

[CONTRIBUTION FROM THE CHEMICAL LABORATORY AND THE RADIATION LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

## Some Exchange Experiments with Radioactive Tracers

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In connection with some experiments on photosynthesis in these Laboratories it was found that the centrally bound magnesium of chlorophylls *a* or *b* does not interchange<sup>1</sup> with  $Mg^{++}$  at room temperature in 80% acetone-water solution. This rather surprising result induced us to study various other metallo-organic exchange reactions. In this paper we report the results of the following exchange experiments: (1)  $Fe^{+++}$  and ferriheme, (2)  $Fe^{+++}$  and ferrihemoglobin, (3)  $Fe^{+++}$  and ferric pheophytin, (4)  $Fe^{+++}$  and ferric tetraphenylporphyrin, (5)  $Cu^{++}$  and cupric pheophytin, (6)  $Fe^{++}$  and ferrous ortho phenanthroline, (7)  $Fe^{++}$  and ferrous  $\alpha, \alpha'$ -dipyridyl, (8)  $Mg^{++}$  and magnesium 8-hydroxyquinolate.

The first 5 pairs (*i. e.*, the metallo porphyrins) showed no measurable exchange even after long periods. The remaining combinations exchanged at a slow but easily measurable rate. These results seem to indicate that structural factors are more important than covalent-ionic bond character in determining the readiness of a metallic ion (in a metallo-organic compound) to undergo exchange.

**Exchange between Magnesium 8-Hydroxyquinolate and  $Mg^{+++}$ .**—Since the magnesium in chlorophyll did not exchange with  $Mg^{++}$ , it was considered of interest to try the exchange with the magnesium salt of 8-hydroxyquinoline. This compound is similar in behavior to the magnesium porphyrins in that they are the only known magnesium compounds which do not form magnesium hydroxide when boiled with concentrated alkali.<sup>2</sup>

Radioactive magnesium,  $Mg^{27}$  (10.2 min. half life) was prepared by bombardment<sup>3</sup> of metallic magnesium with 8 m. e. v. deuterons for ten minutes in the 37" cyclotron. The magnesium was dissolved in concentrated hydrochloric acid, and excess sodium hydroxide added to precipitate magnesium hydroxide. The precipitate was filtered, washed, dissolved and reprecipitated three times in order to remove the last traces of radioactive sodium ( $Na^{24}$ ) which is also produced by the deuteron bombardment. The final magnesium hydroxide, rid of  $Na^{24}$ , was converted into aqueous magnesium chloride and used in this form.

A fresh precipitate of magnesium 8-hydroxyquinolate (carefully freed of excess 8-hydroxyquinoline) was shaken with  $Mg^{+++}$  for thirty minutes at room temperature. The quinolate was filtered off, thoroughly washed and found to be strongly radioactive. Indeed, although the

exchange took place in a two-phase system, the quinolate contained ~60% of the radio-Mg calculated for complete interchange.

**$Fe^{+++}$ -Ferriheme and  $Fe^{+++}$ -Ferrihemoglobin.**—Because of the above result it seemed pertinent to investigate metalloporphyrins other than chlorophyll. As a result of their investigations of the magnetic properties of hemoglobin and its many derivatives, Pauling and Coryell<sup>4</sup> have concluded that the forces holding  $Fe^{+++}$  in ferriprotoporphyrin and ferrihemoglobin are ionic. These compounds readily lend themselves for investigation, particularly since a radioactive isotope of iron,  $Fe^{59}$ , of forty-seven days' half-life<sup>5</sup> is known.  $1.5 \times 10^{-4}$  mole of  $Fe^*Cl_3$  in 0.001 molal hydrochloric acid was added to  $3 \times 10^{-4}$  moles of ferriprotoporphyrin dissolved in 95% ethanol. After standing for about two months at room temperature the alcohol was evaporated off. The  $Fe^*Cl_3$  was removed from the solid residue with 0.001 molal hydrochloric acid solution, the insoluble porphyrin being filtered off. The heme was only slightly active, containing ~2% of the  $Fe^*$ . This small activity could be due to incomplete removal of  $Fe^*Cl_3$ .

In a separate experiment,  $10^{-5}$  mole of  $Fe^*Cl_3$  was added to an aqueous solution containing  $10^{-5}$  mole of ferrihemoglobin<sup>6</sup> in a slightly acid solution. After thirty hours at room temperature the hemoglobin was precipitated with excess trichloroacetic acid and filtered off. The bulky precipitate was carefully washed and ignited to yield mainly ferric oxide. This preparation was found to be inactive, containing less than 1% of the radio-iron used. This is in agreement with the findings of Hahn, *et al.*<sup>7</sup>

**Iron and Copper Pheophytins.**—The magnesium of chlorophyll can be replaced by other metallic ions under suitable conditions without removal of the phytol or methanol. Such compounds are called pheophytins. We have found no exchange between  $Fe^{+++}$  and iron pheophytin or between  $Cu^{+++}$  and copper pheophytin, even after two days in 80% acetone solution.

**$Fe^{+++}$ -Ferric Tetraphenylporphyrin.**—This experiment was carried out in a 0.02 *N*  $HClO_4$ -water-ethanol-benzene solution in which both ferric and the ferric tetraphenylporphyrin<sup>8</sup> salts are soluble. No exchange of iron (<3%) was found after five days at room temperature.

**$Fe^{++}$ -Ferrous Ortho Phenanthroline.**—The (presumably) octahedral arrangement of the six nitrogens in the orthophenanthroline complex forms a rather stable configuration around the central iron ion. It was of considerable interest to compare the lability of the metallic ion in this compound with the various organo-metallo com-

(4) Pauling and Coryell, *Proc. Nat. Acad. Sci.*, **22**, 159, 210 (1936).

(5) Livingood and Seaborg, *Rev. Mod. Phys.*, **12**, 30 (1940).

(6) We are indebted to Professor C. D. Coryell for the ferrihemoglobin and for helpful advice regarding its use.

(7) Hahn, Bale, Ross, Hettig and Whipple, *Science*, **92**, 131 (1940).

(8) This compound was synthesized by Dr. S. Aronoff, and we are indebted to him for his cooperation and generosity.

(1) Ruben, Frenkel and Kamen, *J. Phys. Chem.*, **46**, 710 (1942).

(2) B. J. Miller, Dissertation, University of Chicago, 1931.

(3) The nuclear reactions are  $D^2 + Mg^{26} \longrightarrow Mg^{27} + H^1$  and  $Mg^{27} \longrightarrow Al^{27} + e^-$ .

pounds reported above. The results are summarized in Table I.

TABLE I

Expt. no.	Molality of		H <sup>+</sup>	Time allowed for exchange	Per cent. <sup>a</sup> of random distribution of Fe <sup>+++</sup>
	FeSO <sub>4</sub>	(C <sub>12</sub> H <sub>8</sub> N <sub>2</sub> ) <sub>2</sub> FeSO <sub>4</sub>			
1	0.05	0.05	0.2	5 days	100 ± 5
2	.026	.022	.05	4 minutes	7 ± 2
3	.022	.012	.05	60 minutes	35 ± 5

<sup>a</sup> NH<sub>4</sub>OH was used to separate ferrous ion and the ferrous phenanthroline.

From Table I it is apparent that in aqueous solution at room temperature iron is exchanged at a slow but easily measurable rate.

**Fe<sup>++</sup>-Ferrous  $\alpha,\alpha'$ -Dipyridyl Sulfate.**—Ferrous  $\alpha,\alpha'$ -dipyridyl is somewhat similar to the phenanthroline complex in regard to structure and stability. It was of interest to study this exchange because ferrous  $\alpha,\alpha'$ -dipyridyl sulfate is known to be diamagnetic and Pauling has concluded<sup>9</sup> that the iron-nitrogen bonds are therefore mainly covalent. Thus one might be inclined to predict no exchange of iron atoms. However, this complex does exchange with Fe<sup>++</sup> in aqueous solution. The results are summarized in Table II.

TABLE II

Molality of		H <sup>+</sup>	Time allowed for exchange	Per cent. of random distribution of Fe <sup>+++</sup>
FeSO <sub>4</sub>	(C <sub>10</sub> H <sub>8</sub> N <sub>2</sub> ) <sub>2</sub> FeSO <sub>4</sub>			
0.016	0.008	0.04	2 hours	23 ± 5

### Discussion

It is interesting to note that in ferritoporphyrin where the iron is held by electrostatic forces, no exchange was observed even after two months. On the other hand, the  $\alpha,\alpha'$ -dipyridyl complex, in which the iron-nitrogen bonds are mainly covalent,<sup>9</sup> undergoes comparatively rapid exchange of iron. Apparently structural relations are more important than bond type.

The compounds discussed above may be separated into two classes: (1) the central metallic ion is surrounded by a fused "ring" and (2) the "ring" consists of two or more separate molecules. The magnesium, copper, and iron compounds of class (1) show extraordinary inertness toward metallic exchange, while the members of class (2) are far more labile in this respect. This difference may be due to the fact that for exchange to occur in the fused ring structures the four<sup>10</sup> metal-nitrogen bonds must be broken *simultaneously*<sup>11</sup> while for compounds of class (2) stepwise dissociation may occur so that in equilibrium with the complex

(9) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1939, p. 109.

(10) Because of resonance.

(11) We are indebted to Professor K. S. Pitzer for this suggestion.

there may be present in varying amounts molecular species in which the metal is held by 4, 2 or fewer bonds.

It would seem that the symmetrical planar electrostatic porphyrin ring is sufficiently strong at room temperature and moderate pH to prevent any reversible equilibrium involving the central metal ion. Indeed, we are tempted to generalize and say that at room temperature all metallic-fused "rings" (porphyrins, porphyrazines, phorbins, phthalocyanines, etc.) will not exchange with metallic ions in slightly acid solution. Accordingly, we would predict that disodium phthalocyanine<sup>12</sup> will not exchange with Na<sup>+</sup>.

The inertness of metal porphyrins (and similar pyrrole condensed ring structures) toward exchange should be of much help in the study of certain problems in nuclear physics, namely, the search for genetic relations between nuclear isomers,<sup>13,14</sup> where it is imperative to find a compound that will not exchange with the daughter (lower state) isomer.

**Acknowledgments.**—We are indebted to Professors K. S. Pitzer and G. Mackinney for helpful discussions, and to Dr. S. Aronoff for his cooperation. We wish to thank Professor E. O. Lawrence and the members of the Radiation Laboratory for making these experiments possible.

### Summary

1. Magnesium 8-hydroxyquinolate exchanges quite readily with Mg<sup>++</sup>.
2. Neither ferriheme nor ferrihemoglobin, both of which are paramagnetic, exchanges with Fe<sup>+++</sup>.
3. Copper and iron pheophytins also do not exchange with Cu<sup>++</sup> and Fe<sup>+++</sup>.
4. Ferric tetraphenylporphyrin does not exchange with Fe<sup>+++</sup> over a period of five days.
5. Ferrous orthophenanthroline exchanges slowly with Fe<sup>++</sup>.
6. Ferrous  $\alpha,\alpha'$ -dipyridyl, which is diamagnetic, also exchanges slowly with Fe<sup>++</sup>.
7. It appears that the rate of exchange depends more on structure than on bond type (*i. e.*, covalent or ionic).
8. It is suggested that metallo porphyrin compounds may be of help in the search for nuclear isomers among the metallic elements.

BERKELEY, CALIFORNIA

RECEIVED JUNE 9, 1942

(12) Barrett, Dent and Linstead, *J. Chem. Soc.*, 1719 (1936).

(13) Segre, Halford and Seaborg, *Phys. Rev.*, **55**, 321 (1939).

(14) De Vault and Libby, *ibid.*, **55**, 322 (1939).