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A STUDY OF THE RELATIVE VITAMIN
B COMPLEX CONTENT OF THE
R AND S FORMS OF
SACCHAROMYCES CEREVISIAE
HANSEN, SAAZ

THESIS FOR THE DEGREE OF M. S.
Harold Buskirk
1934

THESIS

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Vitamin
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Mr. Laban

A STUDY OF THE RELATIVE VITAMIN B
COMPLEX CONTENT OF THE R AND S FORMS OF
SACCHAROMYCES CEREVISIAE HANSEN, SAAZ

THESIS

Respectfully Submitted to the
Graduate School of Michigan State College
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

By

Harold Buskirk

1934

1935

The author wishes to express his indebtedness and gratitude to Dr. C. A. Hoppert, Associate Professor of Chemistry, for his advice and supervision in the planning of this project and his assistance in carrying out the vitamin assays, and to Dr. F. W. Fabian, Associate Professor of Bacteriology, for his invaluable assistance and supervision in growing the yeast.

This opportunity is taken to express my thanks to Dr. N. Halliday of the Home Economics Department for her advice in conducting vitamin B assays.

INTRODUCTION

In recent years a great deal of emphasis has been placed on vitamins and their role in nutrition. As a result, extensive studies have been made of the distribution of vitamins. Yeast has long been recognized as one of the best sources of vitamins B and G and recently has been used extensively in the manufacture of ergosterol which can be converted into an antirachitic substance by treatment with ultra violet light. Extracts of yeast containing the vitamins as well as other nutrient parts have been prepared. These extracts are added to foods, medicines etc. Dried yeast has also been irradiated, thereby producing a material of high antirachitic potency. A milk of high vitamin D content is produced by feeding this yeast to milch cows.

Studies have been made by various workers on the variation of vitamins B and G in different species of yeast and in different strains of the same species. A quantitative study has also been made of the amount of ergosterol produced in different species of yeast. In these studies no attention was given to the dissociated variant or form of the yeast which was used. In view of more recent work it is evident that yeasts, like bacteria, undergo dissociation. It has been shown that the normal smooth, S form, may be induced by various

stimuli to dissociate into a rough, R form, and a gonidial, G form, and also various transitional forms. These three types are distinctly different from each^{other} both culturally and morphologically, although they are derived from the same cell of the S form.

Since the previous studies were made with yeast in which the forms were not known or recognized, they did not take into consideration the possibility that there might be a variation in the vitamin content of the dissociants of the same strain. Accordingly it was considered feasible to make an investigation of this phase of the subject.

HISTORICAL

Little work has been done on the variation of vitamins B and G in different species of yeast. Quinn, Whalen and Hartley (1930) however, studied five samples of American and Canadian yeast for their vitamin B and G content. Among them were two samples of brewers yeast, two samples of dry powdered yeast, and a sample of fresh starch-free baker's yeast. One of the samples of brewers yeast was obtained from Canada while the other was from New York. The Canadian was "a top yeast" that had been dried at the plant in steam heated drums and then ground to a coarse powder. The New York

sample was a "bottom yeast" that was obtained in a moist condition and dried in the laboratory. They found that the vitamin B content of the two samples of brewers yeast was definitely greater than any of the other samples, the difference being at least ten fold. The Canadian "top yeast" was slightly more potent than the New York "bottom yeast", but the difference was very slight. The vitamin G content of all the samples was very uniform.

Bills, Massengale and Prickett (1930) made an investigation of the ergosterol content of twenty-nine cultures of yeast. Eighteen different species and eleven different strains were used. They found that different species of yeast similarly cultured, may differ enormously in ergosterol content. Saccharomyces logos contained but a trace, while Saccharomyces carlsbergensis yielded 2.0 per cent. Different strains of one species, Saccharomyces cerevisiae, ranged from 0.2 to 1.4 per cent. They observed that runs giving heavier yields of yeast also showed a higher percentage of ergosterol. The fact that aeration during cultivation greatly influences the yield may have some bearing on the ergosterol content. Previously, the difference in the cultural conditions had been used in explaining the variation in ergosterol content, however these workers found that different yeasts have different capacities for the production of ergosterol. These

capacities may vary favorably or unfavorably according to the cultural conditions.

In view of the fact that Bills et al, found that the ergosterol content could be increased or repressed by manipulating the conditions under which the organisms were grown and also in the light of the recent work of Fabian and McCullough (1934) on dissociation in yeasts, it was suggested that there might be considerable variation in the vitamin content of the different dissociated forms of the same yeast.

Since this work has been completed, one of Dr. Fabian's students, L. J. Wickerham, has found that one of the distinguishing characteristics of the R form of all yeasts studied so far is the presence of highly refractile bodies which he has demonstrated to be fat globules.

DESCRIPTION OF CULTURES USED

The cultures used were: Saccharomyces cerevisiae Hansen, Saaz strain, American type culture No. 2352, an industrial beer yeast, producing a bottom alcoholic fermentation. No scum is produced on beer wort. The temperature limits for budding in beer wort are from 3° to 40°C. The cells are spherical and produce from two to four ascospores. Agar slant cultures have a smooth, white glistening appearance. For morphological

and cultural characteristics see figures 3 and 4.

Sacch^aromyces cerevisiae Saaz rough (R). This form was produced by Fabian and McCullough (1934) by converting single cell isolations of the normal S form by means of chemicals such as lithium chloride and brilliant green added to suitable media and also by physical influences such as desiccation and by abnormal temperatures. The R form produces rugose, dull, wrinkled colonies with filamentous projections extending from one to two mm. from the colonial mass. The cells are elongated averaging 14 to 20 microns in length. Highly refractile bodies were observed in the R cells and tests with Sudan 3 and osmic acid showed them to be fat. However, an extensive analysis has not yet been made. See figure 3.

Sacch^aromyces ellipsoidus Hansen is a yeast producing a bottom fermentation. In beer wort either round or elliptical cells are produced. The temperature limits for budding in beer wort are from 0.5° to 40°C. Scum formation may occur on liquid media. The cells in the scum may be greatly elongated. Ascospore formation occurs; the asci are usually small and ellipsoidal and enclose from one to four ascospores. Agar slant cultures appear dull, white and often slightly wrinkled.

CULTIVATION

It is a rather simple task to grow small amounts of yeast in the laboratory, but when larger quantities are needed the problem becomes more difficult and commercial methods must be adapted to a laboratory scale in so far as possible.

Through the courtesy of a commercial yeast producer a formula for a culture medium and some helpfull instructions were obtained. The formula recommended for the medium was as follows:

100 parts	-----	sugar beet molasses
2 "	-----	mono basic ammonium phosphate
2 "	-----	urea

This material was diluted with tap water to five brix which is equivalent to five per cent sucrose by weight. The acidity was adjusted to pH 5.0. As alternatives, three parts of ammonium sulfate may be used in place of urea, but this necessitates considerable more neutralizing. Aqua ammonia may also be used by adding it each hour and adjusting the acidity. However, urea is recommended as it gives the least trouble. Other formulae were used but the above gave good yields and proved very satisfactory in all other respects.

APPARATUS

To insure greater yields, it was necessary to aerate the growing yeast. Therefore an apparatus had

to be constructed for this purpose. An eight liter wide-mouth bottle was chosen for the receptacle. The aerating device consisted of first;- a ten inch cotton filter which filtered the air from the compressed air line rendering it sterile. The filter was made by packing cotton into a pyrex tube one inch in diameter by ten inches long. One-holed rubber stoppers, with $\frac{1}{4}$ inch tubing inserted, were fitted into each end. (See Fig. 5) The filter was sterilized for 30 minutes at 15 lbs. pressure. Second;- a closed pyrex tube, $\frac{1}{2}$ inch by 18 inches long, with four $\frac{1}{4}$ inch tubes sealed on the closed end at right angles to each other. These tubes were cut off leaving them about $\frac{5}{8}$ inches long. To these projections were attached $\frac{1}{4}$ inch pyrex jets two inches in length containing small holes on the upper side. The jets were connected with gum rubber tubing which made them flexible enough to be placed into the bottle through the four inch mouth. This part of the apparatus was used to bubble air up through the medium. It was sterilized by autoclaving for 30 minutes at 15 lbs. pressure.

An incubator was constructed to accomodate the apparatus. This was regulated to maintain a temperature of 30 C.

GROWING OF SMOOTH YEAST

The medium was prepared according to the formula given previously. Usually from 500 to 800 grams of molasses was made up at one time yielding from six to ten liters of liquid. The acidity was adjusted with sulfuric acid to slightly below the desired pH to prevent the precipitation of phosphates during the sterilization process. Sulfuric acid was used in order that sulfates might be supplied to the medium. The material was placed in two liter flasks and autoclaved at 12 lbs. pressure for 30 minutes. The reaction was readjusted to pH 5 just before it was used. All pH determinations were made electrometrically with a quinhydrone electrode.

The bottle was sterilized chemically with cleaning solution, rinsed with hot water and with the aerating device inserted, was placed in a steamer and heated. The hot medium was poured into the bottle filling it about $\frac{3}{4}$. This was again heated to make sure that the bottle and contents were sterile. After cooling, the bottle was inoculated with about 100 cc. of yeast culture grown in molasses medium for two or three days or until a good growth was produced. Each inoculum was prepared by washing a 24 hour growth from an agar slant into 100 cc. of molasses medium. Before transferring into

the bottle, however, each inoculum was examined microscopically for freedom from bacterial contamination. A few drops of corn oil was added to prevent foaming. The aerating device was connected and air was allowed to bubble moderately through the liquid. The temperature was kept between 28° and 30°C . Although the directions called for a pH determination at hourly intervals, it was found unnecessary from experience because no change was observed. Accordingly the pH was adjusted to the proper point at the beginning and checked at the close of each run.

HARVESTING AND DRYING

When a sufficient amount of yeast was produced which was indicated by the turbidity of the medium, the air was turned off and the yeast was allowed to settle for several hours. At the end of this period the yeast had completely settled out leaving a clear supernatant liquid which could be easily syphoned off. The remaining liquid was removed by placing the material in centrifuge bottles and centrifuging. The yeast was washed twice with physiological salt solution and removed by centrifuging. Before harvesting, the yeast was again examined microscopically for bacterial contamination. All contaminated yields were discarded.

The yeast was placed in an evaporating dish in which it was dried at 37°C . This was accomplished in about 24 hours. The dried yeast was ground in a hand mill and finally in a ball mill which

pulverized it to a fine powder.

One hundred or more grams of each kind was necessary for the vitamin assays.

GROWING OF ROUGH YEAST

An attempt was made to grow rough yeast in the same manner as the smooth, but so much difficulty was experienced that the method had to be discarded. The rough type of yeast grows on the surface, where as the smooth type grows near the bottom. In aerating the rough forms a foam was produced which could not be checked permanently with corn oil as was done in growing the smooth. This foam kept rising until it pushed the cork from the bottle and ran over the side.

The roughs were, however, successfully grown in two liter flasks containing from one to one and one-half inches of molasses medium. The flasks were inoculated, shaken occasionally and allowed to grow for two or three days or until sufficient growth was obtained. Then the contents of all the flasks was poured into one and allowed to settle. Some of the yeast remained on top so that not as clear a separation could be made by syphoning as was obtained with the smooth. Therefore, more of the yeast had to be separated by centrifuging. In all other details the process of harvesting and drying was the same as for the S form previously described.

ASSAYS FOR VITAMIN B COMPLEX

The purpose of this investigation was to make a comparison of the vitamin B complex in the S and R forms of Saccharomyces cerevisiae, Hansen, Saaz strain with that found in other species of yeast. For this purpose, they were compared with Saccharomyces ellipsoidus S and a commercial dried yeast.

The method, in brief, involves feeding young healthy rats a diet complete in all vitamins except the ones to be studied. The material to be tested supplements the diet and is evaluated according to its ability to induce certain rates of growth in the animal. Since both vitamins B and G are required for growth, rats confined to a diet lacking of either vitamin in a short time start losing weight, but when the deficient diets are supplemented with materials containing these vitamins, the rats respond by gaining weight in proportion to the amount of vitamin given. The change in weight of the experimental animal is therefore taken as the chief criterion for the measurement of vitamin potency.

Rats weighing from 60 to 70 grams were selected for the assay. Great care was taken in choosing animals that were uniform in weight, as this is a very important factor in the animal's response

toward various amounts of vitamin B complex.

The animals were fed a basal ration essentially similar to that devised by Barnett Sure (1933) which follows:

Purified casein.....	20
Agar agar.....	2
MacCollums salt #185.....	4
Filtered butter.....	10
Dextrin.....	64
	<u>100</u>

A modification of Steenbock's salt #42 was used in place of MacCollums salt #185.

As some vitamin B complex is stored in the bodies of the rats, it was necessary to deplete the animals of these vitamins before starting the assay. This was accomplished by feeding the basal ration unsupplemented. The point of depletion was recognized when the animals failed to gain weight. This required from two to three weeks. At the end of this period, the animals were placed in individual cages with raised screen bottoms. Litter mates were used on corresponding levels of the R and S forms, but due to some irregularities in some of the litters, this plan could not be followed throughout the experiment. Various samples of yeast were supplemented in the diet. A week's supply was given at one time. The yeast was put in an earthenware cup, mixed with a little basal ration and placed in the cage. The cup containing the basal ration was not removed. This was kept well filled, so that the animals could

eat from this at will. Some animals from each group were selected for controls. They received the basal ration only. The animals were weighed once a week on the day that they received their supply of yeast.

A preliminary experiment was run on Saaz R to determine what levels to feed the yeast. The levels selected proved to be correct but as there was such a wide variation in the rats, it was thought advisable to discontinue this series and select a more uniform group of animals. (See table 1) The animals chosen proved to be very satisfactory.

DISCUSSION

As the vitamin B unit, defined by (Sherman), is given in terms of a daily dose, all levels of yeast fed were listed as grams per day, although it was actually administered in the form of a week's supply. For Saaz R and S, the levels of 0.25, 0.50 and 0.75 grams per day were found to be in the correct range. This also served for Sacch. ellipsoidus but with the commercial dried yeast, lower levels were necessary. Levels were chosen that were multiples of each other, in order to simplify calculations. Thus the levels were 0.0833, 0.1667 and 0.25 grams per day.

By inspection of the tables the results can be readily visualized. In table 1 a comparison of the vitamin B complex in Saaz R and S was made. Some irregularities were noted among the data, but they seem to be minor and most of them can be accounted for. In the case of the 0.50 and 0.75 gram levels of Saaz S, one animal on each refused to eat the yeast in the allotted time, however, the animals finally became accustomed to it. Upon close observation of table 1 and 4, it can be easily seen that there is practically no difference between the Saaz R and S, however, the data seems to indicate the Saaz S to be slightly more potent. The difference however is not great enough to be significant and could easily be in the range of experimental error.

In tables 2 and 3 a comparison of the vitamin B complex was made with a commercial dried yeast (Northwestern brand) and Saacharomyces ellipsoidus. In the case of the commercial yeast, the levels were considerably lower. The 0.0833 gram level gave as good a growth as the 0.25 gram level of Saaz R and S and much better than the Saccharomyces ellipsoidus S,. This indicates that the commercial dried yeast has a potency three times greater than Saaz R and S. It also indicates that its potency is greater than Saccharomyces ellipsoidus. The tests show that the R and S types of Saccharomyces

cerevisiae are slightly more potent than Saccharomyces ellipsoidus.

When the tests were completed, the animals on the lower levels, at which they did not gain very readily, or were losing weight, were fed 0.25 grams per day of the Northwestern brand of dried yeast to determine if they had lost the ability to respond to the vitamin B complex. All of the animals gained from 10 to 20 grams per week. This indicated that the rats were normal in their response to the vitamin B complex, and their failure to grow was due entirely to a low content of this factor in the yeasts fed during the first part of the experimental period.

SUMMARY AND CONCLUSION

As the result of these experiments, the following observations seem warranted:

1. There is no significant difference in the vitamin B complex content of the S and R forms of Saccharomyces cerevisiae Hansen, Saaz strain.
2. The commercial dried yeast contained appreciably more of the vitamin B complex than the S and R forms of Saccharomyces cerevisiae and Saccharomyces ellipsoidus S form.
3. The order in which the various yeasts arranged themselves in their potency of the vitamin B complex, is as follows: Sacch. ellipsoidus, the least potent; Sacch. cerevisiae, Saaz R and S forms the next most potent; Commercial form of dried yeast the most potent of all.

TABLE I

Comparison of the Vitamin B Complex of R and S Forms

Saccharomyces cerevisiae Hansen, Saaz as Shown by

Growth Records of White Rats.

		<u>Saccharomyces cerevisiae</u>					Saaz R		
Rat No.	Gms. Yst per day	Initial weight	1st week	2nd week	3rd week	4th week	5th week	Total Gain	Av. Gain per wk.
2	0.25g	95g	93g	95g*				0	0
4	0.50	98	108	118 *				20	10
5	0.75	79	87	95 *				16	8
10	0.25	91	89	83 *				-8	-4
13	0.50	75	78	75 *				0	0
11	0.75	94	104	116 *				22	11
3	0	91	86	79 died				-12	-6
9	0	82	72	54 died				-26	-13
15	0.25	72	79	83	84	81	85	13	2.6
16	0.50	67	75	75	77	79	-	12	3.0
21	0.25	61	66	71	73	72	-	11	2.75
22	0.50	60	71	77	76	79	-	19	4.75

		<u>Saccharomyces cerevisiae</u>					Saaz S		
Rat No.	Gms. Yst per day	Initial weight	1st week	2nd week	3rd week	4th week	5th week	Total Gain	Av. Gain per wk.
18	0.25	70	71	79	77	80	87	17	3.4
19	0.50	72	74	92	102	106	112	40	8.0
20	0.75	73	90	109	121	130	141	68	13.6
24	0.25	59	63	70	70	70	70	11	2.2
25**	0.50	61	61	65	65	72	77	16	3.2
26**	0.75	56	60	66	70	80	83	27	5.4

* Animals were discontinued because they were not uniform.

** Animals did not eat all of their yeast supply in a week.

TABLE II

The Vitamin B Complex in the Commercial Dried
Yeast as Shown by Growth Records of White Rats.

Commercial Dried Yeast (Northwestern Brand)

Rat No.	Gms. Yst per day	Initial weight	1st week	2nd week	3rd week	4th week	5th week	Total Gain	Av. Gain per wk.
27	0.25g	68g	85g	91g	98g	108g	115g	47g	9.4g
31	0.50	82	90	100 *				18	9.0
33	0.75	80	102	123 *				43	21.5
34	0.25	76	94	103	107	127	142	66	13.2
35	0.50	67	87	98 *				31	15.5
38	0.75	72	100	117 *				45	22.5
28	0	84	77	66 died				-18	-9.0
36	0	83	78	66 died				-17	-8.5
40	0.0833	63	64	70	73	75	78	15	3.0
41	0.1667	67	70	78	90	93	110	43	8.6
43	0.0833	81	78	87	88	89	94	13	2.6
44	0.1667	65	70	82	90	95	100	35	7.0

* Animals were discontinued because the levels were too high.

TABLE III

The Vitamin B Complex Content of Saccharomyces
ellipsoidus as Shown by Growth Records of White Rats

Saccharomyces ellipsoidus Hansen S

Rat No.	Gms.Yst per day	Initial weight	1st week	2nd week	3rd week	4th week	5th week	Total Gain	Av.Gain per wk.
39	0	59g	45 died					-14g	-14.0g
45	0.25	64	60	57	51	58	61	-3	-0.6
46	0.50	50	58	65	73	80	80	30	6.0
47	0.0833	73	60	55	55	(67)	(77)(82)		
48	0.25	77	72	73	72	74	77	0	0
49	0.50	61	67	74	82	100	103	42	8.4
50	0.0833	62	57	57	57	(73)	(90)(98)		

() 0.25 grams of the Northwestern was given per day in
place of the one indicated.

TABLE IV

SUMMARY OF DATA

Showing the Daily Yeast Consumption and the Average
Weekly Gains of Each Series of Rats Fed
on the Different Types of Yeast

Yeast	Gms. Yeast per day	Average gain per week
<u>Sacch. cerevisiae</u>	0.25gms.	2.67gms.
Hansen, Saaz R	0.50	3.87
	0.75	9.50
<u>Sacch. cerevisiae</u>	0.25	2.80
Hansen, Saaz S	0.50	5.60
	0.75	9.50
<u>Sacch. ellipsoidus</u>	0.25	-.30
Hansen S	0.50	7.20
Commercial Dried Yeast	0.0833	2.80
(Northwestern Brand)	0.1667	7.80
	0.25	11.30
	0.50	12.10
	0.75	22.00

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87, 259-64
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Fig. 1
Photomicrograph
of S. cerevisiae
Hansen, Saaz showing
typical S forms of
the yeast.
(360x)

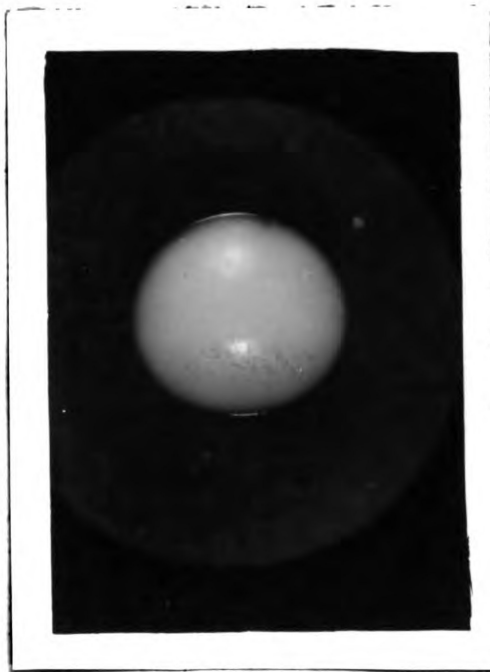
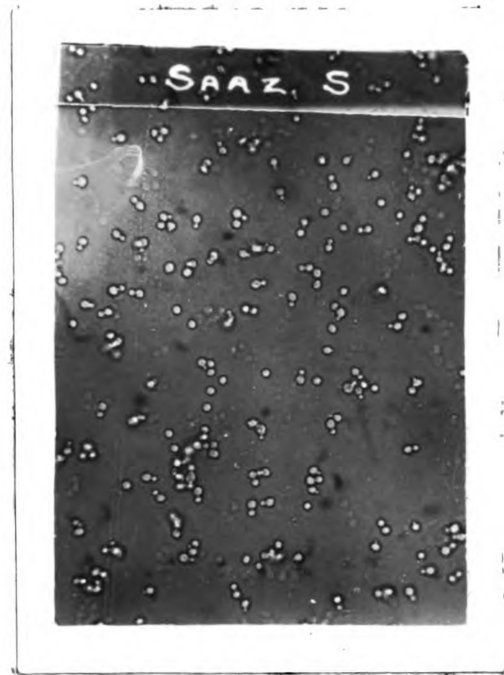
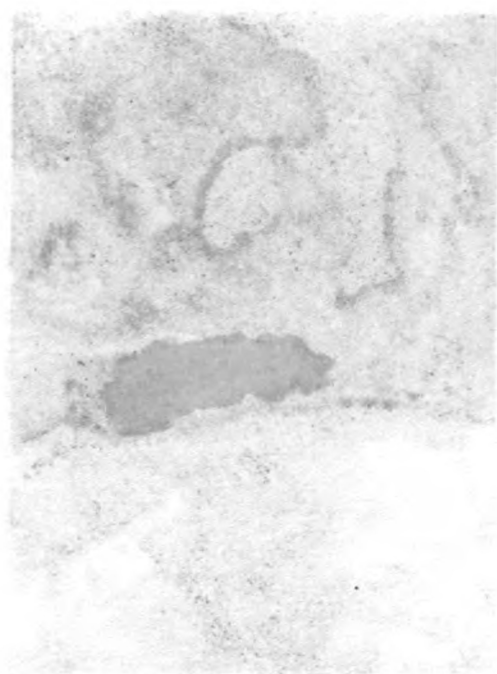


Fig. 2
Photograph of a
typical S colony
of S. cerevisiae
Hansen, Saaz.
(8.5x)



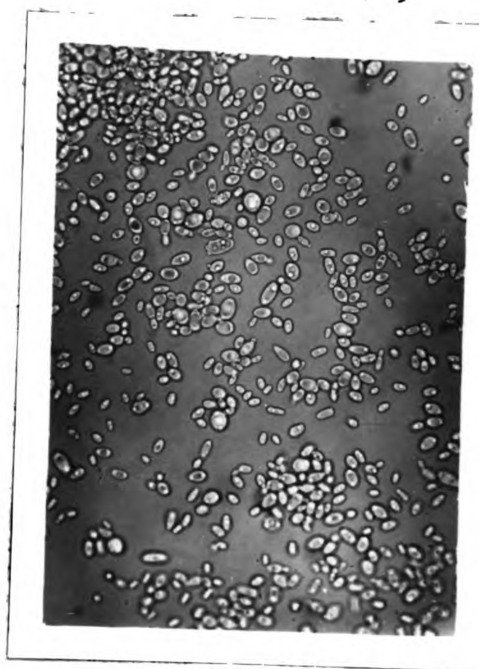


Fig. 3
Photomicrograph
of S. cerevisiae
Hansen, Saaz showing
typical R forms of
the yeast.
(360x)

Fig. 4
Photograph of a
typical R colony
of S. cerevisiae,
Hansen, Saaz.
(13x)

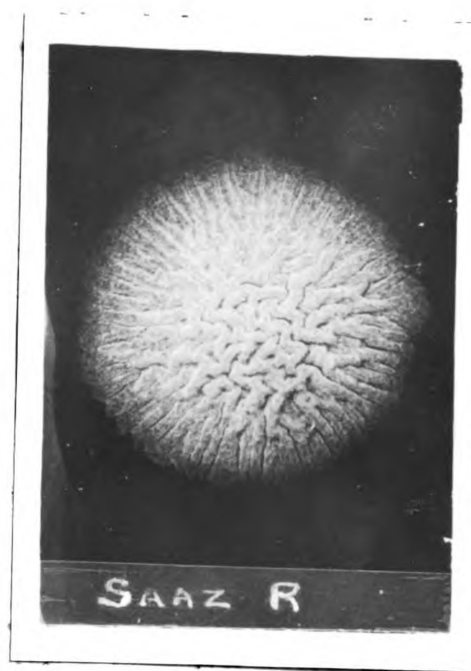
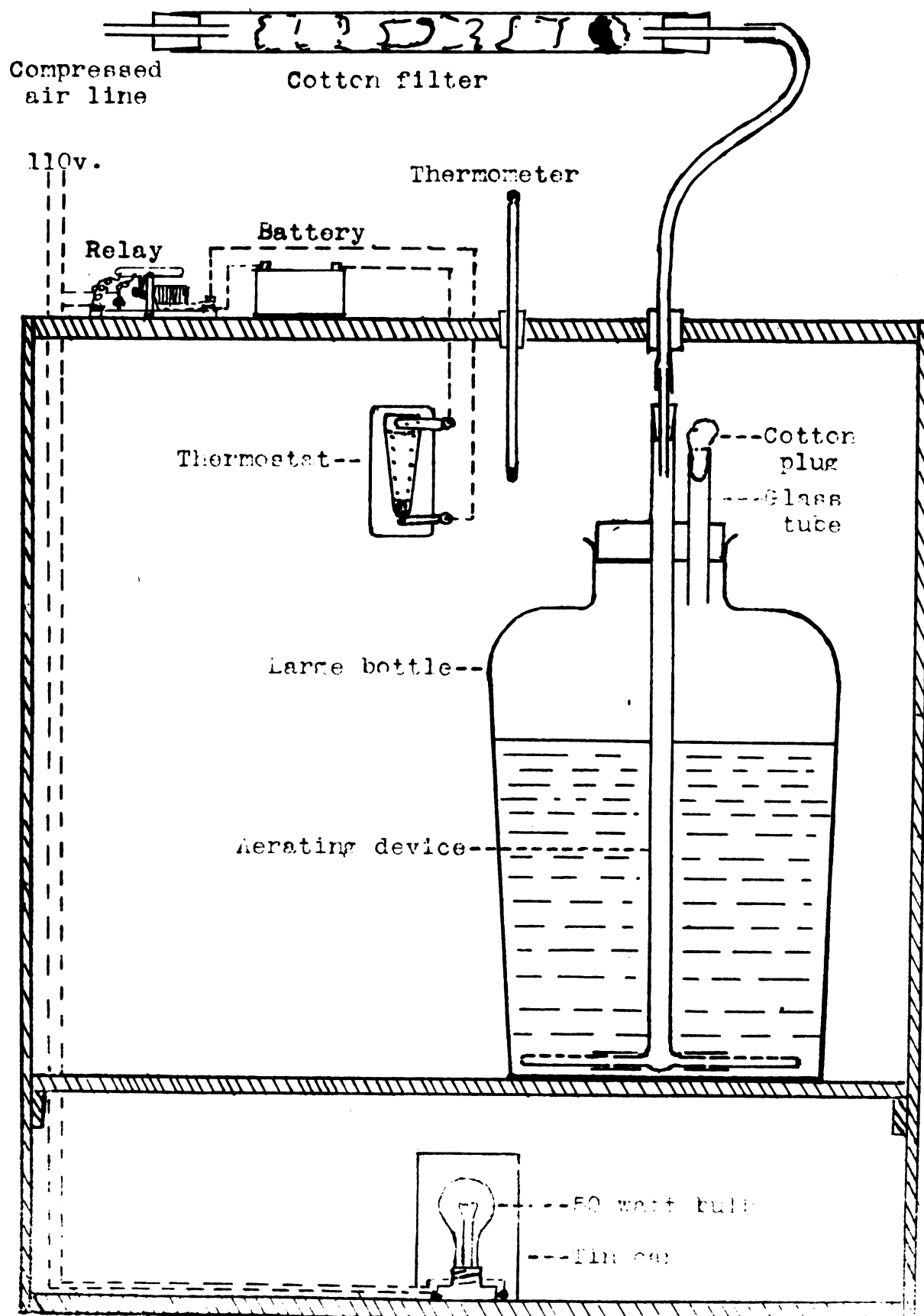
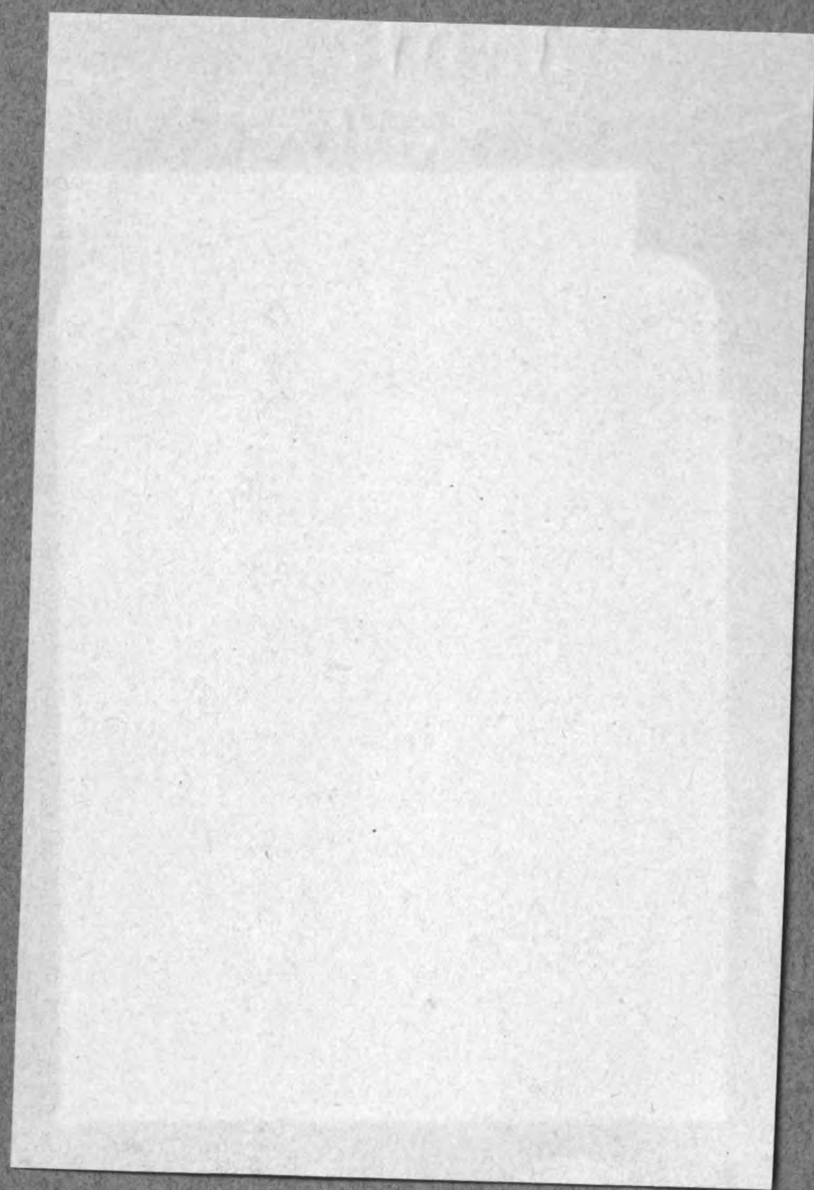


Fig. 5 Schematic drawing showing apparatus used to grow large quantities of yeast.

APPARATUS FOR GROWING YEAST





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