Table II
Total blood lipids, blood cholesterol and total area lipoprotein in 4 groups of mice

Types of Animales	Total Lipids (mg/100 ml)	Blood Cholesterol (mg/100 ml)	Total Area Lipoprotein (cm^2)
Thin Littermates .	632 ± 148	116 ± 10	130
Obese Hyperglycemic	955 ± 239	170 ± 24	240
Thin Swiss	872 ± 71	112 ± 41	145
Goldthioglucose Swiss	1214 ± 55	125 ± 24	340

Duplicate strips were stained with Sudan Black B to locate the lipides. Table II gives data on total lipids, stainable lipides and cholesterol in the blood of these different groups. Methods and data on total lipids and cholesterol have been published[9,10]. The distribution of the lipides (Figure) is similar to that of the rat: lipide materials are found throughout the complete serum protein pattern. The picture is thus in contrast to the sharp α- and β-peaks seen in strips from human sera. There are again differences between groups of mice. The strips from the serum of the thin littermates of obese-hyperglycemic mice showed little lipide in the albumin region but 3 rather distinct bands in the globulin area; 2 in the β-region and 1 in the α-2. The strips from the swiss mice showed heavy lipid staining in the albumin region with light bands in the β- and α 2-globulin regions. Strips from both types of obese mice showed much heavier lipide staining than did strips from thin mice. The albumin fraction was particularly heavy in the obese hyperglycemic mice, somewhat lighter and more mobile in the goldthioglucose obese mice. No distinct bands were observed in the β-or α-region for either type obese mice.

In summary, electrophoresis reveals well-defined patterns of blood protein and blood lipids in mice; these are in many ways intermediary in appearance between patterns obtained for rats and patterns obtained for man. There are strain differences and also differences between obese mice and non-obese mice. The latter appear to be non-specific for the type of obese syndrome, differences due to the increase in fat transport in obese animals overshadow specific differences such as the hypercholesterolemia of obese-hyperglycemic mice contrasted to the normal cholesterol of goldthioglucose mice[1].

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Résumé

L'électrophorèse sur papier permet un fractionnement des protéines et des lipides du sang chez les souris. La distribution des diverses fractions est intermédiaire entre celle du rat et celle de l'homme. Il existe des différences entre diverses souches de souris, ainsi qu'entre souris obèses et non obèses. Les méthodes employées ici, quoique faisant ressortir les anomalises accompagnant l'obésité, ne permettent pas de différencier entre les divers types d'obésités.

[9] J. MAYER and D. J. SILIDES, Exper. *14*, 96 (1958).
[10] J. MAYER, C. ZOMZELY, and F. J. STARE, Exper. *13*, 1 (1957).

The Photoelectric Theory of Photosynthesis IV. The Chromophore Area of Chlorophyll

The Photoelectric Theory of Photosynthesis. It has been postulated by the author in the photoelectric (or photoconductive) theory[1] of photosynthesis, that light-activated chlorophyll transfers electrons to an appropriate oxidant and removes electrons from water. It was pointed out that the primary process of photosynthesis may thus be viewed as a flow of electrons activated by light, with the chlorophyll functioning as a sort of conducting bridge between two half cells, in one of which a water molecule is oxidized by loss of two electrons ($H_2O \rightarrow 2\,e^- + 2H^+ + 1/2\,O_2$), and in the other an oxidant[2], intimately associated with the chlorophyll in the chloroplast, is reduced by gaining two electrons. Stated in an alternative way, a stream of photons strikes the solid chlorophyll phase and creates a stream of conduction electrons which reduce the oxidant. The electron holes left in the chlorophyll phase are constantly eradicated by electrons flowing from water to the chlorophyll[3].

This theory has proved useful in explaining certain experimental results, such as, for example, the recently observed quenching of chlorophyll fluorescence by triphenyl tetrazolium chloride in the presence of hydrazine[4]. Here the organic chloride acts as the electron acceptor and hydrazine replaces water as electron donor. The photoconductive theory has recently been placed on a much stronger experimental basis by measurements on the electron spin resonance of photoactivated chlorophyll by COMMONER, HEISE, and TOWNSEND[5].

Electronic Spectrum of Mg in Chlorophyll. It has been demonstrated by the author[6] that there is an extremely close correspondence of the chlorophyll absorption bands with the emission lines of electronically excited states of the Mg^0 atom and the Mg^+ ion in the visible and near ultraviolet regions of the spectrum. Tables I and II show these comparisons.

These spectroscopic considerations provide excellent evidence that the actual site of initial electron loss by the chlorophyll molecule is at the *Mg atom.* Such electron loss would result in the intermediate conversion of the chlorophyll molecule to a positively charged radical-ion representing an oxidized chlorophyll species:

$$\mathrm{Chl-Mg^{\circ}} \xrightarrow{h\nu} \mathrm{Chl-Mg^{+}} + e^- . \qquad (1)$$

In addition to this *direct photoelectric process*, the following equations representing the photosynthetic mechanism were proposed (Ox represents the electron acceptor):

$$\mathrm{Chl-Mg^{\circ}} \xrightarrow{h\nu} \mathrm{Chl-Mg^{*}} \qquad (2)$$

$$\mathrm{Chl-Mg^{*} + Ox} \longrightarrow \mathrm{Chl-Mg^{+} + Ox^{-}} \qquad (3)$$

$$\mathrm{Chl-Mg^{*} + Ox^{-}} \longrightarrow \mathrm{Chl-Mg^{+} + Ox^{=}} \qquad (4)$$

$$\mathrm{2\,Chl-Mg^{+} + H_2O} \rightarrow \mathrm{2\,Chl-Mg^{\circ} + 2\,H^{+} + 1/2\,O_2} \qquad (5)$$

$$\mathrm{Chl-Mg^{+}} \xrightarrow{h\nu} \mathrm{Chl-Mg^{+*}} \qquad (6)$$

$$\mathrm{Chl-Mg^{+*} + Ox} \longrightarrow \mathrm{Chl-Mg^{++} + Ox^{-}} \qquad (7)$$

$$\mathrm{Chl\text{–}Mg^{++} + H_2O} \rightarrow \mathrm{Chl\text{–}Mg^{\circ} + 2\,H^{+} + 1/2\,O_2} \qquad (8)$$

[1] L. S. LEVITT, Science *118*, 696 (1953).
[2] Possibly the oxidant is 6,8-thioctic acid, the prosthetic group of pyruvic oxidase.
[3] L. S. LEVITT, Abstracts of Papers, Minneapolis Meeting of the Amer. chem. Soc., p. 67C (1955).
[4] E. FUJIMORI, J. Amer. chem. Soc. *77*, 6495 (1955).
[5] B. COMMONER, J. J. HEISE, and J. TOWNSEND, Proc. nat. Acad. Sci., Wash. *42*, 710 (1956).
[6] L. S. LEVITT, Science *120*, 33 (1954).

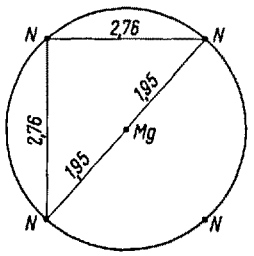
Table I

Comparison of near infrared[7] and visible[6] absorption bands* of ether solutions of chlorophyll with emission lines[20] of Mg^0 and Mg^+. Wavelengths are in mμ. Principal bands of the chlorophylls are in italics

Mg^0	Mg^+	Chl a	Chl b	Chl c
881		875[c]		
	655	*660*	655[fv]	
	635	613	*642*	*635*[a]
			594	
			567	
553		574	553	
			540	
		530		
517		511		
501		498	501	
457		464	457	
	448		*452*	*450*
	439	440[v]		
			435[v]	
435		*430*	428	
406		409		

* Complete bibliography available in RABINOWITCH[14], pp. 606, 668, 736, 801, 826; [f] Fluorescence value; [v] *In vivo*; [a] Value for both absorption and fluorescence; [c] In CCl_4.

The correlation of the chlorophyll and Mg spectra has led MARCUS[8] to the concept of chlorophyll as, essentially, a neutral Mg atom 'solvated' by pheophytin. On this basis, stabilization of the univalent Chl – Mg^+ ion can be explained[9] in the same manner as the stabilizing effect of solvation on the Mg^+ ion produced during the anodic oxidation of Mg[10].



The Molecular Size of Chlorophyll and the 'Chromophore Area'. The molecular area of the planar porphin square of chlorophyll is about 105 square Ångstroms[11], and the molecular area of the entire chlorophyll molecule, including all side chains on the porphin square has been estimated[12] at around 240 $(Å)^2$. The bond distance, r, between the Mg atom and any of the four adjacent pyrrole nitrogen atoms in the very center of the chlorophyll molecule has been calculated[13] to be around 1·95 Å. This then is the radius of the 'hole' between the four central N nuclei (see Figure), and the area[14] of the 'hole' may be calculated to be $\pi r^2 = 12{\cdot}0$ $(Å)^2$.

[7] L. S. LEVITT, Abstracts of Papers, New York Meeting of the Amer. Chem. Soc., p. 68 C (1957).

[8] R. J. MARCUS, Science *123*, 399 (1956).

[9] B. J. ZWOLINSKI, R. J. MARCUS, and H. EYRING, Chem. Rev. *55*, 157 (1955).

[10] R. L. PETTY, A. W. DAVIDSON, and J. KLEINBERG, J. Amer. chem. Soc. *76*, 262 (1954).

[11] E. RABINOWITCH, *Photosynthesis and Related Processes* (Interscience, New York 1945), p. 448.

[12] J. A. KETELAAR and E. A. HANSON, Nature *140*, 196 (1937).

[13] L. S. LEVITT, J. chem. Phys. *28*, 515 (1958).

[14] Calculated as a square area instead of a circle, one gets $4(1{\cdot}95)^2 = 15{\cdot}2$ $(Å)^2$.

Table II

Comparison of ultraviolet[6] absorption bands of chlorophyll with emission lines[20] of magnesium. Wavelengths in mμ (data of HARRIS and ZSCHEILE[21]).

Mg^0	Mg^+	Chl a	Chl b
384	385	380	375
333			334
323		326	325
309	310	312	310
307			306
292; 294	294; 297	296	290
278; 285	280	282	

Now the photon capture cross-section ('chromophore area') of a light-absorbing molecule has been defined[15,16] as the localized area in a molecule where a photon of a given wavelength is effectively absorbed. This area may be calculated[15,17] from the molar extinction coefficient (absorptivity) of a substance at a given wavelength by the equation,

$$\sigma = k\, a_M \qquad (9)$$

where σ is the capture cross section in $(Å)^2$, a_M is the molar absorbtivity, and k is a constant given by

$$k = 10^3 \cdot \frac{(2{\cdot}303)\ (10^8\ Å/cm)^2}{(6{\cdot}023 \times 10^{23})/3} = 1{\cdot}15 \times 10^{-4}, \qquad (10)$$

the factor 1/3 arising from the fact that only this fraction of the molecules in a solution are, on the average, oriented in the proper direction for absorption of a photon[15,17].

For the main (blue) absorption peak of chlorophyll[18] at 430 mμ, $a_M = 1{\cdot}20 \times 10^5$ and therefore $\sigma = 13{\cdot}8$ $(Å)^2$. Since this cross section is for chlorophyll's highest peak[19], it is the largest area over which a photon of any wavelength can be absorbed by chorophyll molecules. Now it is seen that this area, 13·8 $(Å)^2$, compares favorably with the value 12·0 $(Å)^2$ calculated above for the $Mg-N_4$ central area of chlorophyll, but it is significantly smaller than the area (105$(Å)^2$) of the square porphin ring.

These size relationships are compatible with the photoelectric theory[1] of photosynthesis since it is postulated therein that it is specifically the Mg atom of chlorophyll which is electronically excited by light absorption.

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Résumé

La superficie de la coupe transversale de captation des photons, calculée à partir de l'absorptivité molaire par l'équation de Braude, est de 13,8 $(Å)^2$ pour la bande d'absorption principale de chlorophylle à 430 mμ. Cette superficie peut se comparer favorablement à la valeur 12,0 $(Å)^2$,

[15] E. A. BRAUDE, J. chem. Soc. *1950*, 379.

[16] F. C. STRONG, Analyt. Chem. *24*, 338 (1952).

[17] F. C. STRONG, Applied Spectroscopy *7*, 1 (1953).

[18] A. S. HOLT and E. E. JACOBS, Amer. J. Bot. *41*, 710, 718 (1954); J. chem. Phys. *22*, 142 (1954).

[19] For chlorophyll's second principal peak at 660 mμ, the photon capture cross section may be calculated to be 10·4 $(Å)^2$.

[20] C. D. HODGMAN, *Handbook of Chemistry and Physics* (Chemical Rubber Publ. Co., Cleveland 1952), p. 2381.

[21] D. G. HARRIS and F. P. ZSCHEILE, Bot. Gaz. *104*, 515 (1943).

calculée pour la superficie centrale circulaire Mg–N_4, de la chlorophylle, mais elle est, très nettement plus petite que la superficie du noyau carré de la porphyrine, qui est de 105 (Å)[2]. Ce résultat est interprété comme preuve supplémentaire pour la théorie photoélectrique de photosynthèse, selon laquelle l'auteur a postulé que c'est l'atome Mg de chlorophylle qui est excité électroniquement par absorption de lumière.

Cytochrome *h* from *Aplysia depilans* L.

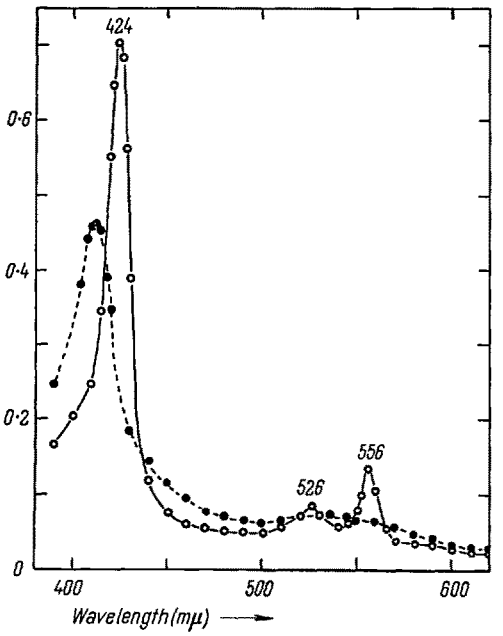
An hemochromogen very similar to Helicorubin has been recently extracted and purified from the digestive gland of *Helix pomatia*[1]. The pigment, which was found to have several properties in common with the cytochromes of the *b* and *c* groups, has been named Cytochrome *h*. According to KEILIN, Cyt. *h* is genetically related to Helicorubin, this latter probably representing its extracellular from.

In the course of an investigation on the cytochrome system of marine Gastropods[2] we have found that, by a method similar to that described for Helix, a pigment can be extracted and purified from the Hepatopancreas of Aplysia giving a spectrum very similar to Cyt. *h* both in the reduced and in the oxidized forms. An account of the isolation and purification of the pigment, which is here indicated as Aplysia Cytochrome *h*, followed by some of its chemical and biological properties, is given in this note.

Aplysia is an herbivorous marine Gastropod which has no oxygen carrier pigment in the blood and no Helicorubin in the intestine nor in any other part of the body[3]. Two species, which are very common in the Bay of Naples, were used: *A. depilans* and *A. limacina*. The best source of material was found to be *A. depilans* and this was therefore preferred for most of the experiments.

The animal was dissected under sea water and the hepatopancreas liberated from its ligaments and from the intestine. For each preparation 500–600 g of tissue from 20–30 animals was used. Fresh organs or acetone powders were used. Acetone powders were found more practical on account of the great amounts of green and yellow pigments which were removed during the preparation. They were obtained by homogenization in a Waring blendor for 1–2 min in cold (– 20°C) acetone, followed by rapid filtration and washing until no color was extracted. The powdered material was extracted for 2 h with alkaline (pH 8) water and centrifuged; the sediment was discarded and the supernatant was treated with 1–2% basic lead acetate. The exact concentration depends upon the dilution of the extract and is determined for each individual experiment. The precipitate was removed by centrifugation and the excess of lead in the supernatant was precipitated at pH 6·0 with sodium sulfate. Treatment with $(NH_4)_2SO_4$ at 65% saturation gives a precipitate which contains the hemochromogen. The precipitate was filtered on a Hyflo Cell bed, washed with 65% $(NH_4)_2SO_4$ and dissolved in water. The purification was repeated several times with ammonium sulfate from pH 5 to 8, followed by negative adsorption on calcium phosphate gel or Cy alumina, and finally the pigment was dialysed against distilled water and concentrated under *vacuo*. Heating at 68°C, as required for the preparation of Helix Cyt. *h*, was not necessary, since Aplysia hepatopancreas does not contain cellulase.

In its oxidized form, the pigment extracted from Aplysia's hepatopancreas shows a peak at 410–412 mμ in the Soret region of the spectrum and a broad band with a maximum around 536 mμ in the visible region. After reduction with dithionite, three bands appear with peaks at 423–424, 526–528 and 556 mμ (Fig.). The *γ* band, both



Absorption spectra of Aplysia Cytochrome *h* in the oxidized (dodded line) and in the reduced (full line) forms.

in the oxidized and in the reduced forms of the pigment, is slightly shifted towards higher wavelengths compared with the values obtained by KEILIN for Helix Cyt. *h* (Table).

Table

Position of absorption bands (mμ) of Cytochrome *h* from Aplysia and Helix Hepatopancreas

		Band	Band	Band
Aplysia	Oxidized	—	536–540	410–412
	Reduced	556	526	424
Helix*	Oxidized	562	536	408
	Reduced	556	526·5	422

* From J. KEILIN[1].

Aplysia Cyt. *h* is reduced by dithionite, lithium hydride, ferrous oxalate, cysteine, ascorbic acid, and glutathione. It is oxidized by ferricyanide, ferric oxalate, and hydrogen peroxide. After treatment of Cyt. *h* with NaOH 0·2 *N* and pyridine, followed by reduction with dithionite, a spectrum is obtained with peaks at 415, 520, and 550 mμ. Attempts to extract iron porphyrin with acidic acetone were not successful, even when treatment with HCl 0·5 *N* had been made previously. The spectrum of the Cyt. *h*-cyanide compound, in its reduced form, has peaks at 424–426, 530–532, and 560–562 mμ.

[1] J. KEILIN, Biochem. J. *64*, 663 (1956); Nature *180*, 428 (1957).
[2] L. TOSI, A. GHIRETTI-MAGALDI, and F. GHIRETTI, R. C. Accad. Lincei *23*, 447 (1957).
[3] E. A. PHEAR, Proc. zool. Soc., London *125*, 383 (1955).