[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE.]

THE NUTRITIONAL REQUIREMENTS OF YEAST. I. THE ROLE OF VITAMINES IN THE GROWTH OF YEAST.[1]

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The composition of the synthetic media used for the growth of yeast has been determined empirically, and as far as the authors are aware, no extensive work has been done in an effort to determine just what kind and concentrations of organic and inorganic substances should be present in the medium for the optimum growth of yeast.

The complexes necessary for the nutrition of animals have been fairly well established and even in the case of the higher plants there is a good deal of information available concerning their nutritional requirements. Unfortunately, our knowledge concerning the nutritional demands of microscopic forms of both plant and animal life is very meager.

Gross analysis of wort or of yeast cells while of much value in determining the constituents necessary for the growth of yeast is by no means final. It may be that certain substances found in such analyses might better be omitted, and as far as the analysis of yeast is concerned it is not safe to say that because a substance is found in the cell it is essential or that the same concentration should prevail in the medium. Furthermore, many substances in wort and yeast are yet unknown. Others, such as proteins, the extractives, lipoids, and so forth, do not lend themselves to individual quantitative determination. That these unknown substances play a marked rôle in the growth of yeast is apparently substantiated by certain investigators, especially Wildiers,[2] who have repeatedly called attention to the fact that certain unknown substances stimulate the growth of yeast and to this class of substances Wildiers has given the name "Bios." Recently Williams[3] claims to have identified this unknown factor as a specific vitamine, Water Soluble B, which is known to be one of the complexes essential for the nutrition of the higher animals. Bachmann[4] likewise has shown that extracts of various substances stimulate the growth of yeasts and is of the opinion that the cause of the stimulation is Water Soluble B. Edday and Stevenson[5] also come to the same conclusion. Willaman[6] claims that vitamines are essential for the growth of *Sclerotinia Cinerea*, the brown rot fungus of peaches and plums. Williams,[7] Bachmann,[4] and Edday and Stevenson[5] have proposed quantitative

[1] Read before the meeting of the American Chemical Society, St. Louis, April, 1920.

[2] Wildiers, *La Cellule*, **18**, 313 (1901).

[3] Williams, *J. Biol. Chem.*, **38**, 465 (1919).

[4] Bachmann, *ibid.*, **39**, 235 (1919).

[5] Edday and Stevenson, *ibid.*, **43**, 295 (1920).

[6] Willaman, THIS JOURNAL, **24**, 549 (1920).

[7] Williams, *J. Biol. Chem.*, **42**, 259 (1920).

measurements for the estimation of Water Soluble B based upon the assumption that this vitamine is essential to the growth of yeast. Currie[1] developed a medium for the growth of *Aspergillus Niger*. This medium was composed of known substances showing that this particular organism did not require any vitamine.

This paper presents data which show that Water Soluble B is not a necessary constituent of a medium for the growth of yeast, that Water Soluble B is not the yeast growth stimulant in the extracts studied, that extracts from alfalfa and wheat embryo contain nitrogenous and inorganic materials which will maintain the growth of yeast, and that the relative potencies of materials in the growth of yeast cannot be arrived at on an equal weight basis.

Experimental.

Method.—The original stock yeast was from a single colony plated from a cake of Fleischmann's yeast and designated by them as *Saccharomyces Cereviseae* Race F. Fifty cc. of wort was inoculated from the colony and the yeast transferred to a fresh portion of this medium every day for a week. The required amount of this yeast was added by pipet to the basal medium to make an initial count of about one.

The concentration of yeast is expressed as "C" (count) and is the number of cells in 16 small squares of the Thoma-Zeiss Chamber multiplied by dilution. C/4 is equal to millions of cells per cc. of medium. By initial count is meant the count when the time is equal to zero for the culture in question.

Into each of the desired number of 125 cc. Erlenmeyer flasks was placed 50 cc. of the medium to be investigated minus the variable constituent. To each flask was added 0 to 3 cc. of a solution of the variable followed by the proper amount of water to bring the volume up to 53 cc. One cc. of the culture was then added, making the final volume 54 cc. The flasks were placed in the incubator at 30°. At the required time 5 cc. of a 5% phenol solution was added to each flask to stop the growth of the yeast and the count made.

The Effect of Extracts from Alfalfa and Wheat Embryo upon the Growth of Yeast.

In order to study this phase of the problem alcoholic extracts of wheat embryo and alfalfa were prepared. The materials were extracted for 6 hours with hot C. P. anhydrous ether to remove fatty substances, followed by extraction for 8 hours with hot alcohol with a continuous extractor. The alcoholic extracts were evaporated to dryness and the residue made up to the desired concentration with distilled water. The solution was sterilized in 10 cc. portions in test-tubes at 7 kg. pressure for 20 minutes

[1] Currie, *J. Biol. Chem.*, **31**, 15 (1917).

The basal medium (this medium differs from that of Williams[1] only in the amount of sugar it contains) had the following composition: 100 cc. of the medium contained 0.30 g. of ammonium sulfate, 0.20 g. of monopotassium phosphate, 0.025 g. of magnesium sulfate, and 10 g. of cane sugar. With this medium as a basis a series of experiments was conducted to determine the effect upon the growth of yeast of various quantities of the extracts.

The results are shown in Fig. 1. Dry equivalent represents the g. of dry alfalfa or wheat embryo in the form of extract added per 100 cc. of medium. The dry equivalent multiplied by 10 is plotted on the abscissa and the count of yeast cells is plotted on the ordinate.

It will be noted that the alfalfa and wheat embryo curves are similar in several respects especially in that both show optimum concentrations. They are unlike in that the optimum concentrations of the alfalfa extract, which is 1.2, dry equivalent, is far more potent than the wheat embryo extract at the optimum represented by 0.06, dry equivalent. In the case of the alfalfa curves the yeast count is not a linear function of the concentration of the extract. The wheat embryo curves up to the optimum have steeper slopes than the alfalfa curves and the wheat embryo curves cross the alfalfa curves at a concentration of extract represented by 0.13, dry equivalent. This leads to the conclusion that the relative potencies of 2 materials cannot be determined by the comparison of the effects of the extracts from equal weights of dry materials. It will be necessary in each case to determine the optimum concentration before such comparisons of materials can be made. For instance, one investigator might compare the stimulating effect of extracts represented by 0.10, dry equivalent, of wheat embryo and alfalfa and come to the conclusion that wheat embryo extract is more effective than alfalfa extract. A second worker might compare the effects of extracts derived from 0.20, dry equivalent, of wheat embryo and alfalfa and come to the conclusion that alfalfa extract is more potent than wheat embryo extract, while a third person, comparing the effects of the extracts represented by 0.13, dry equivalent, of wheat embryo and alfalfa would report the materials equally effective. Even if it be granted that the stimulant in these extracts is Water Soluble B, conflicting results will be obtained in any method which utilizes the stimulating power of extracts upon the growth of yeast as a quantitative method.

One of the curves illustrates the yeast count obtained with various concentrations of purified extract of wheat embryo. This extract was prepared according to the method of McCollum and Simmonds.[2] The wheat embryo was first extracted with C. P. anhydrous ether, then with benzene, and finally by 95% alcohol. The alcoholic extract was placed

[1] Williams, *loc. cit.*

[2] McCollum and Simmonds, *J. Biol. Chem.*, **33**, 55 (1918).

on dextrin and dried. This latter material was extracted with benzene. The benzene was evaporated off and the extract dissolved in distilled water.

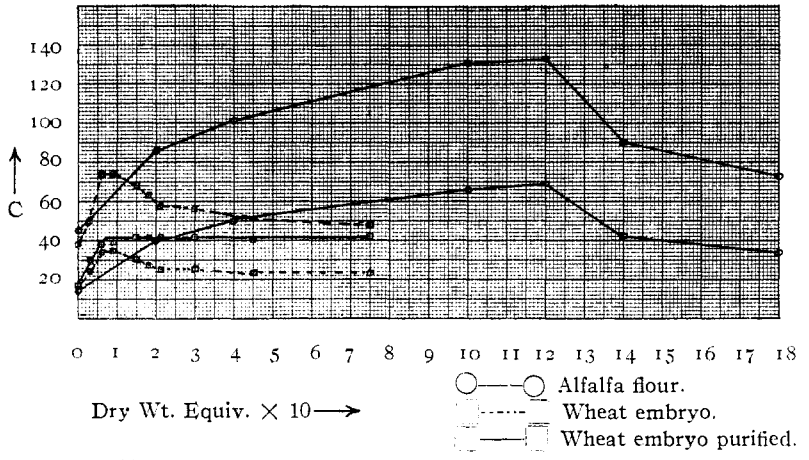

Fig. 1.—The effect of various concentrations of extracts from alfalfa and wheat embryo upon the growth of yeast.

The results obtained by the use of the above extract are plotted in Fig. 1. It will be seen that the optimum concentration of the purified wheat embryo extract is identical with that of the unpurified extract. Not only has the optimum concentration been unchanged, but the potencies of the extracts are the same at the optimum concentration. The curves are dissimilar in only one respect, in that in the case of the purified extract there is no drop in the curve at the dry equivalent 1.0, indicating that something toxic was removed by the purification.

Is the Yeast Growth Stimulant in the Extracts from Alfalfa and Wheat Embryo Water Soluble B?

McCollum and Simmonds[1] have shown that Water Soluble B is readily destroyed by dil. alkali. If the stimulating effects of these extracts is due to Water Soluble B treatment with alkali should impair their efficiency. The alcoholic extracts of alfalfa and wheat embryo were heated for one hour with 5% sodium hydroxide solution at 7 kg. pressure. The alkali was neutralized with hydrochloric acid, and the amount of sodium chloride formed was determined. To the controls the same amount of sodium chloride was added. Optimum concentrations of the extracts were used. The figures in Table I show that the wheat embryo and alfalfa extracts, after being treated with alkali, are as good as the untreated extracts, considered as yeast stimulants.

In the first experiment the basal medium gave a count of 20. Addition of the optimum concentration of alfalfa extract raised the count to 75, while the same concentration of alfalfa extract treated with alkali

[1] McCollum and Simmonds, *J. Biol. Chem.*, 33, 55 (1918).

TABLE I.

The Effect of Alkali upon the Yeast Growth Stimulant in Alfalfa and Wheat Embryo Extract.

Basal medium.	Alfalfa extract untreated.	Alfalfa extract treated.	Wheat emb. extract untreated.	Wheat emb. extract treated.	Wh. pur. extract untreated.	Wh. pur. extract treated.
20	75	77	33	32	34	33
53	186	197	69	71	73	72

gave a count of 77, showing that the stimulant in the alfalfa extract is not Water Soluble B, since this particular vitamine is destroyed by alkali. The optimum concentration of wheat embryo extract when added to the basal medium gave a count of 33, while the same concentration of this material when treated with alkali gave a count of 32, showing that in the case of the wheat embryo the stimulant is not Water Soluble B. Like results were obtained with the purified wheat embryo extract (designated in the table as "wh. pur.").

Do Extracts of Alfalfa and Wheat Embryo Contain Nitrogenous and Inorganic Constituents for the Growth of Yeast?

In one series of experiments the medium was composed of the optimum concentration of alfalfa extract and 10% sugar. In the second series of experiments the optimum concentration of wheat embryo extract and 10% cane sugar were used. In both series the yeast was transferred every other day to a fresh medium, thus diluting to a negligible quantity the constituents of the original medium in which the yeast was grown. The yeast has been growing in these media for the past 3 months. Other investigators have not taken cognizance of the fact that alcoholic extracts of plant tissue contribute to the nitrogen and inorganic nutrition of the organism in question.

Does Yeast Require Vitamines for Growth?

In the paper immediately following the authors have developed a synthetic medium free from unknown constituents. The method of the development of the medium is given in detail in that paper. Yeast has been growing in a satisfactory manner in this medium, being transferred to a fresh medium every other day for a period of 10 months. It is normal in appearance and is growing well. This shows that yeast can grow without the addition of vitamine to the medium.

In Table II the above medium, referred to as Medium F, has been compared with Williams'[1] medium with and without the optimum concentrations of extracts of alfalfa and wheat embryo.

In the first experiment the count in Williams' medium was 130, the count being increased to 188 by the optimum concentration of wheat embryo extract and to 286 by the optimum concentration of the alfalfa extract. In Medium F, which is composed of known constituents, the count was

[1] *Loc cit.*

TABLE II.

Williams.	Williams' + wheat emb. extract.	Williams' + alfalfa extract.	Medium F.	Medium F + alfalfa extract.	Medium F + wheat emb. extract.
130	188	286	288	284	281
140	176	253	260	256	253

288, or the same as that in Williams' medium plus the optimum concentration of alfalfa extract. Since we have constructed a medium of known constituents which gives as good results as Williams' medium plus alfalfa extract, it cannot be concluded that the stimulating effect of the extract is due to Water Soluble B or to any other unknown substances. Furthermore, when the optimum concentration of alfalfa extract was added to Medium F a count of 284 was obtained, when the optimum concentration of the wheat embryo extract was added to Medium F the count was 281.

From the above data it is evident that we have developed a medium, namely Medium F, composed of known constituents, which is not improved by the additions of vitamine-containing extracts. Therefore, vitamines are not essential as constituents of media for the growth of yeast.

Summary.

The relative potencies of 2 materials as yeast growth stimulants cannot be arrived at on an equal weight basis.

Treatment with alkali does not impair extracts of wheat embryo or alfalfa as yeast growth stimulants. Evidently the stimulant is not Water Soluble B.

Extracts of alfalfa and wheat embryo contain sufficient nitrogenous and inorganic material for the growth of yeast.

A medium of known constituents is developed which promotes the growth of yeast without the addition of vitamines. The addition of Water Soluble B does not improve the above medium.

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THE NUTRITIONAL REQUIREMENTS OF YEAST. II. THE EFFECT OF THE COMPOSITION OF THE MEDIUM ON THE GROWTH OF YEAST.[1]

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In the preceding paper data are presented on the rôle of vitamines in the growth of yeast. In the following paper a study has been made on the influence of the nature and concentration of known components of the medium on the growth of yeast.

Experimental.

Method.—The method was identical with that outlined in the pre-

[1] Read before the meeting of the American Chemical Society at St. Louis, April, 1920.