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West Virginia University Agricultural Experiment Station

MORGANTOWN, W. VA.

Departments of Chemistry and Plant Pathology

The Effect of Pressure on Certain Micro- Organisms Encountered in the Preser- vation of Fruits and Vegetables

TECHNICAL BULLETIN

BY

B. H. Hite, N. J. Giddings and Chas. E. Weakley, Jr.

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The Effect of Pressure on Certain Micro-Organisms Encountered in Preserving Fruits and Vegetables

INTRODUCTION.

A preliminary report of the effects of pressure in the preservation of milk was published as Bulletin 56 of this Experiment Station. The work therein reported was continued until hope of preventing certain slow changes in the milk had to be abandoned. The changes were due to enzymes which the pressure could not destroy. The writers are now studying the effect of pressure on enzymes and have shown that the activity of some milk enzymes is not destroyed by a pressure of 100,000 pounds per square inch, at room temperature for seven days, nor by the same pressure applied for an hour each day for more than a week. Cultures of bacteria have not been obtainable from any samples of milk so treated.

It is not always necessary to apply such pressures so long or to repeat them so often in order to obtain sterile samples. A single application probably destroys all non spore-forming bacteria and all spore formers in the vegetative stage; certainly no non sporeformers hereinafter mentioned except *B. prodigiosus* have survived such a pressure for half a minute. The destruction of bacteria in a sample of old milk is usually a most striking demonstration of the effect of pressure. An original count of thirty or forty millions per cc. may be cut down to a few hundred, or a few dozen, by an application of 100,000 pounds for ten minutes.

Such sweeping reductions in the bacterial content of milk are believed to be quite as desirable as the originally contemplated preservation of milk in salable condition. It should be observed that the good effect of pressure is not entirely a matter of the number of bacteria destroyed; for milk is not ordinarily suspected and has never been convicted of being the frequent carrier of sporeforming pathogenic organisms, while non sporeforming pathogenic bacteria known to thrive in

FIG. 1.—Equipment for preserving fruits and vegetables in ordinary tin cans at moderate temperatures and pressures.

FIG. 2.—Tin and glass containers for cultures.

milk (See *B. diphtheriae* and *B. typhosus*) are among the organisms that are the easiest to destroy by pressure.

The changes taking place in compressed samples of milk, fruit juices, etc., are, of course, but a measure of what can be accomplished by the organisms and enzymes surviving the pressure, during such time as they can survive the media. Milk and vegetables are very suitable media for many different organisms, including sporeformers, against which a single application of pressure is useless. In the case of ripe fruits, the yeasts and other organisms having most to do with decomposition are very susceptible to pressure, while other organisms not so susceptible do not long survive the acid media.

FRUIT JUICES UNDER PRESSURE.

The equipment and method of procedure were essentially the same as were used in the work on milk and may be

FIG. 3.—Section through center of cylinder with lead packing and pistons in place.

described briefly as follows. A collapsible block tin tube, Fig. 2, A, was filled with the fruit juice, closed with a metal screw cap and placed in a lead tube (packing) which was filled with water, closed with a lead lid and placed in a heavy steel cylinder C, Fig. 3, between solid steel plugs (or pistons) O. P. Fig. 3. The cylinder was placed between the bed plate, and the ram of a heavy hydraulic press, Fig. 4, and the pressure applied to the lead tube and its contents. There is no trace of leakage while the pressure is being applied and the pressures attainable are limited only by the strength of the cylinder. On

relieving the pressure the lead packings (stretching to their original capacity) are often pulled in two. They never regain their original shape, so can be used but once.

The greater part of the work was done with cylinders seven inches long, six inches outside diameter and one and

one-fourth inch bore. This is a very convenient size, and if made of good tool steel, properly tempered and ground to fit the pistons, may be used indefinitely for pressures up to 100,000 pounds per square inch. The hydraulic press (with cylinder in place) is shown in Fig. 4. The press was intended for a maximum pressure of 100 tons, but has been forced to higher pressures.

The pressures were measured with a gauge as shown.

FIG. 4.—Hydraulic press used for subjecting small samples to very high pressure.

The gauge was graduated in tons and was checked from time to time against the "Picnometer Gauge" shown in Fig. 5 and consisting of a little bottle filled with water and provided with a perforated glass stopper expanded into a cup containing mercury. Submerged in water under pressure, a quantity of mercury, depending on the pressure applied, passes through the capillary perforation into the bottle. This method of measuring pressures is capable of much refinement.*

FIG. 5.—Picnometer bottle for checking the accuracy of the gauge.

Grape juice was among the first materials to be compressed. Some of the samples were fresh when compressed, others were in various stages of fermentation, or inoculated with fermenting samples or with yeast. A pressure of 100,000 pounds for ten minutes stopped fermentation, in all cases, but the tin tubes soon destroyed the color. Glass tubes (homeopathic phials) sealed with plugs of soft wax (Fig. 2F.)

were substituted for the tin tubes, the wax serving as a movable piston to take care of the contraction and expansion of the juice on applying and releasing the pressure. The wax does not make a reliable seal and many tests were lost. On long standing the purple pulp was deposited leaving the clear juice, a fine deep wine color. Working in glass, a very small bubble of gas would have been detected easily, but the pressure quickly puts an end to the formation of gas. Two samples inoculated with yeast were examined at the Boston Biochemical Laboratory as follows: An old sample fermenting rapidly when subjected to 75,000 pounds for thirty minutes; a fresh sample receiving 25,000 pounds for sixteen hours. In each case an extended series of inoculation tests gave negative results. Inoculated with yeast after standing several years, the compressed samples fermented rapidly. Various combinations of lower pressures at higher temperatures, up to 65° C. gave a large number of samples of grape juice in good condition, but the results as a whole were not regular. Temperatures high enough to make an appreciable difference frequently stopped fermentation at ordinary pressures. A pressure of 30,000 pounds came to be regarded as the lowest that would probably be worth applying in practice.

*Engineering News, Vol. XLIV, No. 14.

Sugar and Yeast. Samples of grape sugar dissolved in water and inoculated with baker's yeast (Fleischman's) never fermented after being subjected to a pressure of 60,000 pounds for half an hour at room temperatures. The tests were made in the tin tubes. The gas in the control samples often burst the tubes. The swelling of the tubes served as a convenient test for fermentation. The glass tubes would have made possible the detection of smaller quantities of gas but the wax stoppers were treacherous. Combination tin and glass tubes (Fig. 2G.) were constructed by painting a gold band around the tops of the glass tubes (as in decorating glass or china) and soldering the tin tubes to the gold bands. A low melting solder (Newtons metal, tin, lead and bismuth) and a quick light touch with a small soldering iron that is not too hot, are essentials. A little practice may be required but good solder joints can be made in this way and if the work is well done the joint may be the strongest part of the tube. The fermenting samples usually burst the tin tops (as shown in the figure) without disturbing the solder joint. Coating the inside of the tin portion with paraffin (to prevent action of tin) gave an almost perfect tube. Working with the transparent tubes quickly set at rest any doubts as to the reliability of the simple test above mentioned, for when gas was formed at all it was formed in quantities sufficient to distend the tubes. The results with sugar and yeast were more regular than those obtained with grape juice, and the necessary conditions, less severe. A sample inoculated for five hours before receiving 100,000 pounds for 30 minutes and a similar sample receiving 100,000 pounds for 105 minutes, were examined by Dr. S. C. Prescott at Boston Biochemical Laboratory who reported as follows: "Examined to see if enzymes were still present. I found as was to be expected, the negative. I also made an extended series of inoculation tests to see if it was possible to rejuvenate any cells and secure the fermentation processes as they were going on at the start, but with negative results."

Two yeasts were submitted by Dr. Alwood of the U. S. Department of Agriculture, and as they were understood to be very hard to kill by ordinary methods, they were given 120,000 pounds for thirty minutes on two successive days. Both samples were reported sterile.

Apple Juice. Samples subjected to 60,000 to 80,000 pounds for 30 minutes in plain tin tubes did not develop gas, and remained sweet, but the pulp was precipitated as a dirty lead colored mass. Two samples a month old were examined

and found to be sterile. The objectionable condition of the pulp was believed to be due largely to the action of the tin, and pressures of 90,000 to 120,000 pounds were applied to 10 samples in glass and wax for 120 to 130 minutes. These samples were all from one quantity of juice which was fresh when the first samples were drawn but was fermenting perceptibly before the last of the samples were compressed. The pressure stopped the formation of gas and all samples remained sweet. After standing a year the pulp was found to be precipitated, or rather gathered up from all sides into a jelly-like mass (of the same shape as the tube) leaving the juice perfectly clear. Five years later the samples were absolutely good. They were as clear as crystal. The color was a fine amber. As for the odor and taste, they would be hard to describe and quite as hard to improve. They were everything that good apples can suggest with all of the rawness of the original cider left out. A finer non-alcoholic beverage would be hard to imagine and the work with high pressures would be well worth while if it had done no more than show that such a product can be prepared from a fruit so easily grown in this state, no matter how far the necessary pressures may be removed from those at present attainable on a commercial scale.

Peaches and Pears required conditions no more severe than grape juice. No samples receiving 60,000 pounds for 30 minutes ever spoiled. Samples five years old were in good condition.

Blackberries and Raspberries usually fermented, and the few samples that remained in good condition were not always those receiving the highest pressures.

Tomatoes required conditions yet more severe than the berries. Only three samples are known to have been sterilized at ordinary temperatures and they received 100,000 pounds for 60 minutes on seven successive days. Of thirty samples subjected to 28,000 to 45,000 pounds at 50° to 70° for from 30 minutes to 24 hours, all but five spoiled. These had received 40,000 pounds at 70° for 60 minutes; 45,000 pounds at 65° for 120 minutes; 40,000 pounds at 65° for 160 minutes; 40,000 pounds at 65° for 180 minutes and 32,000 pounds at 60° for 24 hours. Other samples receiving higher pressures for longer periods spoiled. There was nothing regular in the work with tomatoes.

Peas, Beans, Beets, and other Vegetables. The work

with vegetables was absolutely regular from start to finish. Every sample spoiled. The compressed samples did not spoil as quickly as the check samples (receiving no pressure) but they spoiled quite as offensively. Repeated applications of high pressures at room temperatures were not thoroughly tested; nor indeed were lower pressures (30,000 to 50,000 pounds) at higher temperatures (55° to 60°); vegetables of all sorts being regarded as hopeless after the first few tests. Spoiled samples of beans, beets, peas, and radishes were used to inoculate samples of 10% solutions of grape sugar in water. The samples so inoculated were subjected to 100,000 pounds for thirty minutes at room temperatures. After standing five days (at room temperature and pressure) cultures were obtained in all cases, but no cultures were obtained after six weeks.

From the above tests it would appear to follow that pressure destroys the organisms largely responsible for the decomposition of sweet ripe fruits. Sporeforming bacteria were doubtless present in all cases, but in the case of the fruits, remained inactive and finally perished on account of the unfavorable media. The same is true of the usual methods of processing, 220° F for a few minutes being sufficient for peaches while the same temperature for an hour does not always prevent the decomposition of corn and vegetables. It is quite possible that in many instances more organisms died on account of the medium than were killed by the pressure, (see later work on pure cultures in various media). Fortunately for the pressure process, it is the sweet ripe fruits that are injured most by the usual methods of processing, while most vegetables must be cooked before they can be eaten and so it makes less difference if the sterilization of the latter is brought about by the cooking process. In all cases it is the higher temperatures that are most injurious. It was believed to be worth while to determine in the case of sweet ripe fruits, how much the processing temperature could be lowered by such pressures as could be applied to commercial quantities, and the equipment shown in Fig. 1 was installed.

The compression chamber was suspended in the large cylindrical iron water bath shown at the extreme right. Near the water bath is a copper tank for holding the mixture of equal parts of water and alcohol ("filler") used in the high pressure parts of the equipment. The tall device with cylindrical base and three vertical rods is the intensifier which multiplied the pump pressures by 7.27. The steam-driven horizontal hydraulic pump is shown at the left. The pump

drew the filler from the tank and forced it into the intensifier which forced another portion of filler into the compression chamber. On releasing the pressure all filler returned to the tank. Water from the city mains was found to work more satisfactorily than steam in operating the pump and was used almost exclusively. The compression chamber was four and one-fourth inches inside diameter and deep enough to accommodate four one quart fruit cans. Outside measurements were twenty-nine by eleven and one-half inches. It was bored out from a solid steel forging and intended to carry a pressure of 30,000 pounds per square inch. The city water pressure was 78 pounds. The piston operated by the water was 10 inches in diameter. The pistons on the hydraulic pump were 1 inch in diameter. Manifestly much higher pressures than 30,000 pounds were possible. The equipment was constructed by the Watson-Stillman Company of New York. It worked perfectly.

The work with the larger equipment was carried on during two fruit seasons. The cans, filled with fruit and water, and the lids soldered on in the usual way, were placed in the compression chamber and the filler (water and alcohol) from the intensifier forced into the chamber, the sides of the cans bending inward until the pressure on the fruits was the same as that of the filler. On relieving the pressure it is the ends of the cans that respond most readily to the expansion of the contents of the cans but the distortion is slight if the cans are completely filled and the bulged ends may be easily pressed back to their original shape.

During the first season a pressure of 30,000 pounds was applied at a temperature of 48° to 50° C. for a few minutes to several hours. More than two hundred cans of fruits and vegetables were treated, but the treatment as a rule was not sufficient. The vegetables promptly spoiled as was to be expected; so did most of the tomatoes and berries. Peaches gave the most promising results; only eight cans spoiled out of six dozen compressed and the spoiled cans were all from one lot. It was very clear that higher temperatures would have to be used and during the second season's work the bath was kept at 60° C. and the pressure 30,000 pounds, was applied for about an hour unless otherwise stated.

Four dozen cans of vegetables, including corn, peas, beans, beets, radishes, and potatoes, promptly spoiled, the gas pressure being sufficient in many cases to rupture the cans. A can of each of the vegetables mentioned, excepting the po-

tatoes, was compressed for an hour on three successive days, but with the same result. Vegetables were again abandoned as hopeless.

A dozen cans each of blackberries and raspberries developed perceptible quantities of gas and were not further examined. A dozen cans of huckleberries were not swollen at the end of two weeks and one can examined at National Cannery Laboratory was reported as having "no evidence whatever of bacteria, yeasts and moulds" and as being "in a perfectly sweet and fresh condition."

Tomatoes usually soured. Only five good cans were obtained out of the four dozen that were compressed. Three of the good cans were opened within a month, one was examined at the Cannery Laboratory and pronounced sterile and sweet. The remaining can was not opened for several years, but it was a very good sample. No explanation is offered for the failure of the five samples to become as sour as the several dozen other samples compressed at the same time. It is possible that the five cans contained no tomatoes that were not thoroughly ripe.

A dozen cans each of apples, pears, and plums may be said to have kept fairly well. A can of each examined for bacteria after standing a month (to note formation of gas) was found to be sterile, although slight changes had undoubtedly taken place. The changes were not always easy to describe, nor indeed were they always easy to detect. One can of pears six years old was in very good condition. The changes taking place in cider were very much more marked than in the apples from which the cider was made. The pulp was always precipitated and gas was always present. As the cider was fermenting when the cans were filled, the presence of the gas was to be expected, but the precipitation of the pulp is not so easily accounted for, as it was not complete within the period that cultures were obtained. The pulp deposited in paraffin-coated cans was similar to that obtained in glass bottles with paraffin stoppers. In ordinary tin cans deposit could have been mistaken for yellow clay. The changes in question left the juice perfectly clear and sweet. The cider was really improved by the process, but not as much improved as the samples receiving very high pressures at ordinary temperatures. A sample a month old examined at Cannery Laboratory was reported sterile. Four types of cans were used as containers for cider. One can of each type was not opened

for several years when a strip two inches wide and extending around the middle of each can, was cut out and photographed (Fig. 6) to show the action of the cider on the containers. The first strip (on the left) is from an ordinary tin can; the second is from an "inside enameled" can; the third is from a can with patented wax lining and the fourth from an ordinary tin can that was coated with paraffin after it had begun to corrode from standing a year in a damp basement. The paraffin coating is almost perfect as (was intended to be) shown by the scratches through the coating. Paraffin is undoubtedly the best lining; unfortunately it cannot be used at the high temperatures of the usual method of processing. The patented inner coated can has a wax lining that withstands processing temperatures, but the coating is evidently applied to the sheet tin and when this is made into cans the coating is injured at the solder joints. The enameled can came out of

FIG. 6.—Sections of four types of linings of tin cans, showing prolonged action of fruit juice.

the test in remarkably good condition. It is a long step in the right direction.

More than a hundred cans of peaches were compressed. No peaches spoiled and so long as they lasted it was the custom to open a can of peaches for any one visiting the laboratory and interested in canning. In the case of peaches, it would appear that the organisms escaping the pressure can accomplish but little in the time they can endure the medium.

The following report of the National Canners' Laboratory on one consignment of samples of fruits and vegetables would serve as a very good summary of the work with commercial quantities: "We have finished the bacteriological work on the greater part of the samples which you recently sent to the laboratory and the results of our tests follow:

- No. D 80 **Radishes**; spoiled; offensive odor; copious growth of bacteria on plates and in tubes.
- No. D 3 **Beans**; sour; under microscope lactic acid bacteria were visible on plate cultures; very marked growth. Also in bouillon tube.
- No. D 48 **Beans**; same report as No. D 3.
- No. D 21 **Beans**; slightly sour; contained lactic acid bacteria which developed on plate and in tube.
- No. D 47 **Beets**; were discolored; under microscope showed the presence of bacilli, probably tomesceus. Liberal growth on plate and in bouillon tube.
- No. D 46 **Beets**; discolored; contained bacteria, probably tomesceus. Growth on plate and in bouillon tube.
- No. 14 **Beets**; good color; slightly sour; no growth on plates or tubes.
- No. D 81 **Beets**; bitter; contained bacteria; growth in tube and on plate.
- No. D 19 **Tomatoes**; were sour; contained bacteria which developed on plates and in bouillon tube.
- No. D 14 **Tomatoes**; sour; contained bacteria which developed on plates and in tube.
- No. D 8 **Tomatoes**; slightly sour; no growth on plates or in tubes.
- No. 103 **Tomatoes**; perfectly sterile; no growth.
- No. 91 **Bananas**; perfectly sterile; no growth.
- No. 100 **Elderberries**; sterile; no growth.
- No. 129 **Plums**; sterile; no growth.
- No. D 12 **Pears**; sterile; no growth.
- No. 76 **Peaches inoculated with yeast**; sterile; no growth.

EXPERIMENTS WITH PURE CULTURES.

The results of the work above reported were so clearly a matter of the media that it was considered advisable to devote such time as might remain for work on this project to a systematic study of the effect of pressure on specific well known organisms.

FIG. 7.—Cylinder ruptured at 130,000 pounds per square inch.

The facts which we wished to determine were:

1. Whether or not a certain organism could be killed by such pressures as we could safely and easily apply.
2. The time-pressure death point curve. That is the relation between amount of pressure and length of time it is applied
3. The effect of high and low temperatures on death point curve.
4. The effect of reaction of the media on death point curve.

The apparatus used was the smaller but much more powerful equipment first described, and a cylinder, designed for much higher pressures than have yet been mentioned, was constructed. This cylinder, half of which is shown in Fig. 7 was seven inches long $5\frac{1}{2}$ inches in diameter, $\frac{7}{8}$ inch bore,

and turned out from one solid tool steel forging. After carrying various pressures up to 140,000 pounds per square inch, it split in two at 130,000 pounds. The lead packing and its contents were in the middle of the cylinder and occupied but half the length of the cylinder. The dark spots shown in the figure are not due to imperfections in the metal, but to contact with the walls of the building and other obstacles encountered on the way. A larger cylinder, (See A, Fig. 12) with smaller ($\frac{3}{4}$ inch) bore was "built up" from three concentric cylinders shrunk together, as in the construction of a modern gun. In the work hereinafter reported this cylinder was used for all pressures exceeding 100,000 pounds per square inch. The cylinder was constructed by Brown & Sharpe, Providence, Rhode Island, according to specifications in the following letter from Mr. Carl F. Jeansen, Ordnance Engineer, U. S. Bureau of Ordnance:

"Below is a sketch of a built up steel cylinder, that I think will about fill your requirements. The finished cylinder is built up of three separate cylinders, one shrunk upon the other.

"The inside cylinder or tube is to be of Halcomb crucible chrome vanadium steel, type 'A', made by the electric process.

"Mr. E. L. Reed, Hartford, Conn., is sales agent.

"This steel when annealed but untreated, should be of at least the following characteristics:

Tensile strength	75,000 pounds
Elastic limit	50,000 pounds
Elongation	35%
Contraction of area.....	65%

"It should be submitted to the following treatment:

- (1) Temper by heating to 1700° F.
- (2) Cool in water.
- (3) Draw back by heating slowly to 400° or 500° F.
- (4) Hold at this heat 30 to 35 minutes or longer according to size of piece.
- (5) Let cool in air.

"After treatment the steel should give the following values:

Tensile strength	200,000 pounds
Elastic limit	160,000 pounds
Elongation	10%
Contraction of area.....	25%

"The specifications for the two outer cylinders are as follows:

Tensile strength	110,000 pounds
Elastic limit	75,000 pounds
Elongation	12%
Contraction of area.....	20%

"The material in the two outer cylinders may either be nickel steel or plain carbon steel.

"The built up cylinder will stand an internal pressure of 184,000 pounds per square inch before the tangential stresses in the tube reach the elastic limit of 160,000 pounds per square inch. At this pressure permanent deformations begin to take place.

FIG. 8.—Sketch of high pressure cylinder.

side layer has on diameter 3.5" a tangential tensile stress of 77,000 pounds. The tangential tensile stress in the second layer is lower than the radial compression and if the second cylinder fails first it will be by crushing. The other two cylinders will in all cases fail by rupture.

"As there is some reserve strength in the two outside cylinders as compared with the tube, it is considered that even higher pressures than 212,000 pounds per square inch might be used before rupture takes place.

The mode of manufacture is:

- (1) Bore second cylinder to size and measure the bore for every half inch.
- (2) Bore tube (first cylinder) to 0.73".
- (3) Turn tube on outside to correspond to measured diameters of bore in second cylinder with prescribed shrinkages added.
- (4) Heat second cylinder to 550° F above the temperature of the room. The bore will then expand to 1.7065"

- giving a clearance of 1.7065"—1.7020" equal to 0.0045". Put the cylinder on the tube and let it cool.
- (5) Repeat the operation for the third cylinder. When the third cylinder is heated to 550° F, the bore expands to 3.5134" giving a clearance of 3.5134"—3.5103" equal to 0.0031". Put the cylinder in place.
 - (6) Finish the bore and turn to size.

"When the second cylinder is shrunk onto the tube the bore contracts 0.0008009" and when the third cylinder is shrunk on, the bore of the tube contracts 0.001626" absolute. Consequently the total contraction of the bore is 0.0022268" corresponding to a tangential compressive stress of 89,000 pounds per square inch.

"In shrinking on the cylinders care should be taken not to expose the material to any draft, nor should any water or artificial cooling be used. The best method would be to place the piece in some heat retaining receptacle or the heating furnace itself with the draft cut off, where it could cool slowly and uniformly. On account of the excessive pressures to which this cylinder will be subjected any internal stresses caused by uneven cooling must be avoided.

"I have gone into the matter rather thoroughly in case a firm, not familiar with shrinkage operations, will be requested to execute the work.

"I hope that the proposed cylinder will stand the work for which you want it."

Culture Media.

The culture media required in this work was carefully prepared, using as a base 5 grams Liebig's extract of beef and 10 grams Witte's peptone in 1000 cc. of distilled water. To such prepared bouillon was added sugar, acid, or alkali, as the case required. All media containing sugar was sterilized by the fractional method, without pressure.

Reaction of media was determined by the methods usually employed in bacteriological work and it was carefully adjusted to the proper acidity with normal sodium hydrate or normal hydrochloric acid. All media was made plus 10 Fuller's scale, unless otherwise noted. Care was exercised to prevent evaporation and resulting changes in concentration, acidity, etc. Tubed media was discarded if not used within three or four days.

Block tin tubes having a capacity of about 2½ cc. were used as containers for the cultures when they were put under

pressure. These tubes were filled with the required medium, the caps placed on loosely, and were then placed in Petri dishes or specially constructed copper containers (See Fig. 9) for sterilization. In some instances it was thought that possibly the medium used might have some chemical action on the tin tubes, and cultures were allowed to remain in the in-

FIG. 9.—Tin tubes (see E. FIG. 2) filled with broth ready for sterilization.

cubator for four to seven days. Plates were then poured to determine the condition of the organism. Not one of the media thus tested was found to have any apparent germicidal action on the bacteria. The following table gives the results of one such test, using *B. prodigiosus* on + 16 beef bouillon. (See also Table XX.)

TABLE I.—*The effect of pressure on B. prodigiosus in plus 16 beef broth.*

Laboratory Number	Date Cultures Were Made	Days Incubated	Date Plates Were Poured	Date Growth Was Noted
839	April 8, 1912	2	April 10, 1912	April 11, 1912
840	April 8, 1912	4	April 12, 1912	April 13, 1912
841	April 8, 1912	9	April 17, 1912	April 18, 1912
842	April 8, 1912	18	April 26, 1912	April 27, 1912
843	April 8, 1912	38	May 16, 1912	May 18, 1912

Three plates were poured on each date and the results clearly show that cultures of *B. prodigiosus* on + 16 beef bouillon are not appreciably injured by standing for several days in sealed tin tubes.

Age and Condition of Cultures.

Cultures three to seven days old were used to inoculate media in the tin tubes during the first few months after these experiments were started. Quite a few contradictory results were secured at this time and some of them were undoubtedly due to the fact that the age of a culture of any given organism is likely to have a pronounced effect on the vitality of the individuals composing it. It was found that cultures two to three days old gave more uniform results, and thereafter practically all of the inoculations were made from two-day-old cultures.

Of course, the exact time required for securing good cultures would vary somewhat with the organism and the incubation temperature. The bacteria and yeasts used in this work developed very rapidly, however, and in nearly all of the final work they were incubated for two days at 30° C.

Preparation of Cultures in Tin Tubes.

It was found far more difficult to make cultures in these small tin tubes than in test tubes. The so-called self-sealing

FIG. 10.—Platinum loops used for inoculating the broth in the tin tubes.

tin tubes (Fig. 2 E) were used in all this work since they were easily handled, very efficient and permitted the media to be in contact with no other material than tin. The caps for these tubes are made with a conical projection on the inside of the top and this, coming down onto the smooth sharp edge of the mouth of the tube, makes a perfectly tight joint. The opening at the top of these tubes was about three millimeters in diameter, and it was found almost impossible to take up a good loop-full (oese) of the test tube culture and carry it into the tin tubes through this small orifice. For some of the earlier work a straight needle was used to make transfers of cultures to the tin tubes. Later some forms of double loop

were used in order to secure larger and more uniform transfers. The two forms of double loop used are shown in Fig. 10. A hypodermic syringe, such as is shown in Fig. 11, was finally found to be very satisfactory for transferring cultures to these tin tubes. The syringes are known commercially as the glass-bestos tuberculin syringe. The barrel is entirely of glass about 22 mm. long by 5 mm., inside diameter, and graduated in 1-100ths of a cc. The plunger is of brass with asbestos packing. The needle is made of platinum-iridium, and is about 90 mm. long with an outside diameter of a little less than 1 mm.

FIG. 11.—Hypodermic syringe used for inoculating the broth in the tin tubes.

The method used in making cultures in the tin tubes was as follows: The inside of the culture room was thoroughly sprayed with water so as to wash all dust and bacteria or fungus spores out of the air and prevent their being set loose from the walls, floor or ceiling. The copper box containing sterile tin tubes and media, (see Fig. 9); the sterile syringe as shown at A in Fig. 11; and the cultures which were to be used were taken into the culture room. The two persons who were going to handle these materials washed their hands carefully in 1-1000 bichloride of mercury, and took their places in the culture room. One of these persons shook up the culture which was to be used, and carefully flamed the end of the test tube, while the other removed a sterile syringe from its container and forced the plunger to the lower end of the barrel. The tip of the hypodermic needle was then dipped into the culture and about 1 cc. of it drawn up into the barrel. The test tube culture was

then put aside and the box containing media in tin tubes was opened. The person handling the tin tubes, took one out of the box, removed the cap and held the tube upright while the person who had the syringe held it with the point of the needle just above the opening in the tube and carefully forced the plunger down till a drop of the culture appeared at the needle tip and became large enough to fall into the tube. The cap was immediately replaced, screwed down firmly and the tube placed in a sterile Petri dish. The amount of culture drawn into the syringe barrel at one time was usually sufficient to make cultures in all the tubes which were to be used during that half day, and a fresh syringe was used, if the first one did not contain enough culture material.

Methods of Handling Cultures in Tin Tubes.

The cultures prepared as just described, were kept in sterile Petri dishes at room temperatures until wanted. The tin tubes, containing cultures, were then placed in a lead tube packing, already described. The lead tube was then filled with water and pressure applied to it. After the pressure was released the tin tubes were removed and placed at once in sterile Petri dishes. They were finally washed in sterile distilled water before pouring plates.

In all cases, unless otherwise stated, three of the cultures were placed in the lead tube so that each test may be said to have been in triplicate.

During the earlier part of the work, plates were poured from the cultures in the tin tubes immediately after the tests for that day had been completed. It was finally found more satisfactory to incubate these cultures for about 24 hours before pouring plates since there might be only a few living organisms present immediately after the removal of pressure, and by leaving them for 24 hours, these would have an opportunity to multiply and the results would be more sharply defined. It was also found that the water surrounding the tin tubes in the press contained numerous spore forms of both bacteria and fungi. Some of the pressures used in this work not being sufficient to destroy all such external forms, they sometimes caused confusion by appearing on the plates. From all indications the organisms were forced back along the threads toward the top of the cap, and remained there ready to be carried into the Petri dish when a drop of the culture fluid was forced out in pouring the plates. In order

to overcome this difficulty, 4% formalin was substituted for plain water in the lead tubes. This was found to be very effective in preventing contamination from that source. As a further precaution against outside contamination, the tin tubes were dropped into 4% formalin for a few minutes just before pouring the plates, and were then rinsed in sterile distilled water by dropping them into one beaker after another until they had passed through at least three such rinsing waters. Our purpose in this thorough rinsing was to remove as thoroughly as possible the formalin which might be around under the outer edge of the cap.

Pouring Plates.

In pouring plates from the tin tubes, the culture room was carefully washed down, and other precautions used as in making the cultures.

It required but one man to pour the plates, and the procedure was as follows: The cap on the tin tube was completely unscrewed, but not removed. While flaming the open mouth of a test tube containing media, the tin tube was inverted over a glass containing 4% formalin, the cap removed by jarring it with one finger, and three or four drops of the culture squeezed out and allowed to fall into the formalin. Then the test tube of media was held upright, the tin tube moved over it and two drops of the culture squeezed into the test tube. The media in the test tube was immediately poured into a sterile Petri dish and the plate was placed in an incubator at 25° C. or 30° C. for one week, unless growth appeared before that time. In most of this work three tin tube cultures were held as checks for each medium or organism used on any day and plates poured from them at the same time as the others of that kind. Sometimes only two such tubes were held and for part of the work only one control tube was used.

Contradictory Results.

Quite a few apparently contradictory results may be noted in looking through the tables found on later pages. There are several good reasons why such variation might be expected. Some of these have been mentioned under other headings. Others, which may have had considerable bearing on this, are the length of time between making cultures and applying pressure to them; temperature at which cultures were held both before pressure and while in the press; and in-

ability to hold the pressure constant within one or two thousand pounds above or below that desired. It is believed that further work might eliminate nearly all of the contradictions shown in these tables. In order to secure such results, however, it would be necessary to have one man devote all his time to bacteriological work on this project, and that has been impracticable in connection with the work reported in this bulletin.

It was thought that the time required for removal of pressure might have something to do with the destruction of the organisms. Twenty seconds to one minute of time were required for bringing the pressure up to the desired point while the release was practically instantaneous. In order to determine this possibility, several tests were made in which the pressure on certain samples was relieved very slowly, while on duplicate samples it was released instantly. There was found to be no difference in the results as between sudden release or slow release, so it is not likely that this factor alone had any important part in the destruction or non-destruction of the organisms used.

Explanation of Tables and Figures.

The general results of these investigations may best be presented in the form of tables and figures, and a brief explanation may aid in their interpretation. The first column of figures at the left gives the laboratory number of the three cultures used in that test. The second column shows the amount of pressure in thousands of pounds per square inch. The third column gives the length of time in minutes during which pressure was maintained. A zero in the third column means that the pressure was released at once after it was attained. In the fourth column is reported the growth or non-growth of the organism in poured plates.

Control sets are not recorded in these tables as there was a great number of them; they would be more likely to confuse the reader than to help; and any series of tests was completely discarded in case the control tubes for that series did not give good growth. The circular black areas on the figures, are points at which the organism in question was killed; the outline circles indicate points at which it lived, and the circles which are half outline and half solid are points where some cultures were killed while others were not.

It was, of course, practically impossible to make deter-

FIG. 12.—Hydraulic press with constant temperature bath in place.

minations at all points along the supposed time-pressure death point curve. It, therefore, seemed desirable, in summarizing, to make use of such a curve, indicating at the same time the points at which determinations were made. In many cases two or more sets were run at the same pressure and for the same length of time. All such duplicate tests are given in the tables, but are not indicated on the curve.

It will be noted that the curve does not always agree with the death point of the organism as shown by a test. The curve is used in this connection, merely to indicate what is believed to be the approximate position of the death point, if this work could be carried on with absolute accuracy and the tests could be made at practically all points. The curve is especially useful for purposes of general comparison.

Mr. G. A. Roush, assistant chemist, who was associated with this work during the year of 1911, and Dr. J. H. Eisland, Professor of Mathematics in West Virginia University, showed that the death points as plotted lie approximately in curves of the form $y = e$ with the exponent $a+bx+cx^2$.

Bacillus Prodigiosus (Ehrenberg) Flugge.

This is a common organism, occurring in water, milk, and on bread, meat, starchy foods, etc. It produces a red pigment, under favorable conditions, and the production of "bloody spots" on bread, and other materials, has often caused consternation in superstitious minds. It has been reported as causing red milk in some cases.

B. prodigiosus does not produce spores, is a vigorous grower, develops well on any of the more common media, and at ordinary temperatures, so that it appeared to be an organism well suited for use in an investigation of this kind.

The first point to be determined was whether or not it could be destroyed by pressure. It was found that this was readily accomplished at moderate pressures, and the relation of time and pressure to the death point—the time-pressure death point curve—was next taken up. This organism was first tested using distilled water and the results are given in Table II and Fig. 13.

It will be noted that this series of tests was quite complete and the results are certainly as uniform as could be expected.

Since the reaction of the medium was believed to materially affect the time-pressure death point, one series of tests was made using this organism on standard broth which had a reaction of plus 5 Fuller's scale (see Table III and Fig. 14), while another series was made on similar broth having a reaction of plus 16 Fuller's scale (see Table IV and Fig. 15). It will be noted that the plus 5 broth showed a slight raising of the time-pressure curve, while the more acid medium lowered it very markedly.

These results were so encouraging that it was decided to try the effect of temperature variations. One series of tests was made at plus 5° C. (see Table V and Fig. 17) and another series at plus 40° C. (see Table VI and Fig. 18). The medium used for all samples was standard broth having a reaction of plus 5.

A special water bath was constructed for this thermal work, and the cylinder was placed therein as shown in Fig. 12. Ice was used to cool the water to plus 5° C. and a burner was placed under one end of the water bath for maintaining the higher temperature. A small stirring apparatus was used to keep the water in circulation and the bath was held constant within less than 1° C. The cylinder, pistons and lead packings were allowed to stand in the water bath, at the required temperature, for one hour previous to applying pressure to samples. This insured the proper temperature throughout all parts of the apparatus surrounding the cultures.

The effects of thermal variation were somewhat of a surprise, as it was thought that raising the temperature would lower the death point and that lowering the temperature would raise it. By comparing Figures 16 and 19, it will be seen that the effect of each of the temperatures tried was to lower the death point by almost exactly the same amount as the plus 16 broth lowered it.

TABLE II.—*The effect of pressure on B. prodigiosus in distilled water.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
34	100	----	No growth
57	95	----	Several colonies
48	90	----	6-8 colonies
77	80	4	No growth
47		----	Good growth
38	75	----	Good growth
50	70	10	No growth
44		----	Several colonies
1	65	60	No growth
52		20	No growth
45		----	Good growth
49	60	10	No growth
43		----	Good growth
51	55	20	No growth
97		15	No growth
60		10	4 colonies 1 plate
6	50	60	No growth
37		40	No growth
36		30	No growth
98		28	No growth
35		20	6-8 colonies
27		10	Several colonies
32		5	Good growth
30		2	Several colonies
28		----	Good growth
19	45	60	No growth
40		50	No growth
39		40	No growth
99		35	5 colonies 1 plate
41		30	6 colonies
78	42	65	No growth
42	40	90	No growth
58		80	4 colonies
53		70	Several colonies
15		60	Good growth
100	37.5	120	No growth
54A	35	150	No growth
59		150	No growth
84		140	Moderate growth
83		130	3 colonies
54		120	Good growth
55		90	4 colonies
17		60	Good growth
101	30	240	3 colonies
9		60	Good growth

BACILLUS PRODIGIOSUS
Distilled Water.

FIG. 13.—Time-pressure death points of *B. prodigiosus* in distilled water. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

TABLE III.—*The effect of pressure on B. prodigiosus in plus 5 broth.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
2144	120		No growth
2138	110		No growth
797	100	5	No growth
733			Good growth
753			Good growth
1043	90	5	No growth
735			Good growth
805	80	5	Good growth 1 plate
806	70	10	Good growth 1 plate
791		5	Good growth 1 plate
764	60	15	No growth
745		10	Good growth 1 plate
755		5	Good growth
1076		5	No growth
792	55	20	No growth
1378		15	No growth
739		15	Good growth 1 plate
1079		10	No growth
737		10	Good growth
1047		5	No growth
1077		5	No growth
1045	50	40	No growth
1046		35	No growth
1377		35	Good growth
807		35	1 colony
794		30	Good growth
793		20	Good growth
1078	45	50	No growth
1044		45	Good growth
808		40	2 colonies 1 plate
743		40	No growth
741		30	Good growth
795	40	100	No growth
751		90	Good growth
749		80	Good growth
809	35	150	No growth
762		120	Good growth 1 plate

FIG. 14.—Time-pressure death points of *B. prodigiosus* in plus 5 beef broth. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

TABLE IV.—*The effect of pressure on B. prodigiosus in plus 16 broth.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
971	90	---	No growth
989	80	---	No growth
1035	75	---	1 colony
966	70	---	Good growth
959	60	15	No growth
960		10	No growth
961		5	No growth
969		5	No growth
967		---	Good growth
970	50	5	No growth
992		5	No growth
963	45	40	No growth
964		35	No growth
965		30	1 colony
991		30	No growth
990		25	No growth
988		20	No growth
1020		20	No growth
1021		10	No growth
1040		10	No growth
1036		5	No growth
1073	40	5	No growth
1143		5	No growth
1039	35	20	No growth
1038		15	No growth
1144		10	No growth
1037		5	1 colony
1221		5	Good growth
1071	30	20	No growth
1145		15	No growth
1070		10	1 colony
1222		10	Good growth
1226		5	Good growth
1146	25	30	No growth
1224		25	Good growth
1223		15	Good growth
2142	20	180	Good growth
2141		150	Good growth
1380		120	Good growth
1225	20	90	Good growth
1075		60	Good growth
1074		45	Good growth
1050		30	Good growth
1049		15	Good growth
1048		5	Good growth

**BACILLUS PRODIGIOSUS
+ 16 Beef Broth.**

FIG. 15.—Time-pressure death points of *B. prodigiosus* in plus 16 beef broth. Ordinate are pressures in thousand pounds per square inch; abscissas, time in minutes.

FIG. 16.—Comparative location of time-pressure death point curves of *B. prodigiosus* in distilled water, plus 5 and plus 16 beef broth. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

FIG. 17.—Time-pressure death points of *B. prodigiosus* in plus 5 beef broth at 5° C. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

TABLE V.—*The effect of pressure on B. prodigiosus in plus 5 broth at 5° C.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
2119	90		No growth
1315.	80	15	No growth
2118			No growth
2111	70		Good growth 1 plate
2097	65		Good growth
1316	60	15	No growth
1417			Mod. growth 1 plate
2088	50	5	No growth
1416			2 colonies 1 plate
2100	45	10	No growth
2081		5	Moderate growth
2089		5	No growth
2106		5	No growth
2112		5	No growth
1458			Good growth
1334	40	20	No growth
1317		15	1 colony
1333		15	No growth
2099		10	No growth
1483		10	No growth
1332		10	No growth
1457		5	Good growth
2082		5	Good growth
2107		5	Good growth 1 plate
1331		5	No growth
1368			Good growth
1419	35	15	No growth
1335		15	No growth
1481		10	No growth
2113		10	No growth
2104		10	No growth
2080		10	Moderate growth
2090		10	No growth
1456		5	Good growth
2121	30	40	No growth
2120		30	Slight growth
2091		20	Good growth
2079		15	Good growth
1484		15	Mod. growth 1 plate
1420		15	No growth
1480		10	Mod. growth 1 plate
1369		10	Good growth
1346		5	Good growth
1455		5	Good growth
2103	25	60	No growth
2110		40	Good growth

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
2139	20	180	Good growth
2108		120	Good growth
2098		90	Good growth
2092		60	Good growth
2078		30	Good growth
1371		15	Good growth
1370		10	Good growth
1345		5	Good growth

TABLE VI—*The effect of pressure on B. prodigiosus in plus 5 beef broth at 40° C.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
2116	80		No growth
2115	75		No growth
2095	70		No growth
1325			No growth
1294			Mod. growth 1 plate
1269			No growth
1310			No growth
2086	65		No growth
1324			No growth
1293			Mod. growth 1 plate
1309			No growth
1251	60		Good growth
1244	50	5	No growth
1245			Good growth
1252			Good growth
1295	45	5	No growth
1253	40	5	No growth
1271	35	10	No growth
1270		5	Good growth
1255	30	30	No growth
1285		25	No growth
1272		20	Good growth
1254		5	Good growth
1246			Good growth
1300	25	60	No growth
1308		55	2 col. 1 plate
1323		50	Good growth
1305		45	Good growth
1296		30	Good growth
1340	20	120	No growth
1414		100	No growth
2094		90	Good growth
1413		80	1 colony
2085		80	Good growth
1467		80	No growth
1466		70	1 colony
2084		60	Good growth

Fig. 18.—Time-pressure death points of *B. prodigiosus* in plus 5 beef broth at 40° C. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

FIG. 19.—Comparative location of time-pressure death point curves of *B. prodigiosus* in plus 5 beef broth at room temperature, 5° C. and 40° C. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

Bacillus Fluorescens Liquefaciens. Flugge.

This is a very common organism, found in soil and water, and is frequently associated with decaying animal or vegetable matter.

It is actively motile, and does not form spores. When grown on ordinary media it produces a greenish yellow fluorescence which is quite noticeable.

This bacillus was tested upon only one medium, distilled water.

TABLE VII.—*The effect of pressure on B. fluorescens liquefaciens in distilled water.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
389	100		No growth
385	90		No growth
421	80		No growth
420	70		Mod. growth 1 plate
381	65	10	No growth
419	66		Good growth
383	55	10	No growth
439		6	No growth
416	50	10	No growth
651		10	No growth
440		5	No growth
476		5	No growth
375	45	60	No growth
377		40	No growth
379		20	No growth
393		10	No growth
417		10	No growth
624		10	14 colonies
441		6	No growth
475		5	No growth
507		5	No growth
474	42.5	10	No growth
460		10	No growth
436	40	35	No growth
438		20	No growth
459		15	4 colonies
412		10	Moderate growth
622		10	No growth

BACILLUS FLUORESCENS. LIQUEFACIENS
Distilled Water.

FIG. 20.—Time-pressure death points of *B. fluorescens liquefaciens* in distilled water. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
435	37.5	35	No growth
391	35	150	No growth
387		120	No growth
418		60	No growth
395		10	Moderate growth
445	32.5	110	No growth
444		101	No growth
443		90	No growth
446	30	150	No growth
647		90	No growth
457	30	90	No growth
472		90	No growth
506		80	No growth
649		80	No growth
473		80	No growth
621		60	4 colonies
415		60	Moderate growth
414		50	Moderate growth
620		50	Moderate growth
401	25	60	Moderate growth
397		10	Good growth
403	20	60	Good growth

Bacterium Lactis Aerogenes. Huppe.

This is an organism which is very common in milk. It is closely related to the colon group of bacteria. *Bacterium lactis aerogenes* ferments milk, producing lactic acid and gas. If considerable numbers of these bacteria are present in milk used for making cheese, they are likely to produce so much gas that the cheese will swell and be ruined. Butter is sometimes injured by their presence, and some writers state that they may cause slimy or ropy milk. They are very undesirable from the dairyman's point of view.

TABLE VIII.—*The effect of pressure on Bact. lactis aerogenes in distilled water.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
80	90		No growth
596			No growth
79	85		No growth
567			15 colonies
595			No growth
594	80	10	No growth
554	70	11	No growth
593		10	No growth
564		10	No growth
3	65	60	No growth
592		10	No growth
591	60	10	No growth
65			Several colonies
137	55	20	No growth
563		20	No growth
106	52.5	20	15 colonies 1 plate
8	50	60	No growth
86		20	6 colonies
562		20	No growth
590		20	No growth
67		10	5 colonies
87	45	30	No growth
68		20	No growth
589		20	No growth
16	40	60	No growth
89		45	No growth
532		45	No growth
531		41	No growth
88		40	No growth
64		30	Slight growth
553	35	65	No growth
528		65	No growth
557		60	No growth
18		60	No growth
61		40	Slight growth
21		10	Slight growth
535	32.5	70	No growth
91	30	150	No growth
90		130	No growth
561	30	130	Slight growth
560		120	Slight growth
138		120	Slight growth
70		110	Slight growth
69		80	Slight growth
11		60	Good growth

FIG. 21.—Time-pressure death points of *Bacterium lactis aerogenes* in distilled water. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

Streptococcus Lacticus. Kruse.

The ordinary souring of milk at temperatures below 70° F is due to the activity of *Streptococcus lacticus*. This is an organism of great economic importance to everyone interested in milk and its products. It plays a very important part in the ripening of cheese, and also in the ripening of cream for making butter. These bacteria are very numerous about dairy barns and on cows. Three per cent lactose bouillon was used as a medium for the culture of this organism, and 3% lactose gelatin was used in pouring plates. All cultures were incubated at 22° C.

TABLE IX.—*The effect of pressure on Streptococcus lacticus in 3% lactose broth.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
1137	110		Slight growth
1024	100		Good growth
1006	95		Good growth
982			Good growth 1 plate
1189	90	5	No growth
948			Good growth
1188	85	5	No growth
954			Good growth
1136	80	5	Good growth
944			Good growth
956	70	10	No growth
1423		5	No growth
955		5	No growth
986		5	No growth
1025		5	Slight growth
1066		5	Good growth 1 plate
945			Good growth
1140	65	10	No growth
949	60	15	No growth
950		10	No growth
1260		10	No growth
1141		10	No growth
1026		5	Good growth
1007		5	Good growth 1 plate
983		5	No growth
1424	55	20	Slight growth
1257		20	No growth
1186		16	Moderate growth
1214		15	Good growth
985		10	Good growth
1215		10	Good growth
1218		10	Good growth

FIG. 22.—Time-pressure death points of *Streptococcus lacticus* in 3% lactose broth. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates.
1350	50	50	Slight growth
1349		40	Slight growth
1328		30	Good growth
1327		25	Good growth
1258		20	Good growth
1259		15	Good growth
1213		15	Good growth
951		15	No growth
1011		15	No growth
1138	50	15	Good growth
1187		15	No growth
946		10	Moderate growth
1190	47.5	30	Good growth 1 plate
1139		20	Good growth
1067		15	Good growth 1 plate
1422	45	180	No growth
1398		150	Good growth
1348		120	Slight growth
1302		90	Good growth
1191		90	No growth
1261		80	Good growth
1027		60	Good growth
1009		45	Good growth
984		40	Good growth 1 plate
952		30	Good growth 1 plate
1329	40	150	Good growth
1303		120	Good growth
1216		90	Good growth
1010		60	Good growth

Bacillus Subtilis. F. Cohn.

This is one of the very common spore forming bacteria. It is usually spoken of as the hay bacillus because of its frequent occurrence on hay. It is also found in the soil in very large numbers. It is generally present on vegetables and root crops of various kinds. *B. subtilis* spores are quite resistant to heat and pure cultures of it may often be secured on pieces of potato which have been subjected to steam at a temperature of 98-99° C. for about 5-10 minutes on each of two successive days. This organism has also been found capable of producing infections in man under certain conditions.

In the pressure tests made using *B. subtilis*, cultures 12 to 18 hours old were used for making transfers to tin tubes. It was hoped in this way to eliminate the spore forms, but the value or certainty of this method is very doubtful.

TABLE X.—*The effect of pressure on B. subtilis. vegetative. in distilled water.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
185	100	----	Good growth
208		----	4 colonies
220	90	30	5 colonies
221		20	7 colonies
184		10	Good growth
219		10	14 colonies
224		----	Several colonies
183	85	20	Good growth
202	80	20	No growth
209		10	No growth
225		----	10 colonies
182	75	32	Good growth
176		20	Several colonies
203	70	20	No growth
173		10	Good growth
210		10	4 colonies
4	65	60	No growth
181		40	Good growth
177		30	Several colonies.
180	60	50	Good growth
205		50	No growth
223		40	8 colonies
222		30	8 colonies
204		20	No growth
172		20	Good growth
179	55	60	Good growth
178		40	Several colonies
187	50	100	Moderate growth
190		80	Moderate growth
175		50	Several colonies
206		50	No growth
216		40	5 colonies
212		20	6 colonies
226	45	10	4 colonies
189		120	Moderate growth
188		100	Moderate growth
174		60	Several colonies
171		35	Good growth
218	40	50	4 colonies
217	40	40	2 colonies
170		40	Good growth
228		20	Several colonies
227		10	Several colonies.

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
169	35	60	Good growth
232	30	80	3 colonies
231		60	7 colonies
230		40	2 colonies
229		20	4 colonies
233	20	80	4 colonies
234		60	Moderate growth
235		45	Moderate growth
236		20	Moderate growth

Saccharomyces Cerevisiae. Meyer.

This is the common brewers' yeast and is of great economic importance. This organism was tested in distilled water tubes, 3% saccharose broth, 3% dextrose broth, 3% lactose broth, and 3% maltose broth. All poured plates were made upon 1% dextrose agar.

From these tables it will be noted that there was not much difference as between the four kinds of sugar, but the use of distilled water probably checked the rapid growth of the cells and raised the pressure death point. (See Fig. 28.)

It is believed that use of cultures incubated at lower temperatures (15°-20° C) would give more uniform results and a higher death point.

TABLE XI.—*The effect of pressure on Saccharomyces cerevisiae in distilled water.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
257	100	----	No growth
265	95	----	No growth
245	90	----	No growth
274	85	----	No growth
239	80	----	8 colonies
288	65	5	No growth
241	60	10	No growth
657		10	No growth
238		----	Several colonies
259	55	20	No growth
258		10	No growth
659		10	No growth
261	50	30	No growth
260		20	No growth
247		10	9 colonies
661		10	No growth
264	45	50	No growth
263		40	No growth
262		30	No growth
275		20	No growth
305		10	6 colonies
244	40	60	No growth
256		60	No growth
266		50	No growth
301		40	5 colonies
300		30	6 colonies
242		20	5 colonies
237		----	Good growth
268	35	60	No growth
278		50	No growth
302		40	10 colonies
303		31	3 colonies 1 plate
250	30	80	No growth
655		80	No growth
269		70	No growth
663		70	3 colonies
249		60	2 colonies 1 plate
304		50	15 colonies
248		40	6 colonies
240		10	Several colonies
286	25	150	No growth
285		140	Several colonies
276		130	1 colony
277		110	5 colonies
270		90	Several colonies

SACCHAROMYCES CEREVISIAE
Distilled Water.

FIG. 23.—Time-pressure death points of *Saccharomyces cerevisiae* in distilled water. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

TABLE XII.—*The effect of pressure on Saccharomyces cerevisiae in 3% cane sugar.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
868	85		Good growth 1 plate
973			No growth
870	75		Good growth 1 plate
994			Good growth 1 plate
872	70	5	No growth
995			No growth
1115			No growth
974	60	10	No growth
876		10	Good growth 1 plate
874		5	No growth
1116		5	No growth
975		5	No growth
1114			No growth
1091	55		Slight growth
2146	50	20	No growth
898		20	Good growth 1 plate
896		15	No growth
894		10	No growth
1059			Good growth
2148	45	5	No growth
998	40	30	No growth
997		20	No growth
996		10	No growth
1060			Good growth
902	35	50	No growth
900		40	No growth
1208		15	No growth
1103		10	Good growth
1117		10	No growth
1207		10	No growth
1206		5	Slight growth 1 plate
1211	30	35	No growth
1118		35	No growth
1210		30	No growth
1094		25	Slight growth
1366		25	No growth
1209		25	No growth
1102		25	Slight growth
1365		20	No growth
1288		20	No growth
1101		15	Slight growth
1321	25	80	No growth
1320		60	1 colony
1364		60	No growth
1291		60	No growth
1363		50	No growth
1290		45	Slight growth
1319		40	Slight growth
1289		30	No growth
2147	20	180	Good growth
2149		120	Good growth

FIG. 24.—Time-pressure death points of *Saccharomyces cerevisiae* in 3% cane sugar. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

TABLE XIII.—*The effect of pressure on Saccharomyces cerevisiae in 3% dextrose.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
923	85		No growth
925	75		No growth
927	70	6	No growth
931	60	10	No growth
929		5	No growth
1131			No growth
1081	55		Good growth
937	50	20	No growth
935		10	No growth
1163	45	5	No growth
1280			Good growth
941	40	40	No growth
939		20	No growth
1164		5	No growth
1057		5	Slight growth
1133		5	No growth
1172		5	No growth
1132	35	5	No growth
1173		5	No growth
1165		5	No growth
2129	30	60	No growth
2130		50	No growth
2131		40	No growth
2128		40	3 colonies 1 plate
2127		30	Good growth
1084		20	Slight growth
1167		15	No growth
1134		15	No growth
1175		10	Slight growth
1083		10	Moderate growth
1056		5	Good growth
1166		5	Moderate growth
1174		5	Moderate growth
1279	27.5	40	1 colony
1360	25	90	No growth
1312		80	Slight growth
1231		60	Slight growth
1230		50	Good growth 1 plate
1229		40	Good growth
1228		30	Good growth
1176		10	Good growth
1361	20	180	No growth
1313	20	150	Good growth
1278		100	Medium growth
1277		80	Slight growth

FIG. 25.—Time-pressure death points of *Saccharomyces cerevisiae* in 3% dextrose. Ordinates are pressures in thousand pounds per square inch, abscissas, time in minutes.

TABLE XIV.—*The effect of pressure on Saccharomyces cerevisiae in 3% lactose.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
922	85		No growth
1031	80		No growth
924	75		No growth
926	70	6	No growth
930	60	10	No growth
928		5	No growth
1126			No growth
1086	55		1 colony in each plate
936	50	20	No growth
934		10	No growth
1087			Slight growth
940	40	40	No growth
938		20	No growth
1054		5	No growth
1128		5	No growth
1127	35	5	No growth
1089	30	20	No growth
1129		15	Good growth 1 plate
1182		15	Slight growth 1 plate
1088		10	2 colonies 1 plate
1181		10	Slight growth 1 plate
1053		5	Good growth
1180		5	Good growth
1236	25	50	No growth
1235		40	Slight growth
1184		30	No growth
1234		30	Slight growth
1183		20	Good growth
1233		20	Good growth
1275	20	120	No growth
1338		110	Slight growth
1404		110	No growth
1337		100	No growth
1403		100	No growth
1274		90	Good growth

FIG. 26.—Time-pressure death points of *Saccharomyces cerevisiae* in 3% lactose. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

TABLE XV.—*The effect of pressure on Saccharomyces cerevisiae in 3% maltose.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
2123	90		No growth
869	85		1 colony
906			No growth
977			No growth
980	80		No growth
871	75		Several colonies
907			1 plate
1000			No growth
873	70	5	1 colony
1001			No growth
1121			No growth
978	60	10	No growth
877		10	1 colony
875		5	No growth
1122		5	No growth
979		5	No growth
1120			No growth
1096	55		Slight growth 1 plate
899	50	20	No growth
897		15	No growth
895		10	No growth
1062			Good growth
1193	45	5	No growth
2124	40	30	No growth
1004		30	Good growth 1 plate
1003		20	No growth
1002		10	No growth
1194		5	Moderate growth
1065		5	Slight growth 1 plate
1063			Good growth
903	35	50	No growth
901		40	No growth
1107		10	No growth
1123		10	No growth
1195		5	No growth
2135		5	Good growth
1124	30	35	No growth
1106		25	No growth
1463		25	1 colony

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
1462	30	20	Slight growth
1239		15	Good growth
1263		15	No growth
1460		15	No growth
1105		15	No growth
1464		15	No growth
1197		10	Good growth
1267	25	80	No growth
1266		60	No growth
1412		50	Slight growth
1265		50	No growth
1411		45	Good growth
1409		40	Good growth
1242		40	Good growth
1241		20	Good growth
1240		15	Good growth
1198		10	Good growth
2134	20	180	Good growth
2133		150	Good growth
2125		135	Good growth
1358		100	Good growth
1357		90	Good growth
1356		80	Good growth
1199		10	Good growth

FIG. 27.—Time-pressure death points of *Saccharomyces cerevisiae* in 3% maltose. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

FIG. 28.—Comparative location of time-pressure death point curves of *Saccharomyces cerevisiae* in distilled water and 3% sugar broth. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

Saccharomyces Albicans. Reess.

This yeast is found very commonly on decaying plants, dung, etc. It is not very active in ordinary fermentation, but is sometimes parasitic in infants, puppies, and kittens. Its pathogenic activities are largely confined to the throat, and cause the disease known as thrush. This organism was tested only upon distilled water and plates were poured upon 1% dextrose agar.

TABLE XVI.—*The effect of pressure on Saccharomyces albicans in distilled water.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
279	90	----	No growth
293	85	----	No growth
611		----	No growth
280	80	----	Several colonies
253	70	10	No growth
299		5	No growth
607	60	10	No growth
298		5	11 colonies
294	55	10	18 colonies
606		10	No growth
295	50	10	Moderate growth
677		10	No growth
282	45	10	No growth
296		10	5 colonies
256	40	60	No growth
324		40	No growth
325		35	No growth
675		30	No growth
254		20	No growth
683		20	No growth
311	35	50	No growth
290		30	7 colonies
283		20	Several colonies
317	30	80	No growth
604		70	No growth
682		70	No growth
605		60	No growth
666		60	No growth
667		50	No growth
668		40	No growth
271		20	Several col. 1 plate
318	25	140	No growth
344		130	Moderate growth
289		60	Good growth
292		40	6 colonies 2 plates

FIG. 29.—Time-pressure death points of *Saccharomyces albicans* in distilled water. Ordinates are pressures in thousand pounds per square inch; abscissas, time in minutes.

Bacillus Typhosus. Eberth.

B. typhosus is the organism which produces typhoid fever in man. This bacillus is usually found in such locations or under such conditions that it is readily associated with the discharges of persons who are sick with typhoid fever, or who are just recovering from it. The ordinary house fly has been found to be an active agent in the dissemination of this bacillus.

This organism was selected to determine the death point by pressure since this is one of the few non sporeforming pathogenic bacteria which is easily cultivated and identified.

TABLE XVII.—*The effect of pressure on B. typhosus in plus 10 beef broth.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Plates
1496	80	10	No growth
1495	60	10	No growth
1494	45	10	No growth
1493	35	10	Good growth
1492	25	10	Good growth

From the above it will be seen that there is a definite point on the ten minute line where the death point curve of *B. typhosus* crosses it. This work was discontinued on account of lack of time, but the results indicate that this organism would give a time-pressure death point curve similar to those worked out for other organisms.

Bacillus Diphtheriae. Löffler.

The organism causing diphtheria, *B. diphtheriae* is quite widespread and appears to occur in several forms. Non-virulent forms are said to be commonly found in the nose and throat of healthy persons. The culture used in this work was said to have been recently isolated from a diphtheria patient, and strongly virulent.

The diphtheria bacillus was chosen as one of the pathogens to test in this work, because of its occasional occurrence in milk. Löffler's blood serum is an especially favorable medium for the growth of *B. diphtheriae* and the pressed cultures were tested upon this serum, and upon standard beef bouillon. The results are given in Table XVIII.

TABLE XVIII.—*The effect of pressure on B. diphtheriae in plus 10 beef broth.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Growth on Beef Broth and Blood Serum
50	100	10	No growth
51	80	10	No growth
52	60	10	No growth
53	40	10	No growth
54	20	10	Good growth

It will be noted that the pressure death point for this organism lies between 20 and 40 thousand pounds, on the 10 minute time line. This would indicate a death point curve at least as low as that of *B. typhosus*.

Effect of Pressure on Living Organisms.

It has been stated by Choplin and Tammann* that pressure as high as 3,000 kilograms per square centimeter (43,000 pounds per square inch) did not kill yeast, moulds, or bacteria. They assert that organisms so treated are forced into a lethargic condition and may even lose their power of reproduction, but that they are still alive, as evidenced by motility.

All of the results given in connection with our work on micro-organisms are based upon the ability of the organisms to reproduce themselves. In order to learn whether or not the work, as conducted here, would yield results similar to those recorded by Choplin and Tammann, a series of 18 tests were made using *B. prodigiosus* on plus 10 beef bouillon. The results are given below:

*Choplin, G. W. and Tammann, G. Eber den Einfluss hoher Drucke auf Mikroorganismen. Zeitschrift für Hygiene und Infektionskrankheiten. Vol. 45: 171 (1903).

TABLE XIX.—*The effect of pressure on B. prodigiosus on plus 10 beef broth.*

Laboratory Number	Pressure (Thousand Pounds)	Time (Minutes)	Days Incubated	Growth On Plates
1016	55	25	1	No growth
1017	55	25	89	No growth
1012	55		1	Good growth
1013	55		89	Good growth
1018	Control		1	Good growth
1019	Control		89	Good growth
917	60	15	1	No growth
918	60	15	42	No growth
915	60		1	Good growth
916	60		42	Good growth
913	55	25	1	No growth
914	55	25	42	No growth
919	Control		1	Good growth
920	Control		42	Good growth
852	55	25	1	No growth
853	55	25	14	No growth
854	Control		1	Good growth
855	Control		14	Good growth

Six tubes were placed in the press at one time so that both sets of three were under the same pressure for the same interval of time. Then plates were poured from one set at the end of 24 hours and from the other set after a certain number of days. Reference to the above table shows that these organisms gave no indications of suspended animation as a result of pressure. Those which showed no growth on the first day failed to revive after intervals of 14, 42 or 89 days.

Sterilization of Culture Media by Pressure.

It often happens that the sterilization of certain culture media by the use of heat so changes some of the constituents that it is no longer desirable for critical work. In many cases a medium which would be looked upon, in its natural state, as ideal may be so altered that the organism in question will make no growth whatever in it. For special work it is entirely practical to effect sterilization of such media by the use of pressure. Of course there may be some changes in the nature of a complex medium such as fruit juice, but the changes are not at all likely to be so pronounced or of the same kind as would take place if heat were used as the sterilizing agent. Some good results have been secured using apple juice and green tomato pulp, but detailed reports on these will be made at another time.