38.4–39.0° corrected, and 600 g. of carbon disulfide. After stirring all day, the reaction mixture was allowed to stand overnight at 25°. The material was then worked up in the usual way. Before fractionating off the solvent, 121 g. of dimethylaniline was added. After removal of the solvent, the residue was heated to 175–185° for three hours, and then distilled. The fraction of b. p. 60–190° was taken up in ether, shaken with dilute sulfuric acid, dried over sodium sulfate and distilled. After several fractionations 2.2 g. of the ketone was obtained of b. p. (751 mm.) 145–148°, n_D^{18} 1.4460.[14]

This material possessed a pleasant odor, reduced potassium permanganate and added bromine in glacial acetic acid instantly; 0.5 g. of the ketone gave 0.5 g. of a semicarbazone, which formed diamond plates from benzene, and after three recrystallizations sintered at 183° and melted at 184–185°.[15]

Anal. (micro) Calcd. for $C_8H_{15}ON_3$: N, 24.9. Found: N, 24.0.

(B). A mixture of 221 g. of butanone, b. p. 78–79°, and 61 g. of acetone was saturated in a freezing mixture with dry hydrochloric acid gas, and then allowed to stand forty-two hours at 0°. The yellow solution was decomposed as usual, steam distilled, dried over calcium chloride and fractionated. Since large quantities of hydrochloric acid were liberated at this point, the material, b. p. (755 mm.) 136–153°, 50.5 g., was boiled with 40 cc. of dimethylaniline for two hours, then washed with dilute sulfuric acid, extracted with ether, dried and distilled. The fraction of b. p. (750 mm.) 144–148°, n_D^{18} 1.4410, 4.5 g. reduced potassium permanganate and absorbed bromine in glacial acetic acid instantly. One gram of the ketone gave 0.65 g. of a semicarbazone which consisted of two individuals, needles and diamond plates. The latter were finally obtained fairly pure after six recrystallizations from benzene melting at 181.5–183.5°, sintering at 180°.

Cyclohexane and Acetyl Iodide.—Cyclohexane when treated with acetyl iodide was recovered unchanged even after 290 days at 25°.

(14) Krapiwin (Ref. 5) reported b. p. (743 mm.) 144–145°, n_D^{18} 1.4378.

(15) Krapiwin (Ref. 5) reported 178–180°.

Summary

1. The reactions between acetyl iodide and butene-2, isobutene, trimethylethylene, cyclohexene, stilbene, benzene, divinyl ether, furan, thiophene, *sym*-dichloroethylene and cyclohexane have been studied.

2. Trimethylethylene, cyclohexene and thiophene alone formed the expected unsaturated ketones.

3. *sym*-Dichloroethylene and cyclohexane failed to react with acetyl iodide.

4. Acetone and butanone condense in the presence of hydrochloric acid to form a mixture of isomeric unsaturated ketones, one of which is dimethylpentenone.

CAMBRIDGE, MASS. RECEIVED OCTOBER 17, 1933

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF ARKANSAS]

The Extractability of Vitamin G (B_2) from Yeast by Various Acetone–Water and Methyl Alcohol–Water Mixtures

BY PAUL L. DAY[1]

In a series of quantitative experiments, Sherman and Sandels[2] found that vitamin G was not appreciably extracted from dried baker's yeast by neutral 95% ethyl alcohol. Alcohol 80% by weight extracted about one-quarter of the vitamin, and 60% alcohol extracted about one-half of the vitamin in the yeast. It thus appears that with ethyl alcohol–water mixtures, the extractability of vitamin G from yeast increases with increased proportion of water in the solvent mixture. In order to find out whether vitamin G would exhibit similar properties toward other solvents miscible with water, experiments have been made on the extractability of the vitamin from dried yeast by 99.5, 80 and 60% (by weight) acetone; absolute, 80 and 60% (by weight) methyl alcohol. The author wishes to report here the results of these quantitative experiments.

(1) With the technical assistance of William J. Darby.

(2) Sherman and Sandels, *Proc. Soc. Exptl. Biol. Med.*, **26**, 536 (1929). *J. Nutrition*, **3**, 395 (1931).

Experimental

The highest purity acetone and methyl alcohol obtainable were used in the preparation of the extracts. For the acetone–water and methyl alcohol–water mixtures, dilutions were made by weighing suitable portions of the solvents and distilled water. Air-dried baker's yeast was treated with the various solvents, using essentially the same technique as was used by Sherman and Sandels[2] with ethyl alcohol. The procedure was as follows: 400 g. of yeast was treated with 1500 cc. of the solvent, stirred and allowed to stand at room temperature for twenty-four hours, again stirred and filtered with suction, and the residue washed with 750 cc. of the solvent in several successive small portions; the residue was treated with another 1500-cc. portion of the solvent, allowed to stand

for twenty-four hours, filtered, and the residue again washed with 750 cc. of the solvent. The residue was dried at room temperature, and enough corn starch added and thoroughly mixed with the residue to bring the weight up to 400 g. The extracts and washings were combined and evaporated on corn starch at a low temperature, using sufficient starch so that 1 g. of the dried preparation contained the extract of exactly 1 g. or simple multiple of 1 g. of yeast.

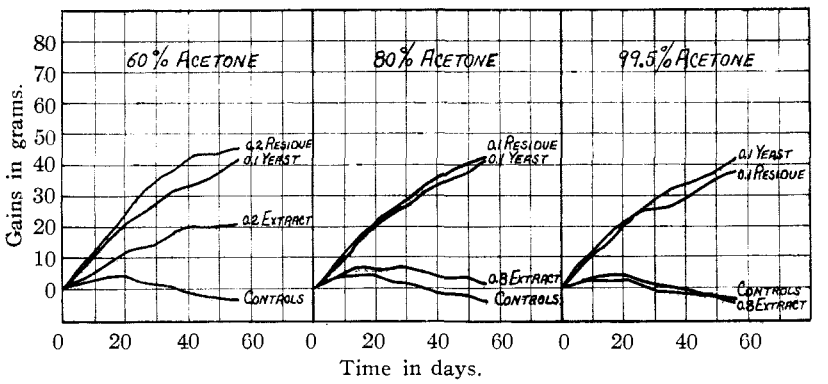

Fig. 1.—Average growth curves of rats showing the extractability of vitamin G from yeast by 60, 80 and 99.5% (by weight) acetone. Each curve represents the average of eight or more rats. The figure at the end of each curve indicates the weight in grams of yeast, yeast residue, or yeast extract fed six times weekly, expressed in equivalents of the original yeast.

The vitamin G contents of untreated yeast, and of the extracts and residues prepared from yeast, were determined by the rat-growth method, using a technique similar to the method of Bourquin and Sherman.[3] The basal vitamin G-deficient diet (No. 625) contained an 80% alcoholic extract of rice polish to supply the vitamin B, instead of a similar extract of whole wheat as employed by Bourquin and Sherman. The details of the method of preparation of this rice polish extract have been described elsewhere.[4] Young albino rats, 35 to 45 g. in weight, were subjected to a preliminary depletion period of two weeks before starting the feeding of the test preparations. The yeast preparations were fed either three or six times weekly in small cups, and in all cases were immediately and completely eaten by the animals. The animals were assigned to the experiments so that each rat receiving an extract was paired by a litter-mate receiving a portion of the corresponding residue.

The results of the feeding experiments upon untreated yeast, extracts and residues from treatment with 60, 80 and 99.5% acetone are shown in Fig. 1. It is seen that the residues from the 99.5% acetone and 80% acetone contained as much vitamin G as the untreated yeast. The extracts from these extractions failed to show appreciable amounts of vitamin even when fed in large quantities. This insolubility of vitamin G in the more concentrated solutions of acetone agrees with the results of Levene,[5] who used acetone to precipitate vitamin G. The residue from the 60% acetone extraction contained about half the growth-promoting properties of the untreated yeast, while the extract contained somewhat less than half of the vitamin, indicating a slight loss of vitamin with this extraction. This loss of growth-promoting activity may have been due solely to errors inherent in the biological assay, although it was probably too great to be accounted for on such grounds. It may have been a real loss of vitamin due to a destruction of the same nature such as has been found due to ethyl alcohol in contact with air.[2,6,7] Numerous investigators have recently reported the existence of new growth factors, so that the possibility that the experiments here reported were concerned with more than one vitamin must be considered. The apparent loss of vitamin by 60% acetone treatment might conceivably have been due to a partial separation of two such growth essentials.

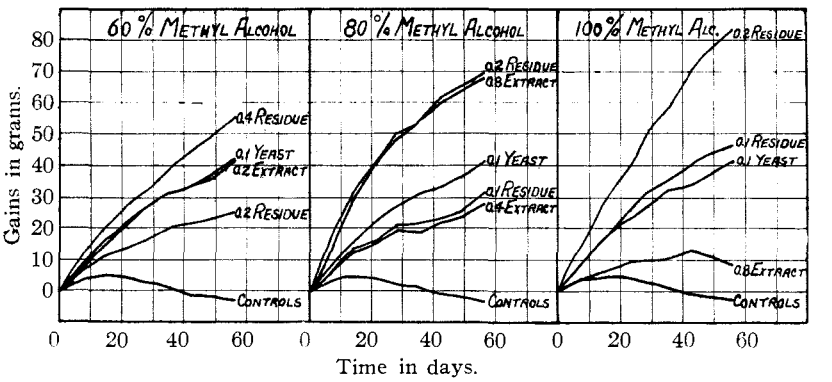

Fig. 2.—Average growth curves of rats showing the extractability of vitamin G from yeast by 60, 80% (by weight), and absolute methyl alcohol. Each curve represents the average of eight or more rats. The figure at the end of each curve indicates the weight in grams of yeast, yeast residue, or yeast extract fed six times weekly, expressed in equivalents of the original yeast.

(3) Bourquin and Sherman, THIS JOURNAL, **53**, 3501 (1931).
(4) Day and Langston, *J. Nutrition*, **7**, 97 (1934).
(5) Levene, *Science*, **71**, 668 (1930).
(6) Chick and Roscoe, *Biochem. J.*, **23**, 504 (1929).
(7) Stiebeling and Alleman, THIS JOURNAL, **55**, 1477 (1933).

Figure 2 gives a record of data obtained on the residues and extracts from treatment of yeast with 60, 80 and 100% methyl alcohol. The residue from 100% methyl alcohol extraction contained vitamin G equal to the untreated yeast, and the extract did not contain an appreciable amount of the vitamin. With the preparations from 80% methyl alcohol extraction, 0.1 g. of the residue (six times weekly) gave approximately the same growth as 0.4 g. of extract (expressed in terms of the original yeast). Similarly 0.2 g. of residue gave the same growth as 0.8 g. of extract. It thus appears that 80% methyl alcohol extracted about one-fifth of the vitamin contained in the yeast, under the conditions employed in the treatment. The extract from 60% methyl alcohol extraction contained approximately half the vitamin, while the residue contained somewhat less than half. All the vitamin contained in the original yeast could be accounted for in the extract plus residue from the 80% methyl alcohol extraction. With 60% methyl alcohol there was an apparent slight loss of vitamin, comparable to the loss resulting from the treatment with 60% acetone, and due possibly to the same cause.

It has been previously reported[4] that an ophthalmia, characterized by keratitis, conjunctivitis and cataract, is the most consistent finding of vitamin G deficiency in rats in this Laboratory. In this series of experiments, not only the negative controls, but also a large proportion of the animals receiving the extracts prepared with absolute methyl alcohol, 80% acetone, and 99.5% acetone, developed cataract. That is, those extracts from yeast that did not promote growth did not prevent the onset of cataract (and the other ocular changes). Conversely, those extracts and residues which promoted growth prevented the development of cataract. It would appear, therefore, that these experiments measured not only the growth-promoting properties of the various preparations, but also their cataract-preventive properties.

Summary

Dried baker's yeast was subjected to extraction with various acetone–water and methyl alcohol–water mixtures. The vitamin G contents of the untreated yeast, the yeast residues and the yeast extracts were determined by feeding experiments. Acetone, 99.5 and 80% by weight, did not extract appreciable amounts of vitamin G. Acetone 60% by weight extracted about half the vitamin G contained in the yeast.

Absolute methyl alcohol did not extract an appreciable amount of vitamin G. Treatment with 80% (by weight) methyl alcohol extracted about one-fifth of the vitamin, while treatment with 60% methyl alcohol extracted approximately half the vitamin G contained in the yeast.

The growth promoting properties of the various yeast residues and extracts were similar to their cataract-preventive properties. Those yeast preparations that promoted growth in experimental animals prevented the development of cataract, while cataract appeared in animals receiving preparations that did not promote growth.

LITTLE ROCK, ARK. RECEIVED OCTOBER 19, 1933