Ferredoxin (Fd) is surface active; both its area/molecule, A, and surface potential, $\Delta V$, (at an air-water interface) vary with the pH of the subphase. From the surface isotherms (at pH 7.7) $A$ and $\Delta V$ are 167 Å$^2$ and 170 ± 20 mV, respectively, when the surface tension is 10 dyne/cm.

Fd and chlorophyll a (Chl) appear to form a mixed monomolecular film. At pH 7.7 a maximum interaction between Fd and Chl is observed when the monolayer contains a mole ratio of Fd/Chl $\approx 2$. Irradiation of Chl-Fd films, in nitrogen atmosphere, results in a bleaching of Chl and an increase of $\Delta V$. The quantum yield for this bleaching is estimated to be 0.4.

Materials and Equipment

The automatic Wihelm y plate surface balance used for this study is essentially the same as that described in detail previously. The main modification is that the trough is fabricated of aluminum and coated with teflon; the barriers are also teflon coated.

Crystalline chlorophyll a is prepared and stored according to AGLION, BROIDE and BRODY. The Fd obtained from Sigma Chemical Company (St. Louis, Missouri) is prepared for them from spinach by the method of TAGAWA and ARNON. These Fd preparations are not equal to the best reported in the literature. According to BORCHERT and WESSELS the ratio of the absorption bands for Fd is $A_{422}/A_{274} = 0.44$. The Fd preparations obtained from Sigma have a ratio of 0.37; on the basis of absorption ratios the purity is estimated to be 81 per cent. The area/molecule, A, calculated from the surface isotherms is based upon the number of Fd molecules on the surface. Assuming that the 19% protein impurity present in the Fd sample is about the same size as Fd then the value measured for A would be 19% too large. Unless otherwise noted all values of A given in this work have been corrected for this error.

In order to obtain a well defined value for the area/molecule of Fd the area is measured at a surface tension, $\pi$, of 10 dyne/cm ($A_10$) instead of at $\pi = 0$ ($A_0$). Determination of $A_0$ requires an extrapolation of the linear portion of the $\pi$-$A$ curve to $\pi = 0$. However, it is found that $\pi$-$A$ curves for Fd are not very linear, thereby making the determination of $A_0$ rather uncertain. The reproducibility of the measurements of $A_10$ is about ±5 per cent. The surface potentials, $\Delta V$, are also measured at 10 dyne/cm, i.e. $\Delta V_{10}$. The accuracy of measuring $\Delta V_{10}$ is ±20 mV.

Unless otherwise noted, all studies were carried out at pH 7.7, phosphate buffer, ionic strength 0.5; the same results are obtained when the ionic strength is decreased to 0.3. The pH of the buffered subphase...
at high ionic strength was measured with a pH meter (Radiometer Modell TTT/C, Copenhagen).

A block diagram of the recording spectrophotometer used to measure spectra of monolayers on an aqueous surface is shown in Fig. 1. Monochromatic light is obtained from a 1000 watt tungsten-iodide lamp in conjunction with a Bausch and Lomb monochromator (1200 lines/mm, dispersion 16 Å/mm) set for a half band width of 2.4 nm. The monochromatic light is chopped at 150 Hz, using mirrors mounted on the shaft of a synchronous motor, to produce two beams which are 180° out of phase with respect to one another. Both beams cross the air-water interface in the Langmuir trough 18 times. This multiple reflection is accomplished with parallel front surface mirrors, one above and one below the surface of the water. The reference beam, \( I_0 \), passes through a clean water surface at the same time the sample beam, \( I \), passes through the pigmented part of the surface. A thin teflon barrier separates the two sections of the trough. With the aid of a lens the two beams are focused on the same area of the photocathode of a photomultiplier tube (Dumont 6911, with S-1 response). The difference in intensity (\( I_0 - I \)), between the two beams is determined with the aid of a lock-in amplifier (Princeton Applied Research, Model HR-8). A reference signal for the lock-in is obtained from a transducer attached to the synchronous motor used to chop the light.

The output of the lock-in is multiplied by the reciprocal of the spectral response of the system, to give \( (I_0 - I)/I_0 \), using a curve follower (Hewlitt Packard model F-3B and model 2 DR-2 A). The curve follower contains a graph of the reciprocal of the experimentally measured \( I_0 \); the \( x \) axis follows the wavelength position on the monochrometer as explained below. The signal, corresponding to \( (I_0 - I)/I_0 \), is fed into a signal averager (Fabri-Tek Model 1062). An improvement in the signal-to-noise ratio is accomplished with the signal averager as spectra are measured repeatedly by scanning with the monochromator in both directions over a range of 512 nm.

A pulse is generated every 2 nm by the monochromator drive. Backlash in the gears amounts to less than 0.5 nm. These pulses, after proper shaping, advance the address in the Fabri-Tek memory; the Fabri-Tek in turn generates a voltage proportional to the address which drives the \( x \) axis of the curve follower (see Fig. 1). After 255 counts (256 addresses are used in the memory of the signal averager) the monochromator drive is reversed. The drive is shut off after a pre-determined number of scans (e.g. 510 counts or 2 scans).

### Results and Discussion

#### A. Ferredoxin Films

The surface isotherm of Fd at an air-water interface is shown in Fig. 2. The aqueous subphase contained phosphate buffer, pH 7.7, ionic strength 0.3. The value of \( A_{10} \) is 167 Å\(^2\) (after correcting for the purity of the Fd preparation). A high ionic strength in the aqueous subphase is necessary in order to keep the Fd on the surface. Only with ionic strengths of 0.3 or greater, are reproducible isotherms obtainable. At ionic strengths of 0.1 the value measured for \( A_{10} \) is decreased markedly.

A typical surface potential curve is shown in Fig. 2. From sample to sample the \( \Delta V - A \) curve could be displaced \( \pm 20 \text{ mv} \) from that shown in Fig. 2. The averaged value of \( \Delta V_{10} \) is 170 mv.
Variability in the purity of the Fd preparations is probably one of the main factors contributing to the low reproducability of $\Delta V$. The $\pi - A$ isotherms, on the other hand, are quite reproducible.

Neither the $\pi - A$ or $\Delta V - A$ properties of the Fd film are modified by irradiation in nitrogen or air. Addition of $10^{-3}$ M ascorbate to the aqueous subphase does not modify the stability of Fd to irradiation.

![Graph](image)

**Fig. 3.** Area/molecule of ferredoxin at $\pi = 10$ dyne/cm, $A_{10}$, and surface potential, $\Delta V_{10}$, as a function of pH are shown by the lower and middle curve, respectively. The aqueous subphase contained either phosphate buffer (shown by $\bullet$ - $\bullet$) or Tris buffer (shown by $\circ$ - $\circ$), at an ionic strength of 0.3. A measure of the reciprocal of the slope of the isotherm ($A_{10} - A_{16}$), is shown by the upper curve, where $A_{10}$ and $A_{16}$ are the area/molecule measured at 10 dyne/cm and 16 dyne/cm, respectively.

Both $A_{10}$ and $\Delta V_{10}$ for Fd are observed to vary with pH, see Fig. 3. Using Tris, as well as phosphate buffers the values of $\Delta V_{10}$ and $A_{10}$ were determined over the pH range 6.2 to 9.2. The ionic strength was held constant at 0.3. A maximum $A_{10}$ of 216 Å² is obtained between pH 7.0 and 7.4. A minimum of 151 Å² is obtained at about pH 8.2. The data for $\Delta V_{10}$ is similar to that obtained for $A_{10}$; a minimum of 140 mv is obtained for $\Delta V_{10}$ at pH 7.4 and a maximum of 210 mv at pH 8.2.

A measure of the reciprocal of the slope of the surface isotherm is given by $(A_{10} - A_{16})$, where the area/molecule is measured at $\pi = 10$ and $\pi = 16$ dyne/cm, respectively. It appears to be rather constant from pH 8.7 to 6.9; below pH 6.9 there is a decrease in the value of $(A_{10} - A_{16})$.

It is apparent that pH has a pronounced effect on the conformational state of the Fd protein. The exact pH which corresponds to the ambient chemistry of Fd in vivo is unknown. To determine which pH brings about the configuration of Fd which is most chemically active will require further study.

Studies of the interaction between Fd and Chl were confirmed to pH 7.7. Chl is stable only at alkaline pH's; at more acidic pH's Chl is rapidly degraded to pheophytin. Consequently interaction studies between Chl and the large area form of Fd, which occurs in the vicinity of pH 7.0, cannot be carried out.

**B. Mixed Films of Ferredoxin and Chlorophyll in Darkness Mixing**

Preparing a mixed film on an aqueous surface presents no problem if the solutes are miscible in the same solvent (e.g. Chl and vitamin K). However, in the case of Chl and Fd where the materials must be added to the surface separately (from benzene and aqueous buffer, respectively) there is some question as to whether the materials have mixed thoroughly on the surface.

If mixing has occurred the optical density of a Chl film (at a given $\pi$) should be decreased quantitatively by the amount of Fd added to the surface. To carry out this test a benzene solution of Chl is added to the surface; the benzene solvent is removed by evacuating and flushing the environmental chamber (housing the Langmuir trough) with nitrogen. At some area of the film, $A_{\text{Chl}}$ (in cm²), the optical density, $O.D_{\text{Chl}}$, of the red absorption band of Chl (at 672 nm) is measured. The film is then expanded to the gaseous state (where the area/molecule is $\gg A_{\theta}$) and Fd is added in small aliquots at several widely spaced loci on the surface. The optical density of Chl in the presence of Fd, $O.D_{\text{Fd+Chl}}$, is measured at a film are $A_{\text{Fd+Chl}}$. If mixing has occurred the following relationship should obtain $A_{\text{Fd+Chl}} \times O.D_{\text{Fd+Chl}} = A_{\text{Chl}} \times O.D_{\text{Chl}}$. A large inequality in this relationship would indicate poor mixing. On the other hand, a small disparity of this relationship could indicate either that the mixing is still not perfect or that the Chl extinction coefficient has been modified. Such a modification could either result from complexation between Chl and Fd or from the effect of the index of refraction and dielectric constant of Fd on Chl.
The O.D.'s and A's for a typical experiment are given in Table I. Both sets of data were measured at a surface tension of \( \sigma = 8 \) dyne/cm; the mole ratio of \( (Fd)/(Chl) \) is 1.3. These O.D.'s, determined from the spectra shown in Fig. 4, are for a light beam crossing a single film 18 times. As can be seen from the data given in Table I the product of O.D. \( \times \) A remains rather constant, thereby showing good mixing between Fd and Chl on the surface. It is assumed mixing occurs at the other mole ratios used in this work.

![Figure 4](https://via.placeholder.com/150)

**Table I. Spectral Properties of a Chl Film and a Mixed Film of Chl and Fd.** * Measurements made at \( \sigma = 8 \) dyne/cm. ** Ratio of absorption bands at 437 nm (Blue) and 672 nm (Red). Interaction: The there is an interaction between Chl and Fd in a mixed monomolecular film may be demonstrated in several ways.

<table>
<thead>
<tr>
<th>Film</th>
<th>O.D. 672</th>
<th>Area [cm(^2)]</th>
<th>O.D. ( \times ) Area</th>
<th>( \varepsilon_{672} ) [cm(^2)/mole]</th>
<th>(Blue/Red)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl</td>
<td>0.063</td>
<td>290</td>
<td>18.2</td>
<td>( 6.6 \times 10^7 )</td>
<td>1.25</td>
</tr>
<tr>
<td>Chl + Fd</td>
<td>0.031</td>
<td>567</td>
<td>17.6</td>
<td>( 6.1 \times 10^7 )</td>
<td>1.47</td>
</tr>
</tbody>
</table>

Good mixing occurs between Fd and Chl providing the total amount of material on the surface is "small". The criteria for "small" is that in the expanded state the presence of Fd and Chl on the surface does not detectably modify the surface tension of pure water; this criteria insures that the surface active materials are in the gaseous phase.

That there is an interaction between Chl and Fd in a mixed monomolecular film may be demonstrated in several ways. Addition of Chl to a Fd film gives rise to a small increase of \( \Delta V \) and a decrease of the slope of the isotherm. If there were no interaction between Chl and Fd the observed \( \Delta V \) would be determined by the relationship:

\[
\Delta V = [Fd] + [Chl] = \Delta V_{Fd} + \Delta V_{Chl}.
\]

The value of \( \Delta V_{Fd} \) and \( \Delta V_{Chl} \) at 10 dyne/cm is 170 and 280 mv\(^{-10}\), respectively. The value of \( \Delta V \) measured experimentally is far smaller than that predicted by the above relationship; consequently, another term is required in the above relationship to account for an interaction or complex between Fd and Chl (i.e. \( \Delta V_{complex} \)). The accuracy of the measurements of \( \Delta V \) was not sufficient to allow an exact determination of \( \Delta V_{complex} \).

At \( \sigma = 8 \) dyne/cm the red absorption maximum of Chl is at 672 nm. The extinction coefficient calculated for the red maximum, \( \varepsilon 672 \) in a pure and mixed monolayer is given in Table I. This value of \( \varepsilon 672 \) is comparable to extinction coefficients for Chl in solution. With Chl–Fd films \( \varepsilon 672 \) varies over a narrow range. Whether this variability is due to the experimental accuracy or is a function of the mole ratio of Chl and Fd in the film is yet to be determined. In Table I it can be seen that \( \varepsilon 672 \) is slightly different for Chl and Chl–Fd films; as mentioned above this might be a further indication of an interaction between Chl and Fd.

The major spectral change observed in mixed film is an increase in the ratio of the blue to red absorption bands; a part of this increase is due to
the absorption of Fd in the blue, and part may be ascribed to an interaction between Chl and Fd.

The wavelength of the red and blue maxima and the blue/red ratio for the Chl a monolayer found in this work agree very well with that previously reported by Jacobs et al.\textsuperscript{11} (i.e. 435 and 675 nm, blue/red = 1.25). The values given by Bellamy et al.\textsuperscript{12} (i.e. 440 and 680 nm, blue/red = 1.19) differ somewhat from those given in Table I. However, this disparity in position of the absorption maxima is to be expected as it is known from the work of Trurnit and Colmano\textsuperscript{13} that both the red and blue absorption maximum are shifted to longer wavelengths as the surface tension or molecules/cm\textsuperscript{2} is increased. Bellamy et al. made their measurements at \(\pi = 15\) dyne/cm, while \(8\) dyne/cm was used to obtain the data in Table I. For the same reason, the \(O.D.\) of the red peak reported in Table I is about one-third the value reported by Bellamy et al.\textsuperscript{12} and about one-half that reported by Colmano\textsuperscript{13}. Since the concentration of Chl on the surface (Chl/cm\textsuperscript{2}) is directly related to \(\pi\) then \(O.D.\), which is also proportional to Chl/cm\textsuperscript{2}, is a function of \(\pi\). Therefore, the difference in \(O.D.\)’s between those given in Table I and those of other workers\textsuperscript{12,13} arise from the lower value of \(\pi\) used to measure spectra in this work.

Additional evidence for an interaction between Chl and Fd is based upon the surface isotherms of mixed films in air. The isotherm of a mixed film of Fd and Chl is labeled EXPER in Fig. 5. In this figure the isotherm is plotted as a function of the total film area, \(A\), in cm\textsuperscript{2}. Surface isotherms of the Chl sample and the Fd sample shown in Fig. 5 are determined in separate experiments. The sum of the individual Chl and Fd isotherms is labeled THEOR. It can be seen that the THEOR isotherm is considerably smaller than the EXPER one. In all the mixed films of Chl and Fd studied the EXPER isotherm is invariably greater than the THEOR isotherm.

The fact that EXPER > THEOR is a sufficient condition to demonstrate an interaction or complexation between Chl and Fd. If there were no interaction between Chl and Fd then it would be necessary that the EXPER and THEOR isotherms equal one another. (Of course the latter equality is not a sufficient condition to demonstrate the absence of an interaction.)

The increase in size upon complexation would be consistent with a conformational change of the protein or a change in orientation of Fd or Chl thereby increasing its projected area on the surface. However, with the data available one cannot distinguish between these two or any other possible mechanisms to account for the increase in surface area upon complexation.

The difference between the two isotherms (EXPER - THEOR) varies with the mole ratio (Fd)/(Chl). In a relationship between (EXPER - THEOR) and (Fd)/(Chl) it is expected that (EXPER - THEOR) will have a maximum at some value of (Fd)/(Chl) where all (or almost all) Fd and Chl molecules are in a complexed form. At values of (Fd)/(Chl) greater or smaller than that for complete complexation, the value of (EXPER - THEOR) will approach zero. A graph of the difference (EXPER - THEOR), in cm\textsuperscript{2}, measured at \(\pi = 10\) dyne/cm as a function of (Fd)/(Chl) is shown in Fig. 6.
Before the size of the complex can be calculated it is necessary to know the stoichiometry of complexation. There are many possibilities for a complex between Fd and Chl, e.g. Chi : Fd : Chi, Chi : Fd, Fd : Chi : Fd. A lower limit for the stoichiometry may be determined from the relationship between (EXPER - THEOR) as a function of (Fd)/(Chi). If the complex consisted of one Fd to one Chl then it would be expected that the largest value for (EXPER - THEOR) would occur at about (Fd)/(Chi) = 1. In Fig. 6 it can be seen that the maximum for (EXPER - THEOR) occurs closest to (Fd)/(Chl) = 2. Thus it appears that the complex contains 2 Fd : 1 Chi [i.e. (Fd) - (Chl) - (Fd)].

If indeed the complex on the surface consists of 2 Fd : Chl then the area may be estimated. From Fig. 6 at (Fd)/(Chl) = 2 it is seen that (EXPER - THEOR) = 120 cm$^2$; the experimentally measured film area at 10 dyne/cm is 651 cm$^2$. So that

\[
\frac{\text{(EXPER - THEOR)}}{\text{EXPER}} = 0.182 \quad \text{or} \quad \frac{\text{EXPER}}{\text{THEOR}} = 1.22.
\]

The $A_{10}$ for Fd and for Chl is 166 and 93 Å$^2$, respectively; therefore, the theoretical $A_{10}$ for the complex is 425 Å$^2$. Substituting this value for (THEOR) in the expression above gives 520 Å$^2$ as the experimental $A_{10}$ for the complex.

C. Mixed Films of Ferredoxin and Chlorophyll in Light

That there is a photoreaction between Chl and Fd in a mixed film, in a nitrogen atmosphere, is shown by: a change in slope of the surface isotherm, a decrease in are of the Chl-Fd film, an increase in $\Delta V$ and a bleaching of the Chl absorption spectrum.

Irradiation of the Chl-Fd films, in N$_2$, invariably results in an increase in the slope of the surface isotherm. In some experiments the slope increased 30 per cent. However, the slope never increased to that obtained in the absence of Chl. In Fig. 5 may be seen the effect of light on a Chl-Fd film, in this case the aqueous phase contained $10^{-4}$ M ascorbate, phosphate buffer pH 7.7, ionic strength 0.3. A measure of the reciprocal of the slope of the isotherm ($A_{10} - A_{20}$) before irradiation is 200 cm$^2$. After irradiation the value decreased to 155 cm$^2$. Associated with the change in slope, the area of the Chl-Fd film also decreased.

The surface potential of the Chl-Fd film, in nitrogen, usually increases after irradiation. The magnitude of the change of $\Delta V$ is about 20 mv, but varies considerably between experiments. The origin of this variability is not yet established.

Previously it was shown$^{14}$ that irradiation of a Chl film in the presence of ascorbate and a nitrogen atmosphere results in a small increase of $A_0$ and a decrease of $\Delta V$. It was presumed that Chl was reduced by ascorbate in light. In the present experiments it seems that Chl is oxidized by Fd in light. It is not yet clear if the presence of ascorbate in the subphase has any influence on this light reaction. Perhaps when Chl is complexed with Fd the sites of the Chl molecule with which ascorbate can react are already complexed with the Fd or the Chl is oriented so that these sites are not in proximity to the aqueous phase.

When the film is irradiated in air there is usually no significant change of the surface isotherm, although in a few experiments a small decrease in $A_{10}$ was observed. A quantitative study of the effect of light on the isotherm as a function of mole ratio of (Fd)/(Chl), intensity of light, etc. was not carried out.

These results are quite different from those obtained when Chl films are irradiated in the absence of Fd$^4$. It was shown that irradiation with bright
light for one hour in nitrogen gave almost no change in the surface isotherm. On the other hand, irradiation in air resulted in a photooxidation of Chl as indicated by a large increase of $A_0$.

The effect of light on the spectral properties of a Chl-Fd film, in a nitrogen atmosphere, is shown by decreases in the ratio of absorption bands ($\frac{A_{672}}{A_{437}}$, $\frac{A_{672}}{A_{499}}$, and $\frac{A_{672}}{A_{631}}$), as well as by a general bleaching of the chlorophyll spectrum (see Table II). The ratios of absorption bands measured for unirradiated films of Chl and Chl-Fd are also given in Table II. While there are differences in the peak ratios between the spectra of irradiated and unirradiated Chl there is no evidence of any new absorption bands or spectral shifts.

Irradiating the Chl-Fd film for 10 min with a low pressure mercury lamp ($7.2 \times 10^2$ ergs/cm$^2$ sec) results in a general bleaching of the Chl absorption spectrum as shown by the value of $\varepsilon_{672}$ (see Chl + Fd + $h\nu$ in Table II). The value of $\varepsilon_{672}$ decreases from 6.4 to $3.4 \times 10^7$ cm$^2$/mole. The calculations for $\varepsilon_{672}$ in Table II are based on the assumption that the total number of Chl molecules on the surface ($9.9 \times 10^{15}$) remains constant.

After the 10 min irradiation the film is left in the dark for 10 minutes. No significant spectral changes are noted after this dark period, indicating that the reaction is not reversible (see Chl + Fd + $h\nu$ + dark in Table II). Additional irradiation for another 10 min results in further bleaching of the Chl film and changes in absorption ratios (see Chl + Fd + $h\nu$ + dark + $h\nu$ in Table II).

The nature of the photoreaction is most likely a reduction of Fd and oxidation of Chl by Fd (and hopefully not by some impurity in the Fd preparation), i.e. Fd + Chl $\rightarrow$ Fd$^+$ + Chl$^\circ$. A proof for this reaction would require the Chl absorption spectrum on the surface changing to that of oxidized Chl and the bleaching of Fd. Bleaching of the red absorption band of Chl is a characteristic of oxidation. There is no evidence of the increase of absorption at 595 or 750 nm which accompanies the oxidation of Chl by ferric chloride in methanolic solution. Nevertheless, it is possible that at an air-water interface and in the presence of Fd oxidized Chl does not have absorption bands around 595 or 750 nm.

An estimate of the quantum yield of photobleaching is made as follows. The total number of Chl molecules on the surface at the start of the experiment, given in Table II, is $9.9 \times 10^{15}$. The decrease in $\varepsilon_{672}$, which results after 10 min light, is presumed to result from a partial bleaching of Chl. The number of molecules bleached or oxidized is readily calculated from the data to be $4.6 \times 10^{15}$ molecules. The possibility of absorption by oxidized Chl at 672 is ignored in this estimate.

To calculate the amount of light absorbed an effective absorption coefficient, $\bar{\varepsilon}$, for Chl is determined for the low pressure mercury lamp. The value of $\bar{\varepsilon}$, equal to $12.6 \times 10^6$ cm$^2$/mole, is obtained by measuring the fraction of mercury light absorbed by a solution of Chl of known concentration. The fraction of light absorbed by the film is $\bar{\varepsilon}$ divided by the area/molecule; this gives $4.6 \times 10^{-4}$.

The intensity of light from the Hg lamp on the surface in quanta is approximately $10^{14}$ quanta/cm$^2$ second. After 10 min irradiation over an area of 450 cm$^2$ the total amount of quanta absorbed by the Chl-Fd film is $1.2 \times 10^{16}$. Dividing the number of molecules of Chl oxidized by the number of quanta absorbed gives a conservative estimate for the quantum yield of about 0.4. This number may even be higher as no account is made in this calculation for the fact that as the film is bleaching less light is absorbed.

### Table II. Special Properties of Chl—Fd Films.

<table>
<thead>
<tr>
<th></th>
<th>$A_{437}$</th>
<th>$A_{499}$</th>
<th>$A_{631}$</th>
<th>$\varepsilon_{672}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl</td>
<td>0.80</td>
<td>—</td>
<td>—</td>
<td>6.6</td>
</tr>
<tr>
<td>Chl + Fd</td>
<td>0.72</td>
<td>4.2</td>
<td>3.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Chl + Fd + $h\nu$</td>
<td>0.63</td>
<td>2.8</td>
<td>3.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Chl + Fd + $h\nu$ + dark$^a$</td>
<td>0.68</td>
<td>1.2</td>
<td>1.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Chl + Fd + $h\nu$ + dark$^b$ + $h\nu$</td>
<td>0.38</td>
<td>—</td>
<td>—</td>
<td>1.5</td>
</tr>
</tbody>
</table>

$^a$ Each irradiation was 10 min with a low pressure Hg lamp ($7.2 \times 10^2$ ergs/cm$^2$ sec). $^b$ The dark period lasted 10 minutes.
I would like to acknowledge the invaluable assistance of MARIE-NOELLE JAUMAIN in carrying out the experimental portion of this work, and of Dr. B. AUBREY and Mr. J. WOODLEY in the construction of the recording spectrophotometer for monomolecular films. This work was supported in part by research grants from NIH (RO-1-EY 00173) and NSF (GB 18 354). The author was supported, in part, by a U.S. Public Health Service Research Career Program award (K3—GM17918).


Freisetzung von $^{51}$Chrom aus markierten HeLa-Zellen nach Poliovirus-Infektion

Release of $^{51}$Chromium from Labeled HeLa-Cells after Infection by Poliovirus

KLAUS KOSCHEL
Institut für Virologie der Universität Würzburg


HeLa-cells labelled with $^{51}$Chromium start to release $^{51}$Cr into the medium 5—6 hours after infection with poliovirus. $^{51}$Cr-release is only observed when the viral genome is intact. Virus particles previously irradiated by UV do not cause chromium-release. Furthermore, protein synthesis must be allowed to proceed for 3—4 hours. For the effect to take place. Viral RNA synthesis is not required. Chromium release, release of viral particles and leakage of cellular proteins into the surrounding medium follow the same time course and have identical requirements. They are therefore considered to reflect a single phenomenon, a leakiness of the cell due to virus infection.

In der Literatur wurden verschiedene Methoden beschrieben, den Eintritt des cytopathogenen Effekts (CPE) virusinfizierter Zellen bis zum Zelltod zu verfolgen:

1. Morphologische Kriterien 1,3, einschließlich der Abrundung von Zellen und ihre Loslösung von Glasperflächen.
3. Freisetzung zellulärer Proteine (teils lysosomaler Enzyme) ins Medium 5,6.

Sonderdruckanforderungen an: Dr. KLAUS KOSCHEL, Institut für Virologie der Universität, D-8700 Würzburg, Versbacher Landstraße 7.

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