ON THE POSSIBILITY OF LONG DISTANCE ENERGY TRANSFER BY RESONANCE IN BIOLOGY

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Energy transfer is usually thought to occur in biochemical and biological systems in general by a collision process in which an energy-rich—or (physically) excited—molecule loses its excitation energy in a collision of the second kind with another molecule. Electron transfer may accompany this process. However, recently another mechanism has gained importance, namely, the energy resonance transfer mechanism. By this is meant the transfer of energy by electrodynamical interaction from an excited oscillator to an oscillator in resonance with it and so close to it that their distance is small compared with the wavelength of the vibrating electromagnetic field emitted by the former. This mechanism has been introduced by J. PERRIN[1] to explain the depolarization of fluorescence of dyes with increasing concentration of the dye. F. PERRIN[2] developed the first quantum mechanical theory of the phenomenon. ARNOLD AND OPPENHEIMER[3] have shown that the mechanism may transfer energy with good efficiency from the pigment phycocyanin to chlorophyll in the photosynthesis of blue-green algae. DUYSENS[4] has reached similar conclusions with regard to the energy from carotenoids in blue and red algae and from chlorophyll *b* to chlorophyll *a* in the green alga *Chlorella*.

FRANCK AND LIVINGSTON[5] have pointed out that the mechanism can, in principle, explain the transfer of energy in proteins. Recently, many biologists (*cf.* WALD[6]) are beginning to think in terms of energy transfer by resonance. MCLAUGHLIN AND SZENT-GYÖRGYI[7] have investigated the quenching of the fluorescence of several aromatic hydrocarbons by some biologically active compounds in the hope of finding evidence for the more general occurrence of resonance transfer in biochemical systems. Such a finding would show the importance of physically excited states for biology. The latter investigators were interested in aromatic hydrocarbons because of the carcinogenic properties of this class of compounds and because the absorption spectrum of the biologically active substance dinitrophenol (LOOMIS AND LIPMANN[8]; CLOWES AND KRAHL[9]) overlaps the fluorescence spectrum of an aromatic hydrocarbon like chrysene. This means that the excited chrysene molecule emits fluorescent "light" containing frequencies which the dinitrophenol (DNP) molecule is able to absorb so that the latter molecule is in resonance with the former. Another striking example of a similar situation is in the oxidation-reduction series; namely, reduced diphosphonucleotide (DPN)-riboflavin-oxidized cytochrome *c*, which, as is well known, is very important for biological energy transfer. As is seen from Fig. 1, in which the absorption and fluorescence spectra of these compounds are plotted, the fluorescence spectrum of the first two compounds in this series is completely or for the most part overlapped

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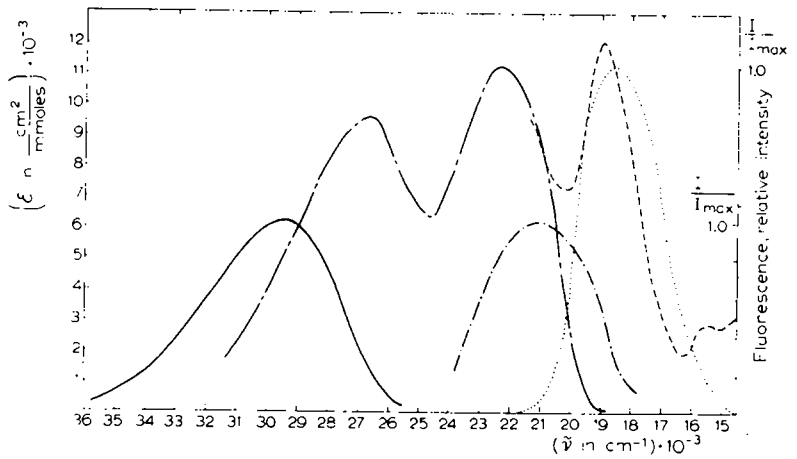

Fig. 1. Absorption and fluorescent spectra for some constituents of the biological redox-continuum, measured in water, pH 7. ——— Absorption spectrum for reduced DPN, – – – absorption spectrum for oxidized riboflavin, – · – · – absorption spectrum for oxidized cytochrome *c*, — · — · — fluorescent spectrum for reduced DPN, and ····· fluorescent spectrum for oxidized riboflavin.

by the absorption spectrum of the compound following it in this series. Recognizing that such a correlation between the spectral distribution and the functional order of the components in the respiratory continuum may be more than fortuitous, we thought it would be interesting to investigate the possibility that, at least on theoretical grounds, energy might move through this continuum (mitochondria, *e.g.*) by resonance transfer.

Of course it should be recognized that spectral shifts which occur when co-enzyme and apo-enzyme combine to form the intact functional enzyme will change the spectral overlaps. However, we do not think that these spectral shifts will change the order of magnitude of the results calculated below. It should be realized that the mere fact of a qualitative overlap does not necessarily mean that in these systems energy will be transferred over large distances by the mechanism of energy resonance transfer. To answer this quantitative aspect of the problem we shall apply the theory developed by FÖRSTER[10]. This investigator has shown that the energy transfer from an excited molecule to another molecule, with a probability of at least 50% (*cf.* DUYSENS[4], p. 81), occurs during the lifetime of the excited state of the molecule when the distance of the molecules is smaller than a critical value R_0. For the case of dipole interaction FÖRSTER (*ref.* [11], p. 226) has derived the next expression for the critical distance R_0:

$$R_0 = \sqrt[6]{\frac{3\,(\ln 10)^2 c}{8\pi^4 N'^2 n^2}\,\frac{\tau J_{\tilde{\nu}}}{\tilde{\nu}_0^2}} \qquad (1)$$

In this formula ln 10 is the natural logarithm of ten, c the velocity of light, N' the number of molecules per cm³ in a one molar solution, n the refractory index of the medium for the frequencies of the fluorescent light, τ the life-time of the excited state of the fluorescent molecule, $\tilde{\nu}_0$ the average of the wave numbers of the peak of the fluorescent and of the longest wavelength peak of the absorption spectrum of the fluorescent substance and $J_{\tilde{\nu}}$ the overlap integral. The latter is defined by (FÖRSTER[11], p. 226):

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$$J_{\tilde{\nu}} = \int_0^\infty \varepsilon_A(\tilde{\nu})\varepsilon_F(2\tilde{\nu}_0 - \tilde{\nu})\mathrm{d}\tilde{\nu} \qquad (2)$$

in which $\varepsilon_A(\tilde{\nu})$ represents the decadic extinction coefficient of the absorbing substance for the wave number $\tilde{\nu}$ and $\varepsilon_F(2\tilde{\nu}_0 - \tilde{\nu})$ that of the fluorescent substance for the wave number $2\tilde{\nu}_0 - \tilde{\nu}$ using the discovery of LEWSCHIN[12] that the absorption and the fluorescent spectrum of a substance are the mirror image of each other when they are plotted versus wave number. The value of $J_{\tilde{\nu}}$, which is a quantitative measure of the overlap of the fluorescent and absorption spectra and that of $\tilde{\nu}_0$ can be obtained from spectral data. To apply formula (1), which, after substitution of the values of the fundamental constants c and N', $3 \cdot 10^{10}$ cm sec^{-1} and $6.02 \cdot 10^{20}$ respectively, assumes the form:

$$R_0 = \sqrt[6]{\frac{(1.69)}{n^2} 10^{-33} \frac{\tau J_{\tilde{\nu}}}{\tilde{\nu}_0^2}} \qquad (3)$$

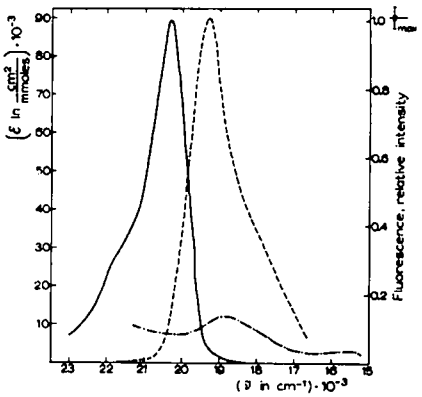

Fig. 2

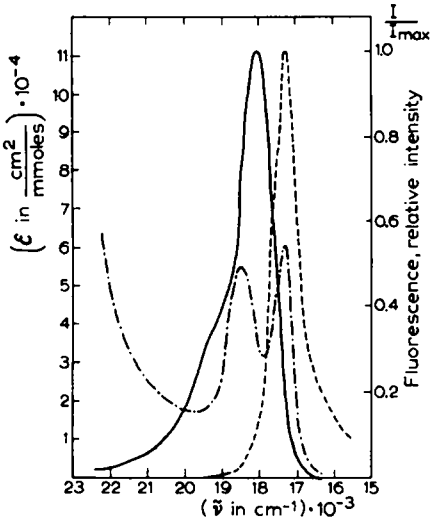

Fig. 3

Fig. 2. Absorption and fluorescent spectra of fluorescein and the absorption spectrum of oxidized cytochrome *c*, in water. ——— Absorption spectrum of fluorescein, – – – – fluorescent spectrum of fluorescein, —·—·— absorption spectrum of oxidized cytochrome *c*.

Fig. 3. Absorption and fluorescent spectra of rhodamine B and the absorption spectrum of oxyhemoglobin (rabbit) all in water. ——— Absorption spectrum of rhodamine B, – – – – – fluorescent spectrum of rhodamine B, —·—·— absorption spectrum of oxyhemoglobin.

Fig. 4. The absorption and fluorescent spectra of fluorescein and the absorption spectrum of oxyhemoglobin (rabbit), all in water. ——— Absorption spectrum of fluorescein, —·—·— absorption spectrum of oxyhemoglobin, and – – – – – fluorescent spectrum of fluorescein.

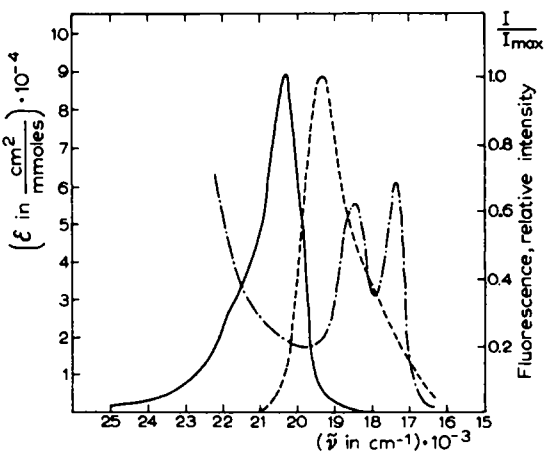

Fig. 4

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TABLE I

System	$\tilde{\nu}_0$ in cm^{-1}	$J_{\tilde{\nu}}$ in cm^3	$\int_0^\infty \frac{(2\tilde{\nu}_0-\tilde{\nu})^3}{\tilde{\nu}}\varepsilon(\tilde{\nu})d\tilde{\nu}$ in cm^{-1}	τ in 10^{-8} sec	R_0 in Ångström units	Reference and/or Figure
3,4-Benzpyrene + DNP in methyl alcohol	25 400	$48\cdot10^{10}$	$336\cdot10^{14}$	$0.58\,\eta_Q$	$40\sqrt[6]{\eta_Q}$	Fluores. spectr.: SPOLSKY *et al.*[29] Absorp. spectr.: FRIEDEL AND ORCHIN[30]
9,10-Dimethyl-1,2-benzanthracene + DNP in methyl alcohol	26 100	$40\cdot10^{10}$	$239\cdot10^{14}$	$0.82\,\eta_Q$	$41\sqrt[6]{\eta_Q}$	Same
3,4-Benzpyrene + riboflavin in methyl alcohol	25 400	$48\cdot10^{10}$	$336\cdot10^{14}$	$0.58\,\eta_Q$	$40\sqrt[6]{\eta_Q}$	Same
9,10-Dimethyl-1,2-benzanthracene + riboflavin in methyl alcohol	26 100	$29\cdot10^{10}$	$239\cdot10^{14}$	$0.82\,\eta_Q$	$39\sqrt[6]{\eta_Q}$	Same
Reduced DPN + riboflavin in water	25 200	$15\cdot10^{10}$	$96\cdot10^{14}$	$2.04\,\eta_Q$	$41\sqrt[6]{\eta_Q}$	Fig. 1
Riboflavin + oxidized cytochr. *c* in water	20 500	$33\cdot10^{10}$	$125\cdot10^{14}$	$1.57\,\eta_Q$	$48\sqrt[6]{\eta_Q}$	Fig. 1
Fluorescein + oxidized cytochr. *c* in water	19 900	$13\cdot10^{11}$		0.51^*	51	Fig. 2 *SZYMANOWSKY[31]
Rhodamine B + hemoglobin in water	17 700	$43\cdot10^{11}$		0.20^{**}	55	Fig. 3 **GAVIOLA[32]
Fluorescein + hemoglobin in water	19 900	$42\cdot10^{11}$		0.51^*	61	Fig. 4 *SZYMANOWSKY[31]

For the first six systems the values of R calculated for several values of η_Q between one and zero are listed in Table II.

we still need the value of the refractory index n and that of the value of the life-time τ of the fluorescent state. As we shall be interested only in systems in water or methyl alcohol solution we have $n = 1.33$ for the frequencies we shall consider.

Substitution of this value for n into equation (3) yields

$$R_0 = \sqrt[6]{(0.95)10^{-33}\frac{\tau J_{\tilde{\nu}}}{\tilde{\nu}_0^2}} \tag{4}$$

When the life-time of the fluorescent molecule is not known we shall use the formula (FÖRSTER[11], p. 158):

$$\frac{1}{\tau} = \frac{8\pi n^2(\ln 10)c}{\eta_Q N'}\int_0^\infty \frac{(2\tilde{\nu}_0 - \tilde{\nu})^3}{\tilde{\nu}}\,\varepsilon(\tilde{\nu})\,d\tilde{\nu} \tag{5}$$

in which η_Q represents the quantum efficiency of the fluorescent light and all other symbols have the above defined meanings. Substitution of the above mentioned values of n, c and N' leads to:

$$\frac{1}{\tau} = \frac{5.1 \times 10^{-9}}{\eta_Q}\int_0^\infty \frac{(2\tilde{\nu}_0 - \tilde{\nu})^3}{\tilde{\nu}}\,\varepsilon(\tilde{\nu})d\tilde{\nu} \tag{6}$$

To calculate τ with the aid of formula (6) the efficiency η_Q has to be known, which is, unfortunately, not often the case. Therefore, we shall, in those cases, calculate the value of R_0 for several possible values of η_Q (the latter all between zero and one).

All the integrals occurring in the application of the formulae (2) and (6) have been calculated with the aid of Simpson's formula. The results of these calculations are shown in Table I in which are listed, for the several systems mentioned, the values for $\tilde{\nu}_0$, $J_{\tilde{\nu}}$, $\int_0^\infty \frac{(2\tilde{\nu}_0 - \tilde{\nu})^3}{\tilde{\nu}}\,\varepsilon(\tilde{\nu})\,d\tilde{\nu}$ and τ, the value of the latter either taken from the references indicated or expressed in terms of η_Q with the aid of equation (6). For known values of τ the resulting values of R_0 calculated with formula (4) are given in Table I, in the remaining cases R_0 is expressed in terms of η_Q. For the first six systems the values of R_0 calculated for several values of η_Q between one and zero are listed in Table II.

TABLE II

R_0 in Ångström units for the systems	η_Q 1.00	0.50	0.10	0.05
3,4-Benzpyrene + DNP in methyl alcohol	40	36	27	24
9,10-Dimethyl-1,2-benzanthracene + DNP in methyl alcohol	41	36	28	25
3,4-Benzpyrene + riboflavin in methyl alcohol	40	36	27	24
9,10-Dimethyl-1,2-benzanthracene + riboflavin in methyl alcohol	39	35	26	24
Reduced DPN + riboflavin in water	41	36	28	25
Riboflavin + oxidized cytochrome c in water	48	43	33	29

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The calculated values of R_0 for the several systems do not differ much for the same value of η_Q. It is unfortunate that the values of η_Q for the systems considered have not been determined yet. If these were already known they would probably be different for the different fluorescent substances in these systems which would result in different values of R_0. The significance of the results shown in the Tables I and II, is that the calculated values of R_0 are such, even for the lower values of the efficiency η_Q, that the energy resonance transfer mechanism might play a role in the transfer of energy in these systems. To investigate this aspect quantitatively we proceed as follows. From Table II we see that for the value 0.05 of the efficiency of fluorescence--which is of the same order of magnitude as that occurring in the photosynthetic pigments (DUYSENS[4], p. 83) R_0 is equal to 29 A for the aqueous system riboflavin + oxidized cytochrome *c*. To calculate the efficiency of transfer of energy by resonance we still have to know the distance between a riboflavin and an oxidized cytochrome *c* molecule. To obtain some estimate of this value we will first calculate the approximate size of each of those molecules. The diffusion constant D of cytochrome *c* in water is (PAUL[13]) $10.31 \cdot 10^{-7}$ cm^2 sec^{-1}. From the Einstein relation, based on the assumption of spherical molecules with radius r:

$$D = \frac{kT}{6\pi\eta r} \tag{7}$$

we find the value of r from that of D after substitution of the value $(1.38) \cdot 10^{-16}$ erg degree^{-1} for the Boltzmann constant k, 293° for T and 0.01 poise for the viscosity η. In this way we find for cytochrome *c* the value 21 A for r. We obtain from the value of the mobility of the DPNH cytochrome *c* reductase (Pig heart) at 20°: $2.5 \cdot 10^{-5}$ cm^2/volt/sec (MAHLER[24]) an estimate of 34 A for the value of r of this flavin containing enzyme. In this way we obtain, if we assume that a molecule of the latter enzyme touches a molecule of the cytochrome (as is, *e.g.*, the case in the model proposed by MAHLER[14]) a value of about 60 A for the distance between a riboflavin and a cytochrome *c* molecule. From FÖRSTER's theory it can easily be shown, that in a solution the efficiency E of energy transfer from an excited molecule of the fluorescent component to any molecule of the absorbing substance by the resonance mechanism is given by:

$$E = \sqrt{\pi}\,\frac{c}{c_0}\,e^{\left(\frac{c}{c_0}\right)^2}\left[1 - \Phi\left(\frac{c}{c_0}\right)\right] \tag{8}$$

in which

$$\Phi(u) = \frac{2}{\sqrt{\pi}}\int_0^u e^{-x^2}\,dx \text{ and } c_0 = \frac{3}{2\sqrt{\pi^3}N'R_0^3}$$

(*cf.* DUYSENS, *loc. cit.*, p. 81, after correcting some misprints.) From this formula we obtain for a distance of 60 A, corresponding in solution to a concentration of $1.8 \cdot 10^{-3}$ moles/l, between a riboflavin and an oxidized cytochrome *c* molecule for which $c_0 = 1.8 \cdot 10^{-2}$ moles/l, 16% for the efficiency E of transfer of energy by resonance. The assumptions made in the calculation of the distance between a riboflavin and a cytochrome *c* molecule have biased the efficiency E towards a lower value than it actually may have. If the efficiency of fluorescence of the riboflavin is 10%, instead of the 5% assumed above, the efficiency of transfer of energy by resonance becomes 22%. In the model proposed by MAHLER[14] the distance between the riboflavin and the

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porphyrin parts (which probably play the active roles in the mechanism considered here) are much closer than the distance of 60 A estimated above. Therefore, we have plotted in Fig. 5 the value of the efficiency of transfer of energy by resonance as function of the distance of the riboflavin and porphyrin parts for different values of the fluorescence efficiency of the riboflavin (5%, 10% and the max. of 100%).

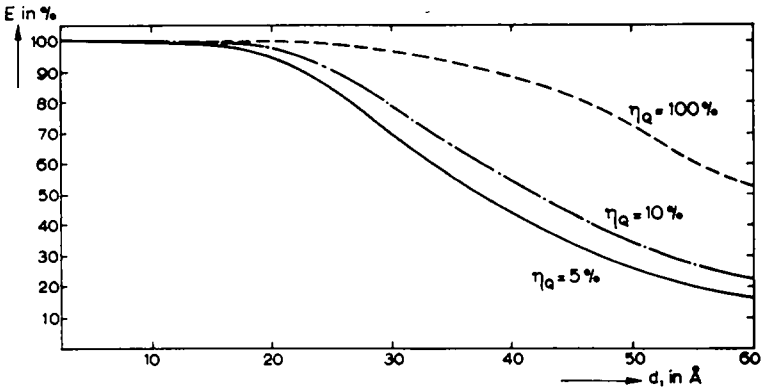

Fig. 5. The calculated efficiency, E, of resonance-energy transfer for the riboflavin-cytochrome c system as a function of the intermolecular distance, d; given for three different values of the fluorescence-yield of riboflavin.

As can be seen from Fig. 5 the efficiency is appreciable already for a distance of the order of 30 A.

In a solution there will also be diffusion of the energy-donor and acceptor molecules. Therefore, we shall compare the relative importance of the energy resonance transfer mechanism and of the diffusion mechanism mentioned in the beginning of this paper. We shall evaluate here the importance of each of these mechanisms for the process of the quenching of fluorescence of the fluorescent compound in these systems. For the evaluation of the diffusion mechanism the diffusion coefficients and the radii of the molecules involved are required and, therefore, we shall study in detail only a system for which those constants are known, namely, the aqueous system fluorescein + hemoglobin, since the lifetime of the fluorescence of fluorescein is also known.

On the basis of the energy resonance transfer mechanism FÖRSTER[15] arrived at the quenching curve given by:

$$\frac{\eta}{\eta_0} = 1 - \sqrt{\pi}\,\frac{c}{c_0}\,e^{\left(\frac{c}{c_0}\right)^2}\left\{1 - \Phi\left(\frac{c}{c_0}\right)\right\} \qquad (9)$$

in which η_0 is the fluorescence yield of the fluorescent substance without any quencher and η that in a solution in which the quencher has the concentration c,

$$\Phi(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-x^2}\,dx^* \quad \text{and} \quad c_0 = \frac{3}{2\sqrt{\pi^3}\,N'R_0^3}$$

* In checking the derivation of equation (9) we found that there are some misprints: $\Phi(x)$ in FÖRSTER's equation should not be $\frac{2}{\sqrt{\pi}}\int_x^\infty e^{-x^2}\,dx$ as FÖRSTER states (ref. [15]; ref. [11], p. 218), but $\frac{2}{\sqrt{\pi}}\int_0^x e^{-x^2}\,dx$, while the exponent of the e power in the right-hand side of (9) should be $\left(\frac{c}{c_0}\right)^2$ instead of $\left(\frac{c_0}{c}\right)^2$ (FÖRSTER[11], p. 218).

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From Table I we see that for the aqueous solution fluorescein + hemoglobin, R_0 = 61 A so that $c_0 = 2 \cdot 10^{-3}$ moles/litre. Substitution of this value into equation (9) leads to the curve plotted in Fig. 6 and marked R.T.

The quenching of fluorescence by diffusion of the excited molecule and a quencher molecule towards each other and deactivation of the former in the resulting encounter has been studied by many investigators, among whom we cite WAWILOW[16], SVESHNIKOFF[17], UMBERGER AND LA MER[18], BOWEN, BARNES AND HOLLIDAY[19], BOWEN AND METCALF; other references can be found in BOWEN AND WOKES[20] and FÖRSTER[11].

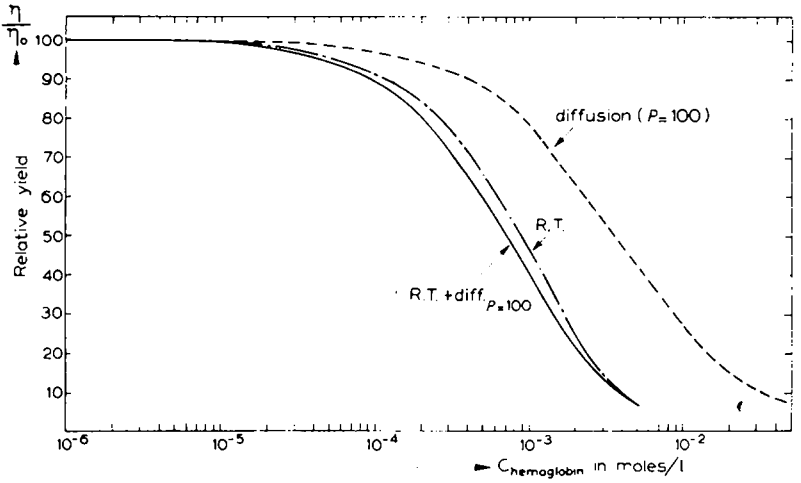

Fig. 6. Theoretical quenching curves for the fluorescence of fluorescein by oxyhemoglobin. Diffusion = diffusional-quenching; R.T. = resonance-transfer quenching; R.T. + diff. = combined quenching by diffusion and resonance-energy transfer; and P = probability of inactivation per encounter.

For the simple case in which the excited molecule either emits its energy of excitation as fluorescence or loses it in a collision with a quencher molecule, the Stern-Volmer law expressed by the equation

$$\frac{F_0}{F} - 1 = k c \tag{10}$$

holds, in which F_0 is the intensity of the fluorescence without quencher, F that in a solution with a quencher at a concentration c and k a constant, the so-called quenching constant (BOWEN AND WOKES[20], p. 53). The latter is determined by the number of encounters between an excited molecule and quencher molecules. This number was derived essentially by v. SMOLUCHOWSKI[21] and is given by FÖRSTER (ref.[11], p. 215):

$$n = 4\pi D R \tau N' \left(1 + \frac{R}{\sqrt{D\tau}}\right) \tag{11}$$

in which D is the sum of the diffusion constants, R the sum of the radii of the fluorescent and quencher molecules, τ the lifetime of the fluorescent molecule and N' the number of molecules per cm^3. The last term in the parentheses is the correction due to the transient solution of the diffusion equation describing the diffusion of the excited molecule and the quencher molecules during the lifetime of the former (FÖRSTER[11], p. 214). Multiplication of the right-hand side expression of equation (11)

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by P, the probability of quenching at an encounter of an excited molecule and a quencher molecule, yields for the quenching constant k the expression (BOWEN, BARNES AND HOLLIDAY[19]):

$$k = 4\pi DR\tau N' \left(1 + \frac{R}{\sqrt{D\tau}}\right)P \tag{12}$$

If we assume that the quenching of the fluorescence of fluorescein by hemoglobin is described by the simple formula (10) we can obtain the quenching constant k from equation (12) using the following data. For fluorescein in water $D = (3.7)10^{-6}$ cm^2 sec^{-1} (HODGES AND LA MER[22]) and for hemoglobin in water $D = (6.9)10^{-7}$ cm^2 sec^{-1} (EDSALL[23], p. 37), so that for the aqueous system fluorescein + hemoglobin $D = (4.4)10^{-6}$ cm^2 sec^{-1}. Using Einstein's relation (7) we find the value of r from that of D after substitution of the value $(1.38)10^{-6}$ erg degree^{-1} for the Boltzmann constant k, 298° for T and 0.00893 poise for η. In this way, we find for fluorescein $r = (6.6)10^{-8}$ cm and for hemoglobin $r = (35.5)10^{-8}$ cm, so that for the system fluorescein + hemoglobin we have $R = (42)10^{-8}$ cm. Substitution of the found values of D and R together with $\tau = (0.51)10^{-8}$ sec and $N' = 6.02 \cdot 10^{20}$ into (10) yields for the aqueous solution fluorescein + hemoglobin:

$$k = 271\, P \tag{13}$$

For the value $P = 1.00$ which means deactivation of the excited fluorescein molecule at each encounter with a hemoglobin molecule, we obtain $k = 271$ cm^3 and substitution of this value into equation (10) gives the quenching curve, marked diffusion ($P = 100$) in Fig. 6. As will be seen from Fig. 6 the resonance transfer mechanism predicts a stronger quenching than the diffusion mechanism with maximum P (= 100%).

As both mechanisms probably act simultaneously in the solution the resulting quenching will be stronger than that due to either mechanism separately. To derive in this case the relative fluorescence efficiency as a function of the concentration of the quencher we proceed as follows (similar to FÖRSTER[15]). The decay of the probability ϱ of excitation is described by the equation

$$-\frac{1}{\varrho}\frac{d\varrho}{dt} = n_e + n_l + n_t + n_c c_Q \tag{14}$$

in which n_e is the number of quanta emitted per sec from one excited molecule, n_t the number of quanta transferred per sec by resonance, $n_c c_Q$ that due to transfer in encounters due to the diffusion of the molecules and n_l that due to other processes such as internal conversion.

Introducing the lifetime τ_0 of the fluorescent molecule without quencher so that

$$\tau_0 = \frac{1}{n_e + n_l} \tag{15}$$

(FÖRSTER[11], p. 157) and,

$$n_t = \frac{1}{\tau_0}\left(\frac{R_0}{R_k}\right)^6 \tag{16}$$

(FÖRSTER[15]), in which R_k is the distance between the fluorescent molecule and the k^{th} quencher molecule, we obtain:

$$-\frac{1}{\varrho}\frac{d\varrho}{dt} = \frac{1}{\tau_0} + \frac{1}{\tau_0}\left(\frac{R_0}{R_k}\right)^6 + n_c c_Q \tag{17}$$

Integrating this equation with the initial condition $\varrho(0) = 1$ we obtain:

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$$\varrho(t) = e^{-\frac{t}{\tau_0}\left[1+\left(\frac{R_0}{R_k}\right)^6\right]\cdot n_c c_Q t} \tag{18}$$

For the average value $\bar{\varrho}(t)$ of $\varrho(t)$ due to all the quencher molecules we obtain, similar to FÖRSTER, from equation (18):

$$\bar{\varrho}(t) = e^{-\frac{t}{\tau_0} - n_c c_Q t - \frac{\sqrt{\pi}\, N R_0^3}{R_g^3}\sqrt{\frac{t}{\tau_0}}} \tag{19}$$

in which R_g is the radius of the volume V (which is assumed to be a sphere) and N the total number of quencher molecules in that volume, so that $c_Q = N/V$. Without quencher we have $N = 0$ so that $\varrho(t) = e^{-t/\tau_0}$ and we obtain for the efficiency η_0 of fluorescence

$$\eta_0 = C\int_0^\infty e^{-\frac{t}{\tau_0}}\,dt = C\tau_0 \tag{20}$$

in which C is a constant of proportionality. With quencher we have similarly for the efficiency η of fluorescence:

$$\eta = C\int_0^\infty \bar{\varrho}(t)\,dt \tag{21}$$

From equations (21) and (20) together with (19) we find after some rearrangements, usual in the case of error functions:

$$\frac{\eta}{\eta_0} = \frac{1}{1+kc}\left[1 - \sqrt{\pi}\left\{\frac{c}{c_0\sqrt{1+kc}}\right\}e^{-\left\{c_0\sqrt{1+kc}\right\}^2}\left\{1 - \Phi\left(\frac{c}{c_0\sqrt{1+kc}}\right)\right\}\right] \tag{22}$$

in which $\Phi(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-x^2}\,dx$ and $k = n_c\tau_0$ is the diffusional quenching constant considered above in equation (12). For the aqueous system fluorescein + hemoglobin the plot of η/η_0 versus C (Hemoglobin) according to equation (22) is also given in Fig. 6 and marked R.T. + diff. ($P = 100$). As might have been expected from what has been said above about the relative strength of quenching due to the diffusion and the resonance transfer mechanism in this system, we see that the curve for the combined mechanisms does not differ much from that for the resonance transfer mechanism alone.

Because physical constants are available for the DPNH-cytochrome *c*-reductase enzyme (from pig heart muscle, MAHLER[24]) we have used it as a prototype for mammalian flavoenzymes. We are aware that for this particular enzyme the bound flavine is non-fluorescent; however, since the characterization of the multiple flavoenzymes in mitochondria is far from complete (see, *e.g.*, CHANCE AND WILLIAMS[25]) it is possible that functional yellow enzymes other than diaphorase may well be fluorescent.

Regarding the presence or absence of fluorescence of the yellow enzymes as respectively supporting or negating the postulated mechanism of energy transfer we would like to emphasize a complimentary mechanism which may be of extreme significance but which we have been unable to treat because of lack of data. Our proposed mechanism completely ignores the possible functional significance of the triplet energy state for the fluorescent molecules. According to BECKER AND KASHA[26], however, if the quantum yield, Φ, for a fluorescent molecule be defined as

$$\Phi = \frac{\text{number of quanta emitted}}{\text{number of quanta absorbed}} \tag{a}$$

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then the total intrinsic quantum yield for a molecule (in the absence of external quenching) may then be

$$\Sigma_i \Phi_i = \Phi_{F^0} + \Phi_{P^0} + \Phi_{\text{int.}} = 1 \qquad \text{(b)}$$

where Φ_{F^0} is the intrinsic quantum yield of fluorescence, Φ_{P^0} is the intrinsic quantum yield of phosphorescence and $\Phi_{\text{int.}}$ is the intrinsic quantum yield for *internal degradation* by thermal steps. The denominator in eqn. (a) would, for biological systems, be replaced by a term such as "number of excited molecules chemically generated". KASHA[27] further emphasizes that, in general, the sum of $\Phi_{F^0} + \Phi_{P^0}$ may approach unity so that $\Phi_{\text{int.}}$ may be negligible in many molecules, at least in rigid solvents. This rigidity probably exists in fixed mitochondrial respiratory components! Of important consequence for biology is the fact emphasized by BECKER AND KASHA[26] that Φ_{F^0} and Φ_{P^0} are complimentary in magnitude. In view of this concept the failure of DPNH-cytochrome *c*-reductase to fluoresce takes on the added functional significance that the singlet excitation state may revert to a triplet state of lower energy than the singlet and, possibly of utmost importance, a longer lifetime, time in which useful biological work can be done (see the discussion by BECKER AND KASHA[26], on the possible significance of the triplet state of chlorophyll for photosynthesis). The fact that the non-fluorescent DPNH-cytochrome *c*-reductase facilitates the energy exchange that its name implies while fluorescent diaphorase will not, may, if our concepts are valid, be revealing a difference in the $\Phi_{F^0} + \Phi_{P^0}$ complementarity in the two mechanisms! It is interesting to point out here that the energy difference between the fluorescent and phosphorescent emissions of flavin is of the order of magnitude of the high energy phosphate bond. In conclusion, we should like to point out that triplet state energy may also be transferred by a resonance mechanism (see, *e.g.*, TERENIN AND ERMOLAEV[28]).

It has been the purpose of this work to bring the energy resonance transfer mechanism to the attention of the biochemists and biologists in general. This work also shows the importance of the determination of the efficiency of the radiations emitted by biological compounds.

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SUMMARY

Calculations have been made of overlap integrals expressing quantitatively the overlap of the fluorescence spectrum of one component and the absorption spectrum of another component in some interesting biochemical systems. From the calculated values the distance is obtained at which the efficiency of transfer from an excited molecule of the fluorescent component to a molecule of the absorbing component is 50%. For the riboflavin–cytochrome *c* system, the efficiency of energy transfer by resonance is calculated for a reasonable value of the fluorescence efficiency. The relative importance of the resonance mechanism and of the inactivation by collision is evaluated for the quenching of the fluorescence of fluorescein by hemoglobin in water.

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After this paper was submitted G. WEBER AND F. W. J. TEALE published (*Trans. Faraday Soc.*, 53 (1957) 646) the quantum yield for several important biological compounds including riboflavin. For this value of the quantum yield of riboflavin (0.26) we have recalculated the efficiency (E) of resonance-energy transfer for the riboflavin–cytochrome c system as a function of the intermolecular distance (d). The results are:

d in A	20	30	40	50	60
E in %	98	89	68	47	30.