We are indebted to the Rockefeller Foundation for financial support of this work.

WILLIAM C. BOYD
JANE BEHNKE

SCHOOL OF MEDICINE,
BOSTON UNIVERSITY

RAW HEN EGG WHITE AND THE ROLE OF IRON IN GROWTH INHIBITION OF SHIGELLA DYSENTERIAE, STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI AND SACCHAROMYCES CEREVISIAE[1]

RAW hen egg white can cause an inhibition of growth of *Shigella dysenteriae* and other microorganisms which is independent of the avidin-biotin phenomenon. Of ten vitamin factors and thirty-one elements tested, iron alone overcame this egg white inhibition.

In the course of work on the stabilization of dysentery bacteriophage during lyophilization, secondary growth of a strain of *S. dysenteriae* failed to appear in the first tube of a d'Herelle titration of a phage sample lyophilized with raw egg white. Titrations run in the absence of phage with varying concentrations of egg white showed that the inhibition of growth depended upon the amount of egg white added to the medium and was not related to phage activity. 0.02 ml of egg white per ml of nutrient broth (0.3 per cent. Lemco meat extract, 1.0 per cent. Bacto peptone, 0.5 per cent. sodium chloride, adjusted to pH 7.2) inhibited the development of $2-20\times10^5$ bacteria for 24 hours at 37° C. Serial dilution of transfers made from the inhibited cultures to fresh egg white-free broth showed that failure to grow was not due to destruction or lysis of the bacterial cells.

Additions of avidin concentrate to the nutrient broth in an amount equal to twice the estimated avidin content of an inhibiting concentration of raw egg white failed to prevent normal growth of *S. dysenteriae*. Conversely, additions of biotin to the egg white-treated broth in an amount estimated to be twice that required to neutralize the avidin content of the contained egg white failed to permit normal growth of the bacteria.

The effect of pH on the inhibition phenomenon by egg white was such that at pH of 5.8 and below, bacterial growth occurred equal to controls without egg white; at pH's between 5.8 and 6.4, partial inhibition was exerted so that as the pH approached 6.4, initiation of growth was increasingly delayed; at pH 6.4 and above, inhibition of growth was complete for at least 48 hours at 37° C.

When raw egg white was dialyzed against distilled water, the dialyzed material retained fully its ability to inhibit the growth of the bacteria while the dialyzate was without effect. Further, heating samples of egg white adjusted to pH 7.3 with phosphate buffer in nutrient broth for one hour at 40° C., 50° C., 60° C. and 70° C. showed that the inhibiting capacity of the raw egg white is stable at temperatures up to 60° C. but destroyed at 70° C. under the conditions employed.

Corn steep liquor, meat extract and yeast extract, when added in relatively large amounts to nutrient broth in the presence of egg white, permitted growth of *S. dysenteriae* to occur equal to controls without egg white. The following known growth factors were tried singly and in combination: thiamin hydrochloride, riboflavin, nicotinamide, inositol, calcium pantothenate, pyridoxine, biotin, para aminobenzoic acid, choline chloride and tryptophane. All were ineffective in overcoming the egg white inhibition.

When yeast extract was ashed, however, the ash after solution in hydrochloric acid was active. Of 31 elements tested, iron alone overcame the egg white inhibition of growth. Soluble ferrous and ferric salts were effective when used in an amount of approximately 20 gammas of iron for every ml of raw egg white, the ferrous iron being consistently somewhat more active than the ferric form. The amount of "free" iron (dipyridyl method) in the 4.5 ml of nutrient broth used routinely was 0.8–1.0 gammas. Since one ml of egg white requires 20 gammas of added iron, 0.04 to 0.05 ml might be expected to make the "free" iron of the broth unavailable to the bacteria for growth. In general, the results obtained justify this expectation.

Ferrous and ferric ammonium sulfate were added to egg white at various pH's and dialyzed overnight at room temperature against buffered saline. Qualitatively, the data obtained showed that at pH 7.0 and above egg white made both forms of iron undialyzable; at pH 6.0 and to a greater extent at pH 5.0, some ferrous iron could be dialyzed, while ferric iron was undialyzable at both pH's. All results were compared to controls of iron alone in buffered saline at the same pH's. The effect of pH on the dialyzability of iron from egg white-iron salt mixtures finds some parallel in the relation of pH to the inhibition of growth of *S. dysenteriae* in egg white-treated nutrient broth.

When ferric or ferrous iron in concentrations employed in this study were added to raw egg white, a tan to brownish coloration appeared. The depth of color varied directly with the amount of iron added. Possible correlation of color production with the biological phenomenon reported here is being investigated.

Preliminary study of the inhibitive effect of raw egg white on the growth of a strain of *Staphylococcus aureus, Escherichia coli* and *Saccharomyces cerevisiae*

[1] A preliminary report.

(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.

The authors wish to thank Dr. Dean Burk, of the National Cancer Institute, Bethesda, Md., for supplying the avidin concentrate used in this work.

ARTHUR L. SCHADE
LEONA CAROLINE

OVERLY BIOCHEMICAL RESEARCH FOUNDATION, INC.,
NEW YORK 1, N. Y.

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.

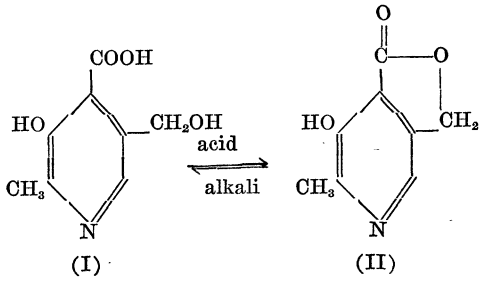
This new fluorescent substance was isolated in crystalline form from urine heated with acid. Since this product was easily reduced (with hydrosulfite) to a non-fluorescent form and reoxidized (with H_2O_2) to the original form it was surmised to be an oxidation product. Employing a slow direct oxidation 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.

JESSE W. HUFF[4]
WM. A. PERLZWEIG

DEPARTMENT OF BIOCHEMISTRY,
DUKE UNIVERSITY SCHOOL OF MEDICINE

[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.