PN 1201

A study of the mode of action of 3-(4-chlorophenyl)-1,1-dimethylurea on photosynthesis

Photosynthetic inhibitors can be classified, according to their site of action, in three broad categories: (a) those acting exclusively on, or very close to, a photochemical reaction, (b) those acting only on a thermal step, and (c) those affecting both photochemical and thermal steps. We know of no inhibitor of the first type. Inhibitors of the second type are numerous, *e.g.* CN^-, N_3^-, H_2S, 2,4-dinitrophenol etc. In the third class are generally included compounds such as phenylurethane, DCMU or NH_2OH (*cf.* refs. 1–3). The theoretical implications for the mechanism of photosynthesis of the high potency of CMU as an inhibitor prompted us to reinvestigate its mode of action.

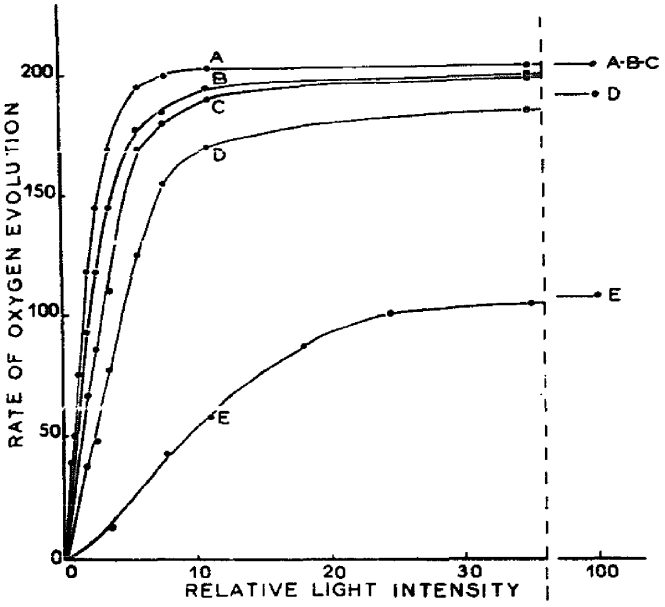

Fig. 1. Effect of CMU on the white-light intensity curve of oxygen evolution in Chlorella. Temperature, 22°. A, untreated cells; B, with $2 \cdot 10^{-7}$ M CMU; C, with $7 \cdot 10^{-7}$ M CMU; D, with $2 \cdot 10^{-6}$ M CMU; E, with $8 \cdot 10^{-6}$ M CMU.

To that end, we measured its effect on the photosynthetic oxygen evolution in *Chlorella pyrenoidosa* with a bare platinum electrode of the HAXO AND BLINKS type[5], the electrical output of which was fed into a recording galvanometer. The cells were suspended in 75 mM phosphate buffer (pH 6.9). Monochromatic light was obtained from a Bausch and Lomb monochromator. Light intensity was controlled with a series of neutral density filters.

Fig. 1 shows the effect of various concentrations of CMU on the rate of oxygen evolution at different light intensities. These results clearly show that, with concentrations below 10^{-6} M, the light-dependent portion of the curve is modified but not the temperature-dependent phase.

Abbreviations: CMU, 3-(4-chlorophenyl)-1,1-dimethylurea; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

From concentrations of about $5 \cdot 10^{-8}$ M–10^{-6} M, CMU behaves as an inhibitor of the first category as defined above. At concentrations above 10^{-6} M, it becomes an inhibitor of the third category, acting both on a photochemical and a rate-determining dark step.

We next sought to determine which light reaction is blocked in the low-CMU-concentration range. There is already much evidence of a different nature[1,6–9] that it is the reaction catalyzed by the "short wavelength" or "system 2"[10] pigment complex. We reasoned that, if such is the case, an action spectrum of inhibition should show a wavelength dependency, inhibition being greatest at wavelengths for which the rate of oxygen evolution is determined by "system 2". In the red, maximum inhibition would then occur at about 690–700 mμ where most of the absorption is due to "system 1", and the rate is determined by "system 2" as shown by the action spectra of the Emerson enhancement effect[11].

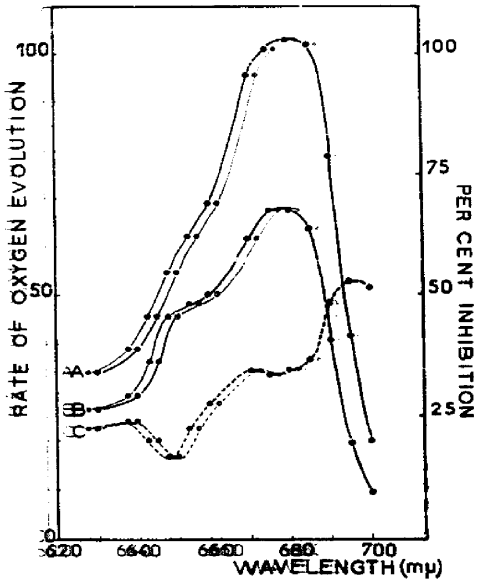

Fig. 2. Effect of CMU on the action spectrum of oxygen evolution in Chlorella. Temperature, 20°. A, untreated cells; B, with CMU; C, action spectrum of inhibition (ordinate: % of inhibition).

Fig. 2 shows the expected wavelength dependency: there is a minimum of inhibition at 650 mμ and a maximum at 695 mμ. In some experiments we have been able to get 60–65 % of inhibition at 695 mμ and no inhibition at all at 650 mμ. These results confirm and extend similar observations made by DUYSENS *et al.*[10] with DCMU on the red alga Porphyridium.

To summarize, we may say that CMU, in the range of $5 \cdot 10^{-8}$ M–10^{-6} M, inhibits the "short wavelength" light reaction, acting on or close to the photochemical step. At higher concentrations, it acts also on a rate-limiting dark step. This step is probably not situated in the Calvin cycle, as evidenced by the $^{14}CO_2$ fixation patterns obtained with and without CMU in Scenedesmus under H_2 (ref. 12).

Conceivably, it is between the two photochemical reactions, at the level of a cytochrome for example. In this respect, KELLIN's observation that urethane inhibits the reduction of cytochrome c during respiration may be relevant[13].

For complete inhibition at saturating light intensity, the molar ratio of adsorbed CMU to chlorophyll is of the order of 1/200 (ref. 4) or of 1/280–1/700 in Euglena[14], i.e. about one CMU molecule per Emerson center. However, our data show that the photochemical reaction is affected at concentrations three or four orders of magnitude smaller than that required to produce full inhibition at saturating light intensity. This high affinity of CMU for the photochemical reaction can be explained by two alternative hypotheses: either it competes with a photo product for an enzyme or it acts as a trap at the energy-transfer level.

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Biosynthesis of ascorbic acid by rats fed a high level of dietary tyrosine under toxic and adapted conditions

That a high level of dietary tyrosine is toxic to rats is well established[1-3], though the nature of this toxicity is not clear. It has also been reported[4] that rats can adapt to a high tyrosine intake. This adaptation is indicated by a return to lower excretion levels of intermediary metabolites of tyrosine and is correlated with an increase in growth rate. However, little is known about the mechanism of this adaptation. Since ascorbic acid is known to have a role in tyrosine metabolism[5], it seemed worthwhile to examine whether the biosynthesis of ascorbic acid by the liver tissues of rats is affected during feeding toxic amounts of tyrosine and after adaptation to these high amounts of tyrosine.

In our experiments, albino rats of either sex, weighing 40–50 g were used. They were fed a basal diet containing 9% casein prepared according to BENTON *et al.*[1],