Brodskiĭ and Miklukhin[4] have shown that when benzophenone oxime is rearranged in aqueous hydrochloric acid enriched with H_2O^{18}, complete exchange of oxygen with solvent takes place. Benzanilide exchanges none of its oxygen under these conditions. Therefore the oxygen atom in the resulting benzanilide molecule is not the same one that was present in the oxime. This is incompatible with Higman's mechanism, unless a highly improbable oxygen exchange takes place in the oxime itself. Such an exchange would involve inversion of configuration and formation of mixed amides, neither of which is observed under the operative conditions.

All the known facts are well interpreted in terms of the ionization mechanism which was lucidly set forth in the article[5] to which Higman referred. It is difficult to see why that theory should now be thought unacceptable, and in point of fact no criticism of it has been advanced.

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Porphyrin Fluorescence in the Livers of Pellagrins in Relation to Ultra-Violet Light

The examination, by ultra-violet fluorescence microscopy, of frozen sections of fragments of livers removed by an improved biopsy procedure[1] from twenty African pellagrins, soon after admission to hospital, revealed the presence in eight cases of an intense scarlet red fluorescence. This fluorescence was localized to the hepatic cells, and, when severe, could involve the entire lobule. When the fluorescent material was dissolved in the fat droplets in the hepatic cells, these assumed an intense crimson colour.

In serial biopsies at weekly intervals of livers of these eight pellagrins maintained on a carbohydrate vitamin-poor diet, it was seen that this fluorescence disappeared first from the central zone and last from the periportal region of the lobules. In so doing, all gradations of colours from red to yellowish-brown background fluorescence, previously described[2], were observed.

Oxidized cytochrome, bilicyanin and porphyrins are, so far as we know, the only likely substances in animal tissue said to give a red fluorescence[3]. We are satisfied that the scarlet fluorescence in the livers of pellagrins is not oxidized cytochrome or bilicyanin for the following reasons: (1) We obtained no fluorescence in concentrated solutions of oxidized cytochrome prepared from beef heart by the method of Keilin and Hartree[4]. (2) Bilicyanin prepared by adding an alcoholic solution of zinc sulphate and iodine to an alcoholic solution of bilirubin gives a yellow-red fluorescence which is rapidly changed to yellow-green on the addition of alkalis and, simultaneously, the spectroscopic bands characteristic of choletelin make their appearance. (3) The red fluorescence of the livers of pellagrins is intensified by acids and alkalis. It is soluble in water and we have found it, in this respect, to react in a manner identical to dilute solutions of protoporphyrin and the red fluorescence we observed in a liver obtained from a case of severe porphyria in a woman seen in the acute stages of the disease.

Unfortunately, the core of liver tissue obtained from pellagrins was too small to allow for the extraction of porphyrin for spectroscopic identification. The porphyrin fluorescence in the pellagrous livers was invariably associated with evidence of active formation of cytolipochrome and cytosiderin. The iron in some of these livers may amount to as much as 5 per cent of dry weight of liver[5]. The transitory character of the scarlet fluorescence appearing in the acute phases of the disease can easily account for the presence of masses of iron pigment so constantly present in the livers of pellagrins[5,6,7] without any visible porphyrin fluorescence.

The association of scarlet fluorescence and iron pigment is further evidence in support of our view that cytosiderosis of the livers of adult pellagrins is an expression of the disruption of an iron porphyrin complex within the liver cell.

Three pellagrins who had lost their porphyrin fluorescence while on a carbohydrate vitamin-poor diet were afterwards exposed to ultra-violet light for ten minutes on five consecutive days. When biopsy was performed on the sixth day, a marked recrudescence in the fluorescence was noted in the livers of all cases, and in two it was even more intense than on the day of admission to hospital; there was no aggravation of the skin lesions or of the systemic reactions generally seen in this disease. Traces of porphyrins were found in the urine of only one of these cases. Examination of the liver of a healthy patient, admitted to hospital for a minor surgical injury, before and after treatment with ultra-violet light under conditions identical to those provided for the pellagrins, revealed the absence of visible scarlet fluorescence.

From these studies we conclude: (1) Porphyrin fluorescence in the liver can occur during the acute phases of pellagra. (2) The great accumulation of iron pigment in many livers of adult African pellagrins is probably caused by the disruption of an intracellular iron porphyrin complex, such as catalase and cytochrome, present normally in the liver cell. (3) Treatment with vitamin B complex is not required to resolve the porphyrin fluorescence in the livers of pellagrins while on a carbohydrate vitamin-poor diet. (4) Ultra-violet light can excite a recrudescence of the porphyrin fluorescence in the liver without causing an exacerbation of the other external manifestations of the disease. Massive quantities of porphyrins can appear in the liver cells without any detectable amounts in the urine. (5) These experiments emphasize the close inter-relationship between the reactivity of the skin to ultra-violet light and the deposition of iron and the appearance of porphyrin fluorescence in the liver in African pellagrins.

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