and cytochrome f and P_{700}. On the basis of the reduction of viologen dyes by illuminated chloroplasts, Kok, Rurainski, and Owens (1965) have proposed the existence of a System I chemical having a reduction potential in the vicinity of -0.7 V. The thermodynamic calculations support this hypothesis.

Less is known about System II, which oxidizes water to molecular oxygen in order to generate a reductant. The usual assumption that the upper-end of System II terminates near plastoquinone is reasonable if one assumes that a powerful oxidant with a potential of greater than +1.0 V. is generated, and some losses are incurred in the oxidation of water. It is also thermodynamically possible that electrons removed from water could be brought to the potential of ferredoxin with a single quantum of light, as has been suggested by Arnon (1966); this is indicated by the dotted line to the far right of Fig. 11. For a third possibility, Kok and Datko (1965) have recently suggested that the

reductant produced by System II has a potential of +0.18 V.; a two electron/photon process between this potential and the water/oxygen potential would be in accord with the thermodynamics.

The authors are grateful to many colleagues in the Laboratory of Chemical Biodynamics for a number of lively discussions which helped greatly in the formulation of the theories presented here. We are particularly indebted to Kenneth Sauer, E. A. Dratz, D. R. Gentner, and I. D. Kuntz, Jr., in this regard.

This work was supported in part by the U.S. Atomic Energy Commission.

Received for publication, 1967.

FOOTNOTES

[1]However, the variation which Clayton (1965a) has obtained between the prompt fluorescence and chemiluminescence spectra of green bacteria indicates that thermal equilibration is not complete.

[2]100 lux = 9.3 foot-candles

[3]For a review of what is known about the electron-transport chain of bacterial photosynthesis, see Vernon (1964); for plants, see Clayton (1965b). For a more recent review of both, see Vernon and Ke (1966).

REFERENCES

ARNON, D. I. 1966. In "Currents in Photosynthesis", J. B. Thomas and J. C. Goedheer, eds., Rotterdam, Ad. Donker, p. 465.

BELL, L. N. 1964. Zh. Eksperim. i Teor. Fiz. 46:1117. [English transl.: Soviet Phys.-JETP 19:756]

BERTSCH, W., J. ASSI, AND J. DAVIDSON. 1967. Private communication.

BUTLER, W. L. 1966. In "The Chlorophylls", L. P. Vernon and G. R. Seely, eds., New York, Academic Press, p. 343.

CLAYTON, R. K. 1965a. J. Gen. Physiol. 48:633.

CLAYTON, R. K. 1965b. "Molecular Physics in Photosynthesis", New York, Blaisdell Publishing Company.

CLAYTON, R. K. 1966. In "The Chlorophylls", L. P. Vernon and G. R. Seely, eds., New York, Academic Press, p. 610.

DRATZ, E. A., A. J. SCHULTZ, AND K. SAUER. 1967. In "Energy Conversion by the Photosynthetic Apparatus", Brookhaven Symposium in Biology No. 19, Upton, N. Y., Brookhaven National Laboratory, p. 303.

DUYSENS, L.N.M. 1958. In "Photochemical Apparatus", Brookhaven Symposium in Biology No. 11, Upton, N. Y., Brookhaven National Laboratory, p. 18.

FULLER, R. C., S. F. CONTI, AND D. B. MELLIN. 1963. In "Bacterial Photosynthesis", H. Gest, A. San Pietro, and L. P. Vernon, eds., Yellow Springs, Ohio, Antioch Press, p. 71.

KATZ, E., E. C. WASSINK, AND R. DORRESTEIN. 1942. Enzymologia 10:269.

KELLY, J. AND K. SAUER. 1965. Biochemistry 4:2798.

KOK, B., AND E. A. DATKO. 1965. Plant Physiol. 40:1171.

KOK, B., H. J. RURAINSKI, AND O.V.H. OWENS. 1965. Biochim. Biophys. Acta 109:347.

LOACH, P. A. 1966. Private communication.

MORTIMER, R. G., AND R. M. MAZO. 1961. J. Chem. Phys. 35:1013.

MURATA, N., M. NISHIMURA, AND A. TAKAMIYA. 1966. Biochim. Biophys. Acta 112:213.

OLSON, J. M., AND E. K. STANTON. 1966. In "The Chlorophylls", L. P. Vernon and G. R. Seely, eds., New York, Academic Press, p. 381.

PARK, R. B. 1966. Private communication.

RABINOWITCH, E. I. 1951. "Photosynthesis", New York, Wiley and Sons, Chapt. 28.

ROSS, R. T. 1966a. J. Chem. Phys. 45:1.

ROSS, R. T. 1966b. Thesis, University of California, Berkeley.

ROSS, R. T. 1967. J. Chem. Phys. In press.

SAUER, K. 1966. Private communication.

SAUER, K., AND J. BIGGINS. 1965. Biochim. Biophys. Acta 102:55.

SAUER, K., AND R. B. PARK. 1965. Biochemistry 4:2791.

STREHLER, B., AND W. ARNOLD. 1951. J. Gen. Physiol. 34:809.

VERNON, L. P. 1964. Ann. Rev. Plant Physiol. 15:73.

VERNON, L. P., AND B. KE. 1966. In "The Chlorophylls", L. P. Vernon and G. R. Seely, eds., New York, Academic Press, p. 569.

WEISS, C. JR. 1966. Biophys. J. 6:261.

FIGURE CAPTIONS

Fig. 1. Multiplication of the absorption spectrum of chlorophyll by the room temperature blackbody curve to compute the wavelength distribution of spontaneous radiative transitions (dotted curve). Arbitrary logarithmic vertical scale: absorption cross-section from the extinction coefficient of monomeric chlorophyll a in CCl_4 (Sauer, 1966); blackbody curve for 295°K in units of quanta/cm^2sec per unit wavelength interval; curve for the distribution of radiative transitions in units of quanta/sec per unit wavelength interval.

Fig. 2. Kinetics and thermodynamics of a photochemical system (a) in the absence of energy storage, and (b) in the presence of energy storage when the thermodynamic activity of the trap is a fraction δ of that in (a).

Fig. 3. Comparison of calculated and experimental luminescence spectra of purple bacteria. Experimental absorption, o, and luminescence, •, data were taken at 100 cm^{-1} intervals from the curves of Olson and Stanton. (luminescence data in (c) from Clayton.) Solid line: experimental

luminescence spectrum; dashed line: luminescence spectrum calculated from the absorption spectrum with the Planck factor for 295°K.

Fig. 4. The luminescence spectrum of spinach calculated with the assumption that plants contain a single photochemical system. The plotted points were obtained by multiplying the tabulated absorption spectrum of Sauer and Biggins (Sauer, 1966) by the Planck law factor for 295°K.

Fig. 5. Partition of quanta between photosystems I and II in spinach as a function of photon energy. Quantum yield of System I as measured by Kelly and Sauer, o; difference from 1 of the quantum yield for System II as measured by Sauer and Park, Δ. Filled symbols indicate corrected quantum yield obtained by extrapolating instrument band width to zero. The solid line indicates the partition assumed in subsequent calculations.

Fig. 6. Activation spectra for the two photosystems of spinach.

Fig. 7. Calculated luminescence spectra of Systems I and II of spinach. Vertical scale is the same as in Fig. 3, but the curve for System II has been magnified by 5 X in order to make the area under the two curves approximately equal.

Fig. 8. Comparison of the calculated and experimental luminescence spectra of spinach chloroplasts. Experimental points from Murata, Nishimura, and Takamiya. Calculated curve obtained by adjusting the amounts of System I and System II luminescence so as to match the experimental luminescence intensities at 685 and at 730 nm. Hatch marks indicate points at which the spectrum was calculated.

Fig. 9. Work stored and quantum yield for loss processes as a function of excited state potential when μ_{max} = 1.30 eV. Losses due to a finite transfer rate are not considered.

Fig. 10. Suggested electron flow diagram for bacterial photosynthesis. The potential change indicated for the light-driven step P_{890} to X was determined thermodynamically.

Fig. 11. Suggested electron flow diagram for plant photosynthesis. The potential changes for the light-driven steps were determined thermodynamically. The solid line indicates the light act of System I and the vertical dashed lines indicate two possible positions for System II.

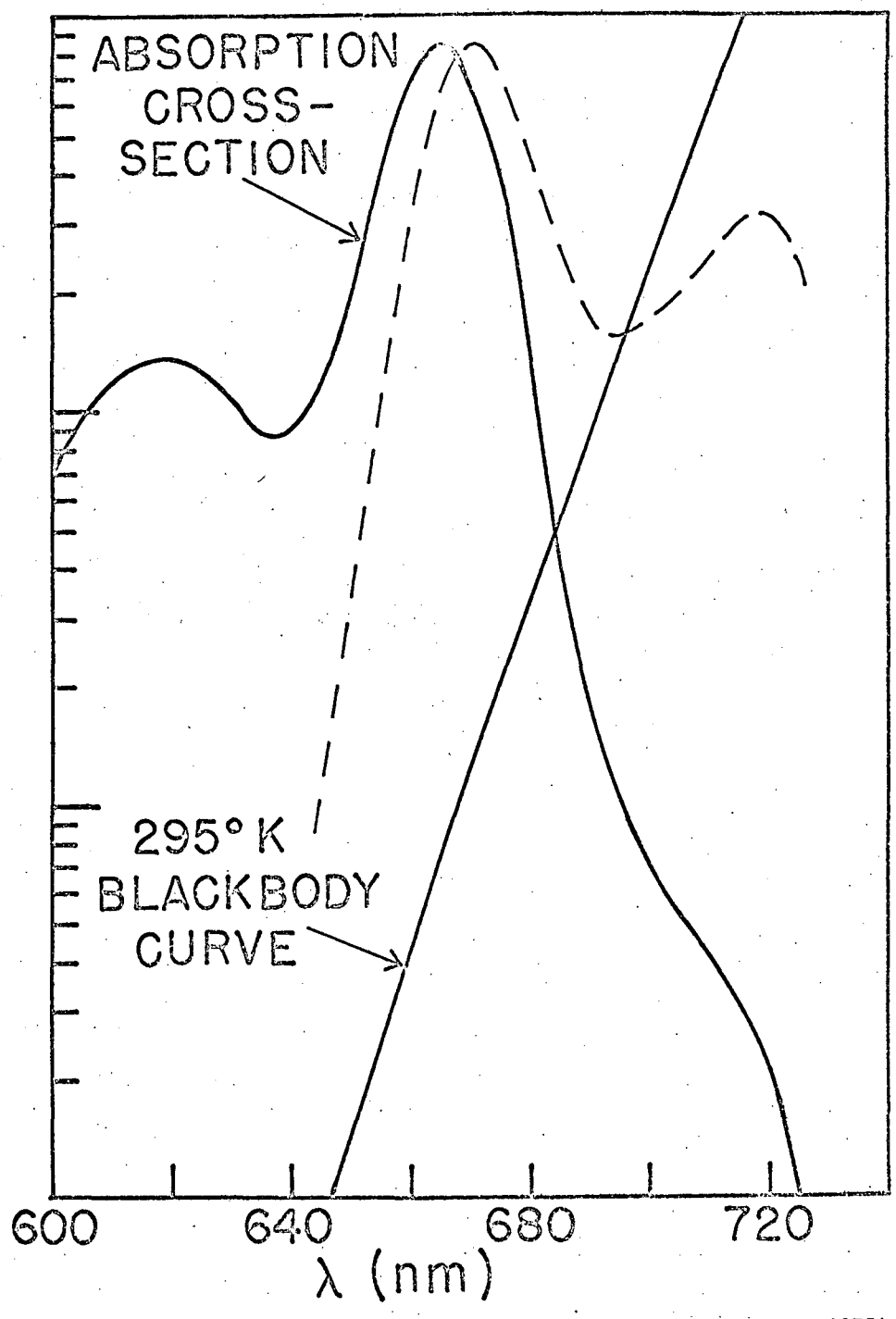
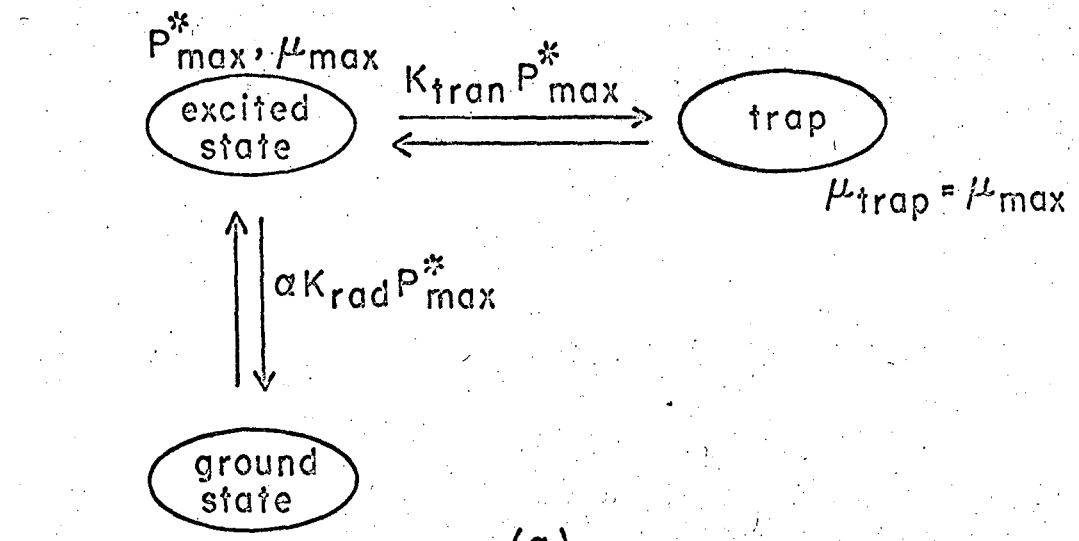
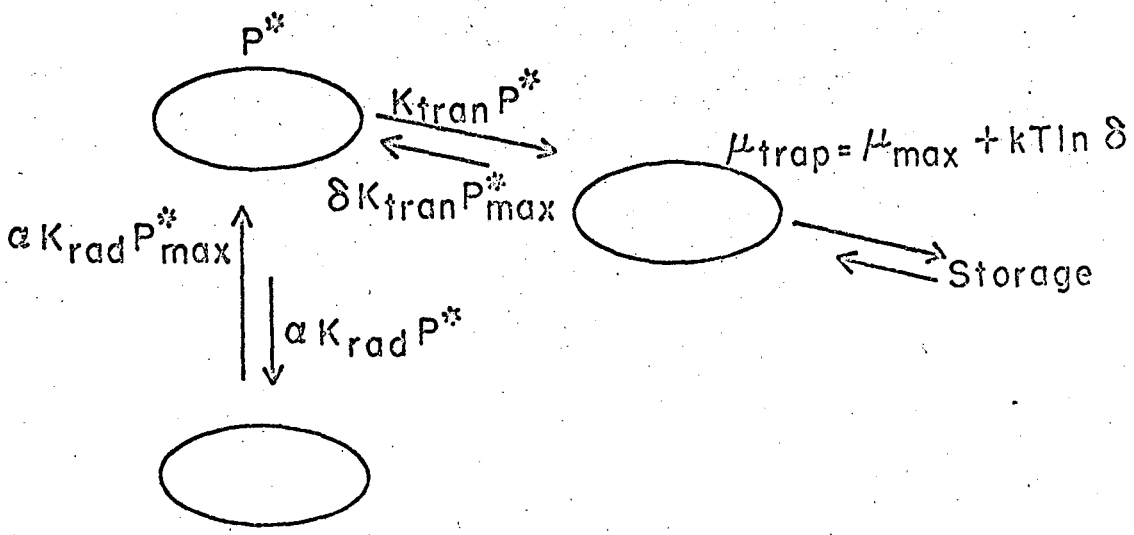

ABSORPTION
CROSS-
SECTION
295° K
BLACKBODY
CURVE
600
640
680
720
λ (nm)
MUB-13751

Fig. 1



(a)



(b)

MUB-11266

Fig. 2

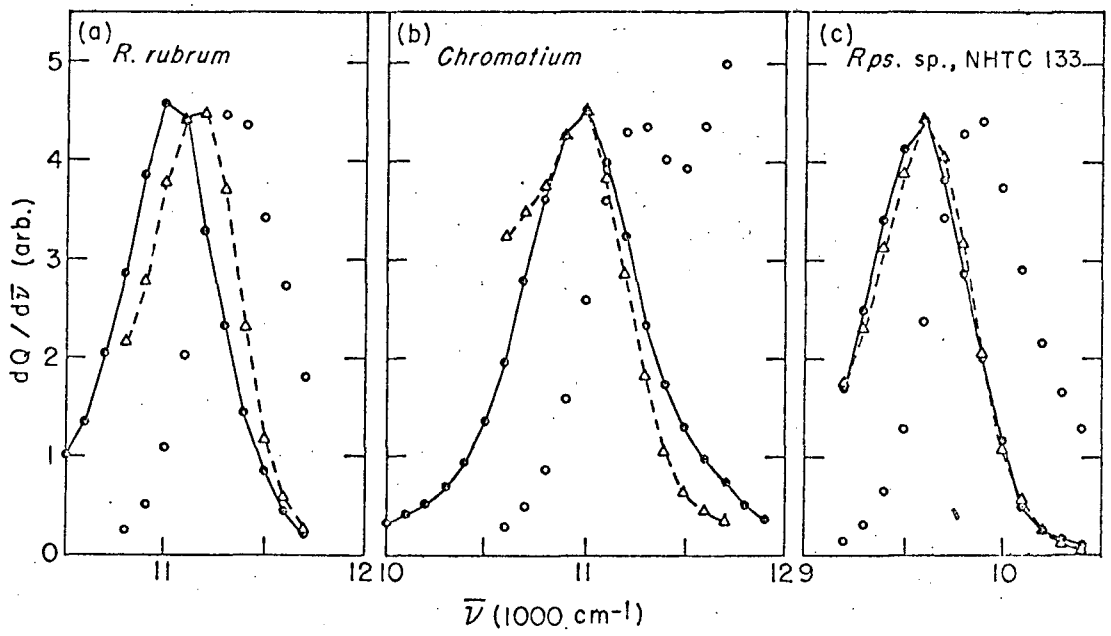
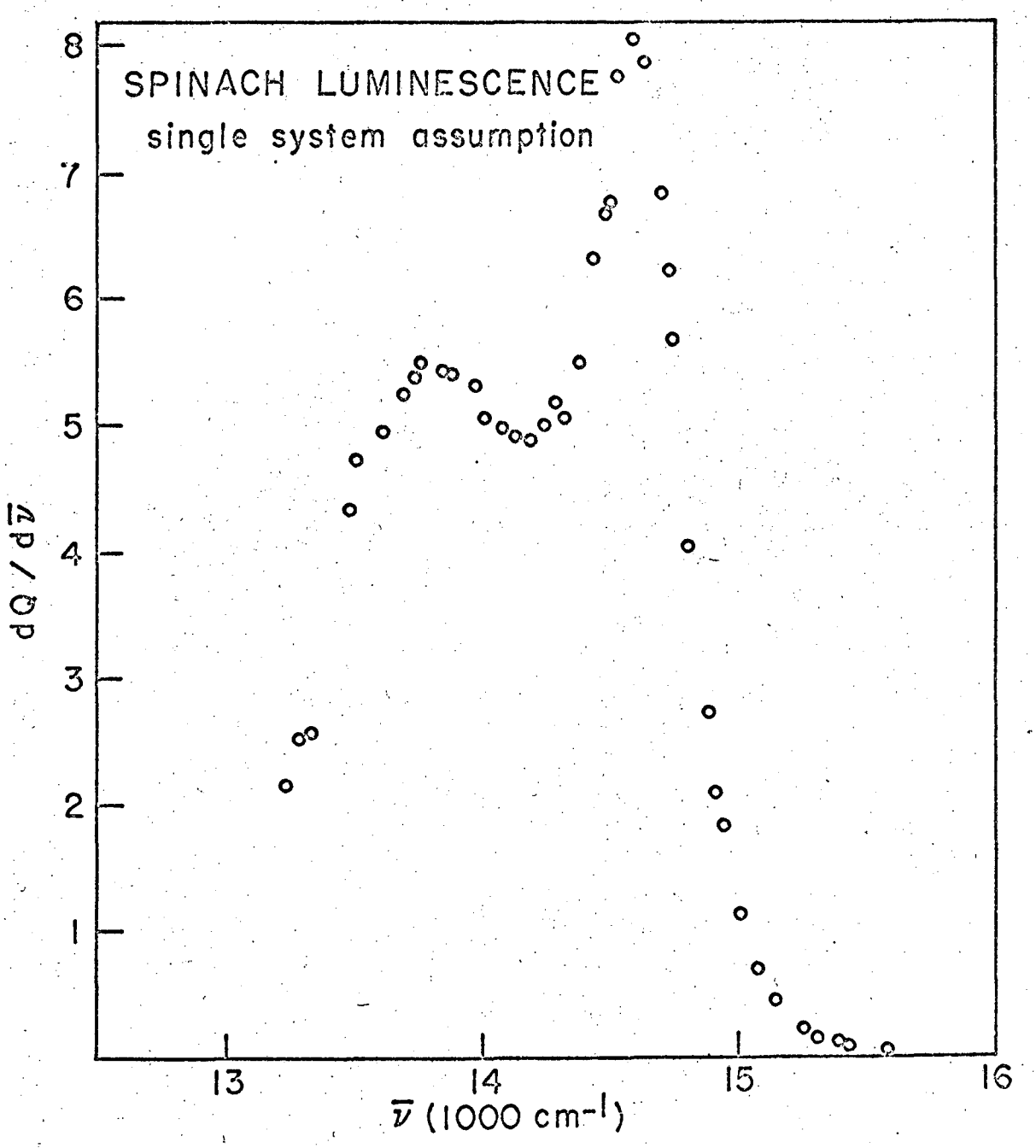

(a)
R. rubrum
(b)
Chromatium
(c)
Rps. sp., NHTC 133
dQ / dν̄ (arb.)
0
1
2
3
4
5
11
12
10
11
12
9
10
ν̄ (1000 cm⁻¹)
MUB-11285

Fig. 3



MUB-11261

Fig. 4

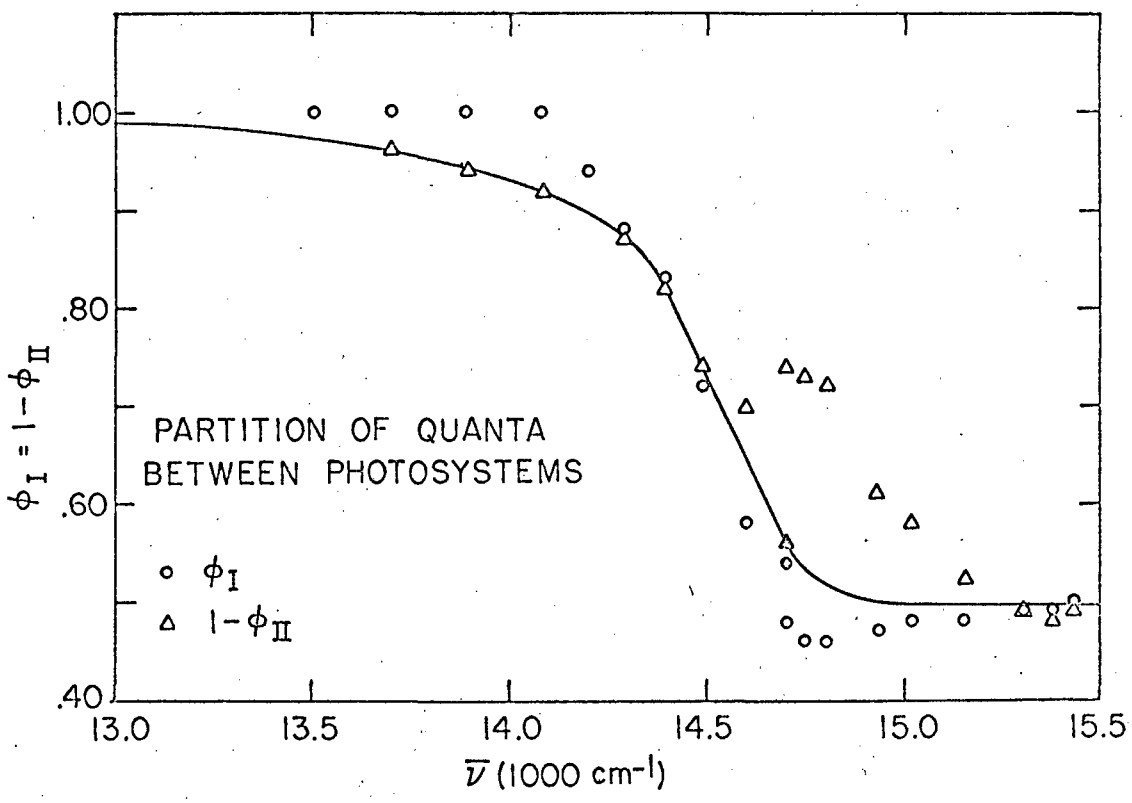

1.00
.80
.60
.40
$\phi_I = 1 - \phi_{II}$
PARTITION OF QUANTA
BETWEEN PHOTOSYSTEMS
ϕ_I
$1 - \phi_{II}$
13.0
13.5
14.0
14.5
15.0
15.5
$\bar{\nu}$ (1000 cm^{-1})
MUB-11265

Fig. 5

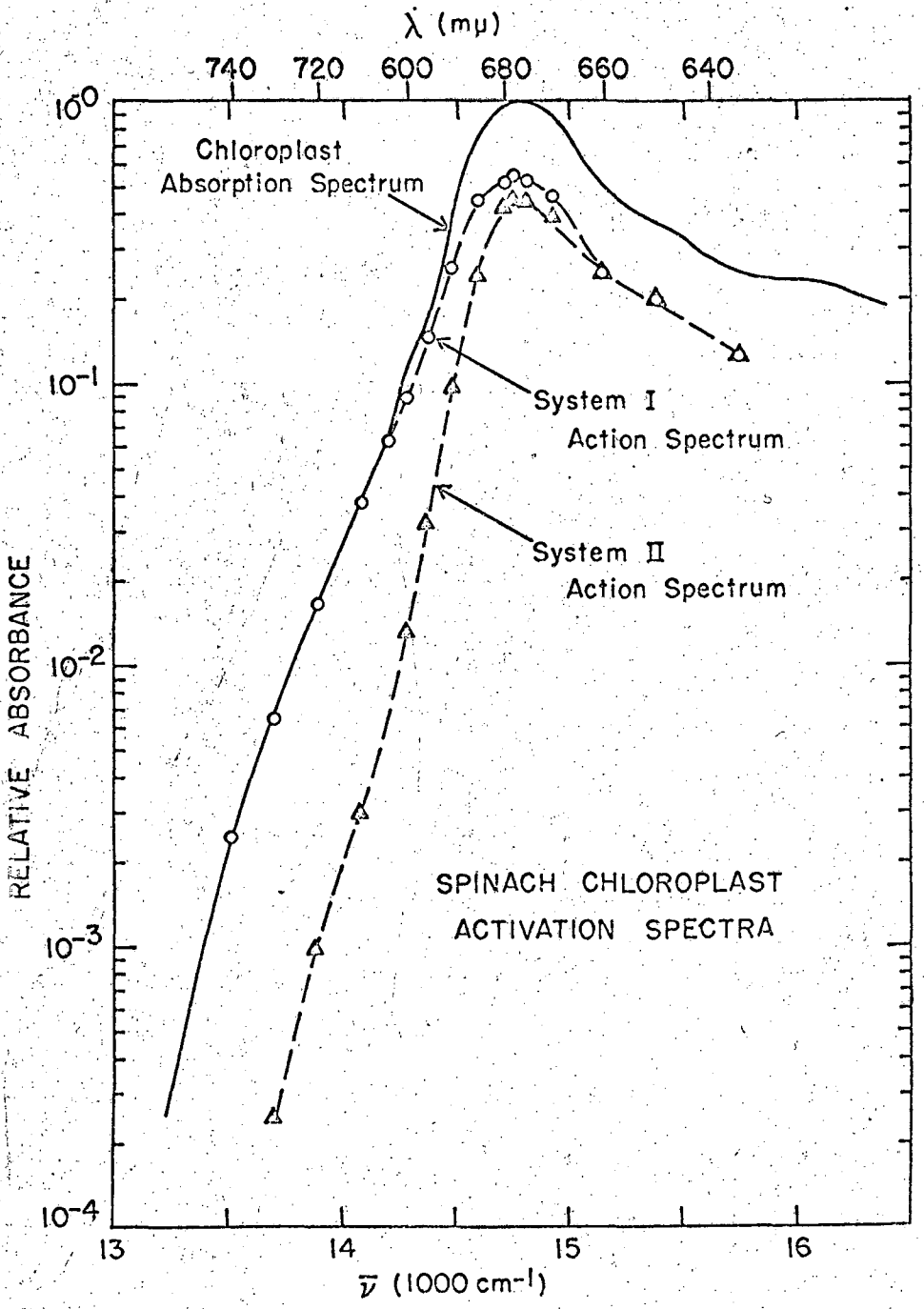
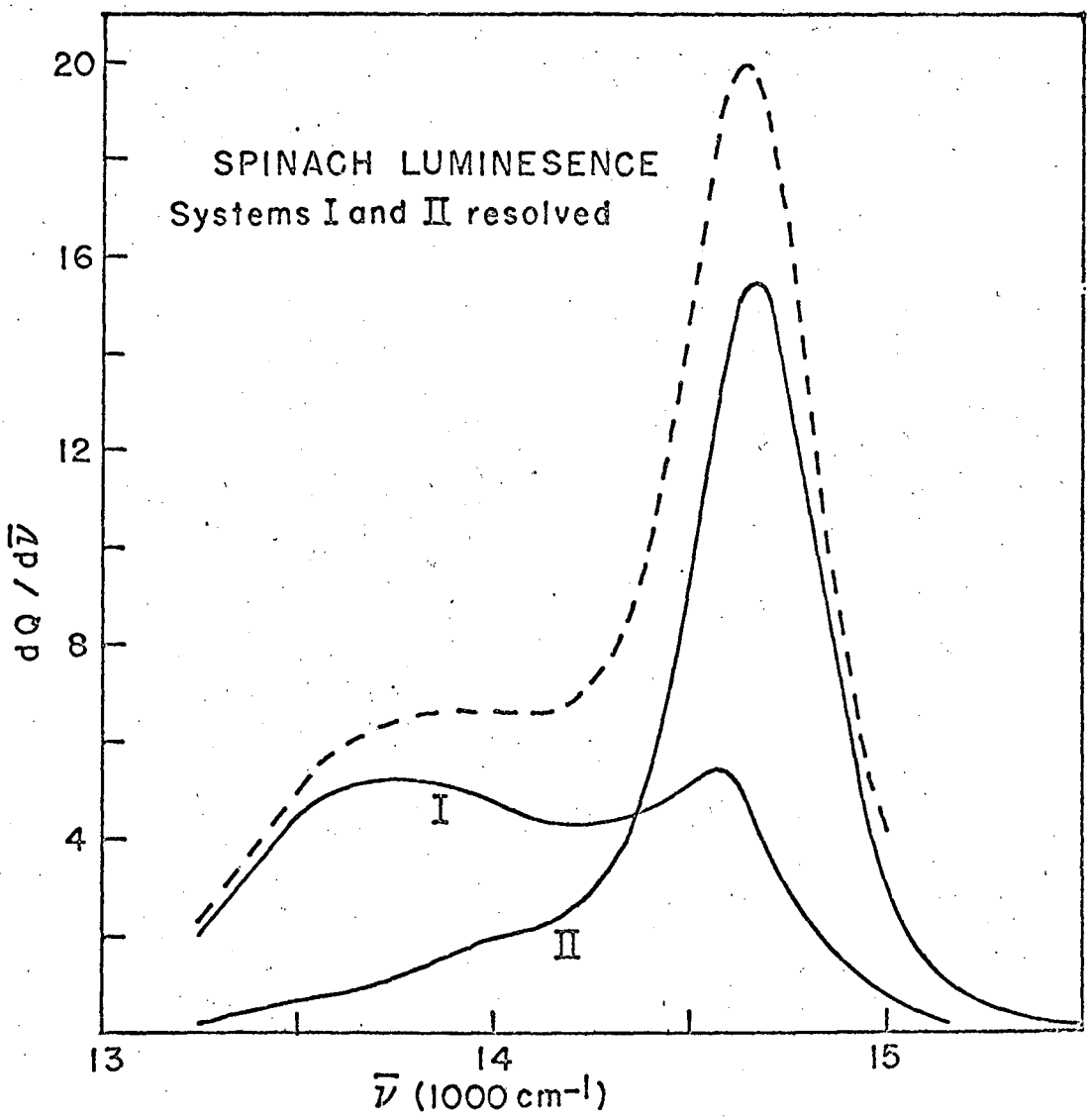

λ (mμ)
740
720
600
680
660
640
10^0
10^-1
10^-2
10^-3
10^-4
RELATIVE ABSORBANCE
Chloroplast
Absorption Spectrum
System I
Action Spectrum
System II
Action Spectrum
SPINACH CHLOROPLAST
ACTIVATION SPECTRA
13
14
15
16
ν̄ (1000 cm^-1)
MUB-9514

Fig. 6



MUB-11260

Fig. 7

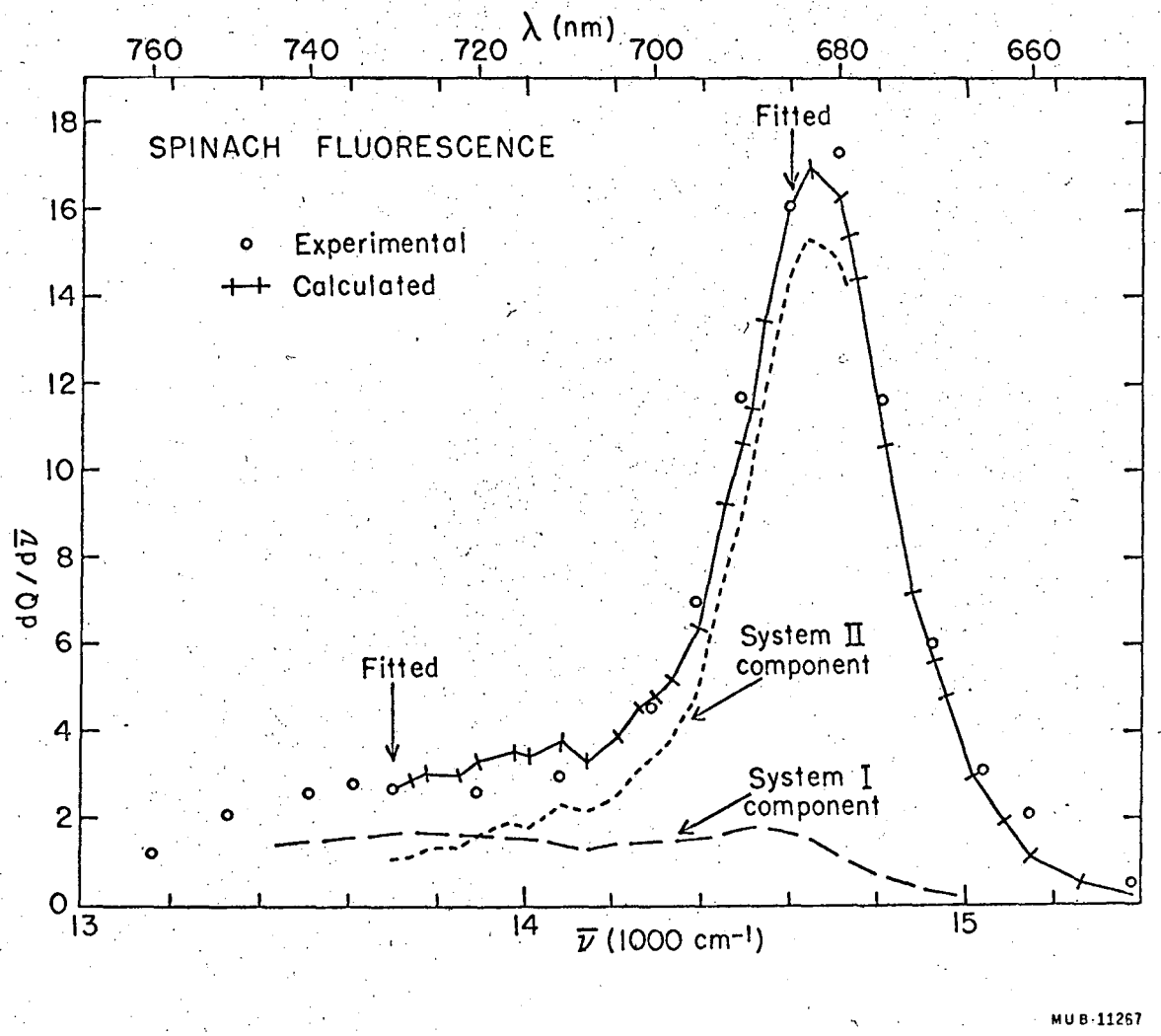

λ (nm)
760
740
720
700
680
660
SPINACH FLUORESCENCE
Experimental
Calculated
Fitted
Fitted
System II component
System I component
dQ/dν̄
ν̄ (1000 cm⁻¹)
13
14
15
0
2
4
6
8
10
12
14
16
18
MUB-11267

Fig. 8

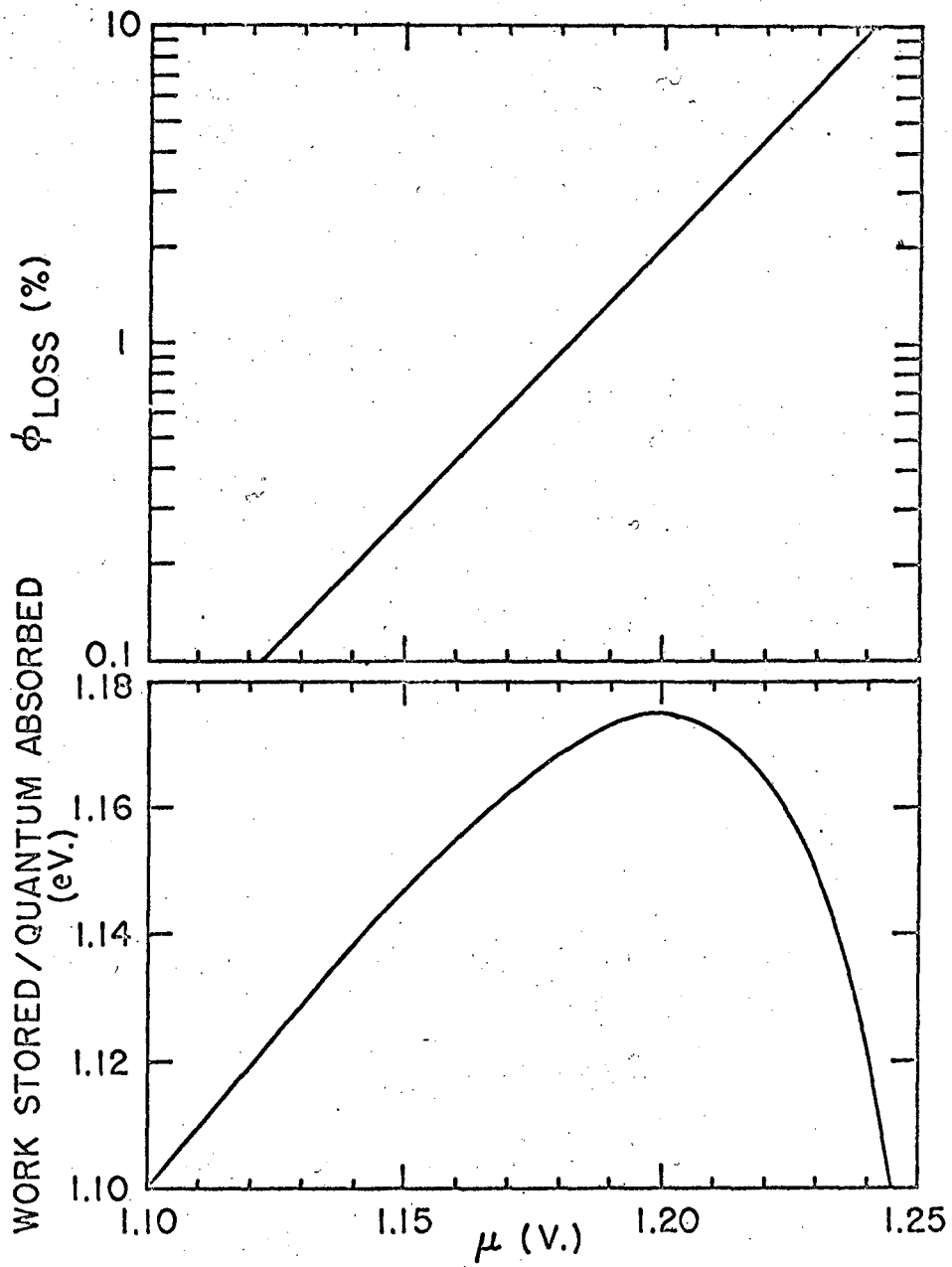
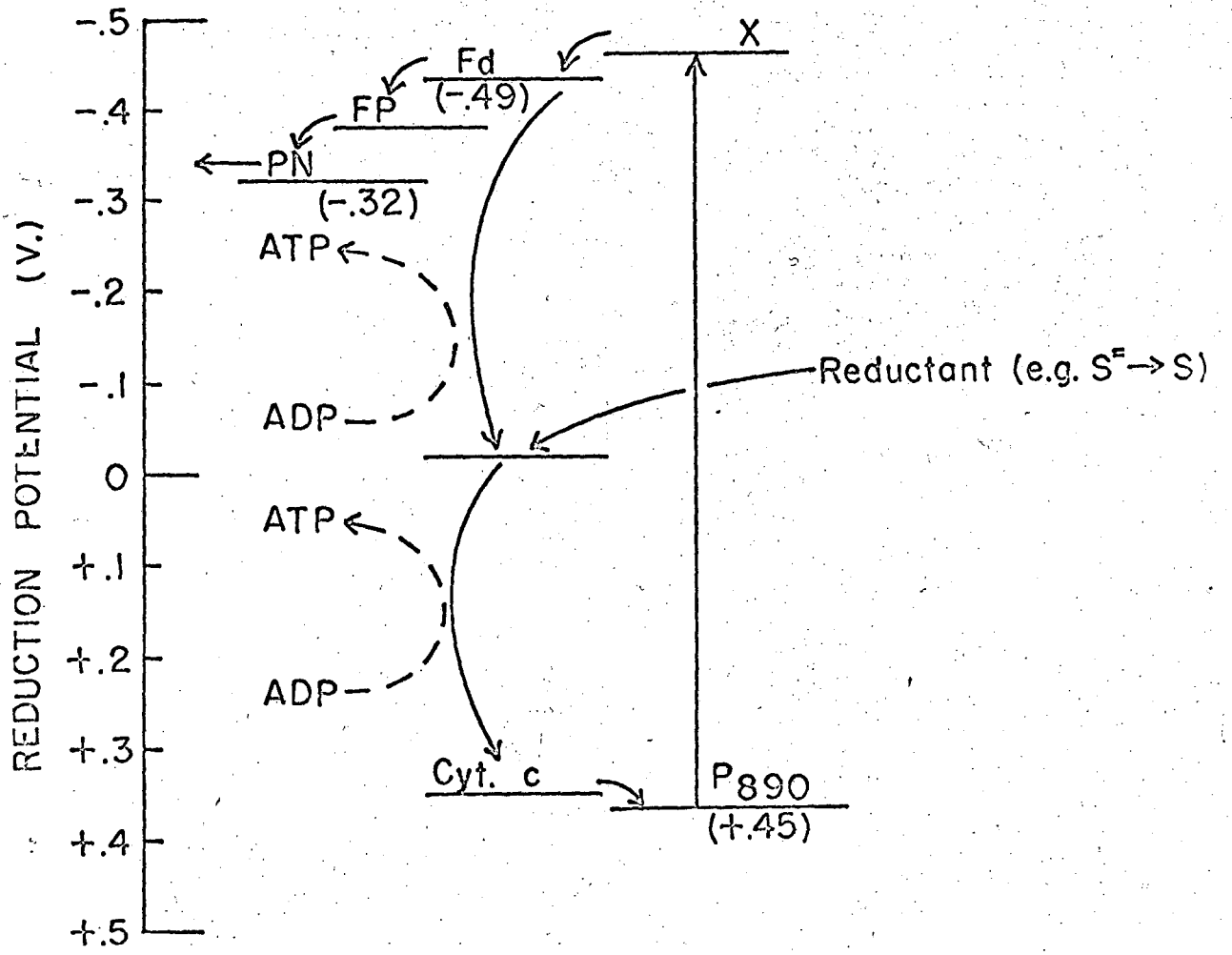

10
1
0.1
ϕ_{LOSS} (%)
1.18
1.16
1.14
1.12
1.10
WORK STORED / QUANTUM ABSORBED (eV.)
1.10
1.15
1.20
1.25
μ (V.)
MUB-11263

Fig. 9



MUB-9519

Fig. 10

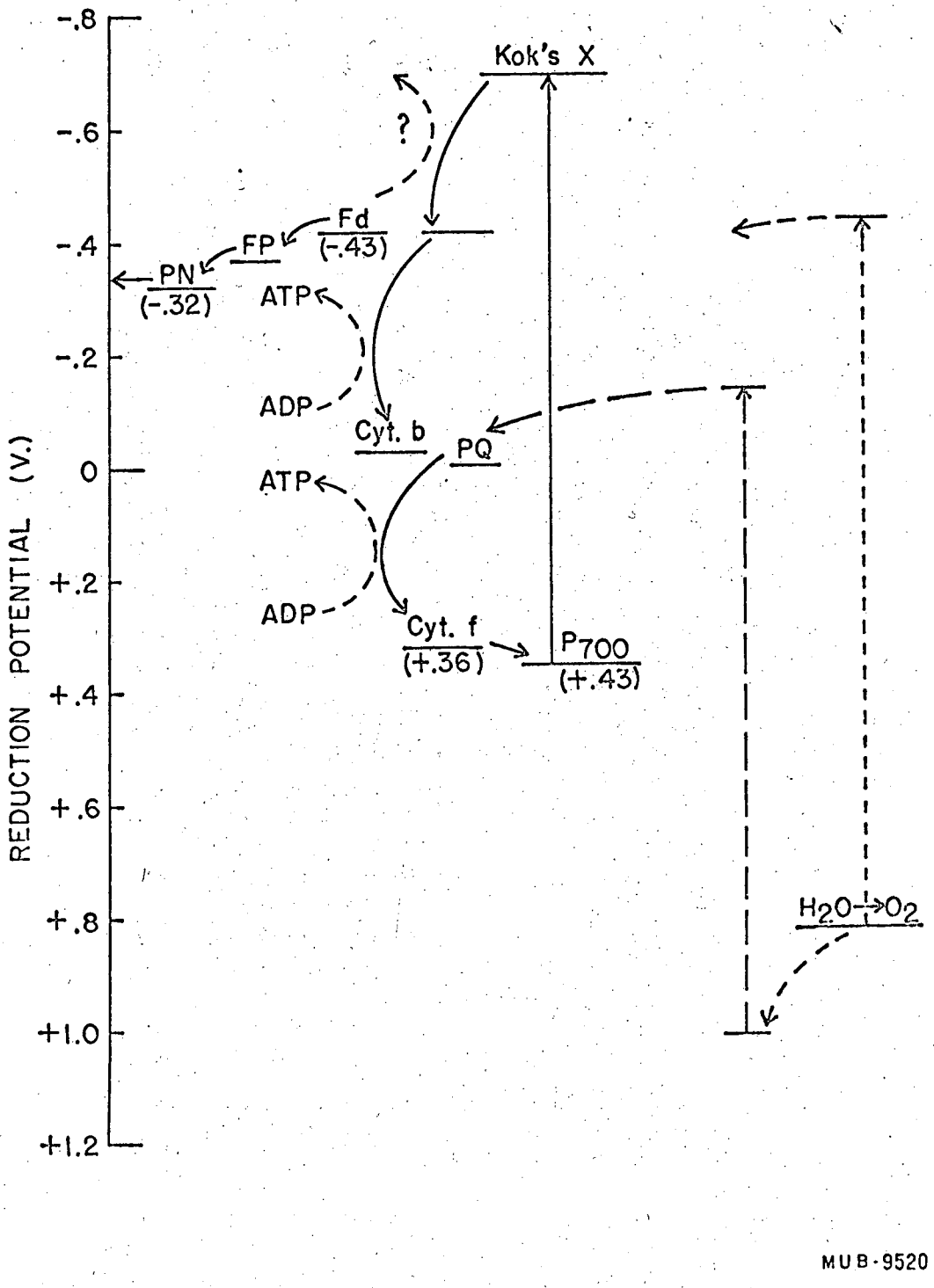

-.8
-.6
-.4
-.2
0
+.2
+.4
+.6
+.8
+1.0
+1.2
REDUCTION POTENTIAL (V.)
Kok's X
?
Fd
(-.43)
FP
PN
(-.32)
ATP
ADP
Cyt. b
PQ
ATP
ADP
Cyt. f
(+.36)
P700
(+.43)
H2O→O2
MUB-9520

Fig. 11