(Fleischmann No. 139) was made. *S. aureus,* non-exacting with respect to biotin, showed an egg white sensitivity similar to *S. dysenteriae.* The less sensitive *E. coli* was only slightly retarded in its rate of growth. *S. cerevisiae,* in the presence of excess biotin in a 1 per cent. sucrose nutrient broth at pH 7.0, was completely inhibited by a 1/20 dilution of egg white. At pH 6.0 this inhibition was strongly marked but not complete. Iron addition, either ferrous or ferric, overcame the egg white inhibition shown by all the organisms tested.

The foregoing results suggest that the ability of raw egg white to combine with iron and make it unavailable to microorganisms for growth may have significance for diagnostic and therapeutic purposes as well as for biological phenomena in which egg white and/or iron are known to, or may, play an important role. Thus, in animal nutrition, the egg white injury syndromes may find explanation in deficiency not only of biotin but also of iron induced either directly in the host or indirectly through modification of the intestinal flora. Egg white might be used to advantage as a tool in the study of various anemias or of perosis and other multiple deficiency diseases with reference to possible involvement of iron.

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AN OXIDATIVE METABOLITE OF PYRIDOXINE IN HUMAN URINE

IN 1941 Singal and Sydenstricker described in a brief note in this journal[1] the appearance of a fluorescent compound in human urine after the ingestion of pyridoxine (B_6). No further studies were reported concerning its nature. We confirmed this observation and also found that upon heating the urine with acid this substance is converted to a new compound possessing a fluorescence of maximum intensity at above pH 8.6 as compared with a maximum at pH 3–4 for the original substance. Moreover, the fluorescence intensity of the new substance is 25 times greater.

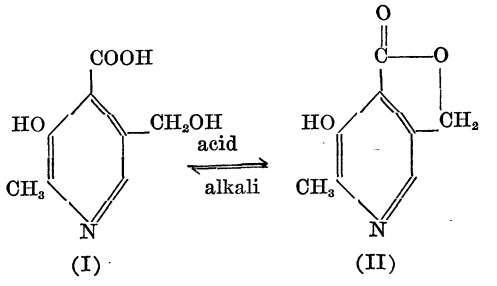
This new fluorescent substance was isolated in crystalline form from urine heated with acid. Since this product was easily reduced (with hydrosulfite) to a non-fluorescent form and reoxidized (with H_2O_2) to the original form it was surmised to be an oxidation product. Employing a slow direct oxidation of B_6 HCl with permanganate in neutral solution a product was obtained which appears to be identical with the urinary substance, as shown by the data in Table 1.

TABLE 1

Compound	M.P. (II)	Mixed M.P.	M.P. of methyl ether of (II)	Mixed M.P.	Fluorescence* (II)	(I)
Synthetic	263–5°	263–5°	111–2°	111–2°	1300	52
Urinary	263–5°		111–2°		1300	52

* The fluorescence is expressed in galvanometer divisions per microgram per cc in the Coleman Electric Photofluorometer employing filters B1 and PC1.

The structure of this compound was shown to be the lactone of 2-methyl - 3-hydroxy - 4-carboxy - 5-hydroxymethyl pyridine (II),[2] which is converted by heating with alkali to the corresponding acid form (I). This is the form (I) in which the oxidative product is excreted in human urine after the ingestion of B_6. The intensity of the fluorescence of the two compounds varies with the pH of the solution according to well-defined curves different and characteristic for each of them.



(I) (II)

After the ingestion of 50 mg of B_6HCl normal human adults excrete 3–8 mg of compound (I) in 4 hours, and 200–300 mg in 24 hours after the ingestion of 1 gram. It is not excreted by dogs and is excreted to a small extent by rats after the administration of the vitamin. Our observations indicate that it is not identical with the metabolites of B_6 studied in these 3 species by Scudi and collaborators.[3]

The details of the isolation and identification of the compounds as well as of a method for their quantitative determination will be published elsewhere.

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[1] S. A. Singal and V. P. Sydenstricker, SCIENCE, 94: 545, 1941.

[2] The isomer of this compound, the lactone of 2-methyl - 3-hydroxy - 4-hydroxymethyl - 5-carboxy pyridine was synthesized and described by S. A. Harris, E. T. Stiller and K. Folkers, *Jour. Amer. Chem. Soc.,* 61: 1242, 1939, in the course of the elucidation of the structure of vitamin B_6. We are greatly indebted to Dr. K. Folkers of Merck and Company for a sample of this isomer which aided us materially in establishing the structure of the metabolite described in this paper.

[3] J. V. Scudi, R. P. Buhs and D. B. Hood, *Jour. Biol. Chem.,* 142: 323, 1942.

[4] Nutrition Foundation Fellow. The authors are also indebted for grants in aid of this study to the John and Mary Markle Foundation and the Duke University Research Council; and to Merck and Company, Inc., for a generous supply of pyridoxine.