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**Authors**
Calvin, M.
Arnoff, S.

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M. Calvin and S. Aronoff

December 30, 1948

Berkeley, California

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# CHLOROPHYLL AND PHOTOSYNTHESIS

By

M. Calvin and S. Aronoff[1]

Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California[2]

December 30, 1948

Experiments designed to test whether chlorophyll transfers non-labile hydrogen during photosynthesis have been concluded. The experimental procedure consisted in the suspension of Chlorella algae in $D_2O$ followed by the exposure of a vessel to light for a time of such duration that the mole ratio of oxygen evolved as the result of photosynthesis to chlorophyll present was 20-1; identical runs without light were also made. The chlorophyll was then isolated, purified and analyzed for deuterium content. The procedure used was applicable only to non-labile hydrogen, since exchange with hydrogens capable of transfer would proceed to the same extent regardless of the presence or absence of light and the isolation and purification of the chlorophyll would result in a loss of all exchangeable deuterium by virtue of copious washing with ordinary water. Thus, the scheme that has been tested is as follows:

$$\text{Chlorophyll} \longrightarrow \text{dihydrochlorophyll (photochlorophyll)} + 2\ H$$

$$\text{Photosynthesized chlorophyll} + HOH \longrightarrow \text{Chlorophyll} + O_2$$

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1 Present address: Atomic Research Institute, Iowa State College, Ames, Iowa

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The result of the initial experiments showed more deuterium in chlorophyll from algae exposed to light than in those kept in the dark, but the ratio of deuterium incorporated was still only one-quarter of that expected. Later work confirmed earlier experiments and noted that in 4 to 5 hour reactions the ratio of deuterium incorporated in the chlorophyll in the light to the deuterium taken up in the dark was approximately 4. Some chlorophyll was apparently formed in the dark. This was further confirmed by the use of $C^{14}$. The ratio of the percent incorporation of $C^{14}$ in the light-synthesized chlorophyll to the dark-synthesized chlorophyll indicates that the greater amount of deuterium in the light synthesized chlorophyll could be accounted for by the formation of newly synthesized chlorophyll. The degradation of light and dark chlorophyll to smaller units (pheophorbide) which should still contain the active hydrogen and, therefore, give a higher analytical result showed no preferential distribution of deuterium within the porphin ring (as compared to phytol).

Analytical methods of conversion of combusted $H_2O$-$D_2O$ to hydrogen-deuterium were shown to be satisfactory. Control exchange experiments with pure chlorophyll in 90% acetone, 10% deuterium water showed the usual number of labile hydrogens. The results of the experiments are, therefore, negative in character and indicate that (a) chlorophyll may act as a sensitizer rather than as a hydrogen transport agent, or (b) if it does not act as a hydrogen carrier then it (1) functions via an enolizable hydrogen, or (2) the fraction of chlorophyll that is fairly active is small compared to the amount of material, or (3) the path of hydrogen from water to chlorophyll proceeds via a large reservoir of non-exchangeable hydrogen.