variation actually found for the hydrogens. The intercheck between the purified helium and the light hydrogen is satisfactory and clearly points to the purity of the light hydrogen used as the standard gas being certainly better than 99.9% with respect to any impurity likely to be present. Thus, the analytical values of the deuterium samples must be correct to within 0.2% of the real absolute value if one accepts Livingston's value for the atomic weight of D. This latter value would certainly seem to be much more accurate than 0.1% so that the deuterium analyses may be accepted within the limits of error found. More discussion of the significance of this point will be forthcoming in another paper in which the results —obtained using this gas balance as an analytical device—of a recent investigation of the electrolytic separation factors for the hydrogen isotopes will be published. The gas balance has proved very useful as a primary standard in preparing samples of the hydrogen isotopes of known deuterium concentration for use in calibrating other analytical devices of the relative type such as thermal conductivity gages of the Farkas form and another form, details of which are published elsewhere.[14] It is of some interest for practical reasons to note two other results in the data of Table II, namely, those obtained from measurements made on ordinary tank helium and hydrogen. The measurements have been expressed in terms of the apparent molecular weights of the tank gases in order to facilitate comparisons with the purified gases. Both gases obviously contain very appreciable amounts of heavier impurities. In the case of the helium this impurity may have been due largely to nitrogen because during the arcing process the brown fumes of nitrogen peroxide were clearly visible. With proper precautions many kinds of two component—as well as three component equilibria of known equilibrium constants—gaseous combinations could be analysed using this balance.

(14) N. R. Trenner, *J. Chem. Phys.*, **5**, 382 (1937).

Summary

1. A simple type of quartz fiber suspended gas density balance which can be constructed easily has been described.

2. The method of adjustment and use of this gas balance as well as its characteristics have been described and illustrated by experimental results.

3. The use of this gas balance as an analytical device and as a primary standard for (making up) samples of the hydrogen isotopes of known deuterium concentration has been illustrated.

PRINCETON, N. J. RECEIVED MARCH 1, 1937

[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, E. R. SQUIBB & SONS]

Crystalline Vitamin B_1 from Natural Sources

BY R. D. GREENE AND A. BLACK

This paper deals with a study of vitamin B_1 which was carried out in this Laboratory during the past three years, a portion of which was referred to in a preliminary report.[1] Several workers[2-7] have developed methods for the isolation of the vitamin from natural sources. Since the beginning of this work two different groups of workers[8-9] have developed processes for the synthetic production of vitamin B_1. While it had been our purpose to provide a less expensive method for the isolation of the naturally occurring vitamin, it is not claimed that the process herein described can meet the synthetic methods on a competitive basis. Rather it is submitted because it contains certain features which have proved successful in the production of pure natural vitamin B_1 and which may be useful in the fractionation of the B complex.

(1) Greene and Black, *Science*, **84**, 185 (1936).
(2) Jansen and Donath, *Mededeel. Dienst Volksgezondheid Nederland Indië, pt. 1*, 186 (1926).
(3) Ohdake, *Proc. Imp. Acad.* (Tokyo), **7**, 102 (1931).
(4) Windaus, *Nachr. Ges. Wiss. Göttingen*, 209 (1932).
(5) Seidell and Smith, THIS JOURNAL, **55**, 3380 (1933).
(6) Kinnersley, O'Brien and Peters, *Biochem. J.*, **27**, 232 (1933).
(7) Williams, Waterman and Keresztesy, THIS JOURNAL, **56**, 1187 (1934).
(8) Williams and Cline, *ibid.*, **58**, 1504 (1936).
(9) I. G. Farbenindustrie A. G., British Patents 456,735, and 456,751 (1936).

Adsorption

Since Seidell's discovery in 1916 of the adsorption of vitamin B_1 by certain agents, such as fullers' earth, this procedure has been used by nearly everyone in their attempts at concentration. We have studied the adsorptive capacities of a num-

ber of substances, among which were fullers' earths, charcoal, kaolin and aluminum oxide, and have found the fullers' earths to be the best. There are variations in fullers' earths and some English earths and Lloyd's reagent were the best of those which were studied.

We have found that a fullers' earth adsorbate prepared according to the general method of Seidell serves as a satisfactory starting point for our scheme of purification. In addition, we have prepared adsorbates of increased potency by first removing impurities from the solutions with charcoal at a *p*H of 4–5, followed by fullers' earth adsorption. The preliminary charcoal step, which is similar to that developed by Peters[6] and coworkers, apparently removes certain substances which interfere with the adsorption of B_1. It removes riboflavin if present in the extract. By this method we have prepared, from extracts of yeast or rice polishings, fullers' earth adsorbates twice as rich in vitamin B_1 as were obtained by direct adsorption. An obvious advantage is the consequent reduction in the scale of later operations. The influence of this procedure on the strength of adsorbates is shown below.

TABLE I

Solution for adsorption	Ratio of Lloyd's reagent to yeast or rice polish	Int. units[a] per g. adsorbate
Aqueous extract of yeast		
(a) Control	1:50	300–400
(b) Same treated with Norit 1:200[b]	1:50	600
(c) Same treated with Norit 1:50[b]	1:50	600
Aqueous extract of rice polish		
(a) Control	1:175	100
(b) Same treated with Norit 1:50[b]	1:175	270–300

[a] Determined by comparative growth tests with International Standard. [b] Tests of the charcoals indicated a negligible quantity of vitamin B_1 present.

Extraction of the Vitamin from Fullers' Earth

Methods for the preparation of pure vitamin B_1 have been handicapped largely by the limitations of the procedures available for the removal of the vitamin from fullers' earth. Studies were undertaken on a few of the known methods of elution[7,10,11] and while not tried exhaustively, they did not in any case give very high yields.

(10) Seidell and Smith, *U. S. Public Health Repts.*, **45**, 3194 (1930).
(11) Stuart, Block and Cowgill, *J. Biol. Chem.*, **105**, 483 (1934).

Observations on the solubility and consideration of the structure[12] proposed for the vitamin and also the Williams[7] method of elution with quinine sulfate led to the belief that salts of certain simple organic nitrogen bases might be useful in its extraction. Although it has been reported[13] recently that such a free base as pyridine extracts the vitamin from fullers' earth, we had previously found it to be quite ineffective, whereas the *hydrochlorides* of pyridine, quinoline or aniline were remarkably effective. Limited data indicate that salts other than hydrochlorides are also effective. An extensive study has been made of the extraction by aqueous and aqueous alcoholic solutions of these hydrochlorides in order to remove effectively the vitamin from fullers' earth with a minimum of inactive matter. Although the solutions of the salts elute some riboflavin, they are much less effective than solutions of the free bases. Solutions of the base hydrochlorides in the normal alcohols, from methyl through amyl, extract vitamin B_1 and exhibit solvent powers which decrease in that order. Solutions of 15–20% pyridine hydrochloride in 88% ethanol or 75% *n*-propanol operating at room temperature were found to be the most practical and yielded 80–90% of the vitamin B_1 present in the fullers' earth.

Consideration of the elution of vitamin B_1 by pyridine hydrochloride suggested that the mechanism is one of replacement, such as the action of quinine sulfate in the Williams process.[7] In confirmation of this view, limited data indicate that there is a definite adsorption of pyridine hydrochloride by the fullers' earth during elution.

Attempts have been made to modify the Seidell[10] alkaline extraction method, by introducing an immiscible liquid which is a good solvent for the vitamin. For example, mixtures of phenol with sodium hydroxide or sodium bicarbonate gave fairly high yields of vitamin; in fact, fair yields of crystals were obtained from such extracts. It was also found that sodium chloride could be substituted for the alkali but that the yields were somewhat lower. In other experiments aniline–butyl alcohol and aniline–butyl alcohol–carbon disulfide were used in place of phenol, and the vitamin was extracted, although the yields were not as good as when phenol was used. In general these methods were more difficult to operate than the methods with salts of nitrogen bases.

(12) Williams, THIS JOURNAL, **57**, 229 (1935).
(13) Cook and Carroll, *Ind. Eng. Chem.*, **28**, 741 (1936).

Distribution of Vitamin B_1 in Immiscible Solvent-Water Systems

This work had its origin in a conviction that the possibilities of available solvents in the role of vitamin B_1 purification had been but incompletely realized. The water miscible alcohols, methyl, ethyl and *n*-propyl, have been employed[6,10] with success in removing inert matter by precipitation. Block and Cowgill[14] have reported the concentration of B_1 by extraction of the free base from alkaline solutions by means of certain immiscible solvents.

Consideration of the NH_2, OH and S groupings in the structure of B_1 suggested the use of a solvent mixture containing them. It is noteworthy that an equal mixture of aniline and butyl alcohol against water shows a coefficient higher than either of the components, and the addition of carbon disulfide, in which the vitamin is almost insoluble, further enhances the solvent power. Because of the well-known reaction between aniline and carbon disulfide this mixture is limited to a theoretical interest. This relation between the reactivity and the solvent power is interesting when compared with that of the mixture of a tertiary amine, such as quinoline, and butyl alcohol, which has a coefficient slightly lower than those of its components.

This preliminary work led to a more exhaustive study of the distribution of vitamin B_1 in immiscible solvent–water systems. The results obtained from the more interesting and useful of these systems are summarized in Table II. These data were obtained in the following manner.

In most cases, 2.0 cc. of a 0.01% aqueous or saturated salt solution of pure vitamin B_1, which was adjusted to the indicated *p*H, was added to 2.0 cc. of the solvent and thoroughly shaken for several minutes. After the layers separated they were measured and an aliquot of each was filtered, freed from solvent with ether, and tested for B_1 by the Kinnersley and Peters[15] modification of the Pauly reaction. Although extensive experience has shown that this test is not specific for vitamin B_1, in these experiments there were no interfering substances. In cases where the coefficient was very low, stronger solutions of B_1 and more solvent were used. In the alkaline solutions the time was limited to ten minutes and there was little or no evidence of destruction.

(14) Block and Cowgill, *J. Biol. Chem.*, **97**, 421; **98**, 637 (1932).
(15) Kinnersley and Peters, *Biochem. J.*, **28**, 667 (1934).

It must be emphasized that the coefficients which are contained in Table II are not necessarily the same as those for the B_1 in crude concentrates, because other substances may have some effect on the solubility.

The high solvent power of phenol and cresol and the effect of sodium chloride were very interesting and were taken advantage of in purification of vitamin B_1. The marked differences in the solubility in phenol at different *p*H's were also very useful.

Incidentally, some of these have been found to be good solvents for riboflavin and some of the other factors of the B complex. This work is being followed up at the present time and the results will be presented in a later publication.

TABLE II

DISTRIBUTION COEFFICIENTS OF VITAMIN B_1 IN SOLVENT/H_2O SYSTEMS AT 22 ± 2°

Solvent	*p*H 5.0 H_2O	*p*H 5.0 Satd. NaCl	*p*H 10.0 H_2O	*p*H 10.0 Satd. NaCl
Phenol[a]	52.0	567	...	..
p-Cresol	48.5	..	..	..
Aniline	0.047	1.0	0.05	0.94
Pyridine	..	0.93	..	1.33
Quinoline	.083	.20	.10	0.13
n-Propanol	..	.39	..	.42
n-Butanol	.073	.18	.16	.21
n-Pentanol	.015	.08	.014	.08
Benzyl alcohol	.18	6.75	.82	7.25
Aniline–BuOH 1:1	.13	1.82	.18	2.0
Aniline–BuOH–CS_2 1:1:1	.60	0.62	..	..
Quinoline–BuOH 1:1	.06	.16	..	..
Pyridine–PrOH	..	.42	..	0.4
Pyridine–BuOH	..	.31	..	.2
CS_2	.0002	..	..	..
Ether	.0001	.0001	.0001	.0006

[a] At *p*H values of 1.0, 3.0, 3.5, and 7.0 the coefficients for phenol/H_2O are 5.5, 3.0, 8.0, and 76.5, respectively. For phenol/saturated NaCl, *p*H 3.0, the coefficient is 170.

Isolation of Vitamin B_1 Crystals

It has been possible to develop a sequence of purification steps which produces high yields of pure vitamin B_1 by taking advantage of the procedures of extraction, distributions between water and immiscible solvents, and readsorptions. The resulting method has been applied in the production of eleven independent lots of crystals.

The fullers' earth adsorbates were prepared by the usual procedure except in a few cases where the preliminary charcoal step was used. In this study we have not been concerned primarily with the maximum yields of vitamin in the adsorption from extracts of rice polish and yeast. The experience of others, as well as ourselves, has shown that it is possible to obtain high yields in this step.

For elution, 250 g. of the adsorbate is treated with a solution of pyridine hydrochloride prepared by mixing 210 cc.

TABLE III

Preparation	Extracting agent	Adsorbate used, g.	Actual yield of crystals, mg.	Calcd. yield, mg.	% yield[a]
B-5 Rice polish	C_5H_5N·HCl, 88% EtOH	200	60.0	111	54.1
B-32 Rice polish	C_5H_5N·HCl, 70% *n*-PrOH	50	11.5	27.8	41.5
B-52 Rice polish	C_5H_5N·HCl, 88% EtOH	60	12.0	27	44.5
B-58 Rice polish	C_5H_5N·HCl, 88% EtOH	50	9.0	22.5	40.0
B-73 Rice polish	C_5H_5N·HCl, 88% EtOH	275	33.0	82.5	40.0
B-54 Yeast	C_5H_5N·HCl, 88% EtOH	245	92.0	331	27.8
B-23 Rice polish	C_6H_5OH, 0.4 *N* NaOH	100	15.5	55.5	28.0
B-30 Rice polish	C_6H_5OH, satd. aq. $NaHCO_3$	100	14.5	55.5	26.0

[a] The crystals are 90 + % pure; the actual percentage yields of pure B_1 are therefore slightly lower than these figures. The vitamin content of the adsorbate was determined by the Smith curative rat test.

of pyridine, 210 cc. of concentrated hydrochloric acid (37–38.5%) and 1200 cc. of absolute ethyl alcohol. After stirring at room temperature for one hour, the mixture is separated by centrifuge and the residue is extracted a second time with a mixture of 130 cc. of pyridine, 130 cc. of concentrated hydrochloric acid and 1050 cc. of absolute alcohol. After centrifugation the residue is discarded and the combined extracts are distilled *in vacuo* to remove alcohol and water. The sirupy mass, which contains the vitamin hydrochloride, is taken up in 300 cc. of *n*-propanol and 430 g. (an excess) of sodium bicarbonate is added gradually. After stirring for one-half hour the mixture is filtered by suction and the salt residue is extracted with two small portions of a 1:2 mixture of pyridine–propanol. The combined pyridine–propanol solutions, which contain the vitamin B_1, may be freed from solvents in either of two ways: (a) by distillation *in vacuo* at 25–30°; (b) extraction by ether or a similar solvent in the presence of a water layer to take up the vitamin. In either case, the last traces of pyridine are removed by ether extraction, after adjustment to a *p*H of about 7.0 with sodium bicarbonate.

The aqueous solution, which at this point usually has a volume of two or three times the weight of the fullers' earth taken, is promptly adjusted to a *p*H of 4.5 and saturated with sodium chloride. The inert precipitate is removed by filtration and washed with a little saturated salt solution. The course of purification from this point may take either of the following two routes. (a) This longer procedure has given somewhat higher yields. The saturated salt solution of the vitamin, which has a volume of about 750 cc., is extracted with four 50-cc. portions of 88% phenol. These phenol extracts contain about 70–80% of the vitamin present in the fullers' earth. At this point, if desired, a salt-free concentrate containing 2.0–2.5% of vitamin B_1 may be prepared readily by washing the phenol extract with small amounts of water, then removing the phenol with ether and adjusting the aqueous solution of the vitamin to a *p*H of 4.5. However, in the preparation of the pure vitamin, the combined phenol extracts are washed three times with 700-cc. portions of 0.001 *N* hydrochloric acid, with separation by centrifuge. Phenol is added to replace that removed by the water. The phenol layer retains certain impurities, including riboflavin, while the aqueous solution now contains nearly all of the vitamin B_1, in a state of 3–3.5% purity. The aqueous solution, after removal of phenol by ether extraction, has a volume of 2250–2500 cc. and a *p*H of 3.5–4.0 and is ready for charcoal[16] treatment. The solution is agitated for one hour with sufficient charcoal to remove most of the yellow-brown color, which usually requires between 20 and 40 mg. per gram of adsorbate. Under such conditions a considerable amount of impurity and a negligible amount of vitamin B_1 is removed. At this point the vitamin B_1 content is 4–5% of the salt free solids. (b) In the shorter alternative procedure the use of phenol is eliminated. The saturated sodium chloride solution of the vitamin is diluted with four volumes of water and adjusted to a *p*H of 4.0. Treatment of this solution with charcoal is carried out about as in (a), except that the required amount of charcoal is somewhat larger.

The charcoal treated solution from either (a) or (b) is now ready for fullers' earth readsorption. In our experimental work we have used Lloyd's reagent for this purpose; one gram of the reagent to 20–30 g. of the original fullers' earth is suitable. After stirring for one hour with the solution, the Lloyd's reagent, which contains the vitamin in a condition of 8–10% purity, is removed by filtration and washed with alcohol. The vitamin B_1 is eluted from the adsorbate by stirring for one hour at room temperature with seven volumes of a 20% solution of pyridine hydrochloride in 88% *n*-propanol, followed by a second treatment with six volumes of the solution. Ethanol or *n*-butanol may be used in place of propanol. The vitamin concentrate is isolated as described in the treatment of the eluate of the first adsorbate, except that sodium bicarbonate is added directly, without first removing propanol.

After freeing from solvents the aqueous solution is promptly adjusted to a *p*H of 4.5 and evaporated nearly to dryness *in vacuo*. At this point the organic solids contain about 11–14% of vitamin B_1. Precipitation of impurities with 95–99% ethanol is now employed, about as described by other workers.[5-7] The absolute alcohol solution is evaporated to a clear sirup, dissolved in two or three times its weight of 88% phenol (1.2 cc.) and then thoroughly mixed with *n*-butanol (10.8 cc.) while warmed to 50–60°. The mixture is then held at 0° for one-half hour and centrifuged to remove precipitated matter, which is taken up in 0.3 cc. of phenol and 2.7 cc. of butanol as before. After chilling and centrifugation the precipitate is discarded and the combined phenol–butanol solutions, which contain vitamin B_1 of about 20–30% purity, are set aside at room temperature to crystallize. The formation of crystals may be hastened by nucleation. We have found that this solvent mixture, in addition to removing impurities, is decidedly superior to ethanol in inducing crystallization of vitamin B_1 from such concentrates. After removing the first crop of crystals, about 15–20%

(16) We have used powdered Norit or Darco with equal success.

more may be obtained readily by reducing the volume of the mother liquor to about one-fifth. Slightly yellow colored crystals of 90 + % purity have been obtained from several lots of fullers' earth in yields of about 28 to 50%. After recrystallization from 97–98% ethanol, pure white crystals melting at 243–244° (uncorr.) are obtained. When tested by a modified Smith rat curative method, such crystals contained an average of 325,000 International units of vitamin B_1 per gram, which is fully as active as the synthetic vitamin. Although most of the work has been done with rice polish, crystals have also been obtained from yeast, but the yields have been lower. Table III contains the results of a number of typical lots, as well as the results of two batches in which phenol and alkali were used in place of pyridine hydrochloride.

Summary

Pure crystalline vitamin B_1 has been prepared from rice polish or yeast by a process which involves two adsorptions on fullers' earth, extraction with acid salts of nitrogen bases such as pyridine, and the use of organic solvents.

A study of the distribution of vitamin B_1 between water and certain immiscible organic solvents has shown that a number, especially phenol, have high solubility for B_1. The presence of sodium chloride in the water in many cases has a marked effect upon the distribution.

Combinations of phenol and butyl alcohol are very useful for the removal of inert material from B_1 concentrates and afford a good medium for its crystallization.

Charcoal has been used to remove substances which interfere with the adsorptions of B_1 on fullers' earth, both before the original adsorption and before the subsequent readsorption.

NEW BRUNSWICK, N. J. RECEIVED MAY 20, 1937

NOTES

The Preparation of Isopropyl *m*-Tolyl Ether from *m*-Cresol and Isopropyl Chloride[1]

BY THOMAS BOYD WITH ED. F. DEGERING

Synthetic thymol[2] is prepared by the rearrangement of isopropyl *m*-tolyl ether which, heretofore, has been synthesized from *m*-cresol and isopropyl alcohol[3] or bromide.[4] Better yields are obtained, however, from *m*-cresol and isopropyl chloride by use of the following procedure:

Place 25 g. of *m*-cresol, 30 ml. of isopropyl chloride, 9.2 g. of sodium hydroxide pellets, and 75 ml. of isopropyl alcohol in a 500-ml., electrically heated and thermostatically controlled autoclave. Slowly during one hour bring the temperature to about 150° and maintain a temperature range of 150–160° for three hours more.

Allow the bomb to cool, then empty and rinse in turn with water and with two 15-ml. portions of benzene. Extract the mixture and washings with three 25-ml. portions of benzene,[5] then wash the benzene extract with three 20-ml. portions of 15% sodium hydroxide solution.[6] Wash the benzene layer with water, dry with anhydrous potassium carbonate, and recover the solvent by distillation. Rectify the residue through a modified Podbielniak column, and collect a fraction at 193–197°; yield, about 23–27 g.

Various factors such as temperature, period of heating, the nature and quantity of the solvent, and the method of preparing the reaction mixture have been quite thoroughly studied. The best results were obtained by the procedure outlined above.

THE CHEMISTRY DEPARTMENT
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LAFAYETTE, INDIANA

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(1) Presented before the Organic Division of the Fourteenth Midwest Regional meeting of the American Chemical Society, Omaha, Nebraska, May 1, 1937.

(2) J. B. Niederl and Samuel Natelson, THIS JOURNAL, **53**, 1928–34 (1931); F. J. Sowa, H. D. Hinton and J. A. Nieuwland, *ibid.*, **54**, 2019 (1932), and **55**, 3402 (1933); Richard A. Smith, *ibid.*, **56**, 717 (1934), and **55**, 849 (1933).

(3) Hashichi Ono and Minoni Imoto, *J. Soc. Chem. Ind.*, Japan, Suppl. Binding, **39**, 170 (1936); M. D. Curwen, *Ind. Eng. Chem., News Ed.*, **14**, 413 (1936).

(4) J. B. Niederl and Samuel Natelson, THIS JOURNAL, **53**, 1928–1934 (1931); **54**, 1063 (1932); F. J. Sowa, H. D. Hinton and J. A. Nieuwland, *ibid.*, **54**, 2019 (1932); Richard A. Smith, *ibid.*, **56**, 717 (1934).

(5) As a check, acidify the water layer with concd. hydrochloric acid. If an oily layer forms, extract with benzene and add to the previous extract.

(6) Acidify the sodium hydroxide extract and washings. Extract with benzene, and distil the solvent. The residue, 3–6 g., is recovered *m*-cresol.