A Study of Yeast.

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[Loaves showing the increasing strength of the loaves.]

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The great general classification of plants is into the chlorophyll bearing and the non-chlorophyll bearing. This second group includes the fungi. Of these there are six principal sub-groups, the saccharomycetes, to which the yeast belongs, being one. All evidence points to their derivation from some higher fungi. Some authorities state that the conidia of many ascomycetes and basidiomycetes, and especially the smuts, will bud extensively in culture solutions and induce fermentations. None of the cultivated yeasts, however, are known to have come from these conidial stages, which are mere passing stages of a more complicated life history.

The yeast plant is of the simplest construction, a single cell comprising

the whole plant. The cells vary in shape under different conditions and may be "spherical, egg-shaped, filamentous, dumb-bell and lemon-shaped"

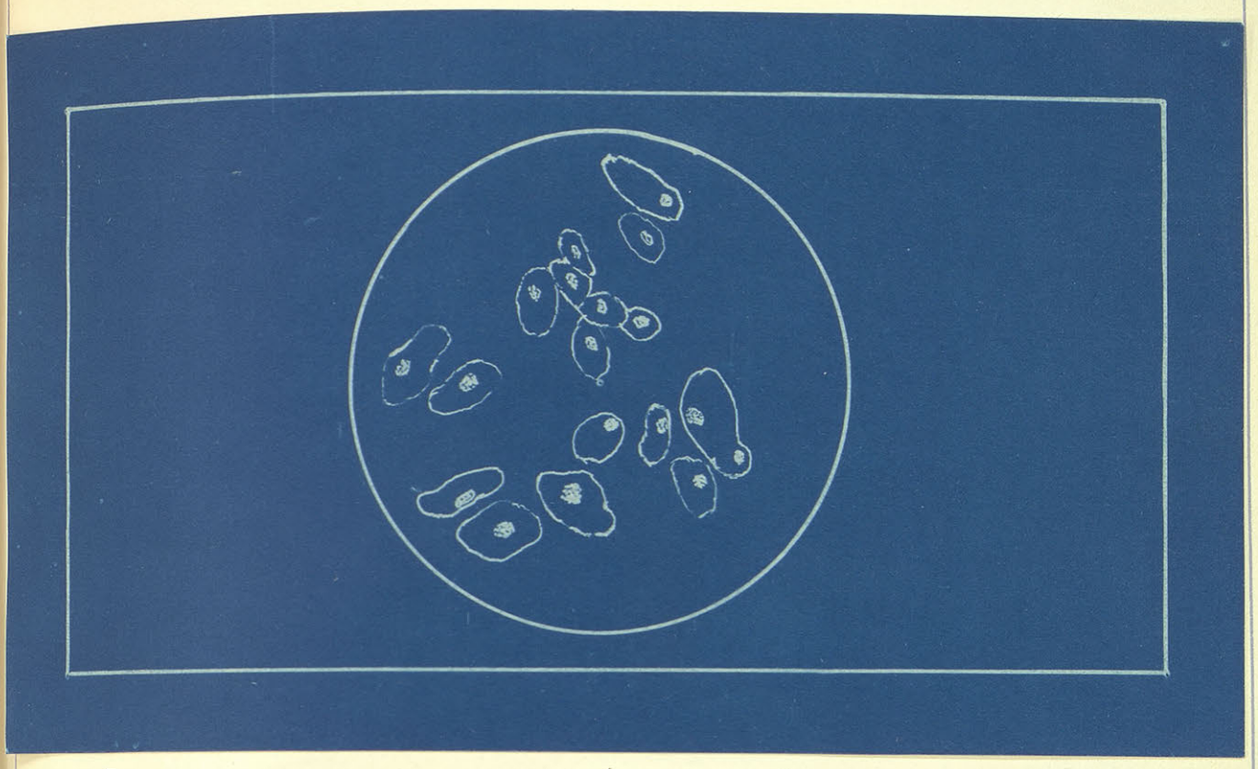
The species are distinguished rather by physiological characters such as maximum and minimum growth-temperatures and the optimum temperature of spore formation. The yeast cell has an outer covering enclosing a proto-plasmic mass which carries on the living processes of the cell.

The protoplasm is disposed in the form of a network, the meshes occupied by a material similar in composition but presumably not living. Its greatest characteristic is lability for it is constantly undergoing decomposition and rebuilding. Some of the decomposition products are used again, others are thrown off. The protoplasm contains albuminoids, with glycogen and fat

present, and ten distinct mineral substances have been found. The glycogen has been found to increase when the plant is grown in a diminished air supply. The outer membrane of the cell is very thin and composed of two layers. It toughens and thickens somewhat as the cell ages. It is probably of pectose or some analagous pectin substance.

The cell has one nucleus that long remained undiscovered because of the difficulty in staining. The method of Janssens and Leblanc and the Heidenhain method were used in the laboratory with good success.

Heidenhain's method is as follows; A cover glass is cleaned and a drop of the culture spread upon it and allowed to dry. Holding the cover glass with the forceps, this is passed through the glass flame quickly, three times. The cover glasses were then allowed to stand for several hours in a solution of



[Yeast Nucleus. (Heidenhain Method.) Drawn by aid of Zeiss-Abbe, Camera Lucida.]

100 c.c. distilled water and 2½ grams of iron alum, then placed over night in a solution of 100 c.c. distilled water and ½ gram haematoxylin. They are decolorised by dipping in distilled water, or if the stain was very heavy, in distilled water to which a few drops of sulphuric acid had been added. This stained the nucleus a brownish-

black, and the membrane of the cell an indistinct yellow.

In the Janowski and Leblanc method, the procedure is somewhat different. A portion of the yeast culture is placed in a solution of one part potassium iodide, 100 parts water, and iodine to saturation. A drop of this culture is then placed on the cover glass and allowed to dry and then placed in the iodine solution and left for 24 hours, from this it is placed, first in water and then run through a series of alcohols; 30%; 50%; 70%; 85%; 95%. The 95% alcohol remains over night or as long as 48 hours if desired. The specimen is then stained by ordinary laboratory methods with carbol fuchsine. It was not found necessary to wash with sulphuric acid there. Both methods were satisfactory.

The knowledge of the food principles

contained in yeast led to the utilization of the yeast residue from fermentation industries. From the yeast cells separated from all refuse has been made an extract called "muk" or "sirio" and used much as Liebig's "Extract of Beef" in the making of bouillon. So far it seems to be more of a novelty and prescribed principally for diabetic patients than as a regular article of diet.

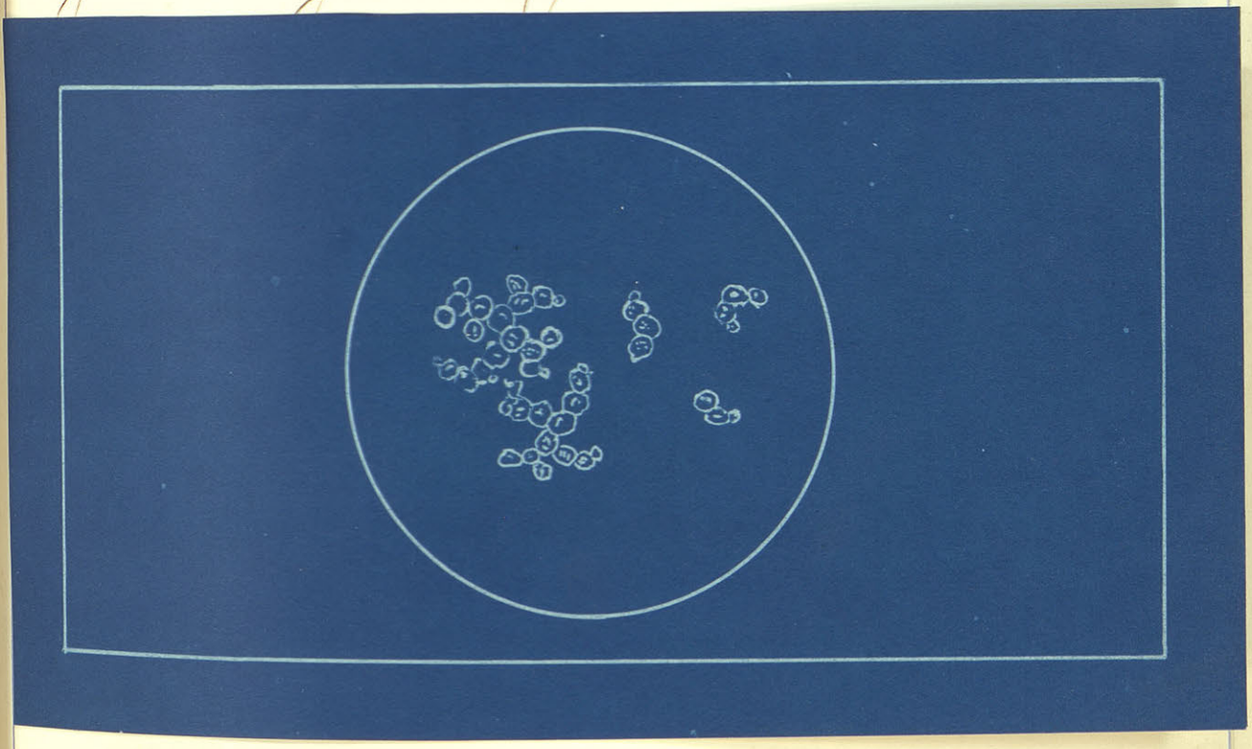
By steeping grain in water and filtering off the extract, and to this filtrate adding alcohol in excess, a white precipitate was found that would convert starch paste into sugar. The substance was named diastase and held to be a kind of ferment but unorganized. These unorganized ferments were found to be associated in some way with the living substance of either animal or vegetable cells and played an important part in the life

of the organism itself. This separated them into two classes - the one a living organism working only during its own process of growth and multiplication, the other a class of substances which could be extracted by solvents from cells and capable of setting up decomposition apart from the life of the cell itself; hence the names organized and unorganized ferments, the latter more often now called enzymes. It is held that an enzyme does not undergo decomposition from its own activity. This activity is lessened and finally paralyzed by the presence of an excess of the products which they form.

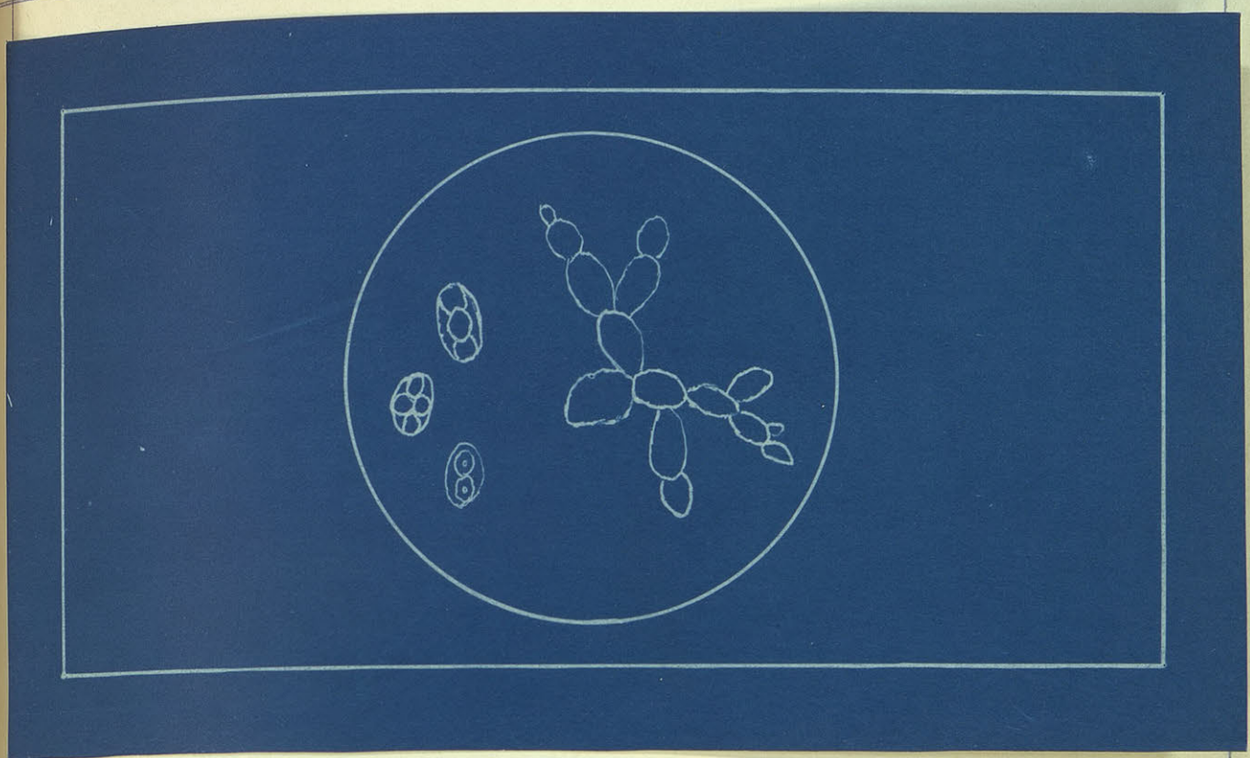
Pasteur in 1858 advanced this enzyme theory of fermentation, his belief being that fermentation is due to the various enzymes contained in yeast and not to the growth of the yeast itself. An interesting theory had been held before this but had not been proved. It was that the plant itself

caused the fermentation by its growth, the want of oxygen in the media causing the plant to take the oxygen from the sugars and thereby causing the decomposition known as fermentation. Buchner substantiated Traube's theory by submitting yeast cells to high pressure and produced fermentation in sugar solutions by the extract so obtained. Formerly, the enzyme, invertin or invertase was recognized as present in yeast and this had the power of breaking down saccharose into dextrose. Now there are others recognized, for one, yeast glucase which turns maltose into dextrose. It is now known that hydrolysis precedes fermentation of all polysaccharides. This enzyme content cannot be changed by differing conditions and so the main characters used in classifying species. The action of fermentation by yeast is one of decomposition. None but saccharine solutions are capable of

undergoing alcoholic fermentation. The living yeast cell forms the enzyme invertase. Lavoisier studied sugar quantitatively and found that the fermentation of it is first by hydrolysis into glucose and fructose, and in the second stage, both of these are decomposed into alcohol and carbon dioxide. The yeast also forms glycerine and succinic acid, about 4% of the sugar being changed into this.



[Slide from undisturbed sponge mixture showing budding of yeast and the vacuoles of the cell.]



[Spore formation and a colony formed from one cell. - From Dicker.]

Yeast reproduction is by budding and by spore formation, the budding taking place when the plant is in a media favorable for its most rapid growth; the spore formation when there is little to nourish it. In budding the cell puts out one or more processes which finally become abstricted from the mother cell, the nucleus at the same time dividing, giving a portion

of itself to the bud. The buds may remain attached for a long time so that they may form an irregular group of cells clinging together.

In spore formation, the protoplasm separates into two or four masses that become walled and lay inside the mother cell. When this spore is returned to nutrient media, the cell wall proper swells and breaks, releasing the enclosed portions that increase in size and reproduce by the vegetative process.

The rate of increase depends upon the temperature, species, aëration and the chemical composition of the culture media. The time required to complete the generation of a cell has been found to be as follows:

20 hours at 40° C.
10 1/2 hours at 18 1/2° C.
6 1/2 hours at 23° C.
5 4/5 hours at 28° C.
9 hours at 34° C.

The temperature at which the common bud yeast, saccharomyces cerevisiae, thrives best is 15° to 20°C. (59°-68° F.) Intense cold does not kill it although its vegetative growth is arrested below 5°C. (41° F.) Heat in the presence of moisture kills it at a temperature above 75°C. (167° F.); if dry, the plant may be heated above 100°C. (212° F.) without killing it.

A comprehensible estimate of the size of the yeast cell would be hard to give but some one has made a comparative estimate and states that the largest it would take 4000 laid side by side to make an inch.

Biologists had kept what we know as yeast or the saccharomyces on the uncertain line of demarkation between the animal and vegetable kingdoms for years, some contending it was a plant, others, an animal. In 1822, Person, a microscopist, after years

of exhaustive study of the debated growth gave to it the name mycoderma (fungoid film) indicating that he had settled for himself, at least, that it was a fungus. Thus Latin, in the '30's, Latour, Schwann and Kützing stated expressly that yeast is a plant. Meyen agreed and gave to the new genus the systematic name saccharomycetes (sugar fungus) and from then the improved laboratory methods and the knowledge that this microscopic organism plays such an important part in practical industries, led to extensive investigations and a fuller knowledge of the history of the plant. The brewing industry has been almost revolutionized by Hansen's investigations with the pure yeast cultures. It found that upon the species of yeast as well as upon the substances fermented, depend the aroma, flavor and color of beer. This would seem to be quite as true of the baker's as the

brewer's products but as yet the baker has done little along pure yeast lines. The reason perhaps is that the breweries are owned by companies, controlling in one place an immense amount of capital, their finished products allowing transportation long distances and keeping for years, while the baker's products are perishable, do not allow of transportation to the consumer and must be manufactured on a comparatively small scale and hence do not control an amount of capital that would warrant the costly investigations that must be made before the knowledge gained returns a monetary consideration for the experiment. As an example of what such investigations cost, Hansen's experiments in the Carlsberg Breweries, Copenhagen, covered years, the laboratory erected and equipped for his use cost thousands of dollars, and his corps of scientifically trained assistants would put to shame the faculty of the ordinary university.

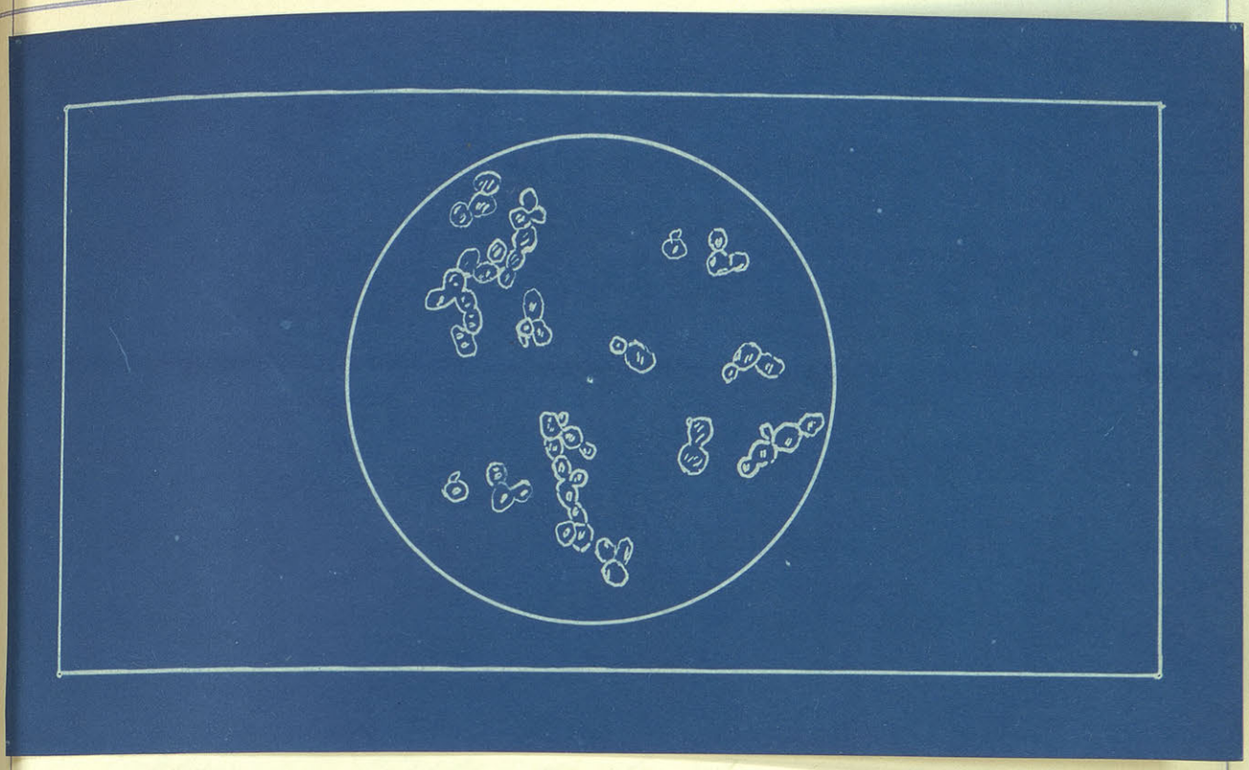
The experiments carried on in the Botany Department of the Agricultural College from January to June, 1905 were satisfactory in that they strengthened the belief that there is no great a future before the pure yeast bread-maker as there was for the pure yeast brewer though the results are comparatively inaccurate from the scientific viewpoint.

The work attempted was the separation of a single, living yeast cell from the dry yeast cake, known as "Yeast Foam," sowing this upon the media suitable for its growth, keeping at the temperature and amount of light conducive to its most vigorous growth and putting to the final test of bread making, and comparing this with bread made from the same flour, under the same conditions, the leavening power furnished by the cake of yeast from which the one cell was obtained. This cake, during the time

of the carrying forward of the experiment was kept in a closed jar to prevent undue infection from other sources.

The method of procedure was as follows: A portion of the cake was taken with a sterile scalpel and mixed with one c.c. of distilled water and this added in a sponge or media made by cooking one potato till done, draining off the water, and adding 1 tspn of flour and 1 tbspn of sugar and cooking for ten minutes and adding to the well-mashed potato. This was allowed to cool before adding the yeast mixture, and though most of the time it was [illegible] temperature, in 60 hours a vigorous fermentation had begun. From this inoculations were made by means of the platinum wire, sterilized in the gas flame, to a tube of gelatin media.

This gelatin media was made in



[Appearance of slide made from the gelatin culture]

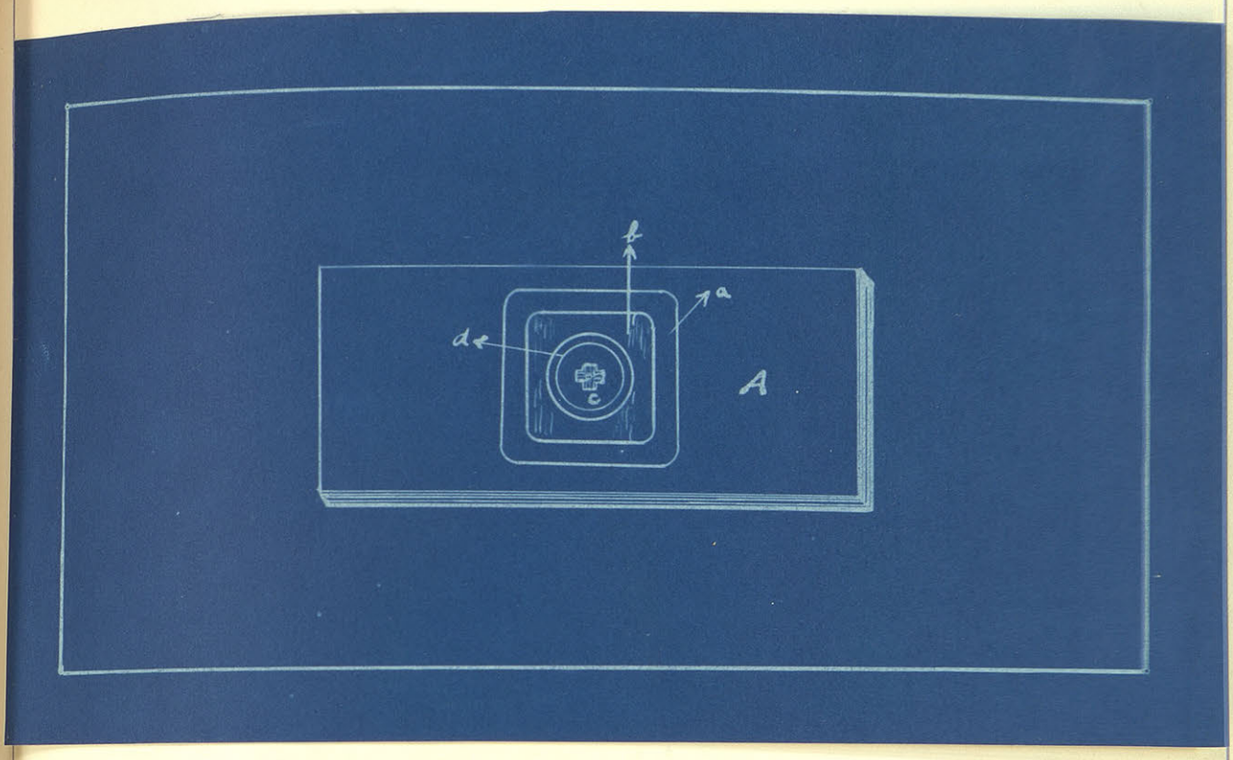
the following manner; One potato was pulid and cooked in 200 c.c. of water till tender, Then mashed, mixed with the water in which it was cooked and strained through a cloth. 200 c.c. of this liquid was put into an agate basin containing 20 grams of shut gelatin clipped in small pieces. 1% of peptone and ½% of sodium chloride was added and then heated

over a water bath with frequent stirring until gelatin was dissolved. Allowed to cool to 50°C. and clarified by stirring in the white of an egg, returning to water bath until egg albumin was coagulated and then filtered through the hot water filter. This was acidulated with sulphuric acid (no definite amount, simply using the litmus test) to prevent the growth of bacteria but not strong enough to kill the yeast. In 48 hours these tubes had vigorous growths of yeast that under the microscope showed no contamination by bacteria.

The task was now to isolate a single cell from this. What is known as Lindner's Dropplet Culture or the Streak Gelatin Method was first tried. The gelatin culture was diluted with the acidulated gelatine media until a possible one cell was in each streak made with a sterilized drawing pen on a roll

-cleaned cover glass. The cover glass was then turned over and fitted with vaseline on the glass-ring chamber of a slide, a drop of moisture being placed in the bottom of the chamber to prevent drying out. These were to be allowed to stand until the one cell had increased to a speck visible to the naked eye and would then be transferred to suitable media, In my hands this method was unsuccessful.

The second trial was made by the dilution method. A tube of gelatine culture was liquefied by slight warmth, care being taken not to heat to such a degree as would kill the yeast and then vigorously shaken. 1 c.c. of this was added to 3 c.c. of dilute sulphuric acid (1 part concentrated sulphuric acid to 10 parts of water) This was subjected to prolonged shaking and a drop taken with a pipette and placed on the centre of a haematimeter, The drop



[Thoma's Haematimeter]

was taken as quickly as possible as there is danger of the cells sinking to the bottom of the liquid and rendering the count inaccurate.

The counting apparatus used was Thoma's haematimeter. The following description is from Klöcker's "Fermentation Organisms;"

"A is a glass slip on which a cover glass (a) is fastened which has a circular hole in the middle and

is .2 m.m. thick. A circular cover glass (c) .1 m.m. thick is fitted centrally in this hole and is also fastened to the glass slip. This annular space (d) is formed. In the middle of (c) two sets of 21 parallel lines are etched which cut each other at right angles. There are thus formed a large square with a side of 1 m.m. and a small square with a side of .05 m.m. The drop of liquid to be examined is placed on this square and enclosed by the cover glass."

The drop was placed on the central part of the haematimeter and the count of the cells taken. Three drops were counted showing 220, 209 and 204 cells in the portion of the drop on the square of the haematimeter. By measurement it was found to take 20 drops of this mixture to make 1 C.C. One

drop of this mixture according to the average found by the counting contained 11,000 cells. One drop of the yeast mixture was added to 500 C.C. of distilled water and vigorously shaken. If evenly distributed this would give 1 cell to a drop. Drops of this were then examined under the microscope and when an isolated cell was found, it was washed from the cover glass with distilled water into a tube containing the gelatin media. These were left at ordinary room temperature in a dark closet from Saturday until Tuesday when a pin point growth was visible to the naked eye. These were left for 4 days when the growth had increased till it covered the surface of the gelatin. A microscopic examination showed two of the cultures infected with bacteria. Inoculations

from the others were made into a sponge mixture made of potatoes, flour and sugar as previously discribed and kept in a baker covered with cotton. The quantity inoculated was equal in weight to one yeast cake. This was allowed to stand four days when two were found to have undergone fermentation. There was no distinguishing characteristic from the ordinary yeast except a slight difference in odor, the pure yeast having a somewhat acetous odor.

Working on the supposition that this amount of yeast material was equivalent to a cake of dry yeast, yeast was started as for ordinary bread making. Four potatoes were boiled until tender the water strained off and 1½ tbspn flour and 4 tbspn sugar added to it and cooked for 10 minutes.

The potato was mashed and added to the potato - water, flour and sugar mixture. When cool the yeast culture was added.

This was allowed to stand at room temperature for 8 hours and then placed in the refrigerator from Saturday till Tuesday.

Tuesday morning a sponge made of 1/2 c. water, 1/2 tsp. salt, 1/2 tsp. sugar, 1/4 c. yeast and 1 c. flour was made. This was allowed to stand over night and the next morning made into a stiff loaf, allowed to rise twice and baked.

The first loaf baked was sweet but lacked leavening. The yeast was allowed to stand 48 hours longer and another loaf made as before. Each time the loaf improved, showing the strength of the leavening

power was increasing. Comparing this with the yeast cake bread, the grain, texture and color were similar but the oder of the pure yeast bread was sweeter.

The experiment led me to believe that in most cases the yeast cake consists of one species with only an insignificant admixture of other organisms and that during fermentation the yeast products destroy any bacteria that may find lodgement and the result will be that of a pure yeast culture bread. What effect different species will have is an interesting question and one that some future investigator may demonstrate.