L. arabinosus to a value equal to that found with *L. casei*. It would appear that there exists in certain enzymatically prepared materials a form of biotin which may be utilized for growth and acid production by *L. casei* but not by *L. arabinosus*. Using yeast extract as an example, it was possible to show that this form of biotin, available to *L. casei* but not to *L. arabinosus*, was avidin-combinable. Acid autoclaving converts this form of biotin to one used equally well by the two organisms. This need not seriously interfere with the use of *L. arabinosus* as an assay organism for biotin since it is well known that there exist in many natural materials combined forms of biotin which are not available to bacteria. Preliminary acid hydrolysis has become an accepted procedure before biotin determination by any method.

The recoveries obtained when biotin was added to several naturally occurring materials are given in Table III. Quantitative recovery of biotin was readily obtained. It is believed that better agreement at varying assay levels for the biotin content of various materials is obtained with the *L. arabinosus* method than is possible with the *L. casei* method of Landy and Dicken.[2]

Since pimelic acid is capable of being utilized in lieu of biotin by the diphtheria bacillus,[15] the ability of *L. arabinosus* to utilize similar dicarboxylic acids was studied. Adipic, pimelic, suberic, azelaic, and sebacic acids were found to possess no biotin-like activity for *L. arabinosus*.

TABLE III.
Recovery of Biotin* Added to Various Materials.

Sample	Biotin content μg/g or ml	Recovery %
Proteose peptone†	0.11	100
Bacto peptone†	0.15	100
Malt extract†	0.050	100
Rat liver‡	0.48	101

* Biotin added to the final solution for assay in an amount which approximately doubled that originally present.

† A solution of these materials assayed without previous treatment.

‡ Autoclaved with 6 N H_2SO_4 at 120°C for 1 hour before determination.

Summary. The microbiological determination of biotin, using *Lactobacillus arabinosus* as the test organism, was studied. The existence of a water-soluble, avidin-combinable form of biotin in certain enzymatically prepared materials which is available to *L. casei*, but not to *L. arabinosus*, was demonstrated. When natural materials are autoclaved with 6 N H_2SO_4, prior to determination of their biotin content, comparable results are obtained with the *L. arabinosus* method and with a method using *L. casei*.

[15] du Vigneaud, V., Dittmer, K., Hague, E., and Long, B., *Science*, 1942, **96**, 186.

[16] *The University of Texas Publication*, 1941, **4137**, 82.

14608

Methionine Deficiency in Yeast Protein.

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The present shortage of proteins has stimulated interest in the production of yeast for use primarily as a source of food (or feed) protein. Work now in progress in this laboratory[1,2] involves the development of methods to produce torula yeasts grown on molasses and fruit wastes, and the evaluation of these yeasts as a source of feed protein.

* One of four regional research laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

1 Stubbs, J. J., Noble, W. M., and Lewis, J. C., in press.

2 Lewis, J. C., Stubbs, J. J., and Noble, W. M., in press.

TABLE I.
Composition of Diets in %.

Diet	Basal mixture	Starch-sucrose 50-50	Yeast extr.	Casein	Brewers' Yeast	Torula Yeast	% crude protein
I	65	19	≅5	16	—	—	13.4
II	65	5	—	—	30	—	13.2
III	65	10	—	—	—	25	13.3

Considerable work, much of it quite recent, has been carried out on the feeding value and amino acid composition of yeast protein. Hock[3] demonstrated the inability of yeast to replace completely a fish meal-casein mixture in diets of 9.3% crude protein when fed to growing rats. Csonka[4] has reported values for tryptophane, histidine, lysine, and arginine in yeast which would constitute adequate amounts of these essential amino acids for the rat, based on the requirements tentatively set by Rose[5] and the presence of at least 13.5% of crude yeast protein in the diet.

Since the work reported here, dealing with the evaluation of yeast protein, was completed, another set of amino acid values has come to our attention. Melnick[6] has cited data obtained from a book in press by Block and Bolling[7] as the source of analyses of yeast powder for all of the 10 essential amino acids. At a 13.5% crude protein level, these values represent adequate amounts for the rat, except in the case of methionine, which would be present to the extent of only 0.27% compared with a requirement of 0.6%. This information substantiates the results of our experiments. To our knowledge no feeding test demonstrating the lack or unavailability of sufficient methionine in yeast for the growing rat has been reported.

Two samples of dried yeast—II, a commercial brewers' yeast, and III, a pilot-plant product of *Torulopsis utilis* grown on molasses—have been fed to 38-day-old rats as the sole source of dietary protein and at a crude protein (6.25 x % nitrogen) level equivalent to that of a 16% commercial casein diet. No corrections have been made for the amount of non-protein nitrogen (purines and pyrimidines) which may represent as much as 15% of the total nitrogen of yeast. However, the conclusions drawn from the experimental data are not changed essentially by the presence of such amounts of non-protein nitrogen.

The basal mixture for the diets described in Table I contained, as percent of the complete diet: sucrose 25, corn starch 25, Wesson (cottonseed) oil 10, McCollum's salt mixture (No. 185) 4, and U.S.P. cod liver oil 1. Vitamin contents, as mg per 100 g of complete diet, were: choline chloride 50, thiamin chloride 0.2, riboflavin 0.5, pyridoxin 0.2, calcium pantothenate 2.5, and nicotinic acid 1.0.

The yeast extract was prepared by extract-

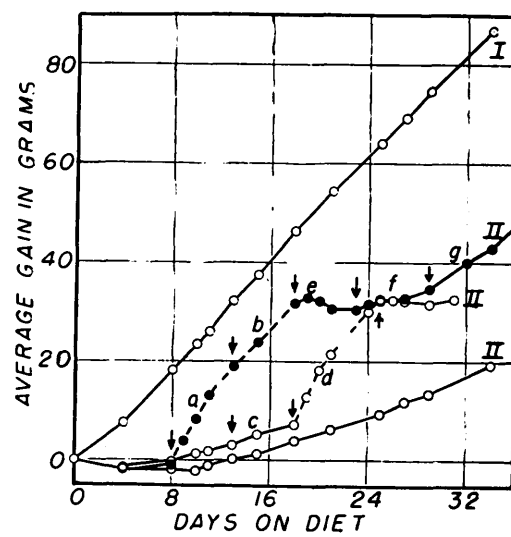

FIG. 1.

The effect of methionine and other amino acid supplements on the growth rate of rats fed brewers' yeast. A change in diet is indicated by a vertical arrow. Amino acid supplements to the brewers' yeast diet II were: (a) methionine, threonine, valine, leucine, and isoleucine; (b) methionine, threonine, and valine; (c) leucine and isoleucine; (d) methionine; (e) threonine and valine; (f) threonine, valine, leucine, and isoleucine; and (g) cystine. I is a reference diet (16% casein).

[3] Hock, A., *Biochem. Z.*, 1942, **311**, 385.

[4] Csonka, F. A., *J. Biol. Chem.*, 1935, **109**, 703.

[5] Rose, W. C., *Science*, 1937, **86**, 298.

[6] Melnick, D., *Wallerstein Laboratories Communications*, 1943, **6**, 167.

[7] Block, R. J., and Bolling, D., in press.

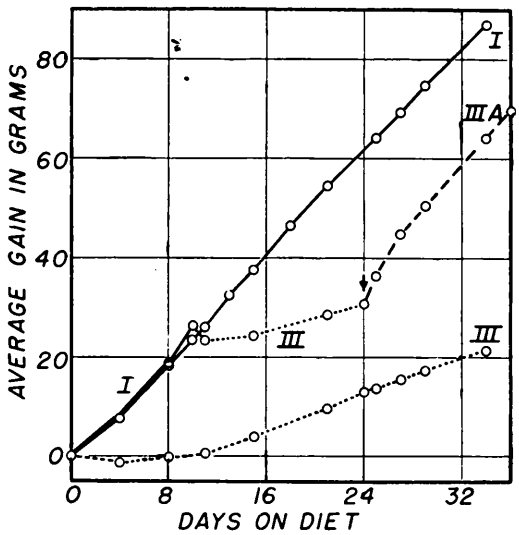

FIG. 2.

The effect of a methionine supplement on the growth rate of rats fed torula yeast. I—16% casein diet; III—25% torula yeast; IIIA—25% torula yeast + 0.5% methionine. A change in diet is indicated by a vertical arrow.

ing 2400 g of brewers' yeast II with 3 successive 6-liter volumes of 50% aqueous methyl alcohol. The extract was concentrated *in vacuo* to a thick syrup and fed in diet I at a level equivalent to 5% whole yeast, in order to make the 3 diets more comparable with respect to all factors except protein.

Diets II and III were supplemented with commercial grades of amino acids as indicated in Fig. 1 and 2. Each of the amino acids was fed at a level of 0.5%, mixed into the whole diet, with the exception of cystine, which was fed at a 1% level.

Six groups of rats were made up of equal numbers of males and females selected from the same litters of approximately equal age, with an average initial weight ranging from 72 to 75 g. A group of 32 rats was placed on diet 1 as a positive control (growth curve in both Fig. 1 and 2); the 5 other experimental groups each contained 16 rats. As indicated in Fig. 1, 3 groups were fed diet II, and two of these were given successively different combinations of amino acids. The broken lines in Fig. 1 indicate where the diet contains a supplement of 0.5% methionine. Fig. 2 shows growth curves for groups on diets I and III, and for one group started on diet I, then changed to diet III, and finally supplemented with 0.5% methionine (heavy broken line).

The rats had been fed a stock diet of Purina Dog Chow supplemented with lettuce and cod liver oil prior to the feeding of the experimental diets. The experimental diets were fed *ad libitum* and feed consumption in the 3 groups, which ranged from 8 to 11 g per rat per day, was correlated roughly with the gains in weight. No deaths occurred during the 5-weeks experimental period.

Both samples of yeast failed to support even moderate growth when fed as the sole source of protein. The addition of 0.5% *dl*-methionine alone to these yeast diets resulted in a growth rate equal to that obtained with a diet containing 16% casein. It appears from these results that, compared with casein and at the levels fed, yeast is seriously deficient for the growing rat in only one amino acid, methionine. (See Fig. 1 and 2.)

Womack and Rose[8] have shown that in diets containing 0.4% cystine, only one-sixth of the requirement of 0.6% methionine for optimum growth can be satisfied by the cystine. The yeast may be estimated to have contributed 0.1[4] to 0.3%[9] cystine to our diets, an amount which theoretically should preclude any significant increase in growth rate due to added cystine.

The methionine deficiency in yeast might conceivably involve the question of the availability of the methionine present, as has been found true for soybean protein.[10,11] However, the digestibility of yeast both *in vivo*[6] and *in vitro*[12] has been measured and found to be quite high. Further work is in progress, both with the rat and with the chick, and will be reported at a later date.

Summary. Brewers' yeast and torula yeast grown on molasses, when either was fed as the sole source of protein to growing rats, were

8 Womack, M., and Rose, W. C., *J. Biol. Chem.*, 1941, **141**, 375.

9 Prunty, F. T. G., *Biochem. J.*, 1933, **27**, 387.

10 Hayward, J. W., and Hafner, F. H., *Poultry Sci.*, 1941, **20**, 139.

11 Almquist, H. J., Mecchi, E., Kratzer, F. H., and Grau, C. R., *J. Nutrition*, 1942, **24**, 385.

12 von Soden, O., and Dirr, K., *Biochem. Z.*, 1942, **312**, 252.

found to be lacking in sufficient available methionine to produce growth equal to that elicited by an equivalent amount of casein. It appears that this fact is one limiting factor which must be considered in evaluating yeast protein as a replacement for animal proteins.

14609

Penicillin Sensitivity of Strains of Non-hemolytic Streptococci Isolated from Cases of Sub-acute Bacterial Endocarditis.

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It is generally recognized that different organisms vary greatly in their sensitivity to penicillin and that even different strains of the same organism exhibit wide variations in susceptibility. In the treatment of human infections the importance of determining the sensitivity of the infecting strain is therefore obvious. In view of the apparent success which has recently been achieved in the treatment of cases of sub-acute bacterial endocarditis with penicillin,[1,2] information on the penicillin sensitivity of strains isolated from this disease is particularly desirable.

Fifty strains of non-hemolytic streptococci obtained from cases of sub-acute bacterial endocarditis were studied.

Gross Description. Of 50 strains examined:

19 were described as "viridans"
12 " " " "indifferent"
3 " " " "resembling enterococci"
2 exhibited 2 types of colonies
1 was described as "weakly hemolytic"
13 were not described, other than as "non-hemolytic"

Serological Grouping. Thirty-nine strains were examined serologically according to Lancefield's procedure. Of these, 10 fell into definite groups as follows:

Group B	1 strain
" C	2 strains
" D	1 strain
" F	2 strains
" H	4 "

In addition, 6 strains showed an antigenic relationship to one or other of the groups but could not be definitely classified. Twenty-three strains showed no relationship to any of the groups for which they were tested. Eleven strains were not tested serologically.

Penicillin Sensitivity. The sensitivity of non-hemolytic streptococci was determined by the following procedure:

The sensitivity of each strain was compared with that of a standard strain of hemolytic streptococcus (C203MV). It had been shown previously that from 0.01 to 0.03 Oxford units are required to inhibit the growth of a 10^{-2} dilution of a 15-hour rabbit's blood broth culture of this strain. Such a dilution contains from 2,500,000 to 3,000,000 organisms. In other experiments it had been shown that the *in vitro* activity of penicillin is influenced by the number of organisms present and by the rate of growth of the organisms. The unknown strains were therefore tested under growth conditions comparable with those of the standard strain. The cultures to be examined were grown for 15 hours in rabbit's blood broth, the original seeding being such as to produce growth of approximately the same density as that of a 15-hour culture of strain C203MV. If the unknown strain grew too heavily the 15-hour culture was diluted with broth to a turbidity equivalent to that of the standard. If the unknown strain grew too lightly, a 10^{-1} dilution was used for the test instead of a 10^{-2} dilution. If extremely heavy or very sparse growth occurred, a more suit-

[1] Loewe, L., Rosenblatt, P., Greene, H. J., and Russell, M., *J. A. M. A.*, 1944, **124**, 144.

[2] Dawson, M. H., and Hobby, G. L., *J. A. M. A.*, 1944, **124**, 611.