

values within experimental error. The statistical and molecular structure calculations, therefore, lead to the necessity of re-labeling the cis and trans isomers of 1,3-dimethylcyclohexane.

Since preparing this manuscript, we have belatedly come upon the work of Mousseron and Granger (5), which seems also to have been overlooked by others. Mousseron and Granger prepared the cis and trans isomers of 1,3-dimethylcyclohexane from appropriate optically active starting materials and found the optically active isomer, which must be trans (6), to be the higher-boiling member of the pair. This completely confirms the conclusion presented above.

For these reasons, a change has been made, as of March 31, 1947, in the naming of the cis and trans isomers of 1,3-dimethylcyclohexane in the tables of physical and thermodynamic properties and in the catalogues of spectrograms issued by the American Petroleum Institute Research Project 44. The change is such that the lower-boiling isomer, formerly labeled "trans," is now labeled "cis," and the higher-boiling isomer, formerly labeled "cis," is now labeled "trans."

The following notation will be used in the tables and catalogues of spectrograms listing these compounds:

cis-1,3-Dimethylcyclohexane.²

trans-1,3-Dimethylcyclohexane.³

In addition to the foregoing changes in connection with the work of the American Petroleum Institute Research Project 44, corresponding changes have been made in the work of the American Petroleum Institute Research Project 6 and in the cooperative program on standard samples of the National Bureau of Standards and the American Petroleum Institute.

All workers in other laboratories dealing with cis-1,3-dimethylcyclohexane or trans-1,3-dimethylcyclohexane are invited to relabel these two compounds in the manner described above. It is also recommended that, whenever either of the two names is written, one or more properties also be recorded for adequate identification apart from the name cis or trans. This latter step will completely eliminate any confusion which may arise from this relabeling.

References

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² This isomer, formerly labeled "trans," has the following properties (1): boiling point at 1 atm., 120.09°C.; refractive index, n_D at 25°C., 1.4206; density at 25°C., 0.7620 g/ml.

³ This isomer, formerly labeled "cis," has the following properties (1): boiling point at 1 atm., 124.45°C.; refractive index, n_D at 25°C., 1.4284; density at 25°C., 0.7806 g/ml.

The Dark Reductions of Photosynthesis¹

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Although green plants have been shown (4) to fix CO₂ in the dark, the conditions influencing that fixation and the compounds formed were unknown. We are investigating these variables.

The conditions of the experiments were as follows: A sample of actively growing algae was split into two parts (approximately 1 cc. algae/15 cc. suspension). One part (I) was kept in the dark, exposed to 4 per cent CO₂ in N₂ for about 8 hours. The other (II) was exposed to the light of a 150-watt tungsten lamp (.7 g.cal./cm.²/min.) for one hour, during which time it was kept free of CO₂ by constant flushing with N₂. The two samples were then evacuated, kept in the dark, and simultaneously exposed to the same gas containing C¹⁴O₂ in N₂ for a period of 5 minutes. At the end of this period, the algae were killed by an acetic acid-HCl mixture and the remaining active C¹⁴O₂ pumped off.

The total nonvolatile radiocarbon content of the two samples was then measured and its chemical distribution determined. The preliminary results are given in Table 1.

From these results alone, it is clear that the reduction of CO₂ to sugars and the intermediates in that reduction does not involve the primary photochemical step itself. This is further substantiated by the appearance of an appreciable fraction (up to 15 per cent) of the radiocarbon in the methylene groups of the succinic acid isolated from sample I of the table (3). It is

TABLE 1
DARK FIXATION OF CO₂ BY *Chlorella*

Pretreatment	I CO ₂ in the dark	II Light in the absence of CO ₂
Total (relative units).....	1	5.5
Succinic acid*.....	70%	6%
Fumaric acid.....	3%	1%
Malic acid.....	—	6%
Cationic substances† (not extractable by ether from pH 1—probably amino acids).....	15%	30%‡
Anionic substances† (not extractable by ether from pH 1).....	9%	10%
Neutral (sugars).....	<.1%	1.5%
Unidentified (extractable by ether from pH 6).....	2%	6%
Unidentified (extractable by ether from pH 1).....	—	25%

* The succinic acid was isolated without carrier and identified by extraction coefficient, equivalent weight, C and H analysis, melting point, and X-ray powder pattern.

† Absorbed by Duolite ion exchange resins C-3 and A-3, respectively.

‡ Largely alanine.

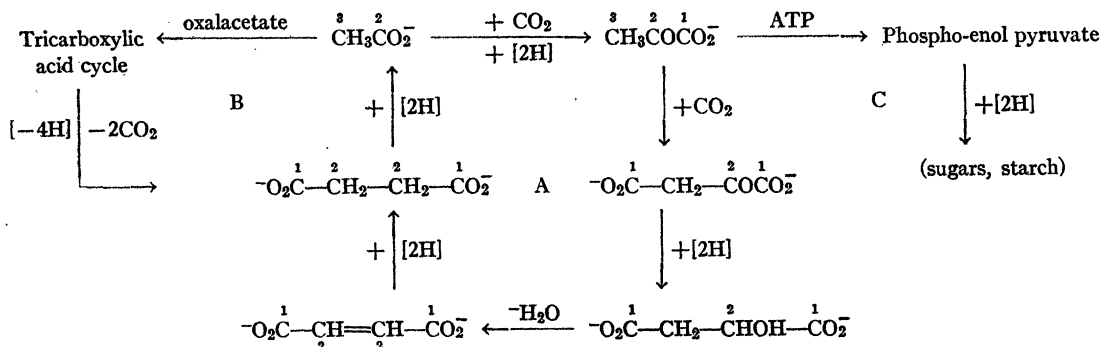
thus confirmed that the photochemical process establishes a reservoir (small, to be sure) of reducing power which can subsequently carry out all of the reduction steps necessary to bring CO₂ to sugar.

Using some of the reactions already established in animal tissue and bacteria (5), it is possible to account for the above

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results as well as the observed distribution of radiocarbon in sugar produced by a short photosynthesis (1).

photosynthesized radioactive sugars has a lower specific activity (per mg. C) than the sugar itself. If respiration in-



Starting with either acetate or pyruvate, the numbers over each carbon atom indicate which carbon atoms are labeled each time around cycle A. The reducing power (indicated as [2H]) is, of course, ultimately derived from the light reaction, and some of it might well be reduced coenzymes I or II. The high-energy phosphate required in these reductions is not explicitly shown in the chart. All or part of it could easily be derived from the combustion of part of the acetate through cycle B.

It should be mentioned that this scheme cannot be a simple reversal of the respiratory system of reactions, since CO₂ derived from respiration of barley leaves (2) containing freshly

volves some of the same intermediates as those shown in the chart, the respiratory system must be physically separated from the photosynthetic system.

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I N T H E L A B O R A T O R Y

The Oxalate Salt of P-Aminodimethylaniline, an Improved Reagent for the Oxidase Test

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The cultural method for the diagnosis of gonococcal infection, now a standard procedure (1) in most public health laboratories, utilizes the so-called "oxidase test" for rapidly distinguishing colonies of the *Neisseria* from non-oxidase-producing colonies of other genera. The oxidase reaction is considered to depend upon the reaction of an oxidative enzyme with an aromatic amine to produce a series of readily discernible color changes ranging from pink to black.

The dye component heretofore recommended for the oxidase test is the monohydrochloride salt of p-aminodimethylaniline. On standing, this agent deteriorates and becomes discolored, thereby reducing its solubility and the clarity of its aqueous solutions. The precipitate which forms interferes with the separation of oxidase-positive from oxidase-negative colonies in mixed cultures.

Because the oxalic acid salts of aromatic amines are, in general, more stable than the corresponding hydrochloride acid salts, the oxalate salt of p-aminodimethylaniline, [H₂N(C₆H₄N(CH₃)₂)₂·(—COOH)₂]¹ was tested for its suitability in the oxidase reaction.

Observations were made on the rapidity with which the dry, crystalline oxalate salt deteriorated at temperatures ranging from 18° to 23° C. One per cent aqueous solutions of the monohydrochloride and of the oxalate salt were compared in the oxidase reaction on chocolate agar plates inoculated with cultures of *N. gonorrhoeae*, with mixed cultures of *N. gonorrhoeae*, streptococci, and diphtheroids, and with cervical and urethral exudates for evidence of gonococcal infection.

A comparative study of the stability of 1 per cent aqueous solutions of each compound was made at temperatures of from 18° to 23° C. Measurements of the oxidative changes in the two dye salts were made at 24, 48, and 72 hours after preparation of the solutions, using a Klett-Summerson colorimeter.

The toxicity of 1 per cent aqueous solutions of the two salts was tested on recently isolated strains of the gonococcus.

The dry, crystalline oxalate salt was more stable than the monohydrochloride salt. After 6 months storage at room tem-

¹ P-aminodimethylaniline oxalate was prepared and supplied by the Research Laboratories of the Eastman Kodak Company, Rochester, New York.