THEORETICAL STUDIES ON PHOTOSYNTHESIS

random walk in the pigment system
the mono cation of bacteriochlorophyll
steady-state approximation in enzyme kinetics

H. A. OTTEN

THEORETICAL STUDIES ON PHOTOSYNTHESIS

random walk in pigment system
the mono cation of bacteriochlorophyll
steady-state approximation in enzyme kinetics

THEORETICAL STUDIES ON PHOTOSYNTHESIS

random walk in the pigment system
the mono cation of bacteriochlorophyll
steady-state approximation in enzyme kinetics

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN
DE WISKUNDE EN NATUURWETENSCHAPPEN AAN
DE RIJKSUNIVERSITEIT TE LEIDEN, OP GEZAG VAN
DE RECTOR MAGNIFICUS DR. A.E. COHEN,
HOOGLERAAR IN DE FACULTEIT DER LETTEREN,
VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN
TE VERDEDIGEN OP WOENSDAG 2 MEI 1973
TE KLOKKE 15.15 UUR

DOOR

HENDRIK ALBERT OTTEN

GEBOREN TE WEDDE,
THANS GEMEENTE BELLINGWEDDE,
IN 1941

1973
BRONDER-OFFSET B.V.
ROTTERDAM

PROMOTOR: PROF. DR. L. N. M. DUYSENS

CO-REFERENT: DR. J. J. C. MULDER

Aan Aniet

CONTENTS

The present work was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

CHAPTER I

GENERAL INTRODUCTION

The first process of photosynthesis is the absorption of a light quantum by one of the pigment molecules, such as chlorophyll a, present in photosynthetic organisms. The major pigment is chlorophyll a (in the case of plants and algae) or bacteriochlorophyll (in the case of photosynthetic bacteria). A small fraction of the total number of pigment molecules present has the ability to convert excitation energy into chemical energy. There is ample evidence (see Duysens (1964) for a review on the primary processes of photosynthesis) that after the light quantum has been absorbed the excitation energy is transferred between pigment molecules until it is trapped by a photochemical active center, a so called reaction center. In this center the primary redox reaction of photosynthesis takes place.

A model containing one trap in the center of a regular array of energy transferring molecules has been treated in several ways. Pearlstein (1966) used a diffusion equation and represented the trap by a suitable boundary condition. Mean trapping times can be obtained out of such an approach. Knox (1968) used a set of master equations to consider the same problem. "Random walk" equations can be used if it is supposed that the energy migration can be considered to be a process in which the excitation hops from one molecule to another.

For large systems containing many traps, Sanders, Ruygrok and Ten Bosch (1970) were able to give an analytic expression between trapping probability and trap concentration. The system was supposed to be periodic with a unit cell which contained one (100% trapping) trap in its center. They used "random walk" equations to describe the system.

The problem of energy migration in a large homogeneous system in which several traps are imbedded was considered again in chapter II of this thesis. Approximate relations between the trapping or fluorescence probability and the concentration of trapping centers could be derived. To that end the mean values of trapping or fluorescence probabilities over all possible systems with different trap distributions but with the same trap concentration were taken. The results were compared with the existing heuristic and intuitive approaches of Vredenberg and Duysens (1963) and Joliot (1964).

If the predictions of theoretical models, provided these are treated

rather exactly, are compared with the results of fluorescence yield measurements, it is possible to obtain information on the structure and functioning of the excitation transferring part of the photosynthetic system.

The trapping of light energy in the photochemical active centers of photosynthetic purple bacteria is accompanied by a number of changes in the absorption spectra of these bacteria. One of these changes is a decrease in absorption around 870 nm accompanied by an increase in absorption around 1250 nm (the wavelength depends on the species that is used). The same changes can be brought about by oxidation with ferricyanide.

In chapter III the hypothesis is tested which states that the afore mentioned absorption changes are due to the disappearance of an electron from the π-electron system of the bacteriochlorophyll molecule. This was proposed earlier on account of evidence from ESR spectroscopy (consult the chapter in question for references).

Semi-empirical π-electron calculations of the MO-SCF-CI type were carried out on bacteriochlorophyll and its mono-cation. We were able to explain the afore mentioned absorption changes with the results of these calculations.

A problem of more general biochemical nature is that of the kinetics of metabolic processes occurring catalysed by enzymes. Often the kinetic equations which are used to describe the behaviour of multi-enzyme systems are not solvable numerically, not even with the aid of large computers. This is caused by the "stiffness" of the set of these coupled differential equations. This difficulty can be avoided if the so called steady-state approximation is used.

Although the steady-state approximation was used for the first time in 1925 by Briggs and Haldane, the question concerning the range of validity of the steady-state approximation still gives rise to new papers in this field.

In chapter IV this problem is considered for a general biochemical system. The study is an improvement of the work of Vergonet and Berendsen (1970) which is closely related to ours.

Chapter V gives an elaboration of the theory of chapter IV on the simplest enzymatic reaction as described by the Michaelis-Menten kinetic equations. A more detailed analysis of the relation between the steady-state approximation and the exact solution is possible for this system.

The different chapters are written as papers for scientific journals.

REFERENCES

Briggs, G.E., and Haldane, J.B.S. (1925) Biochem. J. 19, 338.

Duysens, L.N.M. (1964) Progr. Biophys. Mol. Biol. 14, 1.

Joliot, P., and Joliot, A. (1964) C.R. Acad. Sci. Paris 258, 4622.

Knox, R.S. (1968) J. Theoret. Biol. 21, 244.

Pearlstein, R.M. (1966) Thesis, University of Maryland, U.S.A.

Sanders, J.W., Ruygrok, Th.W., and Ten Bosch, J.J. (1971) J. Math. Phys. 12, 534.

Vergonet, G., and Berendsen, H.J.C. (1970) J. Theoret. Biol. 28, 155.

Vredenberg, W J., and Duysens, L.N.M. (1963) Nature 197, 355.

CHAPTER II

A MATHEMATICAL ANALYSIS OF THE RELATION BETWEEN (BACTERIO)CHLOROPHYLL FLUORESCENCE YIELD AND THE CONCENTRATION OF REACTION CENTER TRAPS IN PHOTOSYNTHESIS

H.A. OTTEN

Biophysical Laboratory of the State University, P.O. Box 556, Leiden, The Netherlands.

Summary - A random walk approach is used to discuss the relation between fluorescence yield and the concentration of reaction center traps.

For the matrix model as used for bacteria, non-negligible deviations are found from the Vredenberg-Duysens relation. An approximately linear relationship between the reciprocal of the fluorescence yield and the trap concentration however remains.

For the unit model as used for system 2, the deviation from Joliot's relation is small. The assumptions of the unit model are however discutable. An alternative matrix model is presented.

1. INTRODUCTION

Excitation spectra of the fluorescence of chlorophyll a in algae and of bacteriochlorophyll in purple bacteria prove that light energy absorbed by various pigments such as chlorophyll b, phycobilins, and carotenoids is transferred to the lowest excited singlet state of chlorophyll a and bacteriochlorophyll respectively (Duysens, 1964). The excitation energy is during the life time of excitation transferred over many hundreds of (bacterio)-chlorophyll molecules until it arrives at a photochemical reactive (bacterio)-chlorophyll molecule P.

First the photosynthetic system in purple bacteria will be discussed. The reactive bacteriochlorophyll molecule P (when excited) is able to transfer an electron to an acceptor X, which forms a complex with P. Because this reaction occurs rapidly compared to the transfer of energy between the bacteriochlorophyll molecules, PX reacts as a trap for the excitation energy. The primary reaction is: $PX + h\nu \longrightarrow P^+X^-$. P^+ and X^- may react with a secondary electron donor or acceptor, which gives rise to the states P^+X

or PX^-. The complex PX in one of its various states is called a reaction center. The long wavelength absorption band of P disappears when P is converted into the form P^+, a reaction which can be followed by absorption difference spectrophotometry. Under experimental conditions under which X presumably remains, because of a rapid reoxidation in state X, it is found that the formation of P^+ is associated with an increase of the fluorescence yield of bacteriochlorophyll; this is explained by the hypothesis that P^+, in contrast with P, does not trap the excitation energy.

In algae appreciable large changes have been observed in the fluorescence yield of chlorophyll a_2, the chlorophyll of the oxygen evolving system 2 (Duysens & Sweers, 1963).

A reaction center PQ has been postulated to explain these changes under "normal" conditions of illumination. P^+ is rapidly reduced, and no absorption changes due to the transition $P \longrightarrow P^+$ are observed. An increase in fluorescence occurs upon the transition $PQ \longrightarrow PQ^-$.

The number of reaction centers in the active state PQ can be measured by measuring the amount of oxygen produced in a very short saturating light flash. For ease of discussion we use the following definition of "photosynthetic unit" (Duysens, 1967). The unit consists of a reaction center and those bacteriochlorophyll molecules that have a higher probability of transferring energy to this reaction center than to another one, when all centers are in the trapping state. Two models of units may be distinguished.

1. *Separate units.* The individual units are at such a large distance from each other that no energy transfer can occur between them.
2. *Energy transferring units.* Excitation energy may be transferred between units when the units are not separated or only slightly so.

In the case of identical separate units the fluorescence yield is a linearly increasing function of the concentration of reaction centers in the non-trapping ("closed") state: if T is the fraction of units in the trapping ("open") state, φ_1 and φ_2 the fluorescence yields of the units in the non-trapping and trapping state respectively, then the total fluorescence yield ϕ is equal to:

$$\phi = (1-T)\varphi_1 + T\varphi_2 = (1-T)(\varphi_1 - \varphi_2) + \varphi_2$$

which is indeed a linearly increasing function of $(1-T)$.

Neither in purple bacteria (Vredenberg & Duysens, 1963) nor in algae

(Joliot, 1964) the fluorescence yield is found to be a linear function of the concentration of the closed reaction centers. Apparently, energy transfer occurs between units. The relationship derived depends upon the assumed model for this energy transfer and the assumptions or approximations made in the calculation.

In this paper the earlier models and calculations given by Joliot and Duysens will be extended and improved and their relationship clarified, in the hope that in the future it will be possible to obtain more insight in the energy transfer and other functional relationships between the photosynthetic units.

2. RELATIONS BETWEEN FLUORESCENCE YIELD AND TRAP CONCENTRATION

2.1. *The model of Vredenberg and Duysens of bacteria*

Experimentally an approximately linear relation between the reciprocal of the fluorescence yield and the concentration of trapping molecules has been found (Vredenberg & Duysens, 1963; Sybesma & Vredenberg, 1963; Clayton, 1966).

It is possible to construct a simple model to explain these measurements. There are two, at first sight different, approaches.

a. *Approach I*

In this approach (Vredenberg & Duysens, 1963) the light emitting system is supposed to be a homogeneous more or less regular lattice of bacteriochlorophyll (Bchl) molecules in which the reaction centers (R.C.) are situated (Matrix model). The active R.C.'s function as traps for incoming excitation energy. After an excitation has been trapped the R.C. is temporary blocked in the form P^+ (see Introduction) for new excitations.

Light incident on the lattice (Fig. 1) will be absorbed by the bulk molecules (the molecules which are not a R.C.) at a rate $d[B^*]/dt = kI$; $[B^*]$ represents the concentration of excited bulk molecules, I means the intensity of the incoming light and k is a rate constant. It is assumed that the suspension has only a negligible absorption. Deexcitation can take place by fluorescence, internal conversion and trapping. We will define the frac-

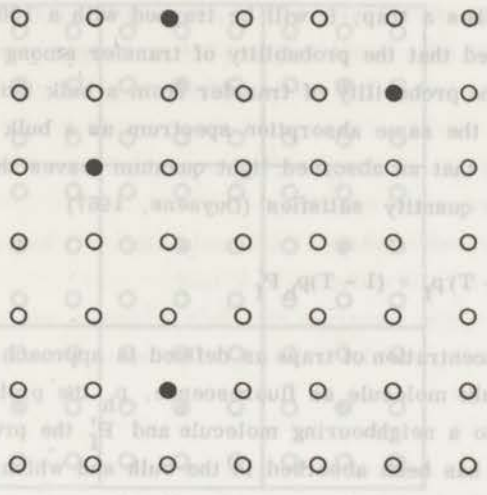

Fig. 1.

A regular lattice as used in the matrix model of bacteria.
● represents a reaction center in the trapping state.

tion of traps T as the number of traps devided by the total number of molecules of the lattice. Then the postulated rate equation reads:

$$d[B^*]/dt = kI - (k_i + k_f)[B^*] - k_t[B^*]\ T \qquad (1)$$

with k_i, k_f and k_t rate constants of deexcitation by internal conversion, fluorescence and trapping respectively. In the steady-state $d[B^*]/dt = 0$ holds. The fluorescence yield P_f, defined as $k_f[B^*]/kI$, is then given by

$$P_f = k_f (k_i + k_f + k_t T)^{-1} \qquad (2)$$

This relation furnishes the linear relationship between P_f^{-1} and T.

b. *Approach II*

In this approach (Duysens, 1967) the excitation transfer to the reaction centers is described as a process in which the excitation hops from molecule to molecule until it reaches a R.C. The homogeneous lattice of Bchl molecules with some traps among them serves again as a picture. If an

excitation reaches a trap, it will be trapped with a 100% efficiency. It is further supposed that the probability of transfer among bulk molecules is the same as the probability of transfer from a bulk molecule to a trap. A trap will have the same absorption spectrum as a bulk molecule. If we call the probability that an absorbed light quantum leaves the system as fluorescence P_f, this quantity satisfies (Duysens, 1967)

$$P_f = (1 - T)\,p_f + (1 - T)p_h P'_f \qquad (3)$$

with T the concentration of traps as defined in approach I, p_f the probability of leaving a bulk molecule as fluorescence, p_h the probability that the excitation hops to a neighbouring molecule and P'_f the probability that an excitation which has been absorbed in the bulk and which has made one jump finally leaves the unit as fluorescence. If it is supposed that $P_f \approx P'_f$, then

$$P_f = \frac{(1 - T)\,p_f}{1 - p_h(1 - T)} \qquad (4)$$

For bacteria $T \leqslant 0.03$ (Duysens, 1952; Nishimura, 1962; Clayton, 1963). Therefore T can be neglected in the numerator (not in the denominator, because $p_h \approx 1$). Then an expression which is analogous to (2) has been obtained.

2.2. *The model of Joliot of system 2*

The relation between fluorescence yield and concentration of R.C.'s in the trapping state of system 2 has been measured by Joliot and Joliot (1964). It is assumed that there exists a linear relation between the concentration of traps and the amount of oxygen deliberated from a short saturating flash of light. In this case no linear relation between the reciprocal of the fluorescence yield and the concentration of traps is observed. To explain the observed results, Joliot assumed the following model: The system is supposed to be composed out of units each containing just one R.C. (Fig. 2). A R.C. can be in the trapping- or in the non-trapping state. If the R.C. of a unit is in its trapping state, each light quantum absorbed in that unit will be trapped by the R.C. with 100% efficiency and no fluorescence will occur. If the R.C. is in its non-trapping state, an absorbed quantum can

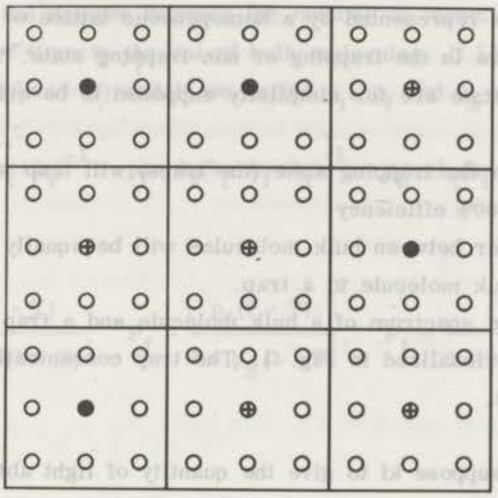

Fig. 2.

A regular lattice as used in the model with units of system 2. —— represents boundaries between units; ● represents a reaction center in the trapping state; ⊕ represents a reaction center in the non-trapping state.

reach another unit with probability p_h. We will define p_f as the probability of a quantum absorbed by a non-trapping unit to leave this unit as fluorescence; T will be the number of trapping units divided by the total number of units.

Joliot used a relative cumbersome summing procedure to obtain the relation between fluorescence yield and the concentration of trapping units. The same result can be obtained using Duysens' simple procedure which leads again to equation (4).

The simple procedure can also be used for more complicated systems. An example, which has been used by Wraight (1972), is given in appendix A.

3. EQUIVALENCE OF APPROACH I AND II

In this section we will prove the mathematical equivalence of approach I and approach II and show the connection between the parameters used. Because the arguments used in the model of Joliot are the same as used in 2.1.b. we will restrict ourselves to the model of Vredenberg and Duysens. We will start to repeat once more the assumptions of the model:

1. The system is represented by a homogeneous lattice of Bchl molecules.
2. The R.C.'s are in the trapping or non-trapping state. The R.C.'s in the non-trapping state are for simplicity supposed to be equal to a bulk molecule.
3. The R.C.'s in the trapping state (the traps) will trap incoming excitation energy with 100% efficiency.
4. Energy transfer between bulk molecules will be equally probable as transfer from a bulk molecule to a trap.
5. The absorption spectrum of a bulk molecule and a trap are the same.

The situation is visualized in Fig. 1. The trap concentration is again defined as in 2.1.

a) Let us suppose kI to give the quantity of light absorbed per unit of time by the system; I represents the intensity of the incident light, k is a constant. A fraction kI(1 - T) of this is absorbed by bulk molecules and can give rise to fluorescence. If we correct formula (1) with the factor (1 - T), it will read:

$$d[B^*]/dt = kI(1 - T) - (k_i + k_f)[B^*] - k_t[B^*]T \qquad (5)$$

Starting from this equation, we will turn to description II.

Let us define $P_t(T)$, $P_i(T)$ and $P_f(T)$ as the probabilities of a light quantum absorbed by the system at a trap concentration T to reach a trap, disappear by internal conversion or leave the system as fluorescence respectively. Then $P_t(T) - T$ gives the probability of a quantum initially caught in the set of bulk molecules to reach a trap. Under steady-state conditions we obtain:

$$kI\,P_i = k_i[B^*] \qquad (6a)$$

$$kI\,P_f = k_f[B^*] \qquad (6b)$$

$$kI(P_t - T) = k_tT[B^*] \qquad (6c)$$

Under steady-state conditions equation (5) gives

$$kI(1 - T) = (k_i + k_f + k_tT)[B^*] \qquad (7)$$

which describes the balance between the number of incoming and outgoing quanta per unit of time in the set of bulk molecules. If $[B^*]$ is eliminated from equations (6) and (7) and if we define p_i, p_f and p_h as:

$$p_i = k_i(k_i+k_f+k_t)^{-1}, \quad p_f = k_f(k_i+k_f+k_t)^{-1}, \quad p_h = k_t(k_i+k_f+k_t)^{-1} \tag{8}$$

we obtain:

$$P_i = \frac{p_i(1-T)}{1-p_h(1-T)}, \quad P_f = \frac{p_f(1-T)}{1-p_h(1-T)}, \quad P_t = \frac{T}{1-p_h(1-T)}$$

or finally:

$$P_i(T) = (1-T)p_i + (1-T)p_h P_i(T) \tag{9a}$$

$$P_f(T) = (1-T)p_f + (1-T)p_h P_f(T) \tag{9b}$$

$$P_t(T) = T + (1-T)p_h P_t(T) \tag{9c}$$

b) We will now start from equations (9) and turn to a kinetical description. To that end we either should introduce a time dependent random walk description or make some assumptions. Here we will assume the validness of equations (6a) and (6b) under steady-state conditions, with k_i and k_f as defined by relations (8). This assumption seems plausible. Equivalently the trapping process of an excitation starting from the set of bulk molecules should be given by a term $k_t^1[TB^*]$ in which $[TB^*]$ denotes the concentration of excited trap neighbours. We will show that the approximation which is inherent to equation (9c) is equivalent to writing the trapping term as a product like $k_t T[B^*]$.

The balance between the total number of incoming and outgoing quanta per second can be written as:

$$\begin{aligned} kI &= kI(P_i + P_f) + kIP_t \\ &= (k_i + k_f)[B^*] + kIP_t \end{aligned} \tag{10}$$

This gives

$$[B^*] = kI(1-P_t)/(k_i+k_f) \tag{11}$$

We are looking for the probability of an excitation which has been caught in the set of bulk molecules to reach a trap. As follows from equation (9c), this probability is given by $(1 - T)\, p_h P_t(T)$. The number of quanta A that will reach a trap per unit of time is then given by $A = kI(1 - T)\, p_h P_t(T)$. With equations (9c) and (11) this gives:

$$\begin{aligned} A &= kI\,(1 - T)\, p_h P_t(T) \\ &= [B^*]\ (k_i + k_f)\ (1 - T)\, p_h P_t / (1 - P_t) \\ &= (k_i + k_f)\, p_h\, [B^*]\ T / (1 - p_h) \end{aligned}$$

With k_t defined from relations (8), this leads to:

$$A = k_t\, [B^*]\ T \qquad (12)$$

4. RANDOM WALK DESCRIPTION

We have shown the equivalence of approach I and II. The essence of both descriptions is expressed by (9) or (10). Starting from a random walk approach, we will now determine the conditions on which (10) is based.

Again the system is defined as in section 3. We will use the following notation:

$\mathscr{B}$ = set of bulk molecules, $\mathscr{T}$ = set of traps,

P_{af} = probability of an excitation which is originally located on molecule a, to leave the system as fluorescence,

p_{ab} = probability of an excitation to go from a to b in one jump,

p_f = probability of an excitation to leave a bulk molecule as fluorescence.

All total probabilities are denoted by capital P's; all one-jump probabilities by small type p's.

If it is further noted that:

$$p_{af} = \begin{cases} p_f & \text{if } a \in \mathscr{B} \\ 0 & \text{if } a \in \mathscr{T} \end{cases} \quad \text{and} \quad \sum_b p_{ab} = \begin{cases} p_h & \text{if } a \in \mathscr{B} \\ 0 & \text{if } a \in \mathscr{T} \end{cases} \qquad (13)$$

the following relations can be inferred:

$$P_{af} = p_{af} + \sum_b p_{ab} P_{bf} \qquad (14)$$

in which the summation extends over all lattice points.

For a given trap distribution the probability matrix p is a known quantity. Then the linear equations (14) are solvable, giving P_{af}.

The mean probability P_f of an absorbed light quantum to give rise to fluorescence is given by $P_f = N^{-1} \sum_a P_{af}$, N being the number of molecules of the lattice. If use is made of equations (14) and (13), this gives:

$$\begin{aligned} P_f &= N^{-1} \sum_a P_{af} \\ &= N^{-1} \sum_a (p_{af} + \sum_b p_{ab} P_{bf}) \\ &= (1 - T) p_f + N^{-1} \sum_{a,b} p_{ab} P_{bf} \end{aligned} \qquad (15)$$

We will now suppose the traps to be distributed at random over the lattice. This condition may be satisfied in the model of Joliot because each unit can be trapping or non-trapping. In the model of Vredenberg and Duysens the traps are distributed at random over the R.C.'s. Whether the R.C.'s are distributed at random over the lattice is questionable.

Each value of P_f depends on the trap distribution of the system S which has been chosen. We will take the mean over all systems with a given trap concentration. This mean value will be denoted by a bar. The total number of systems with a given trap concentration will be denoted by N_s. We then obtain:

$$\begin{aligned} \overline{P}_f &= N_s^{-1} \sum_s P_f \\ &= (1 - T) p_f + N_s^{-1} \sum_s N^{-1} \sum_{a,b} p_{ab} P_{bf} \\ &= (1 - T) p_f + N^{-1} \sum_{a,b} \overline{(p_{ab} P_{bf})} \end{aligned} \qquad (16)$$

To obtain formula (10) we have to make the following assumption:

$$\overline{p_{ab} P_{bf}} = \overline{p_{ab}} \cdot \overline{P_{bf}} \qquad (17)$$

In fact the summation should be included. For an infinite system the term $\overline{p_{ab}\, P_{bf}}$ is the same for all a and b ($a, b \in \mathscr{B}$). Therefore (17) may be used if all boundary effects are neglected. The assumption (17) will be discussed in the next section. We will further suppose that energy transfer only takes place between nearest neighbours. In that case p_{ab} can be written as:

$$p_{ab} = c_a^{-1} p_h \delta_{\langle ab \rangle} \quad \text{if a is not a trap}$$

$$p_{ab} = 0 \quad \text{if a is a trap} \tag{18}$$

$\delta_{\langle ab \rangle} = 1$ if a and b are nearest neighbours; $\delta_{\langle ab \rangle} = 0$ otherwise, c_a = number of nearest neighbours of a.
As we supposed the system to be large, $c_a = c$ = constant. Then $\overline{p_{ab}}$ can be written as:

$$\overline{p_{ab}} = c^{-1} p_h \delta_{\langle ab \rangle} (1 - T) \tag{19}$$

Combination of (16), (17) and (19) gives

$$\overline{P}_f = (1 - T) p_f + N^{-1} \sum_{a,b} c^{-1} p_h (1 - T) \delta_{\langle ab \rangle} \overline{P}_{bf}$$

$$= (1 - T) p_f + (1 - T) p_h \overline{P}_f \tag{20}$$

With this we have obtained the relation on which the models of Joliot and Vredenberg and Duysens are based. It depends on the validity of relation (17).

5. AVERAGING OVER TRAP DISTRIBUTIONS

In this section we will consider the quantity $\overline{p_{ab}\, P_{bf}}$ in detail. Systems with the same trap concentration differ in their distribution of traps. We will classify the systems into four groups:

1) All systems S_1 with $a \notin \mathscr{T}$ (a is not a trap) and $b \in \mathscr{T}$ (b is a trap). Then $p_{ab}\, P_{bf} = 0$ holds, because $P_{bf} = 0$. The number of these systems is given by $N_{s_1} = T (1 - T) N_s$.

2) All systems S_2 with $a \in \mathscr{T}$ and $b \notin \mathscr{T}$. Then $p_{ab}\, P_{bf} = 0$, because

$p_{ab} = 0$. The number N_{s_2} of these systems is given by $N_{s_2} = T(1 - T)N_s$.

3) All systems S_3 with $a \in T$ and $b \in \mathcal{T}$. Then $p_{ab} P_{bf} = 0$ because $p_{ab} = 0$ and $P_{bf} = 0$. The number of these systems is given by $N_{s_3} = T^2 N_s$.

4) All systems S_4 with $a \notin \mathcal{T}$ and $b \notin \mathcal{T}$. Then $p_{ab} P_{bf} \neq 0$ and the number of systems is given by $N_{s_4} = (1 - T)^2 N_s$.

When use is made of (18), $\overline{p_{ab} P_{bf}}$ can be written as:

$$\begin{aligned}\overline{p_{ab} P_{bf}} &= N_s^{-1} \sum_s (p_{ab} P_{bf}) \\ &= N_s^{-1} \sum_{s_4} (p_{ab} P_{bf}) \\ &= c^{-1} p_h \delta_{\langle ab \rangle} N_s^{-1} \sum_{s_4} P_{bf} \end{aligned} \tag{21}$$

Further

$$\begin{aligned}\overline{P_{bf}} &= N_s^{-1} \sum_s P_{bf} \\ &= N_s^{-1} \sum_{s_2 + s_4} P_{bf} \end{aligned} \tag{22}$$

If we combine (19), (21) and (22), we obtain

$$\begin{aligned}\overline{p_{ab}} \cdot \overline{P_{bf}} &= (1 - T) c^{-1} p_h \delta_{\langle ab \rangle} N_s^{-1} \sum_{s_2 + s_4} P_{bf} \\ &= c^{-1} p_h \delta_{\langle ab \rangle} N_s^{-1} \sum_{s_4} P_{bf} - c^{-1} T p_h \delta_{\langle ab \rangle} N_s^{-1} \sum_{s_2 + s_4} P_{bf} + \\ &\qquad + c^{-1} p_h \delta_{\langle ab \rangle} N_s^{-1} \sum_{s_2} P_{bf} \\ &= \overline{p_{ab} P_{bf}} - c^{-1} p_h \delta_{\langle ab \rangle} N_s^{-1} \left(T \sum_{s_2 + s_4} P_{bf} - \sum_{s_2} P_{bf} \right) \end{aligned} \tag{23}$$

With some manipulations, (23) can finally be transformed into

$$\overline{p_{ab} P_{bf}} = \overline{p_{ab}} \cdot \overline{P_{bf}} + c^{-1} p_h T (1 - T)^2 (\bar{P}_{bf}^{(4)} - \bar{P}_{bf}^{(2)}) \qquad (24)$$

with $\bar{P}_{bf}^{(4)} = N_{s_4}^{-1} \sum_{s_4} P_{bf}$ and $\bar{P}_{bf}^{(2)} = N_{s_2}^{-1} \sum_{s_2} P_{bf}$

The averaging process is represented in Fig. 3.

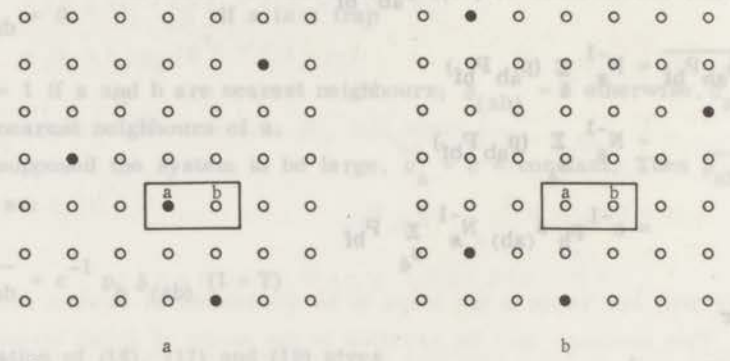

Fig. 3.

Representation of the averaging process of $\bar{P}_{bf}^{(2)}$ and $\bar{P}_{bf}^{(4)}$ at fixed T. $\bar{P}_{bf}^{(2)}$ and $\bar{P}_{bf}^{(4)}$ are obtained by taking the mean value of P_{bf} over all possible trap distributions outside the indicated squares of Fig. a and Fig. b respectively.

We have already supposed the system to be large in order to be able to neglect boundary effects. Increase of the number of traps by one will have a negligible influence on the T value. It is then possible to take simultaneously the same trap distribution outside the indicated squares in Fig. 3 and compare the values of $P_{bf}^{(4)}$ and $P_{bf}^{(2)}$. This gives

$$P_{bf}^{(4)} = P_{bf}^{(2)} + P_{ba} P_{af}^{(4)} \qquad (25a)$$

$$P_{ba} = p_{ba} + \sum_{k_1} P_{bk_1} P_{k_1 a} + \sum_{k_1, k_2} P_{bk_1} P_{k_1 k_2} P_{k_2 a} + \ldots \qquad (25b)$$

The quantity P_{ba} equals the sum of the probabilities to reach a for the first time in 1, 2, 3, ... jumps, when starting from b.

We will write (24) like

$$\overline{P_{ab}\,P_{bf}} = \overline{P}_{ab} \cdot \overline{P}_{bf} + \Delta_{ab} \qquad (26)$$

If use is made of (25) an upper and lower bound of Δ_{ab} can be obtained.

1) Because $p_{ba} = c^{-1} p_h$, we can write

$$\overline{P}^{(4)}_{bf} \geqslant \overline{P}^{(2)}_{bf} + c^{-1} p_h \overline{P}^{(4)}_{af} \qquad (27)$$

For Δ_{ab} this implies: $\Delta_{ab} \geqslant c^{-2} p_h^2 \delta_{\langle ab \rangle} T(1-T)^2 \overline{P}^{(4)}_{af}$.

To estimate the magnitude of Δ_{ab}, we use $\overline{P}^{(4)}_{af} \approx \overline{P}^{(4)}_{bf}$ (which is true for an infinite system).

Because $\overline{P}^{(4)}_{bf} \geqslant \overline{P}_{bf}$, we will find:

$$\Delta_{ab} \geqslant c^{-1} p_h T(1-T)\, \overline{P}_{ab} \cdot \overline{P}_{bf} \qquad (28)$$

2) $P_{ba} \leqslant \lambda_0$, if λ_0 represents the largest value of $P_{ba}(T)$. This maximum value of P_{ba} equals the probability of an excitation to be trapped when it starts from b and if there are no other traps in the lattice except one at position a. It is possible to determine the value of λ_0 when the value of p_h is given. To that end we use the exact solution of the random walk problem as can be given for the periodic lattice with the traps in the centers of the unit cells (Sanders, Ruygrok & Ten Bosch, 1971). λ_0 is given by the probability to reach a trap from a neighbour in the limitting case $T \to 0$. The value of λ_0 can be obtained by extrapolation from the values obtained for finite T. In the model of Joliot a value between 0.5 and 0.6 has been given to p_h. Taking $p_h = 0.55$ we obtain $\lambda_0 = 0.1537$ for the square lattice. In the case of bacteria with $p_h = 0.988$ (see section 7) we obtain $\lambda_0 = 0.5259$. Some other $p_h - \lambda_0$ combinations for the square lattice are given in the table below.

p_h	λ_0	p_h	λ_0
0.988	0.53	0.9999	0.72
0.992	0.55	0.99999	0.85
0.996	0.59	1.0	1.0
0.9992	0.66		

The following inequality can now be given:

$$\bar{P}_{bf}^{(4)} \leqslant \bar{P}_{bf}^{(2)} + \lambda_0 \bar{P}_{af}^{(4)} \tag{29}$$

and from this follows

$$\Delta_{ab} \leqslant c^{-1} p_h \delta_{\langle ab \rangle} T(1-T)^2 \lambda_0 \bar{P}_{af}^{(4)} \tag{30}$$

Consider $\bar{P}_{bf}$:

$$\bar{P}_{bf} = N_s^{-1} \sum_{s_2+s_4} P_{bf}$$

$$= N_{s_2} N_s^{-1} \bar{P}_{bf}^{(2)} + N_{s_4} N_s^{-1} \bar{P}_{bf}^{(4)}$$

$$= T(1-T) \bar{P}_{bf}^{(2)} + (1-T)^2 \bar{P}_{bf}^{(4)} \tag{31}$$

Therefore

$$\bar{P}_{bf} \geqslant (1-T)^2 \bar{P}_{bf}^{(4)} \tag{32}$$

If we again make the approximation $\bar{P}_{af}^{(4)} \approx \bar{P}_{bf}^{(4)}$ and if (32) is used into (30), we will find:

$$\Delta_{ab} \leqslant \lambda_0 T \bar{p}_{ab} \cdot \bar{P}_{bf} \tag{33}$$

If the inequalities (28) and (33) are combined, we obtain

$$c^{-1} p_h T(1-T) \leqslant \Delta_{ab} / (\bar{p}_{ab} \cdot \bar{P}_{bf}) \leqslant \lambda_0 T$$

With this inequality relation we are able to discuss relation (17). As can be seen from equation (26), the maximum and minimum deviation of equation (17) are given by the maximum and minimum value of $\Delta_{ab}/(\bar{p}_{ab} \cdot \bar{P}_{bf})$. Below these upper and lower bounds are given for some T-values and with p_h fixed (the p_h-value determines λ_0).

i Matrix model with $p_h = 0.988$

	T = 0	T = 0.015	T = 0.03
$(\Delta_{ab}/(\bar{p}_{ab}.\bar{P}_{bf}))_{max}$ .100%	0	0.788	1.577
$(\Delta_{ab}/(\bar{p}_{ab}.\bar{P}_{bf}))_{min}$ .100%	0	0.036	0.072

ii Units model with $p_h = 0.55$

	T = 0	T = 0.25	T = 0.50	T = 0.75	T = 1.0
$(\Delta_{ab}/(\bar{p}_{ab}.\bar{P}_{bf}))_{max}$ .100%	0	3.84	7.68	11.53	15.37
$(\Delta_{ab}/(\bar{p}_{ab}.\bar{P}_{bf}))_{min}$ .100%	0	2.58	3.44	2.58	0

As shown now, relation (17) itself may be considered to be a good approximation for the model of Vredenberg and Duysens. It remains to show that it may be inserted into (16). To that end we will rewrite (16):

$$\bar{P}_f = (1-T)p_f + N^{-1}\sum_{a,b}\bar{p}_{ab}.\bar{P}_{bf} + N^{-1}\sum_{a,b}(\overline{p_{ab}P_{bf}} - \bar{p}_{ab}.\bar{P}_{bf})$$

$$= (1-T)p_f + (1-T)p_h\bar{P}_f + N^{-1}\sum_{a,b}\Delta_{ab}.$$

or

$$\{(1-p_h) + p_h T\}\,\bar{P}_f = (1-T)p_f + N^{-1}\sum_{a,b}\Delta_{ab} \qquad (34)$$

Inequality (28) with $T \ll 1$ gives:

$$N^{-1}\sum_{a,b}\Delta_{ab} \geqslant c^{-1}p_h T N^{-1}\sum_{a,b}\bar{p}_{ab}.\bar{P}_{bf} \approx c^{-1}p_h^2 T\bar{P}_f \qquad (35)$$

In the model of Vredenberg and Duysens p_h has a value in the neighbourhood of 0.99 (see section 8). That means that the left hand side of (34) is of the same order of magnitude as the term $N^{-1}\sum_{a,b}\Delta_{ab}$ on the right hand side. Therefore, the insertion of (17) into expression (16) is not justified.

In the case of Joliot's model the term $N^{-1}\sum_{a,b}\Delta_{ab}$ in (34) is less than $\lambda_0 T N^{-1}\sum_{a,b}\bar{p}_{ab}.\bar{P}_{bf}$ which equals $\lambda_0 T(1-T)p_h\bar{P}_f$. The factor $T(1-T)$

has its largest value at T = 0.5. If we take $p_h = 0.5$ and $\lambda_0 = 0.15$ it follows that the term $N^{-1} \sum_{a,b} \Delta_{ab}$ is less than 2.5% from the term at the left hand side in (34). Therefore in the case of Joliot's model the *use* of (17) into (16) turns out to be a good approximation while (17) itself may be less well satisfied.

6. IMPROVED $\bar{P}_f(T)$ RELATION

As shown in the last section, relation (20) is not a good approximation for the model of Vredenberg and Duysens. We will now replace (20) by a better one. To that end (16) and (24) are combined giving:

$$\bar{P}_f = (1 - T)\, p_f + (1 - T)\, p_h \bar{P}_f + c^{-1} p_h T (1 - T)^2 N^{-1} \sum_{a,b} \delta_{\langle ab \rangle} (\bar{P}^{(4)}_{bf} - \bar{P}^{(2)}_{bf}) \tag{36}$$

We further use (25a). The quantity P_{ba} satisfies $P_{ba} \geqslant p_h/c$. We may expect the value $\lambda = p_h/c$ to contain the largest part of P_{ba} for not too small T values. In the approximation $P_{ba} \approx p_h/c$ we will find

$$\bar{P}_f = (1 - T)\, p_f + (1 - T)\, p_h \bar{P}_f + c^{-1} p_h T (1 - T)^2 \lambda \sum_{a,b} \delta_{\langle ab \rangle} \bar{P}^{(4)}_{af} \tag{37}$$

If we use $\bar{P}^{(4)}_{af} \approx \bar{P}^{(4)}_{bf}$, combination of (25a) and (31) gives $\bar{P}^{(4)}_{bf}$ expressed in $\bar{P}_{bf}$:

$$\bar{P}^{(4)}_{bf} = [(1 - T)\,(1 - \lambda T)]^{-1} \bar{P}_{bf} \tag{38}$$

After substitution into (37) this gives:

$$\bar{P}_f = (1 - T)\, p_f + (1 - T)\, p_h \bar{P}_f + \lambda p_h T\, (1 - T)\, (1 - \lambda T)^{-1} \bar{P}_f \tag{39}$$

or, if we solve for $\bar{P}_f$:

$$\bar{P}_f = \frac{(1 - T)\, p_f}{1 - p_h (1 - T) - \lambda p_h T (1 - T)\, (1 - \lambda T)^{-1}} \tag{40}$$

This expression gives a lower bound for $\bar{P}_f$ as a function of T (upper bound for $\bar{P}_f^{-1}$ as a function of T).

The approximation $P_{ba} \approx \lambda = p_h/c$ is too crude for T-values close to zero. In the limiting case of $T \to 0$, P_{ba} will equal the value λ_0 as discussed in section 5. If this value is substituted into (40) we will obtain the upper bound of $\overline{P}_f$ as a function of T.

An approximate T-dependence of λ has been obtained, which looks like:

$$\lambda(T) = \lambda_0 - (\lambda_0 - p_h/c)\,\frac{\{1 - p_h + p_h(1 - p_h/c)\}T}{1 - p_h + p_h(1 - p_h/c)\,T} \qquad (41)$$

The derivation of this relation is given in Appendix B. In section 8 the curve obtained for the matrix model with this expression for λ will be compared with $\overline{P}_f$-values as obtained for the periodic lattice.

7. A SIMPLE RULE TO OBTAIN A FIRST IMPROVEMENT ON THE FLUORESCENCE YIELD OF THE MATRIX MODEL

We will give a simple rule to derive a first improvement on the fluorescence yield (P_f) for the matrix model, which is also easily interpreted.

We will start to write the $P_f(T)$ relation like:

$$P_f(T) = (1 - T)\,p_f + (1 - T)\,p_h\,P_f' \qquad (42)$$

P_f' would be equal to P_f if after one jumping time the probability distribution of the excitation would have remained homogeneous. The neighbours of the reaction centers however have only c-1 neighbours themselves to obtain the excitation from, while other bulk molecules have c. That means that after a time equal to the jumping time the probability to find the excitation on a particular neighbour of a reaction center will be smaller than to find it on a particular other bulk molecule. This effect causes P_f' to be different from P_f.

The probability distribution would have remained homogeneous if from the reaction centers excitation could have flown out with the probability p_h. If we imagine this amount to be supplied to the neighbours of the reaction center, the probability of all excitation then present in the system to give fluorescence equals P_f. With this supply the total probability to find the excitation in the system after one jump equals p_h instead of $(1 - T)\,p_h$. That means an increase of $p_h T$, which is situated on the neighbours of the reac-

tion centers. If we call the mean value of the probability to give fluorescence of an excitation located on a neighbour of a reaction center P_f^n, we can write:

$$P_f(T) = (1 - T)\, p_f + p_h P_f - p_h T P_f^n \tag{43}$$

For the matrix model with T small most molecules are far from a reaction center. The P_f value is therefore mostly determined by these molecules. In the zero order approximation in T we obtain

$$P_f \approx p_f + p_h P_f \tag{44a}$$

$$P_f^n \approx p_f + p_h (c - 1) P_f / c \tag{44b}$$

For an excitation which is localized on a neighbour of a reaction center only (c - 1) ways are open to remain a probability to give fluorescence. For a bulk molecule there are c. This difference causes the factor $(c - 1)/c$ in (44b). Subtraction of the second equation from the first leads to: $P_f^n = P_f - p_h P_f / c$. So we finally obtain the approximate expression:

$$P_f = (1 - T)\, p_f + p_h P_f - p_h T (P_f - p_h P_f / c)$$

$$= (1 - T)\, p_f + p_h (1 - T) P_f + (p_h^2 / c) T P_f \tag{45}$$

This equals the improved relation (40) (with $\lambda = p_h / c$) except for the factor $(1 - T)/(1 - \lambda T)$ in the right hand term. For the matrix model with $T \ll 1$ this factor only plays a role in the next order in T. In it is also the contribution from the averaging process over all kinds of systems.

A still better result can be obtained. If P_{nt} denotes a mean value of the probability of an excitation to reach a trap when it starts initially from the neighbour of the trap, we can approximate P_f^n as:

$$P_f^n \approx P_f - P_{nt} P_f \tag{46}$$

In words: P_f^n equals the difference between the fluorescence probability if the trapping neighbour is not present and the amount which will be intercepted by the trap if it is present. The quantity P_{nt} corresponds to λ. If

(46) is inserted into (43) we obtain relation (40) except for the factor $(1 - T)/(1 - \lambda T)$. This factor can be obtained too, if (43) is written as:

$$P_f(T) = (1 - T)p_f + p_h(1 - T)P_f + p_h T(P_f - P_f^n)$$

If in this equation the mean value over all possible trap distributions is taken, it can be shown that the result is the same as is expressed by (36).

8. NUMERICAL RESULTS

a. *The bacterial system.* The experimental ratio between maximum and minimum fluorescence is approximately 3 (Vredenberg & Duysens, 1963; Clayton, 1966). Using this ratio, (40) with $\lambda = p_h/c$ gives a relation between p_h and the number of nearest neighbours c. This relation is given in Fig. 4.

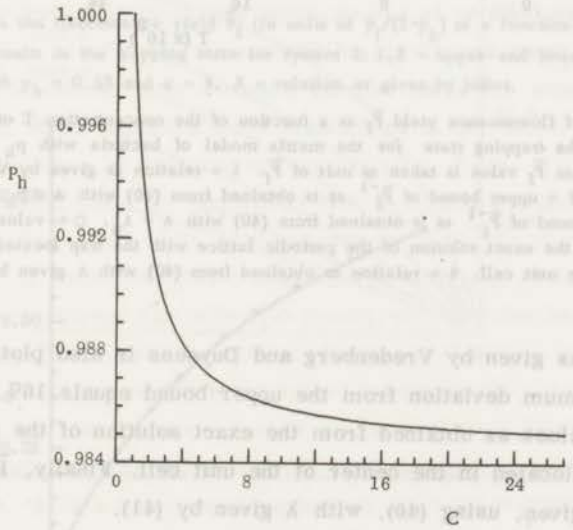

Fig. 4.

Relation between the number of nearest neighbours c and the jump probability p_h from formula (40) with $\lambda = p_h/c$ and a maximum to minimum fluorescence equal to 3.

The maximum value of T is taken to be 0.03. We will consider a two dimensional lattice with c equal to 4. This corresponds to a p_h-value of 0.9880. The upper and lower bound of $\bar{P}_f^{-1}$ as a function of T as obtained from (40) with $\lambda = p_h/c$ and $\lambda = \lambda_0$ respectively, are given in Fig. 5.

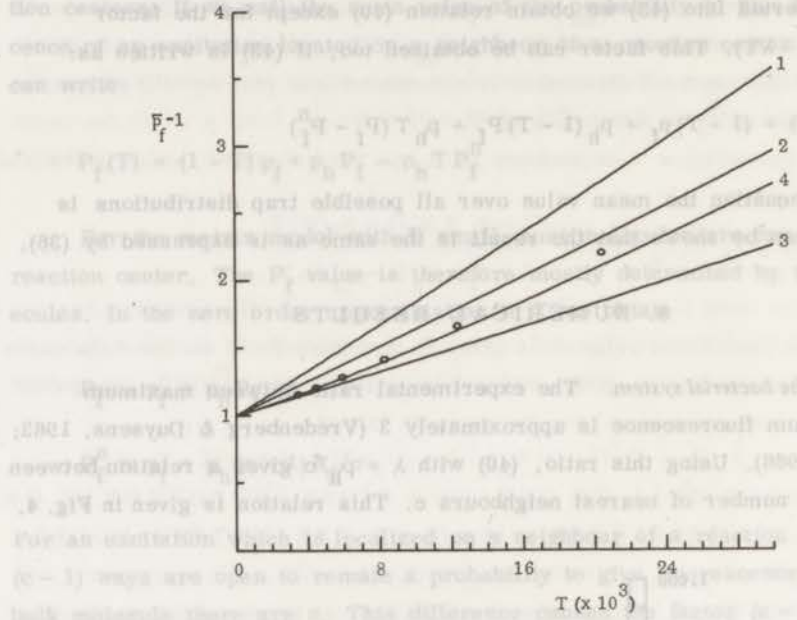

Fig. 5.

Reciprocal of fluorescence yield $\bar{P}_f$ as a function of the concentration T of reaction centers in the trapping state for the matrix model of bacteria with $p_h = 0.988$. The maximum $\bar{P}_f$ value is taken as unit of $\bar{P}_f$. 1 = relation as given by Vredenberg & Duysens. 2 = upper bound of $\bar{P}_f^{-1}$ as is obtained from (40) with $\lambda = p_h/c$. 3 = lower bound of $\bar{P}_f^{-1}$ as is obtained from (40) with $\lambda = \lambda_0$. ○ = value as obtained from the exact solution of the periodic lattice with the trap located in the center of the unit cell. 4 = relation as obtained from (40) with λ given by (41).

The formula as given by Vredenberg and Duysens is also plotted in this figure. Its maximum deviation from the upper bound equals 16%. We have also given some values as obtained from the exact solution of the periodic lattice with the trap located in the center of the unit cell. Finally, $\bar{P}_f^{-1}$ as a function of T is given, using (40), with λ given by (41).

b. *Photosystem 2.* In Fig. 6 we have compared formula (40) and the expression as given by Joliot. As p_h and c values we have chosen 0.55 and 4 respectively. The parameter λ in (40) has been given its minimum value p_h/c (0.1375) as well as its maximum value λ_0 (0.1537). This lower and upper bound of $\bar{P}_f$ coincide within the linewidth of the plotter. Therefore this curve can be considered to give the exact solution of the model of Joliot. Joliot's curve does not deviate more than 3% from it.

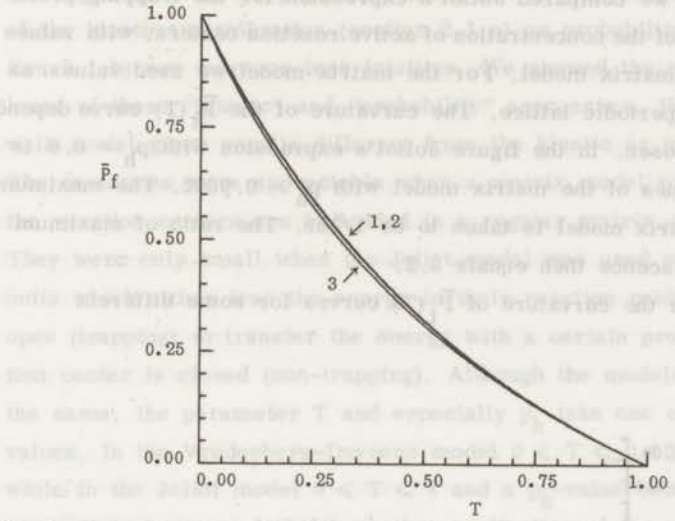

Fig. 6.

Relation between the fluorescence yield $\bar{P}_f$ (in units of $p_f/(1-p_h)$ as a function of the concentration T of units in the trapping state for system 2. 1,2 = upper and lower bound of $\bar{P}_f$ from (40) with $p_h = 0.55$ and $c = 4$. 3 = relation as given by Joliot.

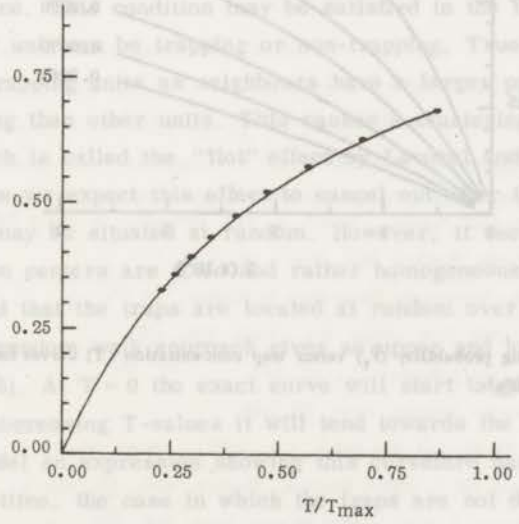

Fig. 7.

Matrix model of system 2 compared with the units model of Joliot. $\bar{P}_t(T)$ = trapping probability, T = concentration of traps (trapping units). ● = point calculated from the matrix model. These points are obtained from the exact solution of the periodic lattice with $p_h = 0.9992$ and a maximum T-value equal to 1/289. ——— curve obtained from Joliot's model with $p_h = 0.6$. This curve has been normalized such that it coincides at $T = T_{max}$ with the point calculated from the periodic lattice with T = 1/289.

c. In Fig. 7 we compared Joliot's expression for the trapping probability as a function of the concentration of active reaction centers, with values obtained from the matrix model. For the matrix model we used values as obtained from the periodic lattice. The curvature of the $\overline{P}_t(T)$ curve depends on the p_h-value chosen. In the figure Joliot's expression with $p_h = 0.6$ is compared with values of the matrix model with $p_h = 0.9992$. The maximum T-value in the matrix model is taken to be 1/289. The ratio of maximum to minimum fluorescence then equals 3.2.

Fig. 8 shows the curvature of $\overline{P}_t(T)$ curves for some different p_h-values.

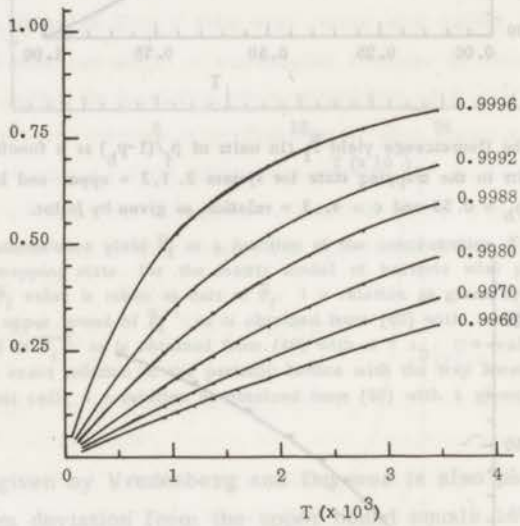

Fig. 8.

Curvature of trapping probability ($\overline{P}_t$) versus trap concentration (T) curves for different jump probabilities (p_h).

9. DISCUSSION

The random walk approach is in our opinion the most exact way to obtain a relation between fluorescence yield and the concentration of trapping centers in a lattice of excitation transferring molecules, since it is most

directly related to the physical situation. Other approaches which make use of the kinetic consideration (section 2.1.a) or probability arguments (section 2.1.b) are more or less intuitive. We showed the mathematical equivalence of these "kinetic" and "probability" approaches. However, the random walk model gives results different from the kinetic or probability approach. The deviations were appreciable when a matrix model was used in which the reaction centers are imbedded in a regular matrix of pigment molecules. They were only small when the Joliot model was used which consists of units which either trap the energy in their reaction center if this one is open (trapping) or transfer the energy with a certain probability if the reaction center is closed (non-trapping). Although the models are mathematically the same, the parameter T and especially p_h take one completely different values. In the Vredenberg-Duysens model $0 \leqslant T \leqslant 0.03$ and $p_h = 0.988$ while in the Joliot model $0 \leqslant T \leqslant 1$ and a p_h-value between 0.5 and 0.6 is used. It is however doubtful whether application of the random walk theory or any other theory to the whole units instead of to the individual molecules is permissible, even if the units would be partly separate.

In our treatment it is assumed that the traps are situated at random in the lattice. This condition may be satisfied in the model of Joliot, because each unit can be trapping or non-trapping. True, trapping units which have non-trapping units as neighbours have a larger probability to become non-trapping than other units. This causes a clustering of non-trapping units, which is called the "lot" effect by Lavorel and Joliot (1972). In the steady-state we expect this effect to cancel out. For the bacterial system the traps may be situated at random. However, it seems more likely that the reaction centers are fixed and rather homogeneously distributed. It may be expected that the traps are located at random over these fixed centers.

The random walk approach gives an upper and lower bound to $\overline{P}_f^{-1}$ (see Fig. 5). At $T = 0$ the exact curve will start tangent to the lower bound, while for increasing T-values it will tend towards the upper bound. For the matrix model an expression showing this curvature has been obtained. The periodic lattice, the case in which the traps are not distributed at random, also shows such a curvature. It seems to be somewhat larger in this last case. For the bacterial systems further experiments are needed to show it.

For small T-values the results of the random walk theory could be obtained with a simple rule. For more complicated systems this simple rule (section 7) may be used as a first starting point if random walk theory be-

comes less manageable.

In order to describe Joliot's data, we used instead of Joliot's model the matrix model with parameters $0 \leqslant T \leqslant 1/289$ and $p_h = 0.9992$. The trapping probability as a function of the concentration of traps was calculated for some T-values using the exact solution of the periodic lattice. The results are compared with those of Joliot's model (Fig. 7) and give a satisfactory correspondence. Thus the matrix model, provided random walk calculations are used, can explain Joliot's data.

Acknowledgements - This investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (ZWO). Thanks are due to Professor L.N.M. Duysens for valuable discussions and to Professor P.W. Kasteleyn for suggestions concerning the notation.

REFERENCES

Clayton, R.K. (1963) Biochim. Biophys. Acta, 75, 312.

Clayton, R.K. (1966) Photochem. Photobiol., 5, 807.

Duysens, L.N.M. (1952) Thesis, University of Utrecht.

Duysens, L.N.M. and Sweers, H.E. (1963) in Studies on Microalgae and Photosynthetic Bacteria, Special Issue of Plant Cell Physiol., Tokyo, p. 353, University of Tokyo Press.

Duysens, L.N.M. (1964) Progr. Biophys. Mol. Biol., 14, 1.

Duysens, L.N.M. (1967) In: Brookhaven Symposium in Biology, 19, 71.

Joliot, P. and Joliot, A. (1964) C.R. Acad. Sci., Paris, 258, 4622.

Lavorel, J. and Joliot, P. (1972) Biophys. J., 12, 815.

Nishimura, M. (1962) Biochim. Biophys. Acta, 59, 183.

Sanders, J.W., Ruygrok, Th.W. and Ten Bosch, J.J. (1971) J. Math. Phys., 12, 534.

Sybesma, C. and Vredenberg, W.J. (1963) Biochim. Biophys. Acta, 75, 439.

Vredenberg, W.J. and Duysens, L.N.M. (1963) Nature, Lond. 197, 355.

Wraight, C.A. (1972) Biochim. Biophys. Acta, 283, 247.

APPENDIX A

AN EXTENSION OF JOLIOT's MODEL

An extension of Joliot's model has been used by Wraight (1972). In his paper a quantum in a trapping unit is assumed to have a probability to leave the unit as fluorescence or to jump to a neighbouring unit. Let us call these probabilities p'_f and p'_h resp. and otherwise remain the same definitions as used earlier. If we use Duysens simple procedure for this more complicated system, P_f can be found from the following relation, which is easily interpreted:

$$P_f(T) = (1 - T)\,p_f + T\,p'_f + (1 - T)\,p_h P_f + T\,p'_h P_f \qquad (A\,1)$$

This leads to:

$$P_f = \frac{(1 - T)\,p_f + T\,p'_f}{1 - p_h(1 - T) - p'_f T} \qquad (A\,2)$$

This is the same expression as would be obtained for a matrix model in which a quantum absorbed in a trap has a probability p'_f to leave it as fluorescence and a probability p'_h to jump to a neighbouring molecule.

To discuss the validness of (A 2) we will start again from the random walk equations. These will read (see section 4 for definitions):

$$\begin{aligned} P_{bf} &= p_f + \sum_a p_{ba} P_{af} && \text{if } b \in \mathscr{B} \\ P_{bf} &= p'_f + \sum_a p_{ba} P_{af} && \text{if } b \in \mathscr{T} \end{aligned} \qquad (A\,3)$$

From this follows:

$$\begin{aligned} P_f &= N^{-1} \sum_b P_{bf} \\ &= (1 - T)\,p_f + T\,p'_f + N^{-1} \sum_{a,b} p_{ba} P_{af} \end{aligned} \qquad (A\,4)$$

If we take the mean value over all systems with a given trap concentration and suppose $\overline{p_{ab} P_{bf}} = \bar{p}_{ab} \cdot \bar{P}_{bf}$ we obtain (A 1).

Let us consider the foregoing relation more closely:

$$p_{ba} = c^{-1} p_h \delta_{\langle ba \rangle} \qquad \text{if } b \in \mathscr{B}$$
$$p_{ba} = c^{-1} p_h' \delta_{\langle ba \rangle} \qquad \text{if } b \in \mathscr{T} \tag{A 5}$$

from which we obtain:

$$\bar{p}_{ba} = (1 - T) c^{-1} p_h \delta_{\langle ba \rangle} + T c^{-1} p_h' \delta_{\langle ba \rangle} \tag{A 6}$$

If we define systems S_1, S_2, S_3 and S_4 as in section 5, $\overline{p_{ab} P_{bf}}$ can be written as:

$$\overline{p_{ab} P_{bf}} = N_s^{-1} \sum_{\substack{s_1, s_2, \\ s_3, s_4}} p_{ab} P_{bf}$$

$$= N_s^{-1} \sum_{s_1, s_4} c^{-1} p_h \delta_{\langle ab \rangle} P_{bf} + N_s^{-1} \sum_{s_2, s_3} c^{-1} p_h' \delta_{\langle ab \rangle} P_{bf}$$

and

$$\bar{p}_{ab} . \bar{P}_{bf} = \{(1 - T) c^{-1} p_h \delta_{\langle ab \rangle} + T c^{-1} p_h' \delta_{\langle ab \rangle}\} N_s^{-1} \sum_{\substack{s_1, s_2, \\ s_3, s_4}} P_{bf}$$

Combining these equations gives:

$$\overline{p_{ab} P_{bf}} = \bar{p}_{ab} . \bar{P}_{bf} + c^{-1} \delta_{\langle ab \rangle} (p_h - p_h') T (1 - T) \{ \bar{P}_{bf}^{I} - \bar{P}_{bf}^{II} \} \tag{A 7}$$

with

$$\bar{P}_{bf}^{I} = (N_{s_1} + N_{s_4})^{-1} \sum_{s_1, s_4} P_{bf}$$
$$\bar{P}_{bf}^{II} = (N_{s_2} + N_{s_3})^{-1} \sum_{s_2, s_3} P_{bf} \tag{A 8}$$

Because for a large system one trap more will not influence the trap concentration significantly, we may consider the same trap distribution outside a

for $(S_1 + S_4)$ as well as $(S_2 + S_3)$ simultaneously. In that case P^{I}_{bf} and P^{II}_{bf} can be compared:

$$\begin{aligned} P^{II}_{bf} &= \mu + P_{ba} P^{II}_{af} \\ P^{I}_{bf} &= \mu + P_{ba} P^{I}_{af} \end{aligned} \qquad (A\,9)$$

μ = probability of an excitation originally on b to give fluorescence, while it does not pass or reach a.

P_{ba} = total probability of an excitation originally on b to reach a in one, two, ... steps.

Taking the mean of (A 4) now gives:

$$\{1 - p_h + T(p_h - p'_h)\}\,\overline{P}_f = (1 - T)\,p_f + T\,p'_f + \Delta \qquad (A\,10)$$

with

$$\Delta = N^{-1} \sum_{a,b} c^{-1}\, \delta_{\langle ab \rangle}\, (p_h - p'_h)\, T(1 - T)\, \overline{P_{ba}\,(P^{I}_{af} - P^{II}_{af})} \qquad (A\,11)$$

If, as in the matrix model, $p_h \approx 1$ and $T \ll 1$, Δ is of the same order of magnitude as the left hand side of equation (A 10) and may not be neglected. Only in the exceptional case $\overline{P}^{I}_{af} - \overline{P}^{II}_{af} \ll 1$ neglectance of Δ will be allowed.

In the unit model $p_h \approx 0.5$. If we define f as $f = \overline{P}_{f\,max} / \overline{P}_{f\,min}$ the following inequality holds

$$\begin{aligned} \Delta &\leqslant (p_h - p'_h)\, T(1 - T)\, \lambda \sum_a \overline{P}^{I}_{af} \\ &\leqslant (p_h - p'_h)\, T(1 - T)\, \lambda\, f\, \overline{P}_f \end{aligned}$$

with λ a value between the minimum and maximum of P_{ba}.

The term $T(1 - T)$ only plays a role for intermediate T-values. It obtains its maximum value for $T = \frac{1}{2}$. With $f \approx 3$ and $\lambda < 0.15$, Δ will be at least a factor 0.075 smaller at $T = \frac{1}{2}$ than the left hand side of (A 10), while it may be expected that the true factor is still some times smaller. Therefore

for the unit model expression (A 2) appears to be justified mathematically. Whether the assumptions concerning the transfer between units are a correct description of the physical situation is doubtful.

APPENDIX B

DERIVATION OF AN APPROXIMATE FORMULA FOR λ.

In this appendix we will give a derivation of an approximate expression for the dependence on the trap concentration of the parameter λ. This parameter was used in equation (40). It was defined from the relation

$$\bar{P}_{bf}^{(4)} - \bar{P}_{bf}^{(2)} = \overline{P_{ba} P_{af}^{(4)}} = \lambda \bar{P}_{af}^{(4)}$$

Consider the periodic lattice with a trap at position 0 and a number of other traps located anywhere in the lattice, except at position 1. Position 1 will be a neighbour of the trap at position 0. Because $\lambda \approx \bar{P}_{ba}$, we are allowed to consider the T-dependence of $\bar{P}_{10}$. The number of traps be N_T and the number of lattice points N. If one more trap is placed into the lattice, say at position i, the following relation holds:

$$P_{10}(N_T + 1) = P_{10}(N_T) - P_{1i}(N_T + 1) P_{i0}(N_T) \qquad (B 1)$$

First of all we will take the mean value over all possible trap distributions over all lattice points except the positions 0, 1 and i. If the mean value of the product is approximated by the product of the mean values, which for the matrix model seems to be justified, we obtain:

$$\bar{P}_{10}(N_T + 1) = \bar{P}_{10}(N_T) - \bar{P}_{1i}(N_T + 1) \bar{P}_{i0}(N_T) \qquad (B 2)$$

The success of section 7 for small T-values implies that the position of an excitation does not matter much as far as its fluorescence probability is concerned, unless it is a neighbour of a trap.

An equivalent reasoning as used for equation (44b) then gives:

$$\bar{P}_{1i} \approx \frac{c-1}{c}\, p_h\, N_T^{-1}\, \bar{P}_t'\,(N_T) \qquad \text{(B 3)}$$

$\bar{P}_t'$ being the mean trapping probability for an excitation starting from the set of bulk molecules. It is supposed in this equation that $N_T \gg 1$ and $T \ll 1$.

To obtain an expression for $\bar{P}_{i0}(N_T)$ we will argue as follows: $P_{10}(1)$ denotes the trapping probability of an excitation starting at position 1 with the trap located at position 0 and if there are no other traps in the lattice. It equals λ_0. If $N_T - 1$ other traps are located at arbitrary positions, $P_{10}(1)$ satisfies:

$$P_{10}(1) = P_{10}(N_T) + \sum_{j \neq 0} P_{1j}(N_T)\, P_{j0}(1) \qquad \text{(B 4)}$$

The summation extends over all trap positions, except the one at position 0. With the arguments mentioned above, this relation can be approximated by:

$$\lambda_0 \approx P_{10}(N_T) + P_{i0}(1) \sum_{j \neq 0} P_{1j}(N_T)$$

If we take the mean value over all trap distributions outside position 1 and 0, this leads to ($N_T \gg 1$):

$$\lambda_0 \approx \bar{P}_{10}(N_T) + P_{i0}(1)\, c^{-1}\,(c-1)\, p_h\, \bar{P}_t' \qquad \text{(B 5)}$$

Further $P_{i0}(1)$ satisfies:

$$P_{i0}(1) = P_{i0}(N_T) + \sum_{j \neq 0} P_{ij}(N_T)\, P_{j0}(1) \qquad \text{(B 6)}$$

This gives with the foregoing approximations:

$$P_{i0}(1) \approx P_{i0}(N_T) + P_{i0}(1) \sum_{j \neq 0} P_{ij}(N_T)$$

or, when taking again the mean value:

$$P_{i0}(1) \approx \overline{P}_{i0}(N_T) + P_{i0}(1)\,\overline{P}'_t(N_T) \qquad \text{(B 7)}$$

Combination of (B 5) and (B 7) gives:

$$\overline{P}_{i0}(N_T) \approx c\,[(c-1)\,p_h\,\overline{P}'_t]^{-1}\,(1-\overline{P}'_t)\,(\lambda_0 - \overline{P}_{10}(N_T)) \qquad \text{(B 8)}$$

This equation and (B 3) can be substituted into (B 2). If $\Delta\overline{P}_{10}$ is defined as $\Delta\overline{P}_{10} = \overline{P}_{10}(N_T+1) - \overline{P}_{10}(N_T)$ (in which the mean value over all positions i can be included), we will get:

$$\Delta\overline{P}_{10} = -N_T^{-1}\,(1-\overline{P}'_t(T))\,(\lambda_0 - \overline{P}_{10}) \qquad \text{(B 9)}$$

The trap concentration is defined as $T = N_T/N$. This implies $\Delta T = \Delta N_T/N$. With $\Delta N_T = 1$, it gives $\Delta T = 1/N$. An expression for $\overline{P}'_t$ can be obtained from equation (40). When $\lambda = p_h/c$ is inserted, we obtain:

$$\overline{P}'_t = \frac{p_h(1-p_h/c)\,T}{1-p_h+p_h(1-p_h/c)\,T} \qquad \text{(B 10)}$$

A factor $1 - T$ has been neglected in the numerator of this formula.

Now it's possible to turn equation (B 9) into a differential equation in T:

$$\frac{d\overline{P}_{10}}{dT} = -\frac{1}{T}\,\frac{(1-p_h)\,(\lambda_0 - \overline{P}_{10})}{1-p_h+p_h(1-p_h/c)\,T} \qquad \text{(B 11)}$$

Integration gives:

$$\overline{P}_{10}(T) = \lambda_0 - \frac{AT}{1-p_h+p_h(1-p_h/c)\,T} \qquad \text{(B 12)}$$

Yet at $T = 0.14$ the function $T/\{1 - p_h + p_h(1 - p_h/c)\,T\}$ deviates less than 8% from it's maximum value $1/\{p_h(1 - p_h/c)\}$. If we suppose our derivation to be valid on a range of T-values till about 0.14, the constant A may be supposed to have a value such, that in (B 12) $\bar{P}_{10} = p_h/c$ at $T = 1$. This gives $A = (\lambda_0 - p_h/c)(1 - p_h + p_h(1 - p_h/c))$. When this is inserted into (B 12), we obtain:

$$\bar{P}_{10}(T) = \lambda_0 - (\lambda_0 - p_h/c)\,\frac{\{1 - p_h + p_h(1 - p_h/c)\}\,T}{1 - p_h + p_h(1 - p_h/c)\,T} \qquad \text{(B 13)}$$

The formula is not valid if $p_h = 1$. This restriction has its origin in the arguments used in the transition from (B 6) to (B 7). There the error made in the approximation $\sum_{j \neq 0} \bar{P}_{ij}(N_T) \approx \bar{P}'_t$ should be negligible compared to $1 - \bar{P}'_t$. This is not the case if $p_h = 1$.

It is interesting to see how the method works when applied to the total trapping probability. If $P_{kt}(N_T)$ denotes the total trapping probability of an excitation starting from the bulk molecule k and if a next trap is localized at position i, the following relation holds:

$$P_{kt}(N_T + 1) = P_{kt}(N_T) + P_{ki}(N_T + 1) - P_{ki}(N_T + 1)\,P_{it}(N_T) \qquad \text{(B 14)}$$

We will take the mean value over all possible trap distributions outside i, approximate the mean value of the product by the product of the mean values and use

$$\bar{P}_{kt}(N_T) = \bar{P}_{it}(N_T) = \bar{P}'_t \qquad \text{(B 15)}$$

If further the mean value over all possible positions i is taken and $\bar{P}_{ki}$ is written as $(N_T + 1)^{-1}\,\bar{P}'_t(N_T + 1)$, we will obtain ($N_T \gg 1$):

$$\frac{d\bar{P}'_t}{dT} = \bar{P}'_t\,(1 - \bar{P}'_t)\,T^{-1} \qquad \text{(B 16)}$$

Integration and insertion of the boundary condition $\overline{P}_t' = p_h$ at $T \to 1$ gives:

$$\overline{P}_t' = \frac{p_h T}{1 - p_h (1 - T)} \qquad \text{(B 17)}$$

This equals the Vredenberg-Duysens relation for an excitation starting from the set of bulk molecules. This should also be expected.

Photochemistry and Photobiology. 1971. Vol. 14, pp. 589–596.
Pergamon Press. Printed in Great Britain

CHAPTER III

ABSORPTION CHANGES IN THE REACTION CENTER OF PHOTOSYNTHETIC BACTERIA AND π-ELECTRON CALCULATIONS ON BACTERIOCHLOROPHYLL, ITS MONO CATION AND ANION

H. A. OTTEN
Biophysical Laboratory of the University of Leiden, Leiden, The Netherlands

(*Received* 9 *November* 1970; *in revised form* 18 *March* 1971)

Abstract—π-Electron calculations of the MO-SCF-CI type in the PPP approximations are carried out for the tetra pyrrole ring of the bacteriochlorophyll molecule, its mono cation and anion.

According to the calculations the formation of the positive ion will be accompanied by the disappearance of the longest wavelength absorption band of the neutral system and the appearance of a new band near 1200 nm. Absorption changes of the same kind are found in the reaction center of photosynthetic bacteria upon illumination, supporting the hypothesis that the primary reaction is an expulsion of an electron from the π-electron system of the reaction center bacteriochlorophyll.

INTRODUCTION

In purple bacteria light energy absorbed by the bulk of the bacteriochlorophyll, the analog of the chlorophyll of green plants, is transferred to a special photochemically active bacteriochlorophyll molecule, discovered by Duysens[1]. This molecule is called a reaction center. Reaction centers are present in a concentration of the order of one percent of the total bacteriochlorophyll. The location of the longest wavelength absorption band of the reaction center of different species of these bacteria varies somewhat. The reaction center is called P890, P870 etc. according to their wavelength. Upon illumination the reaction center changes its absorption. This light-induced spectral change is furthered by oxidizing conditions. The same change can be brought about in extracts from purple bacteria by adding ferricyanide. ESR measurements[2, 3] now suggest the formation of the positive ion of the P870 bacteriochlorophyll molecule during the light reaction.

The spectral changes in bacteria upon illumination are complex (Fig. 1, lower curve) and have only partly been identified. Some of the changes are mentioned here:

(1) Bleaching of the longest wavelength absorption band. (2) Appearance of a new absorption band at 1250 nm. (3) Blue shift of the bands situated at 800 and 590 nm. (4) Changes caused by cytochrome oxidation. (5) In some bacteria small shifts of the absorption bands of the bulk of the pigment system.

The spectral changes in the i.r. region are the most interesting from our point of view. They have been attributed to the oxidative bleaching of the reaction center molecule together with a shift of the absorption band of a bacteriochlorophyll absorbing at 800 nm[4, 5], photodissociation of a dimer[6] and to the oxidation together with a decoupling of a trimer[7]. Thus far it was not clear whether the appearance of the band at 1250 nm was caused by the oxidation of P870.

The first successful description of the spectra of neutral porphyrins was given by Gouterman[8]. By assuming a degeneracy of the two top filled orbitals obtained from

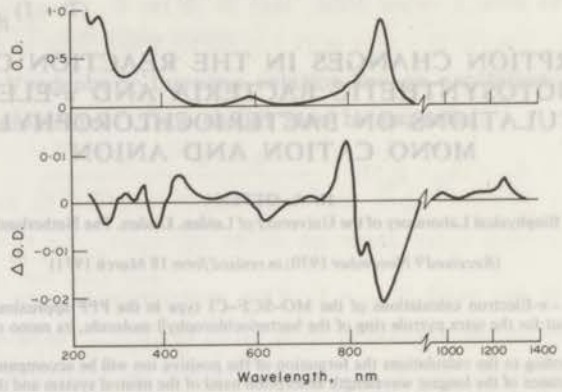

Fig. 1. Absorption and difference spectra of chromatophores from blue-green mutant *R. spheroides* (after Clayton[4]). Upper curve: absorption spectrum of a dry chromatophore film. Lower curve: spectrum of reversible light-induced absorbancy changes in the same film. (Light minus darkness).

a Hückel theory, he was able to interpret qualitatively the spectra of most porphyrins and their reduced forms with a four orbital model. SCF–CI calculations with Weiss and Kobayashi[9] some years later confirmed his ideas and gave a very nice account of many porphyrin spectra.

Following their work, we performed a SCF-CI calculation of the Pariser–Parr–Pople type for the tetra pyrrole ring of the neutral bacteriochlorophyll molecule, its mono cation and anion. Our calculations indicate that the formation of the positive bacteriochlorophyll ion should lead to bleaching of the i.r. band and the appearance of a band near 1250 nm.

METHODS

Because of the great resemblance between the absorption spectrum of bacteriochlorophyll and other reduced porphyrins[10], it is assumed that it is due to the π-electrons of the common skeleton. Figure 2 gives the structure of the bacteriochlorophyll molecule.

If, as for the neutral system, the doublet ground state of the ion is represented by a single determinant of one-electron functions, the variational principle does not give SCF equations with a single SCF operator, as for the closed shell. Roothaan however showed how to construct a single SCF operator[11].

After solving the SCF equations an extensive configuration interaction was performed. Figure 3 gives the path of conjugation and the coordinates of the model.

Geometry. We used the values obtained by Webb and Fleischer[12] for the geometry of porphin. Geometrical changes as induced by the reduction of the two pyrrole rings were not accounted for.

The Mg-atom. The effect of the Mg-atom is two-fold. Firstly it supplies two electrons to the ring system, making it a system of 20 conjugated atoms with, for the neutral system, 22 π-electrons. Secondly, it makes the four-nitrogen atoms equivalent

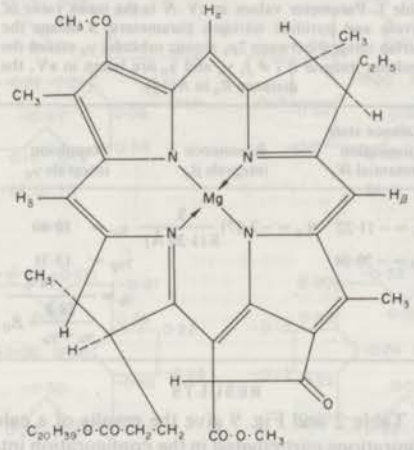

Fig. 2. Structure of bacteriochlorophyll.

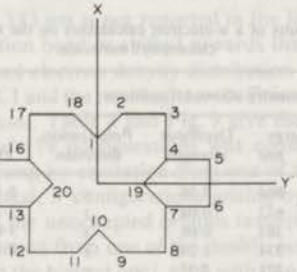

Fig. 3. Coordinates and path of conjugation of the model.

This we took into account by averaging the pyrrole and the pyridine nitrogen parameter values.

Parameter choice. Our choice of parameters for both the neutral molecule and the positive and negative ions is the same as that used by Weiss *et al.*[9]. Table 1 gives the parameter values for valence state ionization potentials (W_i), resonance integrals (β_{ij}) and repulsion integrals (γ_{ij}).

Computer programs. Programs for closed and open shell calculations were originally written by Dr. W. Th. A. M. v.d. Lugt and Dr. J. J. C. Mulder. For the present calculation the symmetry of the model was built in, in order to perform an extensive CI with as many as 179 configurations. The calculations were executed on the IBM 360/50 computer at the Centraal Rekeninstituut of the University of Leiden.

Table 1. Parameter values in eV. $\bar{N}$ is the mean value of pyrrole and pyridine nitrogen parameters; S means the overlap integral between $2p_z$ atomic orbitals; γ_{ij} means the repulsion integral if $i \neq j$; γ_{ii} and γ_{jj} are taken in eV, the distance R_{ij} in Å [14].

Valence state ionisation potential W_i	Resonance integrals β_{ij}	Repulsion integrals γ_{ij}
$W_c = -11{\cdot}22$	$\beta_{ij} = -2{\cdot}371 \dfrac{S}{S(1{\cdot}39\ \text{Å})}$	$\gamma_{cc} = 10{\cdot}60$
$W_{\bar{N}} = -20{\cdot}04$		$\gamma_{NN} = 13{\cdot}31$
		$\gamma_{ij} = \dfrac{14{\cdot}4}{\dfrac{28{\cdot}8}{\gamma_{ii}+\gamma_{jj}} + R_{ij}}$

RESULTS

Neutral molecule. Table 2 and Fig. 9 give the results of a calculation in which all 99 singly excited configurations participated in the configuration interaction (CI).

In agreement with Gouterman's four orbital model [8], the essence of the results can be interpreted in terms of electron transitions between the two orbitals 10, 11 and the orbitals 12, 13 (Fig. 4). The coefficients of these orbitals are shown in Fig. 5.

Table 2. Results of a π-electron calculation on the neutral bacteriochlorophyll molecule.

Symmetry allowed transitions					Symetry forbidden transitions		
Excitation energy eV	cm^{-1}	nm	Oscillator strength	Polarization direction	eV	cm^{-1}	nm
1·54	12431	804	0·56	y	3·12	25139	398
1·96	15828	632	0·04	x	3·75	30217	331
3·24	26103	383	0·08	x	3·84	30963	323
3·72	29982	334	2·24	x	4·10	33091	302
3·87	31189	321	2·07	y			

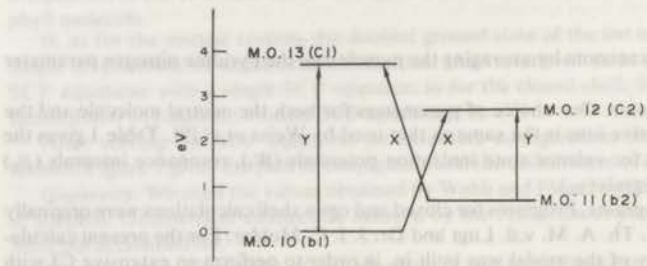

Fig. 4. Four level diagram for the infrared and soret bands. The X- and Y polarized excitations are combinations in the way shown in Table 3. Level assignments are those of Gouterman [8].

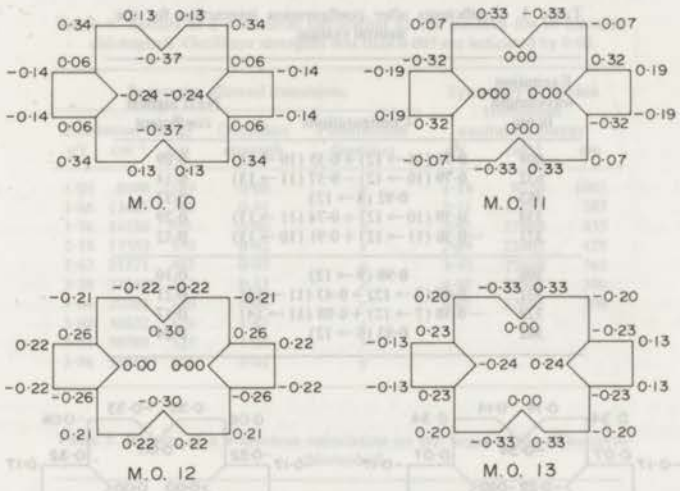

Fig. 5. Orbital coefficients of neutral bacteriochlorophyll.

The band calculated at 383 nm is not reported in the literature. It may perhaps be hidden in the soret absorption band or shifted towards the u.v. by solvent interaction. Figure 6 gives the calculated electron density distribution in the ground state; Table 3 gives the coefficients after CI and the next highest coefficient.

Positive and negative ions. Table 4 and Fig. 9 give the results of a calculation for the positive ion. A total of 179 configurations was considered, namely: the ground state, 10 configurations arising by excitation from one of the doubly occupied orbitals to the singly occupied orbital, 8 configurations arising by excitation from the singly occupied orbital to one of the unoccupied orbitals (except the highest one), 160 configurations arising by excitation from one of the doubly occupied orbitals to one of the unoccupied orbitals (except the highest one), each with two spin functions.

Only the two transitions of lowest energy allow a simple interpretation. For the transition at 1189 nm the configuration and next highest coefficient are: 0·92 (11 → 12) and 0·25 and for the transition at 1085 nm: 0·95 (10 → 11) and 0·23. MO coefficients of these orbitals are given in Fig. 7.

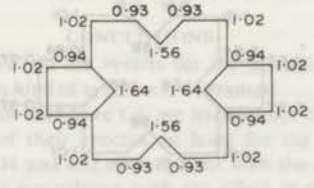

Fig. 6. Electron density distribution in the ground state of neutral bacteriochlorophyll.

Table 3. Coefficients after configuration interaction for the neutral system.

Excitation wavelength in nm	Configurations	Next highest coefficient
804	0·92 (11 → 12) + 0·35 (10 → 13)	0·09
632	0·79 (10 → 12) − 0·57 (11 → 13)	0·14
383	0·92 (8 → 12)	0·32
334	0·59 (10 → 12) + 0·74 (11 → 13)	0·29
321	−0·36 (11 → 12) + 0·91 (10 → 13)	0·12
398	0·98 (9 → 12)	0·10
331	0·87 (7 → 12) + 0·43 (11 → 14)	0·21
323	−0·38 (7 → 12) + 0·88 (11 → 14)	0·17
302	0·93 (6 → 12)	0·24

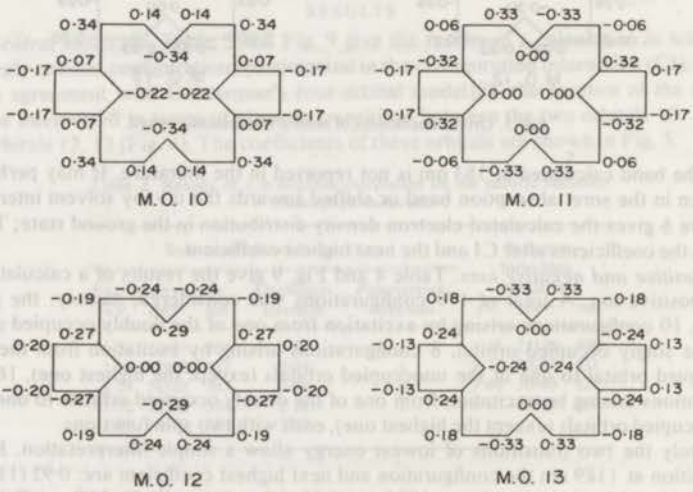

Fig. 7. Orbital coefficients of the positive ion of bacteriochlorophyll.

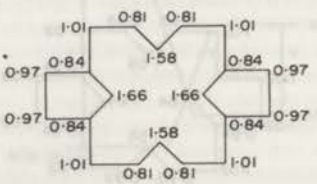

Fig. 8. Electron density distribution in the ground state of the positive ion of bacteriochlorophyll.

Table 4. Results of a π-electron calculation on the positive ion of bacteriochlorophyll. Oscillator strengths less than 0·005 are indicated by 0·00.

Symmetry allowed transitions					Symmetry forbidden transitions		
Excitation energy			Oscillator	Polarization	excitation energy		
eV	cm^{-1}	nm	strength	direction	eV	cm^{-1}	nm
1·04	8408	1189	0·08	y	1·14	9210	1085
1·66	13425	745	0·01	x	2·11	17051	587
1·76	14180	705	0·00	x	2·85	22989	435
2·18	17552	570	0·02	x	2·89	23302	429
2·67	21571	463	0·03	y	3·43	27668	361
3·38	27244	367	0·33	x	4·05	32691	306
3·64	29382	340	0·00	x	4·05	32695	306
3·80	30622	326	1·47	x			
3·82	30789	325	1·04	y			
3·96	31915	313	0·01	y			

Table 5. Results of a π-electron calculation on the negative ion of bacteriochlorophyll.

Symmetry allowed transitions					Symmetry forbidden transitions		
Excitation energy			Oscillator	Polarization	excitation energy		
eV	cm^{-1}	nm	strength	direction	eV	cm^{-1}	nm
1·04	8414	1189	0·19	y	1·12	9061	1104
1·38	11161	896	0·03	x	2·54	20465	488
1·57	12669	789	0·01	x	3·04	24512	408
2·28	18413	543	0·03	y	3·05	24576	407
3·10	24979	400	0·02	x	3·34	26941	371
3·71	29886	335	1·23	y	3·72	30005	333
3·73	30097	332	0·84	x	3·92	31620	316
3·75	30220	331	0·67	x			
3·85	31043	322	0·12	y			
4·06	32720	306	0·17	x			

Figure 8 gives the electron density distribution in the ground state of the positive ion before CI. The coefficient of this state after CI is 0·97 and the next highest coefficient is 0·14. Therefore, the values given in Fig. 8 are a good approximation for the ground state of the positive ion as obtained after CI.

An analogous calculation was performed for the negative ion. The results are given in Table 5.

CONCLUSIONS

Gouterman's calculations and the results for the neutral molecule show that the use of the PPP theory for this kind of systems is warranted.

Because we performed an extensive CI, we may accept the results for the ions to be a good approximation of their spectra, at least for the lowest transitions. The bleaching of the bands at 804 and 632 nm, together with the appearance of two weak transitions in between, is in accordance with the effect of one-electron oxidation of bacteriochlorophyll in methanol as measured by Fuhrhop and Mauzerall[15].

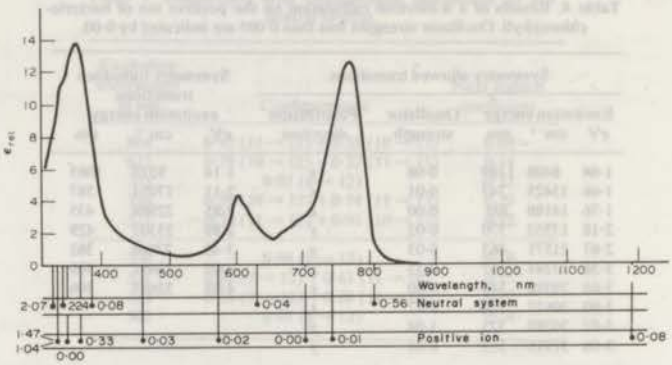

Fig. 9. Spectrum of bacteriochlorophyll in methanol (after Goedheer[13]). The calculated transitions with corresponding oscillator strength of the neutral system and positive ion in the indicated rows. Oscillator strengths less than 0·005 are indicated by 0·00.

Therefore, the photobleaching of the i.r. band of the reaction center bacteriochlorophyll as well as the appearance of the new absorption band at 1250 nm is explained by the formation of the mono cation. The observed weaker bands at 980 and 1140 nm may perhaps be interpreted as vibrational satellites of the 1250 nm excitation.

A model of the reaction center as suggested by Beugeling[6] is not supported by our calculation. His model implies the existence of an absorption band of the positive ion at about 800 nm with nearly the same oscillator strength as the 804 nm band of the neutral system. Our calculation does not give evidence for this.

Acknowledgment—The author is indebted to Prof. L. N. M. Duysens for suggesting the problem and to Prof. L. J. Oosterhof for hospitality.

The investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

1. L. N. M. Duysens, *Progr. Biophys. Mol. Biol.* **14**, 1 (1964).
2. R. K. Clayton, J. R. Bolton and D. W. Reed, *Photochem. Photobiol.* **9**, 209 (1969).
3. K. Cost, J. R. Bolton and A. W. Frenkel, *Photochem. Photobiol.* **10**, 251 (1969).
4. R. K. Clayton, *Photochem. Photobiol.* **1**, 201 (1962).
5. L. N. M. Duysens, W. J. Huiskamp, J. J. Vos and J. M. van der Hart, *Biochim. Biophys. Acta* **19**, 188 (1956).
6. T. Beugeling, *Progr. Photosynthesis Res.* **2**, 1101 (1969).
7. K. Sauer, E. A. Dratz and L. Coyne, *Proc. Natl. Acad. Sci. U.S.* **61**, 17 (1968).
8. M. Gouterman, *J. Mol. Spectry.* **6**, 138 (1961).
9. C. Weiss, H. Kobayashi and M. Gouterman, *J. Mol. Spectry.* **16**, 415 (1965).
10. C. D. Dorough and J. R. Miller, *J. Amer. Chem. Soc.* **74**, 6106 (1952).
11. C. C. J. Roothaan, *Rev. Mod. Phys.* **32**, 179 (1960).
12. L. E. Webb and E. B. Fleischer, *J. Chem. Phys.* **43**, 3103 (1965).
13. J. C. Goedheer, Thesis, Utrecht 1957.
14. N. Mataga and K. Nishimoto, *Z. Phys. Chem. Frankfurt* **12**, 335 (1957).
15. J. H. Fuhrhop and D. Mauzerall, *J. Amer. Chem. Soc.* **91**, 4174 (1969).

CHAPTER IV

AN EXTENSION OF THE STEADY-STATE APPROXIMATION OF THE KINETICS OF ENZYME CONTAINING SYSTEMS*

H.A. OTTEN and L.N.M. DUYSENS

Biophysical Laboratory of the State University, P.O. Box 556, Leiden, The Netherlands.

Summary - An extension of the steady-state approximation of multi-enzyme systems is obtained, which can also be applied when enzyme concentrations are of the same order of magnitude as substrate concentrations. This extension contains as a first order approximation the extension given by Vergonet and Berendsen. The mathematical procedure however is different and avoids difficulties inherent in their theory.

1. INTRODUCTION

For describing the kinetics of biochemical systems, one usually has to solve one or more coupled differential equations. The relaxation times belonging to this set of equations (Eigen, 1960) often differ largely. In that case the solution is difficult to obtain and the set is called stiff (Cooper, 1969). A system showing these features is e.g. glycolysis (Garfinkel and Hess, 1964).

Numerical integration of this kind of equations demands special techniques and requires a substantial amount of computer time. To avoid this difficulty, the steady-state approximation for the components of which the kinetical equations lead to the smallest relaxation times, is often used. Within this approximation it is possible to express the concentrations of these components in the concentrations of the others. In that way the differential equations leading to the stiffness of the system of coupled rate equations are eliminated. The remaining equations can be integrated without difficulties with the usual numerical methods.

The steady-state approximation for a biochemical system is considered to be appropriate if the concentrations of the enzymes are small compared to the concentrations of substrates and products. An attempt to evaluate the applicability of the steady-state method has been made by Vergonet and Berendsen (1970). Their work gives an extension of the steady-state method.

* To be published in the Journal of Theoretical Biology.

There is no discussion however about the error introduced by their way of approach.

This article gives an analysis of the steady-state approximation of multi-enzyme systems. It shows how an extension of the method can be given. The correction formula as given by Vergonet and Berendsen is contained in it as an approximation. Finally a numerical example of the different methods is given.

2. KINETICS OF MULTI-ENZYME SYSTEMS

The equations describing the kinetics of systems that contain several enzymes. will contain as variables the substrate and product concentrations $c_i(t), (i = 1,2, \ldots, n)$ and the enzyme and enzyme-complex concentrations $x_j(t), (j = 1,2, \ldots, m)$ A conservation equation holds for each enzyme species. It states that the sum of the concentrations of the enzyme and its complexes is a constant. The c_i and x_j are supposed to be independent-that means no conservation equation has been left between the variables chosen. The general form of the equations which describe the kinetics of the system is supposed to be:

$$\begin{aligned} \frac{d}{dt} c_i(t) &= \sum_{j=1}^{m} M_{ij}(c_1, \ldots, c_n)x_j + m_i(c_1, \ldots, c_n) \quad (i = 1, \ldots, n) \\ \frac{d}{dt} x_j(t) &= \sum_{k=1}^{m} A_{jk}(c_1, \ldots, c_n)x_k + a_j(c_1, \ldots, c_n) \quad (j = 1, \ldots, m) \end{aligned} \tag{1}$$

The equations (1) are assumed to be linear in the concentrations of enzymes and enzyme complexes. If written in vector notation. the set of equations (1) becomes:

$$\dot{c} = Mx + m \tag{2a}$$

$$\dot{x} = Ax + a \tag{2b}$$

in which a dot denotes a time derivative.

The Michaelis-Menten kinetics can be considered as a special case of (2). This kinetics is characterized by the following reaction scheme

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (3)$$

S = substrate, P = product, E = enzyme and ES = enzyme - substrate complex. With $c = [S]$, $x = [E]$, $x_0 = [E] + [ES]$ = constant and $[P] + [S] + [ES]$ = constant, the equations describing the kinetics of (3) are:

$$\begin{aligned} \dot{c} &= -(k_1 c + k_{-1})x + k_{-1} x_0 \\ \dot{x} &= -(k_1 c + k_2 + k_{-1})x + (k_2 + k_{-1})x_0 \end{aligned} \qquad (4)$$

If we define: $M = -(k_1 c + k_{-1})$, $m = k_{-1} x_0$, $A = -(k_1 c + k_2 + k_{-1})$ and $a = (k_2 + k_{-1}) x_0$, equations (4) can be written as

$$\begin{aligned} \dot{c} &= Mx + m \\ \dot{x} &= Ax + a \end{aligned} \qquad (5)$$

This is a special case of (2) in which c, x, m, a, M and A are scalar functions.

3. STEADY-STATE APPROXIMATION

The steady-state solution $\{c^S(t), x^S(t)\}$ of (2) is obtained by setting in equation (2b) $\dot{x}$ equal to zero and then integrating the resulting equations.

$$\dot{c}^S = Mx^S + m \qquad (6a)$$

$$0 = Ax^S + a \qquad (6b)$$

Equations (6b) form a set of algebraic equations from which x^S can be solved as an expression in c^S.

$$x^S = -A^{-1} a \qquad (7)$$

This expression is substituted into (6a) to give a set of differential equations which only contain the unknown functions $c_i^S(t), (i = 1, \ldots, n)$. This set can be integrated numerically using the given initial conditions $c^S(0) = c(0)$. Substitution of the solution $c^S(t)$ into (7) gives $x^S(t)$ as a function of time.

The initial conditions c(0) and x(0) must be given to determine the

solution of (2). In the case of equations (6) only the initial condition $c^S(0) = c(0)$ can be used. Therefore the steady-state approximation only selects that special approximate solution of (2) for which $x(0) = -A^{-1}(0)a(0)$.

4. EXTENSION OF THE STEADY-STATE APPROXIMATION

We now make an extension of the steady-state approximation. To that end we eliminate x from (2a) with (2b). The new set of equations equivalent to (2a) and (2b) reads:

$$\dot{c} = -MA^{-1}a + m + MA^{-1}\dot{x} \tag{8a}$$

$$x = -A^{-1}a + A^{-1}\dot{x} \tag{8b}$$

If we put $\dot{x} = 0$ the steady-state equations are obtained. However, $\dot{x}$ is not equal to zero but a function which differs little from zero. We may expect that setting $\dot{x}$ equal to the time derivative of the steady-state expression, i.e. $\dot{x} = dx^S(c)/dt$, is a better approximation than setting $\dot{x} = 0$.
If we express $\dot{x}$ in components we obtain:

$$\dot{x}_i \approx -\frac{d}{dt}\left(\sum_{j=1}^{m} A^{-1}_{ij}\, a_j\right)$$

$$= -\sum_{k=1}^{m} \frac{\partial}{\partial c_k}\left(\sum_{j=1}^{m} A^{-1}\, a_j\right)\dot{c}_k$$

If we define the matrix T^S as

$$T^S_{ij} = -\frac{\partial}{\partial c_j}\left(\sum_{l=1}^{m} A^{-1}_{il}\, a_l\right) \tag{9}$$

$\dot{x}$ can be written as

$$\dot{x} = T^S \dot{c}$$

We substitute this expression into (8a) and rewrite $\dot{c}$ in quantities which depend exclusively on c:

$$\dot{c} = (1 - MA^{-1}T^S)^{-1} (-MA^{-1}a + m) \qquad (10)$$

This system of equations can be integrated numerically with initial conditions c(0). Let us call the solution $c_1(t)$. To find $x_1(t)$ we can use the relation $x_1(t) = x^S(c_1)$ in which $c_1(t)$ is substituted.

If the system is started with initial concentrations c(0) and x(0), usually a fast relaxation phenomenon will first occur (see the mathematical analysis further on). A measure of the relaxation times is given by the reciprocals of the eigenvalues of A at t = 0. Good results of the given method may be expected if one takes as initial conditions c(0) values as measured or as estimated after the relaxation has died out.

A more thorough discussion of the steady-state approximation and its extension will be given in section 5. It is not necessary to study this section before proceeding to section 6 on numerical results.

5. MATHEMATICAL ANALYSIS

A. STEADY-STATE APPROXIMATION

To gain insight into the steady-state solution, we will consider equations (2) more closely. In the general solution of (2), $\{c(t), x(t)\}$, we will write x(t) as a sum of a particular solution $x^P(c)$ which depends exclusively on c, and a complementary solution $\xi(t)$:

$$x(t) = x^P(c) + \xi(t) \qquad (11)$$

In the case of the Michaelis-Menten kinetics such a splitting of x(t) has been given by Wong (Wong, 1965) and has been discussed by Otten (Otten, 1973). It can be shown (see appendix A) that if $x^P(c)$ is chosen as an arbitrary solution of the set of partial differential equations:

$$\sum_j \frac{\partial x_i^P}{\partial c_j} \{(Mx^P)_j + m_j\} = (Ax^P)_i + a_i, \quad (i = 1, \ldots, m) \qquad (12)$$

and if the matrix T is defined as

$$T_{ik} = \frac{\partial}{\partial c_k} x_i^p(c) \tag{13}$$

equations (2) can be replaced by:

$$\dot{c} = M x^p(c) + m + M\xi \tag{14a}$$

$$\dot{\xi} = (A - TM)\,\xi \tag{14b}$$

In these equations $x^p(c)$ is supposed to be a known function of the components of c; the matrix A - TM depends exclusively on c; T is determined by the choice of $x^p(c)$.

Criteria under which the steady-state approximation gives a good description of the kinetics as defined by (2), can now be formulated.

a. Equation (7) of the steady-state approximation furnishes an expression $x^s = k(c)$, with

$$k(c) = -A^{-1}(c)\,a(c) \tag{15}$$

The function k(c) has to be considered as an approximate solution of (12). For that to be true the left-hand side of (12) with $x^p(c) = k(c)$ has to be small compared to the terms on the right-hand side.

b. For the steady-state solution to be able to describe the kinetic problem, the term $M\xi$ in (14a) (and ξ in (11)) has to be negligible. Two possibilities can be considered. (i) The function ξ decreases rapidly and the change in c during this time is negligible. This is the case if the largest relaxation time of the set (14b) is small compared to times in which substrate and product concentrations change noticeable. A measure of these relaxation times is given by the reciprocals of the eigenvalues of the matrix A - TM at time zero. Let us call the largest one τ. The change in c_i during this interval starting from $t = 0$ is given by:

$$\delta c_i(0) \approx \sum_j M_{ij}(0)\, x_j(0)\, \tau + m_i(0)\, \tau$$

$$\leqslant \tau \left(\sum_j |M_{ij}(0)|\, x_j^0 + |m_i(0)| \right)$$

x_j^0, represents the total enzyme concentration belonging to x_j, that means the sum of the concentration of the enzyme and its complexes. If now

$$\tau(\sum_j |M_{ij}(0)| x_j^0 + |m_i(0)|) \ll c_i, \quad (i = 1, \ldots, n) \tag{16}$$

holds, then ξ disappears fast enough to be negligible in (14a). Then because of the short duration of this relaxation phenomenon we can disregard ξ in equation (11) as well. If we are interested in this relaxation term, ξ can be found by means of (14b) after the steady-state solution $c^S(t)$ has been substituted. (ii) The function ξ decreases rapidly but the change in c during this time is significant. Then a steady-state calculation has to be started with a c(0) - value which has been measured after the relaxation phenomenon has ceased.

B. EXTENDED METHOD

Consider again equations (11), (12), (14a) and (14b). We now try to find a solution of (12) which is better than the steady-state solution. To that end we first define T_{ij} as $T_{ij} = \partial x_i^p / \partial c_j$. Then (12) becomes:

$$T(Mx^p + m) = Ax^p + a$$

This set of partial differential equations can be transformed as follows

$$MA^{-1}T(Mx^p + m) = Mx^p + MA^{-1}a$$

or

$$(1 - MA^{-1}T)Mx^p = -MA^{-1}a + MA^{-1}Tm$$

or

$$Mx^p = -(1 - MA^{-1}T)^{-1}MA^{-1}a + (1 - MA^{-1}T)^{-1}\{(MA^{-1}T - 1) + 1\}m$$

Since M is not a square matrix, use is made of the identity $(M^{\sim}M)^{-1}M^{\sim}M = 1$, in which $M^{\sim}$ is the transposed matrix of M. This gives:

$$x^p = -(M^{\sim}M)^{-1}M^{\sim}(1-MA^{-1}T)^{-1}MA^{-1}a + (M^{\sim}M)^{-1}M^{\sim}\{(1-MA^{-1}T)^{-1}-1\}m \quad (17)$$

The following approximations of (17) and successively (14a) can be given if we assume that the relaxation phenomenon ξ is negligible or already died out:

1 zero order in $MA^{-1}T$:

$$x^p(c) = -A^{-1}a$$

$$\dot{c} = -MA^{-1}a + m$$

This equals the steady-state method;

2 first order in $MA^{-1}T$ and $T_{ij} \approx T^s_{ij} = -\frac{\partial}{\partial c_j}(A^{-1}a)_i$

$$x^p(c) = -A^{-1}a - A^{-1}T^sMA^{-1}a + A^{-1}T^sm$$

$$\dot{c} = -MA^{-1}a + m + MA^{-1}T^s(-MA^{-1}a + m)$$

This equals the method of Vergonet and Berendsen (see appendix B);

3 $T_{ij} \approx T^s_{ij}$

$$x^p(c) = -(M^{\sim}M)^{-1}M^{\sim}(1-MA^{-1}T^s)^{-1}MA^{-1}a + (M^{\sim}M)^{-1}M^{\sim}\{(1-MA^{-1}T^s)^{-1}-1\}m$$

$$\dot{c} = (1-MA^{-1}T^s)^{-1}(-MA^{-1}a + m)$$

This equals the present method.

After integration of the differential equations of c the relation $x(c) = -A^{-1}a$ will be sufficiently accurate to find x(t).

6. NUMERICAL RESULTS

We carried out, using the extended steady-state method as discussed in section 4 and more thoroughly in section 5, a numerical calculation by means of a PDP-9 computer for the Michaelis-Menten kinetics according to equations (4). The relaxation phenomenon is supposed to have died out and the steady-state value is chosen as the initial condition for the enzyme concentration.

If initial concentrations c(0) and x(0) not related by the steady-state condition $x(0) = x^s(c(0))$ occur, a good estimate of the concentrations immediately after the relaxation phenomenon has died out can be obtained in this case. Because we will choose $k_2 \ll k_1$ and $k_2 \ll k_{-1}$ we may say that during

the relaxation phase the equilibrium of the first reaction, $S + E \rightleftharpoons ES$, rapidly becomes established. The changes Δx in x and Δc in c during this period are approximately equal, $\Delta x = \Delta c$, and satisfy $(c(0) + \Delta c)(x(0) + \Delta x)/(x_0 - x(0) - \Delta x) = k_{-1}/k_1$ From these equations the value of Δx and Δc follow.

In order to make an exact numerical calculation feasible, the choice of parameters was such that the system is weakly stiff. The following values were given to the parameters: $k_1 = 10$, $k_{-1} = 10$, $k_2 = 0.1$, $c(0) = 1$ and $x_0 = 1$. In Fig. 1 the results have been plotted by a Calcomb-plotter connected to the computer. The exact solution is compared with the one obtained with the steady-state method, the method of Vergonet and Berendsen and the proposed method. In all approximate methods we calculated the enzyme concentration from $x(c) \approx -A^{-1}a$.

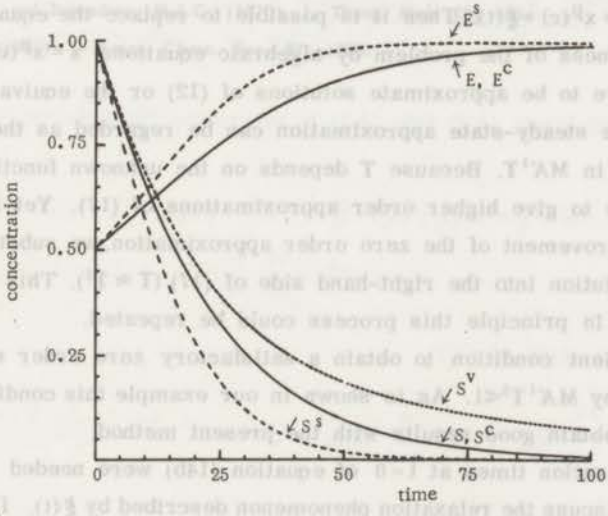

Fig. 1.

Calculation of the Michaelis-Menten kinetics. Parameter values are given in the text. E and S are enzyme and substrate concentrations respectively. Without index: exact; with index S: calculated by the steady-state method; with index V: calculated by the method of Vergonet and Berendsen; with index C: present method.

The calculation shows that even for large total enzyme concentration the proposed method gives good results.

7. DISCUSSION

Fig. 1 shows that for large enzyme concentration the convential steady-state method as well as the method of Vergonet and Berendsen yield a solution which deviates substantially from the exact solution. Our method gives a solution which deviates less than the linewidth of the plotter. (The deviations in the substrate concentration are everywhere less than 0.06%.) The example suggests that the method can give better results than may be inferred from the mathematical analysis. A measure of the appropriateness of the solution can be obtained by substituting it back in the original equations.

From the mathematical analysis we have seen that the problem caused by the stiffness of equations (2) can be bypassed if functions $x^p(c)$ and $\xi(t)$ can be found such that $\xi(t)$ describes a fast disappearing relaxation phenomenon and $x(t) \approx x^p(c) + \xi(t)$. Then it is possible to replace the equations which cause the stiffness of the problem by algebraic equations $x = x^p(c)$. The functions $x^p(c)$ have to be approximate solutions of (12) or the equivalent equations (17). The steady-state approximation can be regarded as the zero order approximation in $MA^{-1}T$. Because T depends on the unknown function $x^p(c)$ it is not possible to give higher order approximations of (17). Yet to be able to find an improvement of the zero order approximation, we substituted the zero order solution into the right-hand side of (17) ($T \approx T^s$). This leads to equation (10). In principle this process could be repeated.

A sufficient condition to obtain a satisfactory zero order solution of (17) is given by $MA^{-1}T^s \ll 1$. As is shown in our example this condition is not necessary to obtain good results with the present method.

The relaxation times at $t = 0$ of equation (14b) were needed in order to be able to discuss the relaxation phenomenon described by $\xi(t)$. If $MA^{-1}T \ll 1$, we also have $A - TM \approx A$. Then the relaxation times can be determined from the reciprocals of the eigenvalues of A at $t = 0$.

Moreover with the given analysis a better understanding of the work of Vergonet and Berendsen (1970) is obtained. Their model decouples in a certain sense the differential equations of enzymes on the one hand and substrates and products on the other by introducing piecewise constant substrate and product concentrations. From a theoretical point of view the lower bound of the time interval of numerical integration, which is needed in their theory, is suspicious. A better approach is given in the above treatment.

ACKNOWLEDGEMENTS

This investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). We thank Mr. J.H.M. v.d. Water and Miss E.L. Huyser for technical assistance in the work on the PDP-9 computer of the Biophysical Institute.

REFERENCES

Cooper, G.J. (1969). FEBS Letters 2, 22.

Eigen, M. (1960). Z. Electrochem. 64, 115.

Garfinkel, D. and Hess, B. (1964). J. Biol. Chem. 239, 971.

Otten, H.A. (1973). To be published in Math. Biosci.

Vergonet, G. and Berendsen, H.J.C. (1970). J. Theor. Biol. 28, 155.

Wong, J.T. (1965). J. Amer. Chem. Soc. 87, 1788.

APPENDIX A

The equations of the kinetics of the enzyme system read:

$$\begin{aligned} \dot{c} &= Mx + m \\ \dot{x} &= Ax + a \end{aligned} \tag{A 1}$$

We will write the general solution $x(t)$ as:

$$x(t) = x^p(c) + \xi(t) \tag{A 2}$$

$x^p(c)$ will be a particular solution of the set of partial differential equations

$$\sum_{j=1}^{m} \frac{\partial x_i^p}{\partial c_j} \{(Mx^p)_j + m_j\} = (Ax^p)_i + a_i , \quad (i = 1, \ldots , m) \tag{A 3}$$

We have to substitute (A 2) into (A 1) to determine the equation for $\xi(t)$:

$$\sum_{j=1}^{m} \frac{\partial x_i^p}{\partial c_j} \{(Mx^p)_j + (M\xi)_j + m_j\} + \dot{\xi}_i = (Ax^p)_i + (A\xi)_i + a_i , \quad (i = 1, \ldots , m)$$

This gives, because of (A 3)

$$\sum_{k=1}^{m} \sum_{j=1}^{n} \frac{\partial x_i^p}{\partial c_j} M_{jk} \xi_k + \dot{\xi}_i = \sum_{k=1}^{m} A_{ik} \xi_k , \quad (i = 1, \ldots , m)$$

If we define the matrix T with $T_{ik} = \partial \dot{x}_i^p / \partial c_k$ we finally obtain

$$\dot{\xi} = (A - TM)\xi \tag{A 4}$$

APPENDIX B

We will prove the equivalence of the method of Vergonet and Berendsen and the improvement on the steady-state method as implied by:

$$\dot{c} = -MA^{-1}\,a + m + MA^{-1}T^{S}(-MA^{-1}a + m) \qquad \text{(B 1)}$$

Let us consider the Euler method for the numerical integration of (B 1) and let us take an integration interval Δt. If we neglect the last term the increase in c during an interval Δt will be given by:

$$\Delta c = \dot{c}\,\Delta t = (-MA^{-1}a + m)\Delta t \qquad \text{(B 2)}$$

The last term in (B 1) will give a correction δc to this. Following the work of Vergonet and Berendsen, we define Δx as

$$\Delta x = x^{S}(c + \Delta c) - x^{S}(c) \qquad \text{(B 3)}$$

This means:

$$\Delta x_i = x_i^{S}(c + \Delta c) - x_i^{S}(c) \approx \sum_{j=1}^{n} \frac{\partial x_i^{S}}{\partial c_j}\,\Delta c_j$$

$$= -\sum_{j=1}^{n} \frac{\partial}{\partial c_j}\,(A^{-1}a)_i\,\Delta c_j$$

or

$$\Delta x \approx T^{S}\,\Delta c$$

The correction term δc then reads

$$\delta c = MA^{-1}T^{S}(-MA^{-1}a + m)\Delta t$$

$$\approx MA^{-1}T^{S}\Delta c = MA^{-1}\,\Delta x$$

This result is the same as obtained by Vergonet and Berendsen.

CHAPTER V

SOME REMARKS ON THE MICHAELIS-MENTEN KINETIC EQUATIONS*.

H.A. OTTEN

Biophysical Laboratory of the State University, P.O. Box 556, Leiden, The Netherlands.

Abstract - A number of properties of the Michaelis-Menten kinetic equations are derived. To that end the enzyme concentration as a function of time is written as a sum of two terms. The first one, f, is chosen such that it depends exclusively on the concentration of the substrate. The second term, ξ, turns out to be given by a decreasing exponential and describes the transient phase. It is not given by the solution of the homogeneous part of the kinetic equation of the enzyme, as has been supposed by Wong. An asymptotic series expansion of f is obtained of which the first term equals the steady-state expression of the enzyme concentration.

INTRODUCTION

The Michaelis-Menten kinetic equations [1] give a good description of chemical reactions in which, under the catalytic influence of an enzyme, a substrate is transformed into a product. It is not possible to give an analytical solution of the corresponding non-linear differential equations. Therefore one often uses the steady-state approximation for theoretical considerations.

The steady-state approximation [2] is considered to be appropriate in those cases in which the enzyme concentration is small compared to the substrate concentration. It does not describe however the transient phase. This transient phase has to be described separately.

It has always been a difficult problem to give the connection between the exact solution of the Michaelis-Menten equations and the steady-state approximation.

Recent attempts to elucidate this connection were made by Wong [3] and Heineken, Tsuchiya and Aris [4]. In the work of Wong the enzyme con-

* To be published in the journal of Mathematical Biosciences.

centration as a function of time has been written as a sum of two terms. The first one was supposed under certain conditions to be approximated by the steady-state solution. He assumed the second term to be given by the solution of the homogeneous part of the differential equation which describes the enzyme concentration. This last assumption is however at fault. In the work of Heineken et al. the exact solution is approximated by fitting together asymptotic expansions for small and large time values.

In this paper we will show that the solution for the enzyme concentration can be built up of a decreasing exponential ξ which depends on the initial condition and a special solution f which is independent of the initial enzyme concentration. An asymptotic series expansion of the function f is given which contains as lowest order term the steady-state expression.

MICHAELIS-MENTEN KINETICS

The Michaelis-Menten kinetics is characterized by the following reaction scheme:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P \tag{1}$$

with S = substrate, E = enzyme, ES = enzyme - substrate complex and P = product. If we put $s = [S]$, $e = [E]$, $e_0 = [E] + [ES]$ = constant, and if we denote a time derivative with a dot, the corresponding rate equations are:

$$\dot{s} = -(k_1 s + k_{-1})e + k_{-1} e_0 \tag{2a}$$

$$\dot{e} = -(k_1 s + k_2 + k_{-1})e + (k_2 + k_{-1})e_0 \tag{2b}$$

The mathematics will become more convenient if we define the dimensionless variables:

$$s' = (k_1 s + k_2 + k_{-1})/k_1 e_0, \quad e' = e/e_0, \quad \tau = k_1 e_0 t \tag{3}$$

If expressed in these new variables, the rate equations become:

$$ds'/d\tau = -(s' - \alpha)e' + \beta \tag{4a}$$

$$de'/d\tau = -s'e' + \gamma \tag{4b}$$

with

$$\alpha = k_2/k_1 e_0,\ \beta = k_{-1}/k_1 e_0,\ \gamma = \alpha + \beta \tag{5}$$

The steady-state approximation $\{s'_a(\tau), e'_a(\tau)\}$ of (4) is obtained if in equation (4b) $de'/d\tau$ is set equal to zero and if the resulting equations

$$ds'/d\tau = -(s'-\alpha)e' + \beta \tag{6a}$$

$$0 = -s'\,e' + \gamma \tag{6b}$$

are solved. Equation (6b) is an algebraic equation from which e'_a as a function of s' is found:

$$e'_a = \gamma/s' \tag{7}$$

This expression is substituted into (6a) to give a differential equation which only contains the unknown function $s'(\tau)$. Integration gives $s'_a(\tau)$ which finally is substituted into (7) to give e'_a as a function of τ.

The initial conditions $s'(0)$ and $e'(0)$ are necessary to determine the solution of (4). In the case of equations (6) only the initial condition $s'(0)$ can be used. Therefore, the steady-state approximation only selects that special approximate solution of (4) for which $e'(0) = \gamma/s'(0)$.

THE GENERAL SOLUTION

In the general solution $\{s'(\tau), e'(\tau)\}$ of (4), $e'(\tau)$ will be written as a sum of a particular solution $f(s')$, which depends exclusively on s', and a complementary solution $\xi(\tau)$.

$$e'(\tau) = f(s') + \xi(\tau) \tag{8}$$

If (8) is substituted into (4) and if we suppose the function $f(s')$ to be chosen as a solution of

$$\{(s'-\alpha)f - \beta\}\, df/ds' = s'f - \gamma \tag{9}$$

the following equation for $\xi(\tau)$ is found:

$$d\xi/d\tau = -\{s' - (s'-\alpha)df/ds'\}\xi \qquad (10)$$

From this we obtain a closed expression for $\xi(\tau)$:

$$\xi(\tau) = A\exp\left[-\int_0^\tau \{s' - (s'-\alpha)df/ds'\}\, d\tau\right] \qquad (11)$$

with A a constant to be determined from the initial conditions $s'(0)$ and $e'(0)$. Apparently $\xi(\tau)$ is not given by the solution of the homogeneous part of (4b) as was supposed by Wong [3]. This stems from the coupling between s' and e'.

For the transient solution $\xi(\tau)$ to decay with time, it is necessary that $s' - (s'-\alpha)df/ds' > 0$. If $f(s')$ can be chosen such that $df/ds' < 0$ this condition is certainly satisfied. In the next section such a choice will be made.

THE TRANSIENT PHASE

In this section we will show that the function $f(s')$ can be chosen such that $df/ds' < o$ for all possible values of s'. Then, the transient phase, as given by (11) decays in time. We first transform equation (9) into the form:

$$df/ds' = \frac{s'f - \gamma}{(s'-\alpha)f - \beta} \qquad (12)$$

1) The extrema of the function $f(s')$ are found in those points at which $df/ds' = 0$. Then two possible situations have to be considered.

i) $(s'-\alpha)f - \beta = \infty$ This implies either $s' = \infty$ or $f = \infty$ or both $s' = \infty$ and $f = \infty$. None of these possibilities satisfies: if only $s' \to \infty$ then $df/ds' \to 1$; if only $f \to \infty$ then $df/ds' \to s'/(s'-\alpha) \neq 0$; if both $s' \to \infty$ and $f \to \infty$ then $df/ds' \to 1$.

ii) $s'f - \gamma = 0$. This possibility cannot be ruled out. Therefore, the extreme of $f(s')$ are found in those points where $f(s')$ intersects the function b/s'. The second derivative d^2f/ds'^2 in those points, is most easily found with (9). If use is made of the definitions of α, β and γ (equation (5)) this leads to:

$$d^2f/ds'^2 = \gamma/\{(\gamma-\beta)s' - \alpha\gamma\} = (k_2 + k_{-1})k_1 c_0/k_1 k_2 s > 0 \qquad (13)$$

From this it follows that $f(s')$ possesses minima only. Then there cannot be

more than one minimum.

2) We will introduce a new variable ζ, defined as $\zeta = 1/s'$. If we transform (9) to this variable, we will find the differential equation in ζ:

$$-\zeta^2 \{(1-\alpha\zeta)f - \beta\zeta\}\, df/d\zeta = f - \gamma\zeta \tag{14}$$

We will try a solution which in a sufficiently small neighbourhood of $\zeta = 0$ can be written as:

$$f(\zeta) = \sum_{n=0}^{\infty} d_n \zeta^n \tag{15}$$

The corresponding recurrence relations are:

$$\begin{aligned} d_0 &= 0 \\ d_1 &= \gamma \\ d_2 &= 0 \\ d_n &= \beta(n-2)d_{n-2} + \alpha \sum_{m=1}^{n-2} m d_m d_{n-m-2} - \sum_{m=1}^{n-1} m d_m d_{n-m-1} \quad , n \geqslant 3 \end{aligned} \tag{16}$$

The series obtained in this way has to be considered as an asymptotic expansion [5].

3) Now we suppose $f(s')$ to be chosen as the special solution of (9) for which (15) together with (16) forms the asymptotic expansion. Then in a sufficiently small neighbourhood of $\zeta = 0$, $df/d\zeta \sim \gamma$. If expressed in s' this gives $df/ds' \sim -\gamma/s'^2$.

Consider the functions $f_1(s') = \beta/(s'-\alpha)$ and $f_2(s') = \gamma/s'$. These two functions are sketched in Fig. 1.

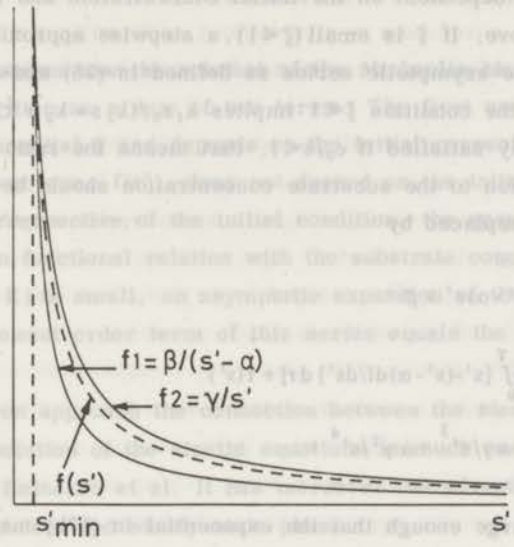

Fig. 1.

Schematic drawing of the functions $f_1 = \beta/(s'-\alpha)$, $f_2 = \gamma/s'$ and the particular solution $f(s')$.

The minimum value of s' is given by $s'_{min} = ((k_1 s + k_2 + k_{-1})/k_1 e_0)_{min} = \gamma$. The functions f_1 and f_2 coincide for this value of s' and have the value 1. As follows from (12) and Fig. 1, a negative value of df/ds' implies that $f(s')$ lies between f_1 and f_2.

As shown in 2), $df/ds' < 0$ if s' is sufficiently large. That means, if $s' \to \infty$, $f(s')$ is situated between f_1 and f_2 and approaches zero with a negative slope. Let us look at $f(s')$ for smaller values of s'. It can not intersect the function $f_1(s')$ because to that end df/ds' would have to become $-\infty$ at the point of intersection (equation (12)). It also cannot intersect the function $f_2(s')$ because at the point of intersection $f(s')$ should have a minimum. Therefore the function $f(s')$ lies between f_1 and f_2 and has a negative slope for all s'-values in the interval $s'_{min} < s' < \infty$. At $s' = s'_{min}$, $f(s')$ coincides with f_1 and f_2 and possesses the value 1.

THE ASYMPTOTIC APPROXIMATION

As we have shown in the last section, the solution of (4) for the enzyme concentration can be written in the form (8) with ξ a decreasing func-

tion in time and dependent on the initial concentration and f(s') the function as discussed above. If ζ is small ($\zeta \ll 1$), a stepwise approximation of f is obtained from the asymptotic series as defined in (15) and (16). Using the definition of ζ, the condition $\zeta \ll 1$ implies $k_1 e_0/(k_1 s + k_2 + k_{-1}) \ll 1$. This condition is certainly satisfied if $e_0/s \ll 1$, that means the ratio of the total enzyme concentration to the substrate concentration should be small. Equations (4) can now be replaced by

$$ds'/d\tau = -(s'-\alpha)e' + \beta \tag{17a}$$

$$e' = A\exp\left[-\int_0^{\tau} \{s'-(s'-\alpha)df/ds'\}\, d\tau\right] + f(s') \tag{17b}$$

$$f(s') = \gamma/s' - \alpha\gamma/s'^3 + \alpha\gamma^2/s'^4 + \ldots \tag{17c}$$

If the time is large enough that the exponential in (17b) may be neglected, we will get for the lowest order approximation:

$$ds'/d\tau = -\gamma(s'-\alpha)/s' + \beta \tag{18a}$$

$$e' = \gamma/s' \tag{18b}$$

This equals the steady-state approximation and leads to the Michaelis-Menten kinetic law $ds/dt = -k_2 e_0 s/(K+s)$, with K the Michaelis-Menten constant, $K = (k_2 + k_{-1})/k_1$. This also corresponds to the zero-order approximation of the "outer solution" of Heineken et al. [4]. The next order approximation will be given by:

$$ds'/d\tau = -(s'-\alpha)(\gamma/s' - \alpha\gamma/s'^3) + \beta \tag{19a}$$

$$e' = \gamma/s' - \alpha\gamma/s'^3 \tag{19b}$$

Equation (19a) has to be considered as an improvement on the Michaelis-Menten kinetic law.

For small time values the exponential in (17b) cannot be neglected. If we take as a first approximation to s' the value s'(0) at $\tau = 0$, we obtain a corresponding first approximation to e' given by $e' \sim \gamma/s'(0) + A\exp(-s'(0)\tau)$. This equals the zero-order term of the "inner solution" as given by Heineken et al. The lowest order approximation of (17b) equals the solution as given by Wong if the second term in the exponential is neglected.

DISCUSSION

We have shown how the solution of the Michaelis-Menten kinetic equations can be written as a sum of two terms. The first one consists of a decreasing exponential ξ and depends on the initial concentration of the enzyme. The second one, $f(s')$, does not depend on the initial condition. That means, that, irrespective of the initial condition, the enzyme concentration tends to a given functional relation with the substrate concentration. If the ratio $\zeta = e_0/(s+K)$ is small, an asymptotic expansion of the function f can be used. The lowest order term of this series equals the steady-state expression.

In the given approach the connection between the steady-state solution and the exact solution of the kinetic equations is much easier obtained than in the work of Heineken et al. It has moreover the advantage of connecting at once the transient and steady-state phase.

Acknowledgements - This investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). We thank Dr. R.A. van Santen for valuable discussions.

REFERENCES

1. L. Michaelis and M.L. Menten, Biochem. Z., 49 (1913) 333.
2. G.E. Briggs and J.B S. Haldane, Biochem. J., 19 (1925) 338.
3. J.T.F. Wong, J.A.C.S., 87 (1965) 1788.
4. F.G. Heineken, H.M. Tsuchiya and R. Aris, Math. Biosci., 1 (1967) 95.
5. P.H. Morse and H. Feshbach, "Methods of theoretical physics, Vol. I, Mac Graw-Hill, New York, N.Y. 1953, pp. 434-437

SUMMARY

In this thesis the results of some theoretical studies on photosynthesis are described.

A model of the process of excitation migration in the pigment system of photosynthetic organisms is given in chapter II. In the model it is supposed that the process of energy migration can be described as a process in which the energy of an absorbed light quantum hops from one pigment molecule to another until it is trapped by a photochemical active center. A homogeneous lattice of molecules in which a number of traps are imbedded was used as a starting point. The traps are thought to be distributed at random. "Random walk" equations of the system have been used to obtain the mean fluorescence probability of a light quantum captured in the system as a function of the concentration of traps. Using the obtained results, it is possible to discuss the approximations which are inherent to the less detailed analysis of the migration process as given by Vredenberg and Duysens for bacteria and in the case of system 2 by Joliot. For the "units model" of Joliot an alternative "matrix model" has been given.

The results of π-electron calculations on both the neutral bacteriochlorophyll molecule and on the mono-cation, that is formed if one electron disappears from the π-electron system, are given in chapter III. The calculations are of the MO-SCF-CI type. The calculations indicate that if the light reaction turns the neutral bacteriochlorophyll into the afore mentioned mono-cation, the bacteriochlorophyll absorption band with the longest wavelength will disappear, attended with the appearance of a new band around 1200 nm. This is in conformity with the observed, light-induced, absorption changes in photosynthetic bacteria. Therefore, the hypothesis (as has been made on evidence from ESR measurements) which states that the primary reaction consists of the expulsion of an electron from the π-electron system of the reaction center chlorophyll, is supported by the calculations.

The relation between the steady-state approximation and the exact solution of the kinetic equations of a system of enzyme-catalysed reactions is studied in chapter IV. An extension of the steady-state method is obtained and its validity discussed.

Finally an application of the theory discussed in the foregoing chapter on the kinetic equations of the Michaelis-Menten reaction is given in chapter V. A more thorough analysis of the relation between the steady-state

approximation and the exact solution is possible because of the relative simplicity of this system. The method that is used leads to a representation of this relation which is simpler than the ones that have been used up to the present.

In each chapter a short summary of its contents is given.

SAMENVATTING

In dit proefschrift wordt het resultaat weergegeven van enige theoretische onderzoekingen op het gebied van de fotosynthese.

In hoofdstuk II wordt een model behandeld voor het proces van excitatie migratie in het pigmentsysteem van fotosynthetische organismen. Er wordt in het model verondersteld dat het migratie proces beschreven kan worden als een proces waarbij de energie van een ingevangen lichtquant van pigmentmolecuul naar pigmentmolecuul springt tot het in een fotochemisch actief centrum gevangen wordt. Als uitgangspunt dient een homogeen rooster van moleculen waarin een aantal vangcentra willekeurig verdeeld voorkomen. Met behulp van de "random walk" vergelijkingen voor een dergelijk systeem wordt de gemiddelde fluorescentiekans voor een ingevangen lichtquant behandeld als functie van de concentratie waarin de vangcentra voorkomen. De benaderingen die inherent zijn aan de minder gedetailleerd opgezette behandeling van het migratie proces, zoals voor bacteriën gegeven is door Vredenberg en Duysens en voor systeem 2 door Joliot, worden besproken. Tevens wordt voor het "units model" van systeem 2 een alternatief "matrix model" gegeven.

In hoofdstuk III worden de resultaten weergegeven van π-electron berekeningen aan zowel het neutrale bacteriochlorofyl molecuul als aan het éénwaardige cation, dat ontstaat wanneer één electron uit het π-electronen systeem wordt verwijderd. De berekeningen zijn van het semi-empirische MO-SCF-CI type. Volgens de berekeningen zou een lichtreactie waarbij het neutrale bacteriochlorofyl door electron overdracht getransformeerd wordt in het genoemde éénwaardige cation, een verdwijning van de langstgolvige bacteriochlorofyl absorptieband teweeg brengen, alsmede het verschijnen van een nieuwe band in de buurt van 1200 nm. Dit is in overeenstemming met de waargenomen, door licht geinduceerde, absorptie veranderingen in fotosynthetische bacteriën. De berekeningen steunen daarom de reeds op grond van ESR-metingen gemaakte hypothese dat de primaire reactie bestaat uit het onttrekken van een electron aan het π-electronen systeem van het reactiecentrum chlorofyl.

In hoofdstuk IV wordt de relatie bestudeerd tussen de "steady-state benadering" en de exacte oplossing van de kinetiek vergelijkingen voor een stelsel van door enzymen gekatalyseerde reacties. Er wordt een uitbreiding van de steady-state methode gegeven en een discussie van de geldigheid ervan.

In hoofdstuk V tenslotte wordt de theorie zoals beschreven in het voorafgaande hoofdstuk toegepast op de kinetiek vergelijkingen van de Michaelis-Menten reactie. Door de relatieve eenvoud van dit systeem is een verdergaande analyse van de relatie tussen steady-state benadering en exacte oplossing mogelijk. Met de gevolgde procedure kan de genoemde relatie eenvoudiger worden voorgesteld dan tot op heden mogelijk was.

Elk afzonderlijk hoofdstuk bevat een korte samenvatting in het Engels.

CURRICULUM VITAE

Overeenkomstig het gebruik in de Faculteit der Wiskunde en Natuurwetenschappen der Rijksuniversiteit te Leiden volgt hieronder een kort overzicht van mijn academische studie.

Na het behalen van het einddiploma H.B.S.-B aan het Gemeentelijk Lyceum te Emmen in juni 1959 begon ik in september van het zelfde jaar mijn studie in de wis- en natuurkunde aan de Rijksuniversiteit te Groningen.

Na het afleggen van het candidaatsexamen (letter d) in februari 1963 vervolgde ik de opleiding met als hoofdvak theoretische natuurkunde. De doctoraalstudie stond onder leiding van de hoogleraren Dr. N.M. Hugenholtz, Dr. H.A. Tolhoek en Dr. H.J. Groenewold en werd in september 1967 beëindigd.

Na de voltooiing van de studie werd ik leraar aan de Analistenschool te Groningen tot december 1968. In december 1968 trad ik in dienst van de Stichting voor Biofysica in de functie van wetenschappelijk medewerker. Vanaf toen tot heden werd, onder leiding van Prof.Dr. L.N.M. Duysens, het werk verricht waarvan dit proefschrift het resultaat is. Gedurende een gedeelte van het onderzoek genoot ik gastvrijheid op de afdeling voor Theoretische Organische Chemie die onder leiding staat van Prof.Dr. L.J. Oosterhoff.

STELLINGEN

I

De wijze waarop Paillotin een halfwaardetijd afleidt voor het conversieproces $ZT^*Q \rightarrow Z^+TQ^-$ in reactiecentra van systeem 2 is onjuist.

Paillotin, G., J. Theor. Biol. 36, 223 (1972).

II

Het door Sauer et al. voorgestelde trimeer-model ter verklaring van het CD-spectrum van reactiecentrum preparaten van R. spheroïdes leidt, indien toegepast op het absorptiespectrum, tot moeilijkheden.

Sauer, K., Dratz, E.A. & Coyne, L., Proc. Natl. Acad. Sci. US. 61, 17 (1968).

III

Er zijn aanwijzingen dat zowel het absorptiespectrum als het in de voorgaande stelling genoemde CD-spectrum van reactiecentrum preparaten van R. spheroïdes te begrijpen zijn m.b.v. een tri-moleculair systeem, bestaande uit twee identieke bacteriochlorofyl moleculen met een absorptieband in de buurt van 800 nm en één bacteriochlorofyl molecuul met een absorptieband in de omgeving van 870 nm.

IV

De door Norris et al. op grond van ESR metingen getrokken conclusie dat het vrije electron in het geoxideerde reactiecentrum van systeem 1 gedelokaliseerd is over twee chlorofyl moleculen, is aanvechtbaar.

Norris, J.R. Uphaus, R.A., Crespi, H.L. & Katz, J.J., Proc. Natl. Acad. Sci. US, 68, 625 (1971).

V

De door Clayton gegeven uitbreiding van het matrix model voor de excitatie-migratie in het pigmentsysteem van fotosynthetische bacteriën is, gezien de resultaten van hoofdstuk II van dit proefschrift, vooralsnog weinig zinvol.

Clayton, R.K., J. Theor. Biol. 14, 173 (1967).

VI

De door Lavorel gegeven uitbreiding van de diffusievergelijking met een wrijvingsterm ter verkrijging van een betere correspondentie tussen theoretische en experimentele uitspraken over de fluorescentie kans van een door systeem 2 ingevangen lichtquant, is uit de lucht gegrepen.

Lavorel, J.,J. Chem. Phys.. 47, 2235 (1967).
Lavorel, J., in: Abstracts of the Seventh International Congress of Biochemistry, Tokyo, 1967.

VII

De toenemende populariteit in Westerse landen van Oosterse rustgevende technieken, zoals yoga en transcendente meditatie, doet ten onrechte het vermoeden rijzen dat er niet een gelijkwaardige Westerse methode bestaat.

Schultz, J.H., Das autogene Training, Georg Thieme Verlag, Stuttgart 1964, 11e druk.

VIII

Het verdient aanbeveling om de weinig gelukkig gekozen benaming van de getallen tussen 10 en 100, zoals reeds tot in negentienhonderddrieënzeventig in gebruik is, nog in tiennegenhonderdzeventigdrie (1973) te herzien.

IX

Het zou van naastenliefde getuigen wanneer ten behoeve van de niet bijbelvaste medemens aan bijbeluitgaven een trefwoordenregister werd toegevoegd.

H. A. Otten

Leiden, 2 mei 1973