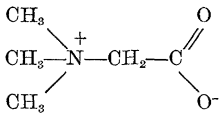
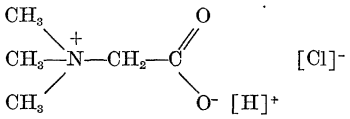


In the case of betaine the hydrochloric acid salt is usually referred to as betaine hydrochloride, and with a great deal more justification than in the case of the chloride salt of choline or of the so-called hydrochlorides of alkaloids, etc. Inspection of the zwitterionic nature of the hydrochloric acid salt of betaine



indicates that the hydrochloric acid is loosely bound; in fact the hydrochloric acid may be titrated as free acid. Pure crystalline betaine hydrochloride may well be used as a stable source of standard hydrochloric acid; doubtless other stable, crystalline salts of betaine might be used as standards for other acids.

The present confusing nomenclature might be avoided by the use of the cumbersome, but adequately descriptive, chemical names, or by the introduction of a more logical system of common names, preferably based on the familiar term "choline" as the root. Since the names which have been proposed so far fail to indicate the onium structure of the compounds, Professor Austin M. Patterson has suggested (in a personal communication) the name *cholinium* for the choline cation; this would permit the use of the terms cholinium hydroxide, cholinium chloride, acetylcholinium bromide, etc., for the salts and derivatives. In such a system betaine aldehyde might become aldocholinium hydroxide, a name which indicates its properties, and betaine aldehyde chloride, aldo-cholinium chloride, a term which suggests its chemical and physiological relationships. The root name *cholonium* would appear theoretically more sound, but might be accepted less readily, since the relation to the term now in common usage is less easily recognized. A satisfactory name for betaine itself is not easily found, its carboxy group being difficult to indicate (carboxycholinium, according to the international system, would indicate the replacement of a hydrogen of the choline cation by a carboxyl group); betainium is the only root name which would appear applicable, and this is far from satisfactory.

The writer will welcome suggestions and comments on this or other systems of nomenclature for the choline compounds. Professor Patterson has suggested that if the workers in this field should arrive at definite proposals regarding terminology, these proposals might be presented to the American committee on biochemical and organic nomenclature of the International Union.

The author wishes to express his appreciation for the advice and suggestions of Professor Austin M. Patterson, Professor C. F. Cori, Profesor H. T. Graham and Dr. T. H. Jukes.

ARNOLD DEM. WELCH

WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE,
ST. LOUIS

BIOLOGICAL DETERMINATION OF VITAMIN B_1 (THIAMIN) IN RHIZOBIUM TRIFOLII

WHILE studying growth factor requirements of the nodule bacteria, it became desirable to determine the ability of the organisms to synthesize vitamin B_1. Since only minute amounts of cellular material were conveniently available, feeding experiments were impractical. The situation required a rapid biological assay, applicable to small quantities of ordinary culture media.

For this purpose, a quantitative method was developed, which is an application of Knight's[1, 2] demonstration that, under suitable conditions, the growth of *Staphylococcus aureus* is proportional to the amount of vitamin B_1 present in the medium. The base medium employed for the estimation of vitamin B_1 is similar to that of Knight[1, 2] and Fildes,[3] but with the substitution of casein hydrolysate for gelatine hydrolysate or known amino acids:

Acid hydrolyzed casein	20 ml (equivalent to 0.4 gm Merck's casein)
Dipotassium phosphate	0.5 gm
Glucose	0.3 gm
Cysteine, HCl	2.0 mgm
Nicotinic acid	0.02 mgm

pH–7.0
Distilled water to 50 ml

Five ml amounts of this medium are tubed, and sufficient distilled water added so that together with the addition of the test material, the final volume is 10 ml. The medium is autoclaved one hour at 15 pounds, vitamin B_1 additions being made under aseptic conditions after sterilization.

A trace of growth from a nutrient agar culture is suspended in 10 ml water (approximately 100,000 bacteria per ml), one drop of which is used for inoculation. No growth occurs in the base medium. However, as shown in Fig. 1, in the presence of as little as .00005 micrograms per ml of vitamin B_1, detectable growth results, the amount of growth increasing rapidly with higher concentrations of the vitamin to .001 micrograms per ml. Growth is determined after

[1] B. C. J. G. Knight, *Brit. Jour. Expt. Path.*, 16: 315–326, 1935.
[2] B. C. J. G. Knight, *Biochem. Jour.*, 31: 731–737, 1937.
[3] P. Fildes, G. M. Richardson, B. C. J. G. Knight and C. P. Gladstone, *Brit. Jour. Expt. Path.*, 17: 481–484, 1936.

incubation for 36 hours at 37° C. by turbidity measurements using the Evelyn electrophotometer.

One gram of pork liver, found by animal assay to contain 36 micrograms of vitamin B_1 per gram, was extracted four times for periods of five minutes each with boiling N/10 hydrochloric acid. This extract was adjusted to pH 7.0 with sterile sodium hydroxide and made up to 100 ml. The actual amount of vitamin B_1 present in dilutions of pork liver extract was calculated by comparing the resultant stimulation with that of known quantities of vitamin B_1 (see Fig. 1). The ordinates represent per cent. increase in light absorption due to turbidity, upon addition of the amounts of stimulant indicated on the abscissae. Those points falling on the steep part of the curve were selected for calculations. By obtaining several points in this region, a convenient check for a given determination is provided. An average from four separate determinations, none of which varied over 3 per cent. from the mean, was 32 micrograms vitamin B_1 per gram. Similar confirmations of the accuracy of the method when applied to biological materials were obtained with standardized samples of pork kidney and ham.

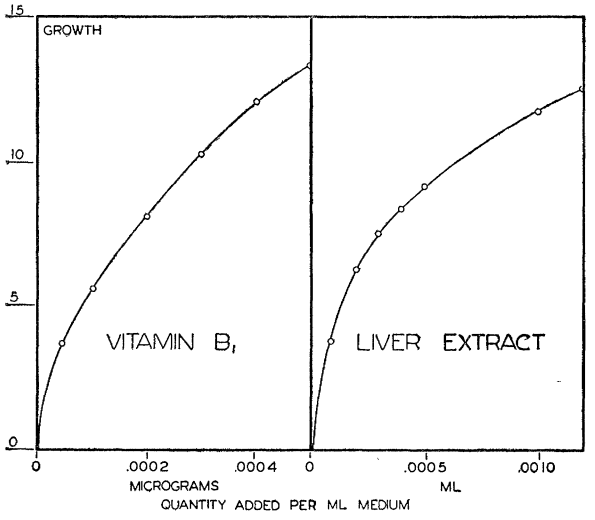


FIG. 1. Influence of vitamin B_1 on growth of *Staphylococcus aureus*. On left, the standard curve for pure vitamin B_1. On right, curve for liver extract to be assayed for vitamin B_1.

Six weeks' old cultures of *R. trifolii*, grown on a vitamin B_1-free synthetic medium were heated 15 minutes at 100° C. with N/10 HCl. This sufficed to liberate the free vitamin from combined form in which it apparently exists in the autolyzed cultures. Various cultures assayed averaged 19.6 micrograms of vitamin B_1 per gram of dry cells. This figure was in agreement with analyses by Meikeljohn's modification of Schopfer's test[4] which, however, is much less sensitive to vitamin B_1 and requires ten days for completion. From the data obtained, it appears that the vitamin B_1 content of *Rhizobium trifolii* cells closely approximates the amount known to be present in yeasts.

P. M. WEST
P. W. WILSON
DEPARTMENT OF AGRICULTURAL BACTERIOLOGY,
UNIVERSITY OF WISCONSIN

SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS TO ASSIST IN PHOTOGRAPHING EXPERIMENTAL MATERIAL[1]

THE apparatus described herewith has proved to be a very convenient device for photographing laboratory specimens, especially small animals such as rats. The advantages of this apparatus lie in the fact that photographs are taken in the laboratory under uniform conditions of distance and light and at about one-tenth the usual cost when made by a commercial photographer, for the individual photographs, and less than one half the usual cost for the pictures finally selected and enlarged for printing. In addition to a saving of time, the animals are not exposed to abnormal conditions outside the laboratory.

The apparatus consists of a boxlike arrangement with an opening at one end for inserting a camera and a hinged door at the opposite end for inserting the specimens to be photographed. A lamp recess is located on each side of the box. The insides of the lamp recesses are painted with white enamel. The inside of the box in which the camera is placed is painted black. A removable glass partition, made of clear window glass 9″ by 18″, with a wing 4″ wide cemented 1″ from each end is used to retain rats within 4″ of the hinged back door. The hinged door carries a gray cardboard, which serves as a background. Gray is desirable as a background for most objects, but since the cardboard is attached with thumbtacks, other colors may be readily substituted as a suitable contrasting background for the particular specimen to be photographed. A yellow cardboard makes a desirable floor when rats are being photographed. A 15-centimeter rule for showing comparative sizes, and a white card with sufficient data to identify the specimen, the date and the number of the particular photograph are attached to the door. These become an integral part of the photograph, but, if placed on the upper part of the door, may be blotted

[1] Published with the approval of the director of the New Mexico Agricultural Experiment Station.

[4] A. P. Meikeljohn, *Biochem. Jour.*, 31: 1441–1451, 1937.