2. The conductivity of the ethylmagnesium bromide solution is the greatest. The order of conductivity is ethylmagnesium bromide, *n*-butylmagnesium bromide, benzylmagnesium bromide, phenylmagnesium bromide, and magnesium bromide.

3. The conductivity in all cases except 2 *M* phenylmagnesium bromide increases with decreased temperature.

4. Molar conductivity of ethylmagnesium bromide decreases with dilution.

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The Relative Extractability of Vitamins B and G by Plain and Acidified Alcohol

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Introduction

By adding gallic or tannic acid to the alcohol used in extracting wheat germ, McCollum and Kruse[1] obtained a more potent extract of water-soluble B than by the use of plain alcohol. They interpreted their results to indicate that the solubility of the salt of the vitamin was greater than that of the vitamin itself, or that the molecular structure of these acids favored the formation of the salt.

Water-soluble B has since been shown to comprise at least two factors, the antineuritic vitamin B, and the more heat-stable vitamin G. Since reviews of the literature have so recently been published by Kruse and McCollum[2] and by Sherman and Smith,[3] a review of the solubility of the vitamins in alcohol is not included here. In general, both vitamins B and G are soluble in alcohol. Vitamin B appears to be soluble in ethyl alcohol of all concentrations, and vitamin G in the more aqueous solutions. However, Sherman and Sandels[4] have found it necessary to consider the physical and chemical nature of the source material as well as the solvent and the method of extraction in evaluating the evidence regarding the solubilities (extractabilities) of vitamins. This paper reports the relative extractabilities of the two vitamins by plain and acidified alcohols from the same source material and under the identical extraction procedures. The original source material and the extracts and residues resulting from the alcoholic extraction were assayed for each of the vitamins.

(1) McCollum and Kruse, *Am. J. Hyg.*, **6**, 197 (1926).
(2) Kruse and McCollum, *Physiol. Rev.*, **9**, 125 (1929).
(3) Sherman and Smith, "The Vitamins," Am. Chem. Soc. Monograph (1931).
(4) Sherman and Sandels, *Proc. Soc. Exptl. Biol. Med.*, **26**, 536 (1929); *J. Nutrition*, **3**, 395 (1931).

Materials and Methods Used

Skim milk powder was chosen as the source material in this study since it contains both vitamin B and vitamin G, and is relatively richer in vitamin G, regarding which the less was known. A composite sample of the milk powder was used throughout the investigation.

Ethyl alcohol 80% by weight was selected as solvent. Both vitamins were known to be soluble in plain alcohol of this strength, but not completely extracted from skim milk powder by it. To study the effect of acidification of the alcohol upon the extractability of the vitamins, the alcohol was used plain and 0.1 *M* with respect to gallic, benzoic and hydrochloric acids. These acids were selected, primarily because of their variety in structure, but also because of the varying hydrogen-ion activity in 0.1 *M* concentrations.

The method of extracting the milk powder with the alcohols was as follows. To 200 g. of skim milk powder was added 500 cc. of purified ethyl alcohol, 80% by weight, plain, or 0.1 *M* with respect to gallic, benzoic or hydrochloric acid. The mixture was thoroughly stirred, allowed to stand at room temperature (20 to 25°) for one hour, stirred and allowed to stand for a second hour. The supernatant fluid was then decanted and filtered, and to the residue was then added 500 cc. of fresh plain or acidified alcohol; the mixture was stirred and allowed to stand for an hour, stirred again, and allowed to stand for a second hour, after which the solution was decanted and filtered, and the residue washed twice by decantation, each time with 200 cc. of the alcohol. The filtrates and washings of both extractions were combined and concentrated to about one-fourth of the original volume by distillation under reduced pressure (temperature about 30°). The concentrated filtrates and the residues were dried separately in evacuated desiccators over sulfuric acid, and were then ground to a fine powder. Identical products were tested in parallel for each of the two vitamins under consideration.

In principle and in most of the details, the methods used for testing the potency of these products in vitamins B and G and thus determining the relative extractability of the vitamins were those described by Chase and Sherman and by Bourquin and Sherman.[5]

Results

The results of this investigation are summarized in Table I.

TABLE I

RELATIVE EXTRACTABILITY OF VITAMINS B AND G BY PLAIN AND ACIDIFIED ALCOHOLS

	Vitamin B		Vitamin G	
	Units[a]	Percentage	Units[b]	Percentage
Source material (skim milk powder), 100 g.	130	100	500	100
Products resulting from treating 100 g. source material with:				
Plain alcohol				
Extract	75	60	50	10
Residue	50	40	175	35
Total recovered	125	100	225	45
Alcohol, 0.1 *M* with respect to benzoic acid				
Extract	55	40	50	10
Residue	45	35	175	35
Total recovered	100	75	225	45

(5) Chase and Sherman, THIS JOURNAL, **53**, 3506 (1931); Bourquin and Sherman, *ibid.*, **53**, 3501 (1931).

TABLE I (*Concluded*)

	Vitamin B		Vitamin G	
	Units[a]	Percentage	Units[b]	Percentage
Alcohol, 0.1 *M* with respect to gallic acid				
Extract	60	45	150	30
Residue	50	40	175	35
Total recovered	110	85	325	65
Alcohol, 0.1 *M* with respect to hydrochloric acid				
Extract	60	45	25	5
Residue	50	40	175	35
Total recovered	110	85	200	40

[a] As defined by Chase and Sherman. [b] As defined by Bourquin and Sherman.

Under the conditions of fractionation here employed, between 35 and 40% of vitamin B was left in the residual matter, whether the alcohol used in the extraction was neutral or acidified. The extracts were somewhat richer in the vitamin than the corresponding residues from the same quantity of source material and were found to contain about half of the vitamin B of the original material. From three-fourths to practically all of the vitamin B of the source material was recovered either in the residues or in the extracts. The apparent loss of vitamin B during the fractionation was greatest in the case of the products prepared by means of alcohol acidified with benzoic acid. This apparent loss may perhaps be attributed to a destruction of the vitamin B by the benzoic acid or to the limitations of the biological method of assay.

About one-third of the vitamin G of the source material was found to be left in the residual matter. With this vitamin as with vitamin B, acidulation of the alcohol had no apparent effect upon extractability. Perhaps these negative results may be due in part to the protein nature of the source material. However, in addition to their reaction with the protein certain acids when added to the alcohol used in the extraction did markedly influence the stability of the extracted vitamin G. The extract prepared by the use of alcohol acidulated with hydrochloric acid contained practically no vitamin G whereas that prepared by the use of alcohol acidulated with gallic acid contained approximately 30% of the vitamin G of the source material. In no case were the extracts as potent in this vitamin as the corresponding residual matter.

Large losses of vitamin G were encountered in every fractionation—over 50% in every case except when the alcohol used in the extraction was acidulated with gallic acid. The least loss was encountered when gallic acid was added to the alcohol; the greatest, when hydrochloric acid was added. The high concentration of vitamin G found in the extract made by the use of alcohol acidified with gallic acid, a phenomenon also observed by McCollum and Kruse for the vitamin B complex, is not due to the greater solubility of the vitamin or its salt as suggested by those workers,

since the increase in concentration of the vitamin in the extract was not accompanied by a decrease in the corresponding residual matter. Apparently the higher concentration is due to a conserving action which the gallic acid has on the vitamin in alcoholic solution and which is related perhaps to the higher reduction potential of the gallic acid present.

Bisbey[6] found that losses of vitamin G in strong alcoholic solution can be considerably diminished by working as nearly as practicable under an atmosphere of nitrogen. Chick and Roscoe and Narayanan and Drummond[7] have also observed inactivation of vitamin G in alcoholic solution. In the investigation here described, the loss of vitamin G in the extracts is probably attributable in part to oxidation catalyzed both by acid and by alcohol.

Summary

1. Under the conditions of fractionation described, about half of the vitamin B of the source material was recovered in the extracts. The vitamin G content of the extracts varied widely, however. The extract prepared by means of alcohol acidified with gallic acid contained about 30% of the vitamin G of the original material; that prepared by means of alcohol acidified with hydrochloric acid contained practically none.

2. Approximately the same proportions of vitamin B and G, from one-third to two-fifths, were left in the residual matter. Acidifying the alcohol with benzoic, gallic or hydrochloric acid did not increase the extractability of either vitamin.

3. Large losses of vitamin G were met in each fractionation but the addition of gallic acid to the alcohol used in the extraction appeared to conserve the vitamin G of the extract.

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(6) Bisbey, Dissertation, Columbia University, 1930.

(7) Chick and Roscoe, *Biochem. J.*, **23**, 504 (1929); Narayanan and Drummond, *ibid.*, **24**, 19 (1930).