with the liver preparations from the phenobarbital-administered rats than with those from the control ones; the increase in rate was much more significant with mevalonate than with acetate (Table II). These data also suggest an intimate relation between the cholesterol synthesis and the microsomal electron transport system. BUCHER *et al.*[7] reported that in the cholesterol synthesis with acetate, a rate-limiting process was the formation of mevalonate. It is probable, therefore, that a reaction involved in the synthesis of cholesterol from mevalonate depends upon the NADPH oxidation catalyzed by the electron transport system. The substrate for the reaction should be a physiological one.

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Effect of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone on chlorophyll fluorescence and photosynthesis

According to current theory[1] relating chlorophyll fluorescence to photosynthetic electron transport, fluorescence is emitted from photosystem II and becomes stronger as the acceptor for system II becomes more reduced. Increased fluorescence after 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) addition is due to blocking of a step between systems I and II—reoxidation of the system II acceptor being prevented. DCMU would not affect photosystem I or its associated dark reactions (including phosphorylation). The theory predicts that uncouplers of phosphorylation will not decrease the strong fluorescence of DCMU-inhibited cells, since they act on the system I side of the DCMU-blocked step and cannot increase the supply of acceptor for system II. In fact, this paper shows that the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) more than halves the fluorescence of DCMU-inhibited cells.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

Chlorella pyrenoidosa and wild-type *Chlamydomonas rheinhardii* were studied primarily; some experiments were performed with *Anacystis nidulans* and *C. rheinhardii* mutants. The blue actinic illumination employed ($5.1 \cdot 10^3$ μW·cm^{-2}; filters: water, Schott KG-1, Corning 4308 and two 4304) was saturating for Chlorella, nearly saturating for Chlamydomonas. The temperature was 25°.

Rates of oxygen evolution and intensity of fluorescence were recorded simultaneously with a two-pen recorder. Oxygen was determined with a platinum rate electrode[3,4]; the electrolyte was culture medium *plus* 0.1 M KCl and was usually equilibrated with 3.6 % CO_2–6 % O_2. Chlorophyll fluorescence, after passing two Corning 2030 and 696 mμ interference filters, was measured with an EMI S-20 multiplier.

Fig. 1 records portions of an experiment with wild-type Chlamydomonas. In Fig. 1A is the response of unpoisoned cells to illumination cycles of 12 min light–3 min dark. Fig. 1B shows the decline of oxygen evolution and the rise of fluorescence after an addition of 4 μM DCMU. After a second addition giving 8 μM, and after full development of inhibition, oxygen evolution was 85 % inhibited and the fluorescence was approximately doubled (Fig. 1C). Also evident is a 5 min-long fluorescence induction after darkness. In Fig. 1D both fluorescence and oxygen evolution decline following an addition of FCCP (10 μM). After full development of FCCP poisoning (Fig. 1E), fluorescence and oxygen inductions are absent, and oxygen evolution is replaced by a small light-dependent oxygen consumption.

Related findings, from other experiments, are summarized as follows. (1) The low-yield, induction-free fluorescent state, seen in Fig. 1E, occurs with 10 μM FCCP whether or not DCMU is present. (2) DCMU does not affect the low fluorescence of cells previously poisoned with FCCP. (3) Similar effects of 10 μM FCCP on fluorescence and oxygen evolution are seen with Chlorella and Anacystis; however, the onset of poisoning is somewhat slower. (4) With Chlamydomonas mutants Ac-115 and Ac-141 (lacking cytochrome *b* (ref. 2)) and Ac-206 (lacking cytochrome *f* (ref. 2)), the steady-

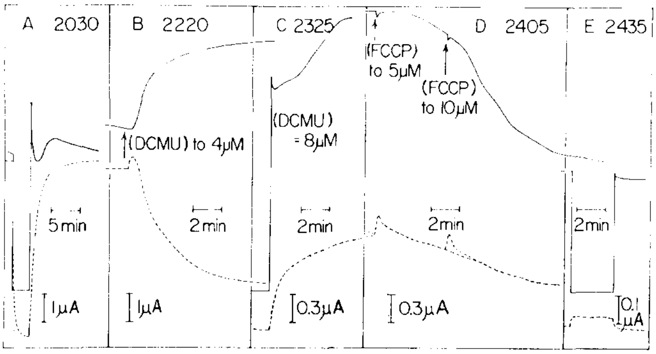

Fig. 1. Effects of DCMU and FCCP on rate of oxygen evolution (– – – – –) and fluorescence (696 mμ) (————) of Chlamydomonas wild-type; 3.6 % CO_2–6 % O_2. Scale for fluorescence same for parts A through E; time and oxygen current scales as indicated. A. Stationary state induction of unpoisoned cells on cycles of 3 min dark–12 min light. B. Development of inhibition following addition of 4 μM DCMU. C. Induction after 3 min dark and after complete development of inhibition by 8 μM DCMU. D. Onset of inhibition following addition of 10 μM FCCP to cells already inhibited by 8 μM DCMU. E. Induction-free response after 3 min dark, 8 μM DCMU *plus* 10 μM FCCP. In B and D, the transient increases in oxygen current immediately following additions of poison are artifacts due to oxygen in the stock solution of the poisons.

state fluorescence of unpoisoned cells was not increased by DCMU, but was approximately halved by FCCP.

The above observations hold for inhibition by 10 μM FCCP, a concentration which completely inhibits oxygen evolution. In contrast, 2 μM FCCP added to continuously illuminated cells, inhibits oxygen evolution only about 35 %, does not affect fluorescence, and does not alter much the induction after 3–9 sec dark periods (Fig. 2). After a dark interval (81 sec), however, induction is drastically slowed—1–3 h being needed to regain the previous steady-state value. Early in this period of "suppressed induction", the fluorescence falls to a minimum, then gradually rises paralleling oxygen evolution. Suppressed induction was seen repeatedly in different experiments with Chlorella and Chlamydomonas; in a given experiment, however, once the hours-long recovery had occurred, additional FCCP was required to repeat the effect. The effect requires a low oxygen concentration (0–1 %) and seems related to the less spectacular lengthening of induction of unpoisoned algae after long, anaerobic dark intervals.

In DCMU-poisoned cells, in which electron transport is blocked between the phosphorylation site and system II, FCCP cannot reduce fluorescence by accelerating oxidation of the acceptor. Alternative explanations for the effect of FCCP on fluorescence are (a) FCCP acts on system II directly and independent of its uncoupling action, and (b) FCCP, acting as an uncoupler, affects system II *via* a path other than electron transport. An example of the first alternative is R. K. Clayton's hypothesis (personal communication) that FCCP accelerates the conversion of fluorescent chlorophyll to non-fluorescent, inactive chlorophyll (EH and E_I of Joliot[5]). Such an acceleration would explain not only the effect of FCCP on fluorescence but also the effects of various uncouplers on delayed light. An example of the second alternative

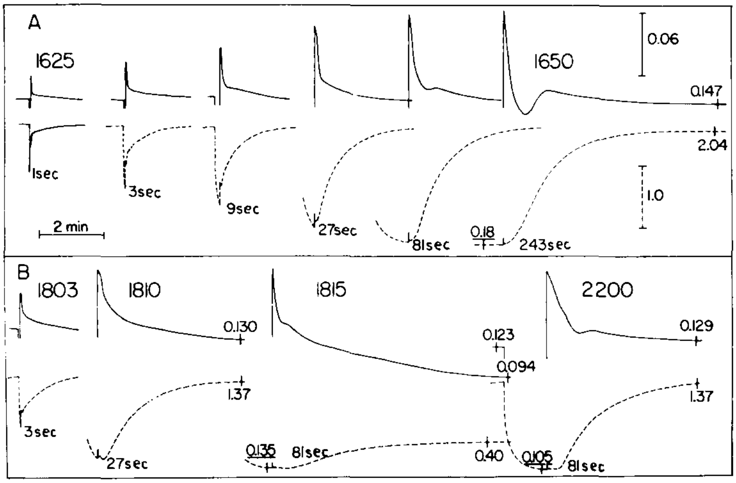

Fig. 2. Inhibition of induction following 81 sec dark, in Chlorella slightly inhibited with 2 μM FCCP; 3.6 % CO_2–0 % O_2. ———, fluorescence; – – – –, oxygen. Time, fluorescence, and oxygen scales same throughout and indicated by bars; fluorescence currents, oxygen dark currents (underlined) and photocurrents indicated at selected points. A. Induction of unpoisoned cells after 1–243 sec dark interruptions. B. cells in presence of 2 μM FCCP; 1803 and 1810: nearly normal induction after 3 and 27 sec dark; 1815: inhibited induction of oxygen and slow decline of fluorescence after 81 sec dark; 2200: induction after 81 sec dark, cells largely recovered from earlier inhibition, during 4 h of subsequent illumination.

would be that the capacity of system II to fluoresce, in response to increased reduction of acceptor, depends on the presence of ATP. FCCP would reduce ATP to a low level and system II would become non-fluorescent whatever the oxidation level of the acceptor. At present, the second alternative seems preferable since it rests on the demonstrated uncoupling action of FCCP.

Three additional points need mentioning. The first is that fluorescence falls slowly during the first 10 min of suppressed induction (Fig. 2), whereas ATP must have already largely disappeared in the preceding dark period. This suggests that the fluorescence capacity of system II responds only slowly to changes in ATP concentration. Secondly, AVRON AND SHAVIT[6] reported that 1 μM FCCP uncouples phosphorylation 50 % in saturating light, but nearly 100 % in low light. This may be correlated with the small inhibition of oxygen evolution by 2 μM FCCP at high light and the much stronger inhibition after 3 min dark. Evidently FCCP effects are intensified in low light or darkness. Thirdly, the several minute-long fluorescence rise of DCMU-treated cells (Fig. 1C) may reflect a slow decay of fluorescence capacity in darkness (due to an ATP drop) and a fast ATP rise when light is restored. In contrast, current theory predicts only a very fast fluorescence rise in the presence of DCMU.

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