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*STUDIES ON THE BIOSYNTHESIS OF CHLOROPHYLL:
CHEMICAL INCORPORATION OF MAGNESIUM INTO PORPHYRINS**

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The incorporation of magnesium into porphyrin is a key step in the biosynthesis of chlorophyll. Previous investigators of this reaction have always found it necessary to employ an activated form of magnesium (e.g., magnesium alkoxides,¹ Grignard reagent,² or magnesium violigen³) and strictly anhydrous conditions. This report concerns the first chemical preparations of magnesium porphyrins under nonanhydrous conditions.

We have found that magnesium is incorporated into the porphyrin nucleus when certain magnesium salts and the tetrapyrrole are heated to reflux in either pyridine or in *n*-propanol. The addition of a small quantity of water (2%) to pyridine or propanol has little or no effect upon either the reaction products or the reaction time, whereas larger amounts of water, while not affecting the over-all outcome of the reaction, do markedly retard the reaction. Furthermore, the addition of a number of other solvents to the reaction mixtures does not influence either the reaction products or the reaction time.

In a preliminary study of the reaction, pyridine was added, without predrying, to a flask containing deuteroporphyrin dimethyl ester⁴ and magnesium perchlorate. The reaction mixture was heated to reflux and, at intervals, aliquots were removed and analyzed with a recording spectrophotometer. The reaction was essentially complete after 6 hr. In later studies, the reaction mixtures were sealed in glass ampules and the reaction was carried out in the autoclave at 18 lb pressure for 2 hr. The reaction was complete in this time. If the ampules are placed in a water bath at 70°C, the reaction is 50 per cent complete in 10 hr.

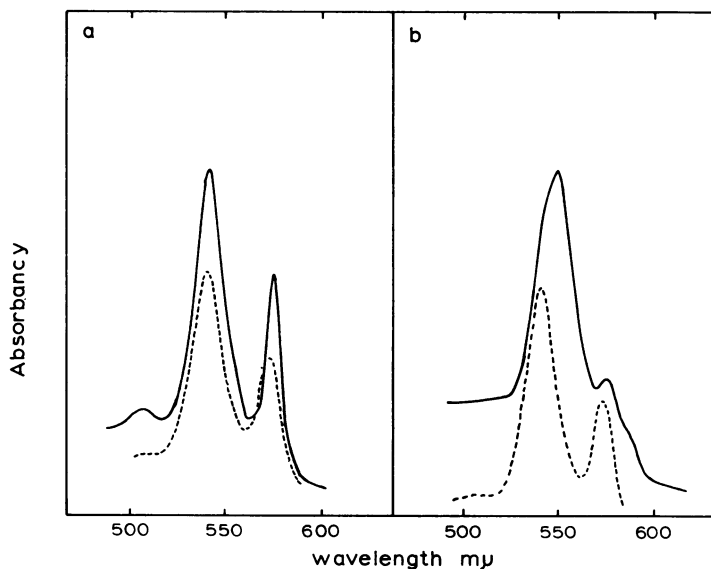


FIG. 1.—(a) Absorption spectra of Mg-deuteroporphyrin dimethyl ester (—) and Zn-deuteroporphyrin dimethyl ester (-----) in diethyl ether, concentrations not equal. (b) Absorption spectra of Mg-deuteroporphyrin dimethyl ester (—) and Zn-deuteroporphyrin dimethyl ester (-----) in anhydrous pyridine, concentrations not equal.

When the pyridine is rigorously dried and the reaction run under strictly anhydrous conditions, the same results are obtained, i.e., magnesium deuteroporphyrin diester is formed at the same rate. The spectrum of the product in dry pyridine consists of three absorption bands with maxima at 577 $m\mu$, 553 $m\mu$, and a Soret at 422 $m\mu$. On the other hand, if the pyridine is not completely dry, there are additional peaks on the short wavelength side of both the 553 $m\mu$ and 422 $m\mu$ peaks. Addition of more water causes a disappearance of the long wavelength peaks with formation of sharp single peaks at 576 $m\mu$, 543 $m\mu$, and 406 $m\mu$. A similar spectral shift is observed when alcohol or acetone is added to the pyridine reaction mixture. Although the spectrum of the product depends on the presence of water in the system, magnesium is incorporated into porphyrins whether or not water is present. The spectral changes must merely reflect a solvent effect upon the spectrum of the magnesium porphyrin.

Magnesium deuteroporphyrin dimethyl ester prepared in the system described above was identified by means of the following criteria: (1) Magnesium deuteroporphyrin dimethyl ester was prepared according to the method of Granick using decomposed Grignard reagent. The spectrum of the latter was compared to our product prepared as described above in both pyridine and, following extraction, in ether. In both solvents the spectra were identical. Absorption maxima in ether for both were at 576 $m\mu$, 543 $m\mu$, and a Soret at 406 $m\mu$. (2) To make certain that the product was not being mistakenly identified, zinc deuteroporphyrin dimethyl ester, which resembles the magnesium complex, was prepared by reacting zinc acetate with the porphyrin in pyridine. Absorption maxima of the zinc complex in pyridine were at 575 $m\mu$, 543 $m\mu$, and the Soret at 414 $m\mu$. After extraction into ether, the spectrum of the zinc complex in ether had absorption maxima at 571 $m\mu$,

540 $m\mu$, and the Soret at 410 $m\mu$. Magnesium deuteroporphyrin dimethyl ester prepared by refluxing in wet pyridine was extracted into ether. The pink ether layer was washed with several volumes of water and then analyzed with a flame photometer. Analysis for Mg was made through use of its emission line at 2852 Å, and for Zn by its atomic absorption at 2139 Å. The results indicated the presence of relatively large amounts of magnesium and no detectable zinc. A reagent blank control was prepared and carried through all steps of the procedure. Neither magnesium nor zinc was detectable in this solution.

The reaction reported here is not specific for deuteroporphyrin dimethyl ester. Magnesium is also incorporated into mesoporphyrin dimethyl ester, hematoporphyrin, hematoporphyrin dimethyl ester, coproporphyrin tetramethyl ester, and protoporphyrin dimethyl ester under the same conditions. Likewise, the reaction is not specific for $Mg(ClO_4)_2$. $MgCl_2 \cdot 6H_2O$, and $Mg(NO_3)_2 \cdot 6H_2O$ can also be employed. However, with these salts the time required for complete reaction is of the order of two days in refluxing pyridine. With $Mg(C_2H_3O_2)_2 \cdot 4H_2O$ the reaction is even slower. Finally, the method is not limited to Mg incorporation, but works also for Cu(II), Zn(II), Fe(II), and Co(II).

Experiments in the Presence of Water.—Fixed amounts of water were added to ampules containing $Mg(ClO_4)_2$ -deuteroporphyrin dimethyl ester in dry pyridine, and the reactions were carried out in the autoclave. Spectra were taken after 2 hr and again after 10 hr. At 2 hr, only for those solutions which contain no more than 2 per cent water was the reaction approaching completion, whereas after 10 hr in the autoclave those solutions with no more than 8 per cent water were nearly complete. Therefore, although water definitely retards reaction, it is clear that water does not prohibit reaction.

Experiments with Other Solvents.—In the same manner as the experiments with water, deuteroporphyrin dimethyl ester in purified, dry pyridine and $Mg(ClO_4)_2$ in purified, dry pyridine were added to a series of ampules. Then a number of different solvents were added, so that each solution contained 25 per cent pyridine and 75 per cent of one of the following: ethyl acetate, anhydrous methyl alcohol, dioxane, chloroform, carbon tetrachloride, benzene, hexane, ether, and acetone. In all cases, the reaction was complete within 3 hr. Experiments were also conducted to contrast pyridine, 2,4-lutidene, and 2,6-lutidene. Under the same conditions (both deuteroporphyrin dimethyl ester and $Mg(ClO_4)_2$ were dissolved in the appropriate solvent, and autoclaved for 2 hr) formation of the magnesium-porphyrin complex in pyridine was complete; in 2,4-lutidene (technical grade—85%) the reaction was 50 per cent complete; in 2,6-lutidene (practical grade—95%) there was no observable spectral change from that of uncomplexed deuteroporphyrin dimethyl ester.

Experiments with Alcohols as Solvents.—As mentioned earlier, we have also found magnesium to be unincorporated into the porphyrin nucleus when the solvent was *n*-propanol instead of pyridine, all other reaction conditions remaining the same. The *n*-propanol can either be dried over CaO and freshly distilled before using, or used without further purification. Again, small quantities of water can be added without altering the reaction products, although the reaction rate is markedly decreased.

A series of alcohols were tested as solvents for the reaction: absolute methanol, absolute ethanol, 95 per cent ethanol, *n*-propanol, isopropanol, and *n*-butanol. It

was observed, from the spectrum of each, that at the time required for complete reaction in *n*-propanol (2 hr), the reaction was only about 50 per cent complete in *n*-butanol and in isopropanol, and there was no reaction in methanol or in ethanol. To be especially noted here is that if a small amount of pyridine is added to the ampules containing methanol, ethanol, *n*-butanol, or isopropanol, the reaction does go to completion within 2 hr to form the desired magnesium porphyrin. This, along with the previously mentioned experiments with other solvents, leads us to believe that pyridine exerts a specific effect upon the reaction rather than merely acting as the solvent for the reaction. Work is now being conducted to prove this possibility and to elucidate other phases of the kinetics and mechanism in both model and biological systems.

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*INTRODUCTION OF SPECIFIC DRUG RESISTANCE
PROPERTIES BY PURIFIED RNA-CONTAINING
FRACTIONS FROM PNEUMOCOCCUS**

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The information-bearing function of messenger RNA is supported by two lines of evidence. On the one hand, the relation of certain RNA components of cells to DNA of the same origin is indicated by complementarities in total composition or certain cases of hybrid complex formation which suggest localized complementary arrangements. On the other hand, the correspondence of certain polyribonucleotides with specific peptide structures (indicating "messenger" capacity) is suggested by specific incorporation of particular combinations, or in a few cases of arrangements, of amino acids, when such RNA's are present in biosynthesizing systems.

It seemed important to seek a system in which proteins of specific nature and function might actually be produced as the result of introduction of specific kinds of RNA into cells. Such an experiment could be done in an intact cellular system by introducing RNA of known potentiality into cells not previously containing it. Certain sulfonamide-resistant properties of pneumococcus^{1, 2} seemed well suited for this work, since for several reasons they should provide a considerably "ampli-