LETTERS TO THE EDITORS

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Formation of Non-Enzymic Hæm

NEILANDS[1] has described the formation of coprohæm from iron and coproporphyrinogen in aqueous solution at physiological pH. Since uroporphyrinogen and coproporphyrinogen, rather than the porphyrins themselves, are now believed to be the true intermediates in the biosynthesis of hæm[2,3], it would be tempting to postulate that protoporphyrinogen is the intermediate which reacts *in vivo* with iron to form protohæm. Further to test this hypothesis, we have incubated aqueous solutions of coproporphyrinogen, coproporphyrin and protoporphyrin with ferrous salts under various conditions and have estimated the hæm formed spectrophotometrically as the pyridine hæmochrome.

Coproporphyrinogen ($1–2 \times 10^{-4}$ M) after 16 hr. incubation at pH 6 and 37° C. in the presence of large excess of ferrous ions ($1–5 \times 10^{-2}$ M) formed a small amount of coprohæm: less than 10 per cent when incubated under nitrogen and much less when incubated in air. However, starting from coproporphyrin, we found up to 25 per cent formation of coprohæm on incubation for 16 hr. at 37° C. under nitrogen and up to 12 per cent on incubation in air. At 100° C. in air the yields from both coproporphyrin and coproporphyrinogen were high and approximately the same. The porphyrinogens are readily oxidized to porphyrins by atmospheric oxygen and the coproporphyrinogen solutions always contained some coproporphyrin, even in those experiments in which incubation was carried out under nitrogen. It cannot be excluded that the formation of hæm observed when coproporphyrinogen was incubated with ferrous iron was derived, in fact, from coproporphyrin.

Protoporphyrin incubated with ferrous salts under the same conditions as were used for coproporphyrin was precipitated from solution and gave no detectable protohæm, but when native globin was added in order to solubilize the porphyrin there was an appreciable formation of hæm (up to 10 per cent with about 10^{-4} M porphyrin and 10^{-2} M ferrous iron). 'Teepol' (sodium alkyl sulphates) was even more effective; it held both the porphyrin and the ferrous iron in solution at all pH values in the range of neutrality (Phillips has used various detergents in order to measure the spectra of porphyrin esters in aqueous media; unpublished work). The yields of protohæm varied from 20 to 45 per cent over the pH range 5–8.

A high degree of reproducibility was not possible, particularly in the presence of ferrous hydroxide, which was probably oxidized to varying degrees depending upon small differences in the conditions. Nevertheless, we feel that our results demonstrate clearly that porphyrins and ferrous iron combine under conditions of physiological pH and temperature.

In a more complete study of this problem at least two points deserve closer examination. We found that in the presence of 'Teepol' the pyridine hæmochrome of the hæm formed from protoporphyrin had an absorption maximum at 550 mμ in place of 557 mμ. We also found difficulty in getting appreciable incorporation at 37° C. (not at 100° C.) of iron into coproporphyrin formed by the autoxidation of coproporphyrinogen, as opposed to incorporation into coproporphyrin added as such.

Goldberg *et al.*[4] have shown that protoporphyrin will serve as a substrate *in vitro* for an enzyme system forming hæm from nucleated erythrocytes. It seems unlikely that protoporphyrin could be reduced to porphyrinogen by a hydrogenase of avian erythrocytes, since even a powerful reducing agent such as dithionite will not bring about this reduction.

Two distinct stages may be observed in the chemical reduction of porphyrins and in the course of re-oxidation. The fully reduced porphyrinogen (hexahydroporphin) is colourless, but an intermediate stage exhibits a yellow colour and a urobilin-like spectrum with a broad absorption maximum in the region of 500 mμ. This may be a dihydro- or tetrahydro-porphin with a dipyrrylmethene-like structure, and it is of interest that addition of dithionite reduced this intermediate to the colourless porphyrinogen (cf. ref. 3). Such reduction might well be accomplished by a reverse reaction of the erythrocyte dehydrogenases and might constitute the mechanism whereby the porphyrinogen intermediates of hæm synthesis are normally prevented from 'escaping' as uro- or copro-porphyrin, porphyrins which can no longer be drawn back into the system of hæm formation. Indeed, it might be wondered whether in pathological conditions associated with production of excess of free porphyrins, for example, porphyria cutanea tarda[5], some derangement had not occurred in these reducing systems.

From our results, and from these considerations, taken in conjunction with the known ease of entry into the porphin macrocycle of divalent ions such as iron, copper and zinc, we suggest that, in the biological formation of hæm, iron reacts with protoporphyrin, not with protoporphyrinogen.

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Evidence for the Involvement of Ferrous Iron in the Biosynthesis of δ-Aminolævulic Acid by Chicken Erythrocyte Preparations

DURING an investigation into the mode of action of 5 : 6-dimethylbenzimidazole as an inhibitor of δ-aminolævulic acid synthesis in particulate preparations of chicken erythrocytes[1], a range of metal ions was examined for effect on the inhibition. The following metals were without any significant effect: cobalt, copper, nickel, magnesium, manganese, lead and ferric iron. Ferrous iron, however, consistently diminished the inhibition; a typical set of results is shown in Table 1. Addition of ferrous sulphate to freshly prepared systems not containing inhibitor produced a depression of synthesis of δ-aminolævulic acid. If, however, the preparation was aged at

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