THE PRESERVATION OF EGGS

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Foreword

To Professor Hilton Ira Jones, from whose wide experience and constant interest this work has profited at every step, I wish to express my obligation and thanks.

Contents.

(Contents — continued)

The Preservation of Eggs.

The first attempt at the preservation of eggs is scarcely antedated by man's first use of eggs. Men have tried to preserve eggs since their food value was known, and some of the methods discovered as the result of accident and experience are in use now. Yet, even at the present time, no method has been discovered of preserving eggs on any and all scales and for any length of time without affecting in any way the properties upon which the selling quality depends. The domestication of chickens and the use of eggs are as old as history but the poor keeping quality of eggs has always made their immediate consumption necessary. The development of the contained germ and the susceptibility of eggs to putrefaction have hitherto precluded long storage or distant shipment.

Chickens produce many more eggs in the spring and early summer than during the fall and winter. It is in winter when the supply of other necessary foods is limited and the early spring when the scarcity of food is most felt that a perfectly preserved stock of eggs would be most welcome, and it is precisely then that eggs are least abundant and consequently most expensive. The equalization of the price of eggs over the whole year and the resulting service which such a prevention of hysterical prices would mean to the family of moderate income were impelling motives in this research.

New methods of preserving eggs have appeared in greatest numbers in the last twenty-five years. Before that the need was not so pressing. The markets were still local. The egg supply of the towns and cities was brought from the surrounding country. But the swelling of city populations and the consequent division of labor which the congestion of peoples in cities entails made the supplying of food an important industry. The food market has become national and even inter-national. A disaster in Turkey affects the price of wheat in Chicago. Preserved fruits, canned vegetables, and dressed meats are sold thousands of miles from the place of their preparation. The greatest part of Armour and Company's output the past year has been shipped to France. Refrigerator cars make the shipment of perishable foods comparatively easy. Although eggs are an important part of the diet of almost every family in this and other countries, their preservation and shipment over long distances has not yet been successfully accomplished. Egg dealers handling millions of eggs a year report losses from spoiling as high as twenty-five per cent.

It is no wonder that much attention has been paid to this problem in recent years. It is surprising, in view of the time that has been spent upon this problem, to note the small number of methods experimenters have confined themselves to.

Historical.

The Arabs used to bury fresh eggs deep in the desert sands. Records show that the ancient inhabitants of the West Indies practiced a similar mode of storing eggs, but in their case the ultimate object was not alone preservation but the "ripening" of the egg. For their standards of taste led them to consider as the greatest delicacy eggs which had arrived at a stage of putrefaction indescribable.

The warm climate of ancient Italy and Greece and the lack of any method of artificial refrigeration made the preservation of eggs among the peoples of antiquity practically impossible. The preservation of eggs at low temperatures in Northern Europe and Scandinavia was naturally feasible and was extensively practiced.

The use of inert gases.

Many a fact in nature has been discovered and made use of before its explanation was known. Perhaps the simplest expedient discovered in this way and one of universal applicability was to bury or pack the eggs in an air tight substance. In more recent times the substitution of some inert gas has accomplished the same end.

F. C. Calvert (France)[1] in 1873 kept eggs either entire or pierced in nitrogen, hydrogen, or carbon dioxide for three months. When kept in dry oxygen the eggs suffered no change, provided the samples were infertile, but in moist oxygen a white, filamentous mold was formed. Pierced eggs in moist oxygen soon putrefied, the more quickly, the moister the medium. This ideal condition of absolute dryness, never exists in practice. Newlaid eggs were well preserved in weak chlorine water, but when the bottle was left open, the eggs became covered with Penicillium Glaucum, a mold of peculiarly rapid

[1]F. C. Clvert, Comptes Rendus, 1873, lxvii, 1024-1026

and extensive growth. In solutions of calcium chloride and of calcium hydroxide and calsium sulphate Penicillium Glaucam soon appeared, but in dilute phenol the eggs were still sweet at the end of eight months though slightly coated with the same mold.

M. Dubrunfaut[1] two years before used calcium hydroxide with better results. Some care was exercised in selecting the eggs for preservation. The eggs were placed in lime-water and those were rejected which did not sink. The alleged reason for this discrimination was that those which did not sink were fertile and fertile eggs did not keep well in this solution. While his statement of the relation of fertility of eggs to their keeping quality in calcium hydroxide solution can not be denied in the absence of firsthand data, we do believe that he has assigned the wrong cause to the buoyancy of eggs. No such uniform correspondence between fertility and buoyancy has ever been found during the course of the present work. Gas may be formed during the growth of the germ, but we have found that many fertile eggs at the end of three weeks in an incubator contained less gas than infertile eggs of the same age and history.

An English patent was issued to H. Jerne[2] for a process of preservation by coating to produce impermeability and maintaining an atmosphere of camphor vapor. The eggs were coated first with gelatin and then with a mixture of nitrocellulose and camphor dissolved in

[1]M. Dubrunfaut, <u>Comptes Rendus</u>, lxxii, 1871, 106

[2]H. Jerne, British Patent Number 2,145, Jan. 27, 1911.

amyl acetate, acetone, or methyl acetate, preferably the first.

Lake[1] in England patented a process in 1888 of keeping eggs in an atmosphere of carbon dioxide. Fernand Lescarde[2] in 1909 used carbon dioxide as the preservative medium, but modified the procedure to include sterilization of the egg. Eggs were subjected to reduced pressure to remove the air and were impregnated with carbon dioxide under pressure in order to kill the bacteria within the shell. They were then placed in an atmosphere of about 94% carbon dioxide and 6% nitrogen, and kept in this atmosphere at very near 0° Centigrade. This method is admirable from the standpoint of completeness, and combines some of the methods used singly by others. Its usefulness is, however, limited by the fact that the atmosphere of carbon dioxide must be carefully maintained around the egg during transportation. The distinctive thing about this method is that it makes some attempt at sterilization of the inside of the egg. Practically all others working in this field have stopped with sealing up the egg in such a way as to smother the germinal cell. It has been demonstrated that the best sealer can not prevent the growth of all the germs which have already gained access to the interior. Many of the organisms present in an egg, although thriving best in the presence of oxygen, can still become facultatively aerobic in the absence of oxygen, and can produce the necessary gas from the nutrient medium of the egg itself. Consequently, all ordinary methods of sealing must be

[1]Lake, British Patent Number 1, 653, 1888

[2]Fernand Lescarde, British Patent Number 7,804, April 1, 1909.

used with fresh eggs.

The United States, France, and Germany have done most work upon this problem. 32% of all the methods studied were developed in Germany, 32% in the United States, and 16% in France.

Comparatively little research seems to have been done in England. Jessen[1] in 1891 advocated wrapping eggs air tight in a rubber-coated paper. The laboriousness of this method makes it an industrial impossibility. In the same year Mills[2] patented a process of coating with gelatin. Two similar patents were issued later (Farquhar and North,[3] 1892; Coakley,[4] 1899). A slight modification in the form of a gelatinous starch solution was patented by Schultz[5] (1902).

All of the methods studied can be grouped in four classes according to whether the eggs are preserved (1) at low temperatures without preliminary treatment, (2) by packing in comparatively air tight substances, (3) in the dry state after coating with various impervious agents, or (4) in solutions without preliminary treatment.

(1) Cold Storage

At the present time storage at low temperatures is most relied upon when the eggs are not to be moved from place to place. The temperature of the storage rooms is maintained at about 1° or just above the freeezing point of the egg. The freezing point of eggs is $.4270^{\circ}$-$.4800^{\circ}$ at first and rises slightly during incubation corresponding to

[1]Jessen, British Patent Number 15,580, 1891
[2]Mills, British Patent Number 17,717, 1891
[3]Farquhar and North, British Patent Number 15,128, 1892
[4]Coakley, British Patent Number 18,816, 1899
[5]Schultz, British Patent Number 1,328, 1902

a rise of osmotic pressure from 5.5 to 7.3 atmospheres.

Fernand Lescarde,[1] whose earlier method of carbonation was noted above, found that eggs could be preserved for as long as thirteen months below their freezing temperature. The essential precaution in this case is the same as for the storage of frozen fowls, namely that the frozen products be thawed out very slowly. Even with this care rapid deterioration subsequent to storage can not be avoided.

Cold storage can only be successfully used when the surrounding atmosphere is dry and the eggs are kept dry. Calvert[2] showed that the least moistness will cause the formation of shell mold and will hasten putrefaction. Even in dry surroundings the temperature of storage is not sufficient to kill the germinal cell. Growth is merely inhibited until the eggs are released for shipment. Soiling is then accelerated, because the long subjection to cold has broken down the white and destroyed the germicidal power which the egg possessed when fresh. The loss, then, occurs not during the preservation but immediately afterwards.

De Loverdo[3] stated that eggs could be kept for months at 1° C., without any loss in weight or change of any kind, provided they were dry. On the other hand, M. de Laroquette[4] found that at the end of four or five months the eggs in storage had evaporated to the extent of four or five per cent of their original mass, and were stale. When wrapped in paper and stored in a cellar, they kept for two or three months but lost 40-50 milligrams a day be evaporation, and there was no protection against

[1]Fernand Lescarde, Ber. II, Internat. Kältekong, Wien,2,1910,373.

[2] ante page 3

[3]De Loverdo, Comptes Rendus Acad. Science (Paris),144,1907,41-43.

[4]M. de Laroquette, L'ind. beurre, 50,1910,600-603, through Mois chim. et electrochim. 8, 1910, 31-32.

infection or mold. This danger, he found, could be diminished by plunging the eggs 50-60 seconds in boiling 10% brine. This treatment formed a millimeter layer of coagulated albumin and sterilized the shell. F. Prall in 1907 reported that eggs kept in a cool cellar spoiled in six weeks.

(2) Preservation by packing in air-tight substances.

Packing in various materials is an old and much used preservative process. Two patents for the use of lime as a packing material were issued in England in 1891[1] and 1898.[2] W. P. Wheeler of the New York State Agricultural Experiment Station in his annual report 1890[3] declared that he had kept eggs in various materials and that he found most of them unsatisfactory. All the eggs were wiped when fresh with a rag saturated with a fat or oil containing an antiseptic. Eggs wiped with cottonseed oil impregnated with boracic acid and packed in salt spoiled in four or five months. Bran packing gave the same result. Wiped with vaseline and salicylic acid eggs kept four to five months without loss. The quality was considered superior to that of ordinary limed eggs. This worker found no difference between the keeping quality of fertile and of infertile eggs, and none between the eggs of different chickens.

The Ontario Agricultural College[4] during the trial of various methods of preservation found that a small percentage of the eggs packed in salt spoiled, and that all suffered from evaporation. Eggs in oats became musty and evaporated to the same extent. Several methods of

[1] McArdle, British Patent Number 3592, 1892.

[2] Wilson and Wilson, British Patent Number 15,461, 1899.

[3] W. P. Wheeler, Report of the First Assistant, N. Y. State Sta., 122-169

[4] Report of the Manager of the Poultry Dept., Ontario Agr. College and Experiment Farms Report, 1899, 130-134.

preserving eggs are quoted by the Queensland Agricultural Journal[1] of 1899. A method of particular value was mentioned. A solution of salt and borax was poured over the eggs to be preserved, which were then covered with vegetable ashes. The eggs were packed in bran, salt, and so forth in air-tight boxes.

A note on the successful preservation of eggs in peat dust appeared in 1897[2]. Prall[3] found that eggs kept in dry sand in closed vessels spoiled in six weeks.

A. A. Brigham[4] of the Rhode Island Agricultural Experiment Station tried a number of methods of preservation, including as the agents water-glass, dry table salt, lime-water, salt brine, vaseline, ashes, gypsum, powdered sulphur, sulphur dioxide, potassium permanganate, salicylic acid, and lime-water and salt brine. The old way of using slaked lime and salt brine was very effective and inexpensive. Smearing with vaseline proved effective for a few weeks. Packing in dry table salt was effective for a few months. Water-glass solution, however, was found to be best, even as weak as 3% and had the advantage of being usable again.

Borax and washing soda have been used under the name of British Egg Preservative[5].

J. Vosseler[6] in an article published in Germany in 1908 stated

[1] Queensland Agri. Journal, 4, 1899, Number 3, 418-419

[2] Land und Centralblatt, Posen, 25, 1897, Number 34, 209.

[3] ante page 8.

[4] A. A. Brigham, Rhode Isl. Expt. Station Rept., 1901, 297-233

[5] J. S. Jeffrey, No. Carolina Sta. Bulletin 191, 11-17.

[6] J. Vosseler, Der Pflanzer, 4, 129-136 also Chemisches Zentralblatt, 1908, volume II, 1214.

that three methods were available for preservation, packing in dry substance such as chopped straw, salt, ashes, and lime; placing in preserving solutions, e. g., lime-water, water-glass, potassium permanganate solution, and salicylic acid; and covering with an airproof or germicidal material. Coating with or packing in water-glass gave best results. The eggs packed in dry materials became musty and tasted of the packing.

L. Prenzlau[1] of Hamburg in 1910 patented the following process. Eggs are subjected to gaseous formaldehyde and enclosed in a packing material. The whole is then surrounded with a filling material saturated with formaldehyde. The fact that formaldehyde has such a strong odor would make its use undesirable in connection with food.

Many of these processes have not been successful. The looser packings allowed evaporation and the growth of mold, while others have merely inhibited the growth of the germ. The greatest difficulty to be overcome in this business of preserving eggs is the effect which most preservatives have upon the quality of the egg. Surrounding materials possessing a strong odor, particularly volatile liquids like ethyl alcohol and formaldehyde solution impregnate the interior with their odors and leave a taste. That objection against these preservatives which can not be met is the slowness of the processes and the necessity of transporting the packing materials with the eggs.

(3) Coating to produce impermeability.

The latter objection, of course, cannot be urged against methods which consist in coating the egg with an impervious and sometimes antiseptic substance. If the eggs can simply be dipped in some solution

[1] L. Prenzlau, British Patent Number 15,309, June 25, 1910.

and stored without further treatment, no fault can be found from the standpoint of labor- or time-saving. But so many processes of coating require that the eggs be individually rubbed or smeared with the material, and, of course, in commercial competition with such can hope for no success.

On of the earliest uses of this general method was made by Adam Good[1] in 1880 when he patented in this country a composition for preserving eggs consisting of a hot solution of borax, sugar, and lime. The eggs were dipped in this liquid and dried, whether by hand or not is not said.

J. H. Thieriot[2] gives a report of tests in Germany of twenty methods of which the most satisfactory were found to be (1) varnishing with vaseline and (2) preserving in lime-water or (3) in water-glass. The latter method was considered preferable as varnishing takes considerable time and the lime-water is liable to give the eggs a disagreeable odor and taste. So much disagreement there is about the value and the effects of calcium hydroxide (lime-water) that this opinion is worth noting.

Vaseline presents itself to most experimenters with varying results and with various receptions. In 1890 Wheeler of the New York Station, it was note above,[3] found that eggs kept four or five months when greased with vaseline containing salicylic acid, and seemed superior to limed eggs. Strauch[4] states that in his work the best results were obtained by coating with vaseline and placing in lime-water and by preserving in water-glass.

[1] Adam Good, U. S. Patent Number 225,518, March 16, 1880.

[2] J. H. Thieriot, U. S. Consular Reports, Dec., 1897, 563-564.

[3] vide ante page 8

[4] R. Strauch, Das Hühnerei als Nahrungsmitte l und die Conservirung der Eier, Bremen M. Heinsius, 1896,50 also Milch. Zeitung,26, No.22, (342

Vaseline was used at the Ontario Agricultural College[1] among other materials during an extended investigation in 1899. The different methods tried were:

1. Immersion in water-glass of different strengths.

2. Immersion in calcium hydroxide solutions.

3. Coating with vaseline.

4. Packing in dry oats.

6. Immersion in water-glass and packing in egg case after drying.

The poor results from water-glass are belied by the favorable reports of others who have used it. Lime-water was found to leave a slight taste in the egg. Vaseline ket the eggs well but imparted to them an undesirable flavor. A small percentage of those packed in salt were bad and all suffered from evaporation. Oats gave less protection and did not prevent molding.

The results obtained from a dry coating of water-glass are especially interesting because the chief drawback to the use of water-glass is that the eggs must always be kept immersed. The report of this work states that all of the eggs dipped and then dried were fairly well preserved but that the eggs lacked flavor. Another writer stated that eggs preserved in water-glass could be kept for several weeks after they had been removed from the solution.

A. Duboux and C. H. Rapin[2] patented a preserving composition in 1911 consisting essentially of vaseline to which were added talcum 10%

[1] vide ante page 8[4]

[2] A. Duboux and C. H. Rapin, U. S. Patnet Number 1, 019,614, Mar. 5,1912

aluminum tannate 1%, and pulverized tragacanth 4%. These constituents were intimately mixed to form a doughlike mass, tasteless, odorless, insoluble in water and alcohol, and soluble in diethyl ether. The resulting substance was intended for application to the outside of the egg with rubbing to stop the pores. Such a process is by nature doomed to failure in a commercial way; a dozen eggs could be treated by our method while one of these was being "rubbed to stop the pores". The solubility of this substance in ether should have suggested to these men dissolving it in some volatile, agreeable liquid and applying by dipping and drying.

K. Reinhard[1] in 1898 proposed dipping in sulphuric acid to form a coat of calcium sulphate. According to his statement, this method is superior to previous processes, which had consisted in mechanical stopping of the pores. A drawback to these methods was the insipid, lixivious taste imparted to the eggs. Preservation in the true sense, he declared, did not take place, because the air was not entirely prevented from entering. By the Reinhard process the eggs were closed air-tight, the germs on the shell were killed, no taste or odor remained, and there was no danger of bursting during cooking. Sulphuric acid is better than oxalic acid because of the antiseptic action. Marks[2] in England patented a similar process. The alleged superiority of sulphuric acid is questionable in view of the experience of A. G. Gilbert[3] of Canada. Many methods were studied among which was this one. Eggs were dipped in sulphuric

[1] K. Reihard, British Patent Number 18,130, 1898. vide autem Scientific American Supplement, 49, 1900, No. 1273, 20405.

[2] Marks, British Patent Number 12,867, 1903.

[3] A. G. Gilbert, Canada Experiment Farms Reports, 1900, 251-277.

acid, washed, and stored in large bottles. Others were dipped in sulphuric acid, washed, and dipped in alkaline ammonium oxalate. All spoiled in a short time.

A new method appeared in France in 1902[1] by which eggs were immersed fifteen minutes in water at 35°, plunged for five seconds in boiling water, and stored in wood ashes, chaff, bran or sawdust. The heat from the water coagulates a thin layer of albumen just inside the shell, and excludes air. M. de Laroquette[2] used this method to prevent subsequent infection of preserved eggs. N. P. Jacobsen[3] patented a process in 1902 of immersing eggs fifteen minutes in water at 35° and then for five seconds in burnt alum solution, cooling in water, and drying. A French patent was issued to Garrigon[4] for a slightly modified method of plunging the eggs in boiling water to coagulate a thin layer, piercing the air-chamber to introduce an inert gas, and finally dipping in a solution of alum-treated plaster and drying at 35°.

In 1903 Christian E. C. Landsperg[5] obtained a Danish patent on treating eggs with a solution of casein or cheese substance. A protecting layer is formed which may eventually be removed by treatment in a weak solution of ammonia or vinegar. The solution of casein or cheese substance may be produced by means of ammonia and by adding a disinfectant to the solution

[1] _Bull. Soc. Vaud. Agr. et Vit._, Lausanne, 1902, No. 151, 28.

[2] _vide ante_ page 7.[4]

[3] N. P. Jacobsen, Danish Patent Number 14,902, November 10, 1910.

[4] Garrigon (Haute Garonne), French Patent Number 394,455, Nov. 26, 1907.

[5] C. E. C. Landsperg, Danish Patent Number 27,267, 1903.

Prall[1] in his work in 1907 found that when eggs were plastered with paraffin or treated with hydrofluosilicic acid they did not keep. Shellac was more effective. Good results followed from coating with potassium permanganate, immersing in water-glass, or from Hanika's method of dipping in hot water and alcohol. Glyerin and lime-water were good preservatives. Keeping in the dry state near the freezing point with a relative humudity of 80% was especially recommended. Similar to the use of paraffin is the proposal of Campanini[2] to coat eggs with lard and store in baskets or boxes so that there is no point of contact between them. J. D. Smithers in Missouri[3] before treating with lard, subjected his eggs to the action of lime (51 grams), sodium chloride (21 grams), sodium bicarbonate (1gram), and potassium bitartrate (8 grams) in one liter of water. They were then dried and dipped in melted lard.

E. W. Fischer[4] sterilized eggs with water, immersed them in gelatinous substance such as gelatin, agar-agar, and the like, in order to exclude air, and protected them during storage with a coating of paraffin.

A. Cihlar[5] patented a process which is worthy of notice only as an example of methods which are commercially impossible and severe in their action on the egg. He immersed eggs for six hours in strong alcohol to form a thin coagulated layer of albumen. In the first place,

[1] F. Prall, Zeitschrift Unters. fur Nahr.-Genussmittel,14,1907,No.7,445., abst. in Zentr. Agr. Chem., 37, 1908, No 7, 486-489, also Hyg. Viande et Lait, 2, 1908, Number 8, 362-367.

[2] Campanini, Bull. It. Min. Agr., through J. Royal Soc. Arts, 56,1908,866.

[3] John D. Smither, U. S. Patent Number 912,909, February 16, 1909.

[4] E. W. Fischer, Swedish Patent Number 31,624, January 21, 1910

[5] A. Cihlar, German Patent Number 245.785, November 5, 1910.

no method can be successful in a commercial way requires such a long time for carrying out. And this use of alcohol would be disagreeable in its effect on the egg. The coagulation could hardly take place without a taste being imparted to the white. Even if no taste were left, the knowledge of the treatment to which the eggs had been subjected would deter the ordinary man from using them.

Another method equally impossible was used by William H. Burns[1] of Naperville, Illinois. After preliminary immersion in alcohol, eggs were wrapped in a loose water-proof cover and placed in a case upon a layer of sulphur. The case was then stored underground. Such a process can not compete with water-glass for local preservation.

P. A. Schmitt[2] preserved eggs in 1908 by immersing in a solution benzoic acid in alcohol of such a concentration that after drying a coating was formed, which thickly covered all the pores. The eggs did not spoil at 30°-40°. A. G. C. Sturley[3] of Hamburg patented an identical process in the United States in 1911.

(4) Preservation in liquids.

Of the class of preservative solutions this much may be said, that no matter how effective they may be, they can not compete commercially with preservative coatings. The necessity of keeping the eggs immersed in the liquids makes shipment burdensome and expensive. The weight of the containers themselves would be an appreciable item in the cost of transportation. For home use solutions are perhaps the most convenient, requiring no care in application and packing, and being either easily

[1] W. H. Burns, U. S. Patent Number 938,965, November 2, 1910.

[2] P. A. Schmitt, German Patent Number 233,971, Nov. 15, 1908.

[3] A. G. C. Sturley, U. S. Patent Number 995,198, June 13, 1911.

made on the spot or else readily purchasable. However, the consensus of opinion and the preponderance of evidence indicate that no solution or liquid has yet been found which is superior to water-glass. Water-glass, or liquid glass, is a silicate of sodium, soluble in water. Its aqueous solution is highly viscous, involatile, and a poor solvent of gases. The high viscosity and the slight occlusion of gases explain its success in keeping air away from eggs. A necessary property of any preservative liquid is that it shall not be volatile, that is, that its vaporization point shall be high. For this reason glycerin and heavy oils make good sealers.

The concentration generally used is a 10% solution, although strengths as low as 3% have been reported satisfactory. The eggs to be preserved must not be stale or contaminated, because this method contains no provision for sterilization or inhibition of bacterial growth. The eggs must be kept immersed during storage, and to accomplish this some kind of a weight may be used to submerge those which float. They can not be kept more than a few weeks out of glass. If they are exposed to a temperature higher than 7°, according to Brown[1] of England, the heat will cause deterioration in spite of the preservation. The method worked out at this laboratory meets this limitation and will preserve the eggs unaltered for a long period at 40°.

The use of water-glass is rather recent, particularly in England. L. Irwell[2], writing in 1904, says that water-glass was new in England at that time. E. Brown[1] in 1903 used water-glass, lime-water,

[1] E. Brown, Journal Bd. Agri. (London), 9, 1903, No.4, 494-497.

[2] L. Irwell, Dietet. et Hyg. Gaz., 20, 1904, Number 8, 457-458

and refrigeration. He recommended that the eggs be treated as soon as possible after they are laid but not before they are cool. Stead[1] had used water-glass as early as 1882.

The North Dakotah State Experiment Station[2] as the result of a large amount of work in 1897 reported that water-glass preserved eggs better than any packing. The yolks did not settle to the side, the taste was unaffected, and the whites could be beaten easily.

H. H. Borntraeger[3] reports the examination of some eggs that had been spoiled by an attempt at preservation in weak (10° Baumé) water-glass. The solution was strongly alkaline and had penetrated the shell. The white and part of the yolk were gelantinous and translucent as horn. Other experimenters[4] with water-glass advise against allowing the solution to contain free alkali. The alkali diffuses through the shell, transforms the white into a yellow, transparent mass, and solidifies the yolk. James Hendrick[5] gives some tables that no change took place in eggs kept in water-glass four years. There was a slow deposition of silica in the shell. After three or four years the white became pink and tasted of soda.

[1] Stead, British Patent Number 4,910, 1882.

[2] Bulletin 103 U. S. Dept. Agr., 1897; N. D. Exp. Sta. Bull., 1897

[3] H. Borntraeger, Oesterr. Chem. Zeitung, 3,1900, Number 12,295.

[4] E. F. Ladd, N. D. Expt. Sta. Bull. 35, 330-332.

J. M. Bartlett, Maine Agr. Expt. Sta., Orig. Comm. 8th Int. Congr. App.Chem. 18,51-56.

R. Berger, Journal of Industrial and Eng. Chem.,3,1911, 493-495.

A. Evéquoz and E. P. Häussler, Zeit. Unter. für Nahr.-Genuss.,25,1913,96.

M. de Keghel, The Industrial Preservation of Eggs, Rev.Chim.Ind.,24,12.

[5] J. Hendrick, Aberdeen and Nova Scotia College of Agriculture Bulletin 8, also J. Agr. Sci., 2,1907, No. 1, 100-105.

Experimental.

Porosity of Egg-shells.

The egg of the common fowl consists of the shell and its membrane, the white, the yolk, the germinal cell, and the membranes separating these parts. The shell of an egg is very porous. A small hand-glass shows it to be full of small holes; when highly magnified, the egg-shell is seen to be covered with holes, just as a sharp razor edge under the same lens looks like a saw. The thickness of most egg-shells varies very little, but the porosity and the brittleness vary considerably with differend kinds of hens and with the feed. Sometimes the food of the chicken is poor in shell-forming elements and a porous, brittle shell results. The brown colored shell is slightly less brittle than the white, but the composition is practically the same.

If it were not for the porosity of the shell most eggs would not spoil. The germ could not grow without a supply of oxygen, and external contamination could not take place. It is upon this fact that most preserving processes depend for their efficacy.

An index of the porosity of any separating wall is the time required for gaseous or liquid diffusion through it. The problem thus becomes one of finding a delicate indicator which shall accurately measure the extent and the rate of diffusion. Two solutions, the one highly colored and the other clear, separated by a membrane would show diffusion by coloring of the colorless liquid, provided the densities of the two liquids were of the same magnitude. Two liquids whose interaction would produce coloration would serve the purpose, such as a dilute solution of phenolphthalein and a dilute solution of an alkali. The objection to liquids is their reactivity with the substances of which the shell and its membrane are composed.

The most delicate and harmless indicator was found to be dry oxygen and freshly prepared nitric oxide. The slightest contact of oxygen and nitric oxide is indicated by the formation of a dense brown cloud of nitrogen dioxide, according to the equation:

$$2\ NO + O_2 = 2\ NO_2$$

An egg was placed with its broad end down in the neck of a bottle which was barely small enough to prevent the egg passing through, and a mark was made on the shell even with the top of the neck. The shell was carefully and evenly cut along this mark, great care being taken to prevent cracking. The presence of cracks which may be invisible by reflected light becomes apparent by transmitted light, as during candling, and any shells which showed cracks were discarded. Oxygen from a potassium chlorate generator was led into bottles inverted in a pneumatic trough. Colorless nitric-oxide was generated by heating arsenious oxide with dilute nitric acid and also by the action of dilute nitric acid on copper. A stop-cock in the outlet prevented access of air to the system. The nitric oxide was collected over water in bottles.

An uncracked half-shell was fitted into the bottle for which it was intended and which had been filled with oxygen, and the neck of the bottle was immersed for several minutes in melted paraffin. The mouth of a nitric oxide bottle in the trough was then covered with a glass square and the bottle was slipped quickly over the first bottle full of oxygen. The mouths of the two bottles were then sealed together with paraffin and the bottles laid on their side. The system than contained reactive gases separated by a porous wall. Maxium diffusion took place in one hour. The oxygen diffused more rapidly than the nitric oxide because of its relatively smaller density.

A piece of the double shell-membrane was next separated from the shell and pasted across a hole in a square of cardboard. The cardboard was then placed between the necks of two bottles of oxygen and of nitric oxide joined as before, and the mouths were sealed together with paraffin. Diffusion through the moist membrane was very slow, about twelve hours being required for noticeable coloration of the nitric oxide.

The shells so far used had had their shell-membranes removed. When a whole shell with its membrane untouched separated the two coloreless gases no noticeable diffusion took place, even in the course of a week. This result must be interpreted as meaning that diffusion was so difficult and consequently so slow that the shell-membrance dried before any diffusion took place. This membrane is always moist with egg-white and the solidification of the albumen stopped the pores and hindered diffusion.

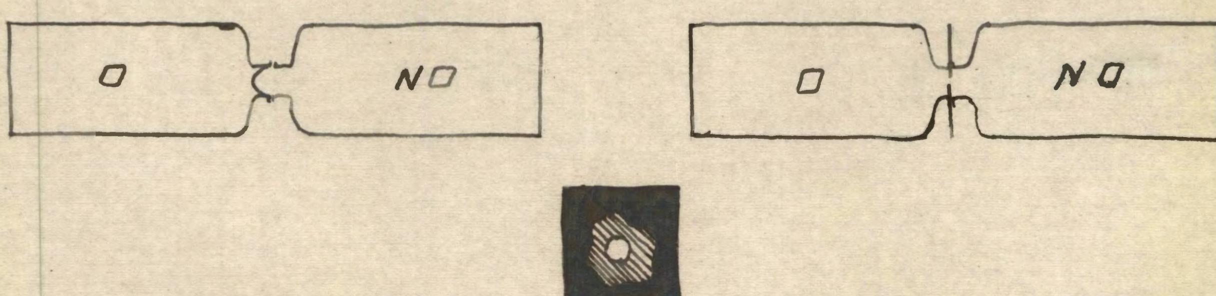

Figure 1, Arrangement for observing diffusion.

The Problem of Sealing.

If an egg were taken when perfectly fresh, at a time, therefore, when few bacteria would have entered the shell, and dipped in a strong disinfectant which could be washed off quickly, the egg would be sufficiently sterile for preservation for a long time, and the problem of defertilization alone would remain. The word defertilization in this work is intended to mean either (1) the immediate destruction of the germinal vesicle so as to preclude growth, or (2) the inhibition of its development without the possibility of its future reactivation, or else (3) its destruction through lack of air. The simplest of all methods of destruction is simply to deprice the germ of air, either by the use of some sealer or by the maintenance of an atmosphere of inert gas around the egg.

The respiration of the germ is very limited for the first few days and increases in amount scarcely perceptibly during the first four or five days. After this period, however, respiration through the shell increases rapidly and at the end of twenty-five days is equal, weight for weight, to that of a live chick. The use of an inert gas for asphyxiation of the embryo is impracticable because of the obvious fact that the atmosphere of gas must always be carefully maintained around the egg during preservation. Moreover, such a medium surrounding the egg would not stop evaporation.

Keeping eggs in a vacuum would deprive the germ of oxygen, but the vacuum would have to be preserved during the process. Aside from the question of convenience, it is difficult to keep a vacuum for any length. Although some methods of preservation have been based upon one of the above principles, the great majority of preservative processes have depended for their effect on the simple exclusion of air, by some sealing agent.

Relative Value of Some Common Sealers.

In order to test the relative efficacy of some of the commonest sealing agents eggs were treated with the sealers and placed in an incubator at 37.5°. The sealing materials used were a gasoline solution of aluminum soap, a gasoline solution of paraffin, water-glass, vaseline, and egg-white.

Aluminum soap dissolved in gasoline in all tests has proved itself superior to every other sealing substance used. Its value as a water-proof coating makes it useful as a protective varnish for army tables, canvas tents, tarpaulins, and aeroplane wings. Aluminum soap consists of the aluminum salts of the fatty acids found in ordinary cheap soap. It can be prepared from any kind of soap, but for our purpose the cheapest soap is the best.

Preparation of the aluminum soap solution.

A large amount of soap is chipped and dissolved in hot water. A hot, saturated solution of burnt potash alum ($K_2\ Al_2\ (SO_4)_4$) or any other cheap aluminum salt is filtered through a fluted filter paper into the hot soap solution. The aluminum slats of the fatty acids present in the solution are insoluble in water and are precipitated instantly. The precipitate floats on the surface as a light, gummy, spongy mass, which is pressed dry. The dry aluminum soap is dissolved in gasoline (about ten grams in 100 cubic centimeters of gasoline). The soap goes into solution rather slowly and only after much heating. The ease with which the soap can be made makes it practical as a household preservative solution. It can be made in any quantity and kept in stoppered bottles.

The paraffin was used as a saturated gasoline solution.

The eggs used to test the sealers were dipped in dilute hydrochloric acid to sterilize the shells and to make them more easily impregnated. They were then washed, dried, and dipped in the sealers, and allowed to dry.

Impregnation by precipitation <u>in ovo</u>.

An attempt was made to seal the eggs with aluminum soap precipitated in the pores of the shell. An egg was allowed to stand in a saturated alum solution. It was then dropped without drying into a dilute soap solution. Another egg was placed in a soap solution and then dipped into an alum solution. Many dyes, including indigo, are fixed in the cloth by precipitation in the interstices of the weave. The cloth is soaked in a solution from which the dye is deposited by treatment in a second bath. This general method of impregnation was considered applicable in this case. Neither attempt was successful, and the reason probably was that the aluminum soap formed on the outside of the shell as the egg touched the second solution prevented access of the reagent to the pores.

Interpretation of results.

All the eggs treated in the course of this work were kept in an incubator at 37.5° C. from two to four weeks. The eggs were kept at a relatively high temperature to hasten spoiling and the growth of the germ. To have found the absolute value of the various treatments, i.e., the actual time of keeping would have required several years, but the circumstances under which the research was conducted did not permit such extended observation. Hence all the results given here are relative, and for relative results no absolute standards of measurement are necessary. Whenever any set of eggs was placed in the incubator, several untreated eggs were put with them as controls to indicate the stage of spoiling and to serve as a standard of comparison. Fresh fertile eggs were found to keep at laboratory temperature (c. 20^{o})for as long as a month without more than a slight enlargement of the germinal cell. It is easily seen that if eggs are treated in such a way as to be kept fresh after the time required for untreated eggs to spoil, both being in the incubator, the treated eggs would have kept longer than the controls at room temperature. In short, incubation simply shortens the time required for the changes taking place without modifying them. Eggs treated in various ways and incubated were compared with eggs immersed in water-glass and kept at the same temperature. This comparison afforded a means of translating all relative results into absolute figures. It was found that eggs in water-glass at 37.5^{o} spoiled in four weeks. Now eggs kept in aluminum soap were perfectly fresh at the end of this time, and the conclusion is that the aluminum soap was more effective than the sodium silicate. Just as the phenol coefficient of disinfectants is obtained, so the water-glass coefficient, or the coefficient with respect to any other preservative agent, can be obtained by finding the number of days in the incubator required for spoiling of water-glass eggs. The coefficient can be expressed numerically by the ratio of the two values. Most preservatives have a water-glass coefficient less than one, a very few, - preeminently aluminum soap - more than one. The determination of the water-glass coefficient affords a ready means of finding the absolute efficacy of any preservative. Unless the coefficient is greater than two, it can be determined in four to eight weeks. The average preservative power of water-glass expressed in days multiplied by the coefficient will give the time of preservation by the method in question.

Results of preliminary tests.

The first set of eggs was examined at the end of two weeks. The controls had spoiled or contained growing embryos. The eggs sealed with water-glass were not good. Spoiling had started as was evidenced by the turbidity and the limpidity of the white and the weakening of the vitellin membrane of the yolk. The paraffined eggs had spoiled completely. The eggs treated with aluminum soap were perfectly preserved and presented the appearance of fresh eggs.

The method of differential evacuation.

The failure of sealing by simple immersion indicated the necessity of developing some effective method of impregnating the eggs with the sealing agents. The porosity of the egg-shell and the fact that respiration takes place through the shell to such an extent as it does suggested applying the sealer by evacuating the egg, restoring the normal pressure, and at the same time immersing the egg in the sealing solution. The evacuation and the subsequent restoration of pressure would create a difference of pressure between the outside and the inside of the egg. If the egg were in the solution, the sealer would be forced into the pores of the shell. The chief cause of the failure of such agents as paraffin and shellac is that they crack upon cooling and drying and the egg is exposed to the air. In the case of aluminum soap this method would be expected to render the coating even more air-proof.

Impregnation by difference of pressure was tried with the gasoline solution of paraffin, the gasoline solution of aluminum soap, and water-glass. The eggs were dipped in dilute hydrochloric acid for ten seconds, washed with water, and dried. They were then placed on the floor of an exhaust dessicator, which was tipped to prevent the eggs from rolling into the calcium chloride chamber previously filled with the sealing agent. The dessicator was exhausted fifteen minutes with a filter pump capable of producing a vacuum equal to the vapor pressure of water at the temperature of the laboratory (c. 20 millimeters). The eggs were then rolled by tipping into the calcium chloride chamber filled with the sealing fluid. The fall was broken by a piece of wire guaze in the bottom of the dessicator. The eggs were subjected to a further evacuation, which brought out the rest of the air in fine streams of bubles. When exhaustion was complete, the cock was opened. At the end of ten minutes the eggs were placed without drying in the incubator with three controls.

After two weeks they were examined, and the method was seen to have produced better sealing. The eggs treated with paraffin and with water-glass were not good but were in much better condition than those sealed with the same agents by simple immersion. The eggs sealed under pressure with aluminum soap were just as fresh as ever. An indication of the large amount of air normally present in an egg and an illustration of the equality of internal and external pressures was the behavior of an egg when subjected to a sudden evacuation. A large cylinder of about six liters capacity was evacuated with the filter pump to a pressure of twenty millimeters. A small bottle of six hundred cubic centimeters volume was provided with a stop-cock, and an egg was put into it. The cylinder and the bottle were connected by their outlets and the stop-cocks were opened. The pressure in the bottle was instantly reduced from about seven hundred and forty millimeters to twenty, and the egg burst.

The effect of maintaining a vacuum around the egg was tried. An egg was placed in a bottle containing calcium chloride and provided with a stop-cock, and the bottle was evacuated for thrity minutes with a Cenco-Nelson three stage, primary pump to a pressure of one millimeter. The bottle was then sealed and placed in an incubator. At the end of two weeks the egg was still in perfect condition as far as the looks and the taste were concerned, but evaporation had taken place to a noticeable extent.

Experiments with aluminum soap.

The superiority of aluminum soap over other sealers as indicated by previous tests seemed to justify its selection for special experiment to determine the necessary conditions for its success, its shortcomings of whatever nature, and the remedies for them. Aluminum soap itself is odorless, tasteless solid and makes an ideal sealer, since it is without effect upon the egg. But the necessity of providing some solvent as a carrier by which it may be applied complicated the problem. The necessary requirements of a solvent for the soap are (1) that it shall dissolve the sealer easily at low temperatures, (2) that it shall be volatile and hence evaporate quickly, and (3) that it shall be without effect upon the egg.

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Many solvents of low boiling point were tried; namely, gasoline, diethyl ether, carbon disulphide, dimethyl benzene, carbon tetrachloride, acetic acid, ethyl acetate, amyl acetate, methyl acetate, dimethyl ketone, and glycerin. Practically the only liquid worth consideration was gasoline, and the absence of any better solvent made it necessary to use it and eliminate whatever objectionable features it had.

Accordingly a set of eggs was treated with aluminum soap with various modifications in order to get further knowledge of its effect on the eggs and to determine in just what way the sealing should done to effect maximum preservation.

Impregnation under pressure seemed no better than ordinary pressure in the first test, and eggs were treated in both ways in order to determine what value, if any, this method had in the case of aluminum soap. It was not considered necessary to evacuate the egg out of the solution, as this arrangement simply quickened the process. The time necessary for complete removal of the air was indicated by the cessation of the flow of small bubbles from the egg, and was found to be about fifteen minutes. In all subsequent tests on vacuums on eggs in solutions this time was considered just sufficient to reduce the internal pressure of the egg to that produced by the pump (c. 1mm.).

It was considered possible that subjection to a vacuum for a long time might have some upon the germinal cell. The germ is a protoplasmic cell in which there exists normally an internal pressure just balancing the pressure of the atmosphere. This cell, though perhaps resistant to increased pressure, may not be able to withstand the disruptive effect of a reduction of pressure. The tests run were designed to show whether low pressure would have completely destructive or only inhibitory effect. And since the use of aluminum soap in connection with this test would obscure the effect of the pressure, eggs were also subjected to reduced pressure without sealing. If this treatment were found to have a markedly repressive effect on the growth of the germ, the use of low pressure in sealing would increase the value of the method.

Two fresh, fertile eggs were sealed by immersion in the soap, two were sealed under pressure with vacuum maintained only long enough to complete evacuate the egg (15 minutes), and two were sealed in the same way with the vacuum maintained fifteen minutes after complete evacuation, or thrity minutes. The effect of evacuation without soap was tried at the same time. Two fresh, fertile eggs were put in the incubator without any treatment, two were subjected to a pressure of one millimeter for fifteen minutes, and two were exhausted for thirty minutes. By comparing eggs of both sets which were evacuated to the same extent further knowledge of the effect of the soap was obtained. The vacuum dessicator for the sealed eggs was previously dried and washed with gasoline. The vacuum dessicator for the unsealed eggs was dried and supplied with anhydrous calcium chloride. This precaution obviates the possibility of increase of pressure during exhaustion due to the vaporization of water.

The physical and chemical changes in spoiling eggs.

The spoiling of an egg is always accompanied by the lowering of the viscosity of the egg-white and of the yolk. Egg albumen itself is limpid, but it is tightly enveloped in a multitude of microscopic sacs, which, being distended and fastened to one another, give the white its viscosity. These sacs tend to disintergrate and lose their firmness as the egg stands, and the white becomes limpid and turbid, sometimes milky. The thinning of the yolk is explained[1] by the transference of water from the white and is a simple case of osmosis. The yolk is separated from the white by the porous vitellin membrance, and because the yolk is denser than the white, it has a greater internal cohesion and a transference of water takes place from wht white to the yolk in accordance with the law of osmosis. The transfer continues until the vitellin membrane breaks and the white and the yolk begin to lose their identity.

Fresh eggs contain a soluble enzyme able to reduce the nitrates present. The amount of the ferment is slight at first but increases at the fourth or fifth day and markedly so after that. Julius Wohlgemuth[2] has shown that there are some ferments localized in the yolk which can split the protein, lecithin, and fat present.

One of the decomposition products of spoiling eggs is hydrogen sulphide. Egidio Pollaci[3] has made experimental determination of the action of hydrogen sulphide on unbroken eggs. When unbroken eggs were kept for twenty-four hours in a closed vessel containing hydrogen sulphide, it was found on breaking the eggs that the white had lost its natural viscosity and had become limpid like water. The whites possessed a yellowish green color and a fetid odor, and the yolk was of a very dark chocolate color due to ferric sulphide. The albuminates, especially of sodium and potassium, present in the white decomposed into the corresponding

[1]A. D. Greenlee, Osmotic Activity in the Egg of the Common Fowl, Journal Amer. Chem. Soc., 34, 1912, 539-545, also Science-new series, 34, 1911, No. 868, 223-224.

[2]Julius Wohlgemuth, Zeit. Phys. Chem. 44, 1905, 540-545.

[3]Egidio Pollaci, Gazetta, 34, 1904, 278-286.

sulphides together with mercaptan substances. When the whites and the yolks were boiled separately with water, they lost a considerable portion of sulphur, an indication that the proteids were broken down and the nutritive value of the egg diminished.

The Bureau of Chemistry of the United States Department of Agriculture[1] working on the effects of cold storage on eggs quail, and chickens found that eggs in storage one year lost by evaporation 10% of the whites. Ins sixteen months the eggs lost their power of cohesion and emitted a characteristic, musty odor a few hours after opening. There was a lowering of the amount of coagulable protein, a change in the reaction, and a lowering of the percentage of lecithin. The lower nitrogen bodies (proteoses and peptones) increase, and there is an increase of amido bodies. All these changes are an indication of the katabolisim and the disintergration proceeding within the egg.

The first indication of spoiling in the eggs kept in our incubator is the turbidity of the white. In the case of infertile eggs physical disintegration and chemical cleavage are the only processes that take place. The eggs treated by us were of two kinds, <u>fresh</u> and <u>old</u>. The old eggs were farm eggs exposed to contamination through handling and bought at the local market several days after being laid. Some were fertile and some infertile, and there was no means of determining the condition of any egg. The fresh eggs were only a day old when used, and were obtained from the poultry farm of this college, where they had been gathered from clean nests, not washed or handled unduly, and packed in clean egg-cases. The eggs for this work were all from pens in which a male fowl was kept, and, according to figures of poultrymen, were probably 95% fertile. The comparison of fertile eggs after

[1]H. W. Wiley et al., <u>U</u>. <u>S</u>. <u>D</u>. <u>A</u>. <u>Bur</u>. <u>Chem</u>. <u>Bull</u>. 115, 1908, 117.

incubation is comparatively easy and in some cases afforded a striking demonstration of the effect of different treatments.

The germinal cell first becomes dark and then commences to grow. After a time an irregularly shaped embryo develops, and the placental veins surrounding the yolk begin to be visible, charged with blood. These blood veins spread out uniformly and by the extent of their growth form one means of comparison. The chick then grows rapidly, the placental veins completely surround the yolk, and the yolk is gradually absorbed by the developing embryo. At the end of two or three weeks the chick becomes well formed.

In previous experiments the eggs had been dipped in dilute hydrochloric acid in order that by the reaction between the shell and the acid ($CaCO_3 + 2HCl = CaCl_2 + H_2O + CO_2$) the pores of the egg-shell might be more exposed to the sealer. Whether or not this preliminary treatment is necessary was determined by running duplicate tests of all eggs without dipping or washing.

All eggs so far treated in this set were fresh and fertile. What value the various agents used on these eggs would have in the case of store eggs of various origins and ages was also to be demonstrated. The "old" eggs used were bought at a local grocery store four days before treatment. The grocer had bought them from a farmer the same day. Although the eggs were probably laid one or two days before this, no surety could be obtained as to their age or the conditions of nests, packing, or handling. However, the nature of the source warrants the assumption of some degree of contamination from these sources.

All the eggs of this set were numbered and treated according to the following table:

Table Number 1.

Preservative effect of aluminum soap and of evacuation.

Number	Kind	Treatment		
21 - 22	fresh	dipped	soap	no vacuum
23 - 24	fresh	dipped	soap	quick vacuum
25 - 26	fresh	dipped	soap	long vacuum
27 - 28	fresh	not dipped	soap	no vacuum
29 - 30	fresh	not dipped	soap	quick vacuum
31 - 32	fresh	not dipped	soap	long vacuum
33 - 34	fresh	dipped	no soap	no vacuum
35 - 36	fresh	dipped	no soap	quick vacuum
37 - 38	fresh	dipped	no soap	long vacuum
39 - 40	fresh	not dipped	no soap	no vacuum
41 - 42	fresh	not dipped	no soap	quick vacuum
43 - 44	fresh	not dipped	no soap	long vacuum
45 - 46	old	dipped	soap	no vacuum
47 - 48	old	dipped	soap	quick vacuum
49 - 50	old	dipped	soap	long vacuum
51 - 52	old	not dipped	soap	no vacuum
53 - 54	old	not dipped	soap	quick vacuum
55 - 56	old	not dipped	soap	long vacuum
57 - 58	old	dipped	no soap	no vacuum
59 - 60	old	dipped	no soap	quick vacuum

(Table Number 1)

Number	Kind		Treatment	
61 - 62	old	dipped	no coap	long vacuum
63 - 64	old	not dipped	no soap	no vacuum
65 - 66	old	not dipped	no soap	quick vacuum
67 - 68	old	not dipped	no soap	long vacuum

When a large number of eggs are treated as these were and incubated at the same temperature for the same length of time, there is afforded a cross-comparison of results and a fulness of interpretation not possible when only one test is run at a time. Thus the effect of exhaustion can be studied on eight sets of eggs in the above table. Numbers 21 to 26 are identically treated in every case except with respect to evacuation time. Numbers 45 to 56, though primarily for testing the effect of dipping on old eggs, will also show relative results from the different evacuation treatments.

Egg number 33 was opened about ten days after setting and was found to contain a chick, which was developed enough to show skeleton, beak, and eyes. The contents were watery. Number 33 and number 34 were exposed to the greatest possible extent to spoiling conditions. They were dipped, so that respiration was easy, and they were not protected by any other treatment. At the end of twenty-one days at 37.5^{o} the eggs were examined and compared.

Results and interpretation.

Number 21 was candled and would have passed as perfect on the market. There was no gas, the germ had not developed, and the yolk had not settled to the side. Number 23 was perfectly preserved but was no better than 21, which had simply been immersed after dipping.

Number 25 was in an equally good condition, and all three were in a strikingly better state than the controls, which had received no treatment. The evacution evidently was not needed. Numbers 27 - 29- 31 had all deteriorated, although no gas had formed. The yolks had fallen to the side, and the whites were slightly watery. The point to be noticed about these is that they were all of the same quality and were not so well preserved as those which had been dipped.

The corresponding old eggs corroborated the conclusion concerning evacuation and dipping. Numbers 45 - 47 - 49, treated with soap and subjected to various degrees of evacuation, were all in good condition, 49 being no better than 45 and all three equal to the fresh eggs thus treated.

The effect of dipping was apparent among the old eggs also. Number 51 was not dipped and although no gas was formed and there was no visible physical deterioration, number 45, which had been dipped, was plainly superior to it. 47 and 53 were equally preserved. Number 49 was in slightly better condition than number 55, which, although presenting a good appearance, was not quite up to fresh eggs in quality. In every case old eggs kept as well as fresh ones. The eggs called "old" were, of course, as good as would ever be found in commerce, but the good results achieved with them shows that our method of preservation is successful with the ordinary market egg, and this fact alone constitutes a point of superiority over most other processes.

Dipping has seemed in most ~~cases~~ to produce better results, and for the best results and under the most rigorous conditions, this should be the initial treatment.

Discovery of the preservative effect of evacuation

The treatment without aluminum soap did not in any case entirely preserve the eggs, but it did afford a test of the effect of varying amounts of evacuation on the growth of the germ. Out of the four groups subjected to this test; namely,

33 - 38	fresh	dipped	no soap	vacuum
39 - 44	fresh	not dipped	no soap	vacuum
57 - 62	old	dipped	no soap	vacuum
63 - 68	old	not dipped	no soap	vacuum

three showed that the evacuation had some effect in repressing the germinal growth. The first group gave results inexplicable by any considerations. Number 33 (vide ante) spoiled in two weeks. Number 35 was in fair condition. The yolk fell to the side and the air-chamber was distended with gas. Number 37 wa an absolute wreck. Of the second group number 39 was in only fair condition. The egg was infertile as was determined by inspection of the yolk, a large amount of gas had formed, and the yolk was stuck to the side. When eggs are sealed the yolk sometimes falls to the side, but when no sealer is applied, the yolk not only falls but because of evaporation sticks fast to the shell. Number 41 contained gas and evaporation had caused the yolk to stick fast, but, although it was fertile, no growth had taken place. No other cause can be reponsible for this condition but the fifteen minute exhaustion. Number 43, evacuated for thrity minutes, was in even better condition than 41. There was not much gas in the air-space, the yolk had stuck only slightly, and the germ had not grown.

The gradual increase in the quality of the eggs corresponding to a longer exposure to vacuum was also seen in the two other groups. In number 57 a chick had begun to develop, the yolk was fastened to the side of the shell, and a slight amount of gas had been formed. No gas was formed in 59, there was no odro, the shell was clean, and the germ had not developed, but the deterioration was evidenced by the wateriness of the yolk. Number 61 was in about the same condition, except that the yolk had not lost its integrity and had stuck to the shell. Number 63 contained a developing chick and was in the same condition as 57. 65 contained gas, the yolk was on the side, and the germ had not grown. Number 67 was in good condition; no gas was formed, and the yolk had not stuck, though it had fallen slightly.

Evacuation seems to be a powerfully contributing factor in the preservation of eggs, being able by itself to inhibit, if not entirely to prevent, the growth of the germ. If this process were ever to be used alone, dipping would not be advisable, since evaporation is so much the more increased.

The even numbered eggs were placed in cold water and boiled, numbers 32 and 50 being pierced at the time. At 75° 54, 22, 26, 28, 24, and 48 cracked lengthwise and were removed. At 80° 56 cracked, at 85° 30, at 90° 52, and at 95° 46. 32 and 50 did not crack at any time. The same objection, then can be maintained against aluminum soap as against water-glass, that the eggs crack when boiled. Piercing the shell prevents this in both cases.

Further experiments with vacuums.

The experiments with aluminum soap having shown incidentally that evacuation was a contributing agent in the preservation, further work with vacuums was done for the sake of confirmation. The reduction of pressure has clearly been demonstrated to have an effect on the germ, but whether or not it would be successful in preserving eggs actually contaminated with gas-producing organisms was still an open question.

In most cases fresh market eggs do not conatain enough bacteria for one to measure the effect of preservative agents upon them. Artificial inoculation was resorted to in the next set of eggs treated with reduced pressure.

As a repetition of the previous tests fresh eggs, both dipped and undipped, were subjected to various periods of evacuation. Two, undipped, were given no treatment, two were exhausted for one hour at a pressure of less than one centimeter, two were kept in the dessicator two hours, and two four hours. Eight more fresh eggs were dipped in 5 N hydrochloric acid and given the same treatment. The eggs used for the rest of this experiment were bought at a local grocery store two days before and were several days old. Four of these were used as controls, and three were exposed to exhaustion for one hour. In order to make a tentative determination of the value of egg-white as a sealer one egg was evacuated for one hour and then dipped in fresh egg-albumen. Four eggs were exhausted two hours and four four hours.

In order to simulate actual conditions of contamination the shells of eggs were impregnated by our pressure method with a strong water suspension of animal feces. The suction was applied for an hour and then suddenly removed. These eggs were exhausted in one, two, and four hour periods. In order to test the effect of evacuation on the congenital flora a bacterial suspension was actually injected into eggs, and the holes were sealed with paraffin. The pressure was then removed for one, two, and four hours. This set was numbered and treated as shown in the table:

Table Number 2

Effect of evacuation on artificially contaminated eggs.

Number	Kind	Inoculation	Evacuation	Other treatment
69-70-71-72	old	none	none	
73 - 75 - 76	old	none	1 hour	
74	old	none	1 hour	egg albumen
77-78-79-80	old	none	2 hours	
81-82-83-84	old	none	4 hours	
85 - 86	old	external	none	
85b - 86b	old	external	none	egg albumen
87 - 88	old	external	1 hour	
89 - 90	old	external	2 hours	
91 - 92	old	external	4 hours	
93 - 94	old	internal	none	
95	old	internal	1 hour	egg albumen
96	old	internal	1 hour	
97 - 98	old	internal	2 hours	
99 - 100	old	internal	4 hours	
101 - 102	fresh	none	none	
103 - 104	fresh	none	1 hour	
105 - 106	fresh	none	2 hours	
107 - 108	fresh	none	4 hours	
109 - 110	fresh	none	none	dipped
111 - 112	fresh	none	1 hour	dipped
113 - 114	fresh	none	2 hours	dipped
115 - 116	fresh	none	4 hours	dipped

Results and Interpretation

After twenty-one days the eggs were examined. Out of the five sets put in together three showed the regular gradation of quality corresponding to more extended evacuation,and these were all eggs which were not contaminated artificially. The results of previous experiment were thus fully corroborated. Egg-white did not seem to be an efficient sealer. It had no effect on number 74. The yolk and the white were both spoiled, there was a strong odor, and a large chick had developed.

Numbers 69 to 84 showed the effect of evacuation. Number 69 was infertile, the yolk was watery and had lost color, there was an odor, and much gas had been formed during the spoiling. Numbers 70, 71, and 72 were likewise spoiled. Number 75 was in practically the same condition as 69 but was better than 74. This fact was probably due to its infertility, as the egg was in other respects bad. Number 76 contained a chick. Numbers 77 to 80 were in very much better condition than 69 tl 76. In number 77 the germ had started to grow. There was a small blood-ring, the yolk had lost color but was not limpid, and the white sacs had broken down. Numbers 78, 79, and 80 contained a small blood-ring around the germ. Numbers 81 to 84, while not perfect in every case, were well kept. In 81 the germ had enlarged slightly, the yolk and the white were in fair condition, and there was no odor. 82 was perfect, 83 nearly so, and 84 showed only a slight blood-spot. The low pressure used was apparently not able to kill the germ but was able to inhibit its growth.

The normal bactericidal power of eggs was evidenced by the failure of the inoculated bacteria to break down the contents. This property of the egg had been known before, and the experiment simply gave firsthand proof of the fact. The inoculated eggs were not much worse than those not so treated. Numbers 85 to 100 were all spoiled. The white was characteristically discolored, the yolks were watery, and gas was formed.

Numbers 101 - 108 and 109 - 116 demonstrated the value of evacuation. 101, 102, 103, and 104 were all spoiled, number 104 containing a developing chick. Number 105 showed a growth of the placental veins and a small embryo. Number 106 also showed a placental ring. Numbers 107 and 108 were well kept, the germs having developed only slightly. Number 109, a control, dipped, contained a chick. In 111 a chick was well formed, and the heart was beating. 112 was bad and contained much gas. 113 and 114, which were evacuated for two hours, were not well preserved but were not in such an advanced state of spoiling as 111 and 112. Both contained slight blood--rings (placental veins), but in neither had the germ developed much. Numbers 115 and 116 (4 hour vacuum) were in good condition. There was no blood, and the white whipped perfectly, i. e., had not lost its original viscosity.

Egg-white as a sealing agent.

A confirmatory test of the value of egg-white was made as follows:

Table Number 3.

Test of egg-white.

Number	Kind	Treatment	
117 - 118	fresh	not dipped	no sealer
119 - 120	fresh	not dipped	egg-white
121 - 122	fresh	dipped	egg-white

Two fresh eggs were used as controls two were dipped in dilute hydrochloric acid and sealed, and two were simply dipped in egg-white. After fourteen days all were found to be equally spoiled. The explanation of the failure of egg-white is probably that it cracks on drying and exposes the shell. Impregnation under pressure is expected to obviate this difficulty.

The Problen of Sterilization.

The bacterial permeability of eggs.

The question of the permeability of eggs to bacteria has received much attention, and researches have definitely proved that bacteria do enter from the outside. R. Lange[1] in 1907 found that when whole eggs were immersed in bouillon cultures of various organisms, Bacillus typhosus and other pathogenic bacteria penetrated the egg as far as the yolk. Another observer[2] in 1893 studied and described sixteen different forms isolated from hen's eggs. According to him these could be placed in two classes, (1) those which cause the production of hydrogen and sulphide as an end-product of their growth upon the egg medium and (2) those which are chromogenic and produce a greenish-blue pigment. When fresh eggs were placed in bouillon cultures and examined at intervals, colonies were found to develop in a few days. This statement, of course gives no indication of the time required for contamination, since the time elapsing between inoculation and maximum growth varies so greatly among different organisms. Thus, Staphylococcus pyogenes aureus, a common pus-producer develops most favorably upon media having a slightly alkaline reaction. It would grow well in the alkaline egg-white and produce well

[1]R. Lange, Arch. Hyg., 62, 1907, Number 3, 201-215.

[2]Zörkendörfer, Arch. Hyg., 16, 1893, 368-401

defined colonies in one or two days. The presence of Staphylococcus colonies after four or five days would indicate the time necessary for contamination as two or three days. Streptococcus pyogenes, another pus-former, and Diplococcus pneumoniae prefer an alkaline medium and would produce colonies in eighteen to twenty-four hours after entrance. On the other hand, Bacillus tuberculosis would not manifest itself in colonies in less than ten days. Zörkendörfer[1] filled blown eggs with nutrient broth, sterilized them, and placed the eggs on a mass of spoiled egg. The colonies were separate, the bacteria having entered at particularly weak places.

Bacteria do not easily infect a dry egg because of the fact that the finishing touch a hen gives an egg is a mucilaginous coating which hardens and prevents for a short time evaporation and contamination. However, undue handling and the too frequent practice of washing soon deprive the egg of its protection. Housewives do not like to buy dirty eggs; a clean egg finds a more ready sale than one not so. While an egg slightly dirtied with hen excrement or feathers may be aesthetically offensive, it is, nevertheless, from this standpoint a better egg than one which has been washed.

G. H. Lamson[2] of the Storrs Agricultural Station Connecticut isolated nine different species of bacteria from a single nest. The number may often be higher, and very old nests are often a source of contamination. The warmth and moisture of the nest provide favorable conditions for the multiplication of bacterial organisms. Lamson dissected hens and found bacteria even in the upper part of the oviduct. While a healthy chicken would probably transmit few or no germs to the egg in the early stages of formation, a diseased condition of the fowl

[1] vide ante page 43.

[2] G. H. Lamson, <u>Conn.</u> <u>Storrs</u> <u>Sta.</u> <u>Bull.</u> 55, 203-214

and of the oviduct might be a dangerous source of infection. Fatal infection has been known to have been due to the bacteria congenitally present in eggs eaten by the infected person.

Bacterial flora of fresh eggs.

We may throw out of consideration among the bacteria in eggs all non sporulating anaerobes, that is, those microorganisms which can only grow in the absence of oxygen and can not form a protective spore in the presence of oxygen. Such bacteria would not live in the dust and straw which eggs might touch. Again, of those which could gain access to the interior of the egg we may disregard all obligatory anaerobes as possible spoiling agents. The porosity of the shell as evidenced particularly by the respiration of the growing germ, always guarantees the presence of oxygen in the interior and would make impossible the growth of such bacteria as requre the absolute absence of oxygen. We may also discount the probability of very many non-motile penetrating an egg-shell.

Mary E. Pennington[1] in 1910, studying the bacteria in fresh eggs found that of 57 eggs examined 7, or 12% were sterile. In the other 50 36 varieties of bacteria were found. However the preponderance of evidence is to the effect that fresh eggs taken immediately after laying do not contain many bacteria within the shells.

Tests were run in this laboratory of the putrefaction of fresh eggs at maximum growth temperatures. The temperature at which most bacteria grow most energetically is about 37.5°, or blood-temperature.

[1]Mary E. Pennington, <u>Chemical and Bacteriological Study of Fresh Eggs</u> <u>Journal of Biological Chemistry</u>, 7, 1910, 109-132, abstracted in <u>Journal of the Chemical Society</u>, 1910, A ii, 224.

The fresh eggs used were dipped in dilute hydrochloric acid in order that all germs on the surface of the shell might be killed and that no contamination might result from this source. The eggs were handled with sterilized hands. Whole eggs were carefully broken and transferred to petri dishes. Whites alone were transferred, and yolks alone. The eggs were incubated at 37.5° for 48 hours. In this time only one colony developed in eight eggs, and a slight amount of gas was formed in one dish. The cause of the spoiling of eggs, then, is not in general the congenital bacteria but those acquired from infected materials. Old nesting material, unclean hands, careless packing in any kind of receptacles, and storage in dirty containers produce an accumulating contamination, so that eggs on the market are almost sure to spoil if kept long enough at moderate temperatures.

The absence of strictly anaerobic organisms from the flora of eggs excludes some of the most dangerous causes of disease - Bacillus tetani, Bacillus anthracis symptomatici, Bacillus oedematis maligni, Bacillus aerogenes capsulatus, and Bacillus botulinus. One experimenter has established the powerlessness of Bacillus dysenteriae to penetrate the egg-shell. If the assumption that non-motile bacteria can not enter an egg is correct, - and it is merely an assumption from purely theoretical considerations, since there has been no work done either in proof or disproof of the belief - many other bacteria are thereby denied entrance to the interior of the egg.

The presence of bacterial flora whose density increases with age contributes the second element to the problem of preservation, namely, sterilization. If it were simply a question of external contamination, the preservation of eggs would not be such a difficult and delicate matter as it now is. For the exterior of eggs could be treated with sterilizing agents which would not affect the interior. The sterilization of the egg is, perhaps, the most difficult part of the problem. The difficulty is forestalled by practically all other experimenters by taking only very fresh eggs for preservation. All methods require that the eggs be perfectly fresh and are helpless in the face of eggs which have been handled and so contaminated by bacteria. The author has never found a single other method which met this problem and escaped this fundamental limitation.

The fact that even old eggs kept perfectly when treated by our process of impregnation under pressure brings us to the tentative conclusion that this method takes care of the sterilization in the sense that it does not permit bacterial growth. Strictly anaerobic organisms cannot live in an ordinary egg. Of the bacteria that may be present at the time of preservation all are almost necessarily aerobes, but some may become facultatively anaerobic under anaerobic conditions. Our process thus reduces to a small amount the number of bacteria which may survive and cause putrefaction. The bactericidal power of ordinary eggs may destroy these. Further experiments, to be described later, were conducted to see whether it was possible to dispose of this residual number, what effect these few may have on the egg, and whether the destruction of all bacteria could be accomplished.

The possibility of destroying bacteria by the use of a vacuum is a question of immediate practical bearing here, but also of great theoretical interest in bacteriology. Professor B. H. Hite[1] of the University of West Virginia has sucessfully destroyed bacteria by subjecting them to high pressures. A large metal cylinder was used and an hydraulic pump capable of giving pressures as high as 100,00 pounds to the square inch. The bacteria varied very much in their resistance to pressure. Many died at 35,000 pounds, and very stisfactory work was done with 30,000 pounds for twenty minutes. The method is chiefly applicable to the sterilization of liquids, milk especially. Another worker[2] attempted to destroy bacteria by pressure. A resumè of the literature on the influence of the electric current on bacteria is given by H. Friedenthal.[3]

Now almost any organism is more resistant to high than to low pressure, and to a sudden raising of the pressure than to a sudden lowering. Compression is less harmful than distention. Every bacterium possesses an internal pressure equal to the pressure it is subject to, and this internal pressure can be accomodated, if slowly enough, to changes in external pressure. That any organism could rapidly and completely adjust itself to a sudden reduction of pressure was considered on theoretical grounds to be impossible. Successful results in demonstrating the truth of this hypothesis were expected to be valuable and applicable in the sterilization of any limpid liquid. Viscous liquids do not permit

[1] B. H. Hite, Sterilization by Pressure, originally published in the Journal of the American Chemical Society, reported in The Literary Digest, 60, 1919, Number 11, 127.

[2] H. Roger, Comptes Rendus, 119, 1894, Number 23, 963-965.

[3] H. Friedenthal, Centralblatt Bakt. u. Par. Med., 19, 1896, No.9-10,319-324.

the evacuation to extend quickly enough to all their parts. An extended experiment was projected to determine the effect of evacuation, both sudden and gradual, on bacterial cultures in liquid media. Sudden evacuation would not be applicable to eggs, but the success of failure of the most extreme method of exhaustion would indicate the success to be expected from gradual treatment.

The method of producing quick vacuums.

The initial problem was to secure some means of instant evacuation of test tubes. Two general methods were worked out. If a small sealed bottle containing a gas at atmospheric pressure is connected to a cylinder of equal volume which is completely evacuated, the resulting pressure will be half atmospheric pressure. If the ratio of volumes be 1 : 10, the final pressure will be 1/11 of an atmosphere. For a given volume now occupies 11 times its original volume. A ratio of 1 to oo would give 0 pressure. The cylinder used for our work had a capacity of 279.08 liters, and the bottle used to contain the tubes had a capacity of 700 cubic centimeters. The volume ratio is 1 : 398.9. The pressure obtained in the bottle was measured by a small manometer consisting essentially of a Torricellian tube, 2cm. in diameter and 10 cm. long, filled with mercury and supported in a small 25 cc. bottle with a 5 cm. neck. The compactness of such a manometer makes possible its use in measuring pressures in small volumes. With atmospheric pressure and absolute vacuum the resulting pressure from this ratio (1 : 400) would be 1.85 mm. of mercury.

The other method of evacuation experimented with is by all means the most direct and effective way of producing a vacuum. It makes use of the combustion in a confined chamber of two gases to form a solid product. The gases were used at atmospheric pressure, but for the effect on the bacteria, would better be used at high pressure. The organisms would then be accustomed to this pressure, and the reduction would be more decided. Thus, if a mixture of ammonia and hydrogen chloride could be compressed without interaction and then exploded, solid ammonium chloride would be formed instantly and a practically perfect vacuum would be produced. Ammonium chloride would be an ideal product because of its low vapor pressure. These two agents, however, can not be used because they react spontaneously.

A mixture of ammonia, hydrogen, and chlorine in the calculated proportions would produce a reduction of pressure, but hydrogen chloride would be left in excess, and the vacuum would be only partial. Chlorine reacts energetically with ammonia to form nitrogen and hydrogen chloride. If the calculated excess of ammonia is used, the hydrogen chloride combines with it to form solid ammonium chloride, but gaseous nitrogen will still remain.

$$2\,NH_3 + 3\,Cl_2 = N_2 + 6\,HCl$$

$$6\,NH_3 + 6\,HCl = 6\,NH_4Cl$$

$$\underline{\hphantom{8\,NH_3 + 3\,Cl_2 = 6\,NH_4Cl + N_2}}$$

$$8\,NH_3 + 3\,Cl_2 = 6\,NH_4Cl + N_2$$

8 volumes 3 volumes 1 volume

The molecular equations show that there is a reduction of 10/11 in the pressure. The reactivity of these gases made the alternative more feasible of using gases whose interaction would form a liquid product which could easily be absorbed.

The gases actually used for experiment were an electrolytic mixture of hydrogen and oxygen. The generator made for this work is shown in the diagram:

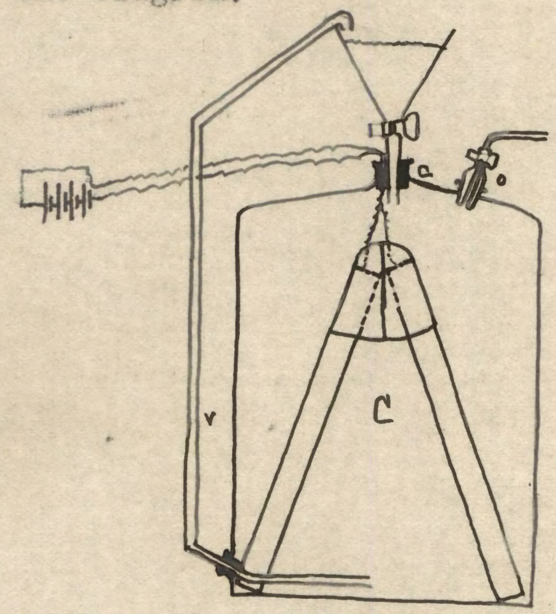

Figure 2. Oxy-hydrogen Generator.

Two carefully insulated lead electrodes reached to the bottom of the large chamber C and are connected to the circuit by wires through the cork c. A large funnel is fixed as an inlet for the acidified water. A stop-cock o furnishes the outlet for the mixture. Escape of the surplus water as the gas is generated is allowed through the tube v. The water is forced by the pressure produced up the tube v and is restored to the funnel. In this case the gas was generated faster than it was used, and an automatic siphon was invented to prevent overflow of the funnel. This siphon is useful in any place where any gradually filling receptacle must be emptied over and over again. It would be particularly useful in drawing off equal fractions of a distillate. It is theoretically and practically impossible to start an ordinary siphon without artificially producing a slight reduction of pressure in the outlet tube.

The self-starting siphon can best be explained by the use of a diagram:

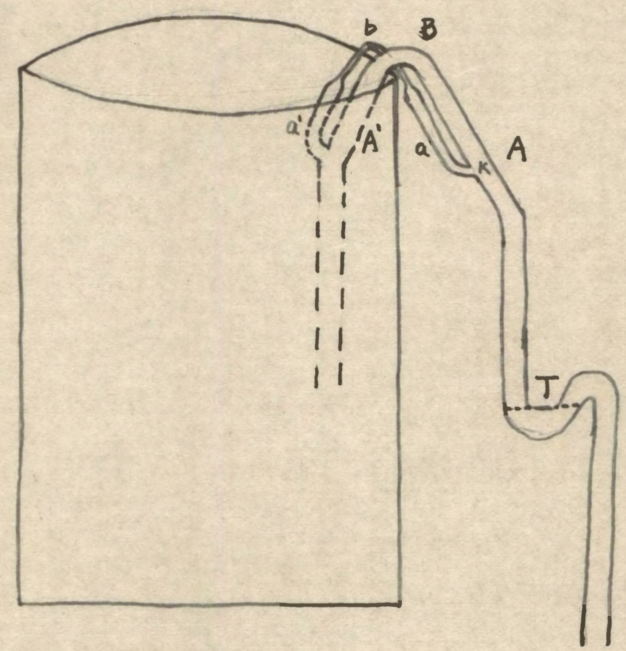

Figure 3. Automatic, self-actuating siphon.

A large glass tube ABA_1, bent at B, is hung on the rim of the receptacle C, which is to be emptied. The amount to be drawn off at one time is determined by the length of the tube A. A smaller piece of glass tubing a is drawn out to form a capillary tube, and the capillary segment is bent at b to the angle aba_1 ($\doteq$ ABA_1). The outlet a1 is sealed into ABA_1 at K, or is connected by the perpendicular arm of a T-tube forming part of BA_1. The large outlet tube is bent as shown to form a trap T. When the liquid filling the container comes to within 1 or 2 cm. of the edge, a part of it will rise in the capillarytube because of the surface tension. A slight further rise of the liquid in the beaker will force the liquid over the bend in the capillary, and the auxiliary siphon begins to operate. When the decending liquid reaches and enters BA_1 at K, the trap T is filled and overflows. A reduction of pressure is created between B and A by the weight of the liquid in D, and the main siphon is actuated. This automatic arrangement will work absolutely without any attention, and it is easily made from ordinary laboratory materials.

The actual explosion of the electrolytic mixture was done in an explosion burette. A pressure of about 10 atmospheres is developed at the time of explosion, and this is instantly succeeded by an almost perfect vacuum. In order to absorb the water vapor formed the tube was filled with concentrated sulphuric acid. The burette was inverted to form a Torricellian tube, and the gases were introduced. The water vapor resulting from the interaction was thus absorbed by the acid on the walls of the burette.

The mechanical problem of greatest difficulty was next to introduce a measured quantity into the burette. Some kind of a cover was needed to prevent the sulphuric acid entering the culture tube. Corking the tube would probably cause the cork to jam tight into the test tube at the instant of explosion. This requirement was met by covering the mouths of the tubes with tight-fitting glazed paper. The acid was denied access and the force of the explosion afterwards blew in the tops. Loose-fitting cotton plugs prevented subsequent contamination. The difficulties of this method prevented its successful use for this purpose at this time, but it must be said that the conditions of experiment just laid down constitute a means of producing better results than can be obtained by the method of differential evacuation.

The general method of determing the effect of reduced pressure on bacteria was by the reduction in gas or acid formation as compared with control tubes. Equal quantities of homogeneous cultures of Staphylococcus pyogenes aureus in lactose-litmus-broth and of Bacillus bulgaricus in litmus milk were measured from a burette into sterile tubes. Some were allowed to grow without treatment and others were used for experiment. For the determination of the effect of bactericidal processes on bacteria by this method of comparison the following organisms may be used:

Table Number 4.

Organisms and Media for Acid and Gas Production Tests.

Organism	Medium	Product
Bacillus coli	lactose-litmus-broth	acid
	lactose, dextrose, mannit broth	gas
	milk	acid
Bacillus lactis aerogenes		
	carbohydrate broth	acid and gas
	milk	acid

(Table Number 4)

Organism	Medium	Product
Micrococcus aureus	dextrose, lactose saccharose	
	broth	acid
	sugar-free proteid media	alkali
	milk	acid
Bacillus typhosus	carbohydrate broth	acid
Pneumococcus	alkaline glucose broth	acid
Bacillus bulgaricus	milk	acid

Staphylococcus aureus and Bacillus bulgaricus were selected. Neither produces spores, both produce acid abundantly, and M. aureus, at least, is very resistant. If reduction of acidity is shown for this organism, it may be expected from other, non-spore-bearing bacteria.

Ten cubic centimer portions of a 24 hour culture of Staphylococcus on lactose-litmus-broth of known reaction were measured into sterile tubes. In order that the same controls might be used, tests of the effect of ultra-violet light on test tube cultures were made. Ultra-violet light has a strong germicidal effect and has been used extensively to sterilize city water supplies. The lamp used in our laboratory is the standard commercial size and is capable of completely sterilizing 1000 gallons of water an hour. Two such lamps in series can handle 5000 gallons an hour. Its success in sterilizing water has caused experimenters to try its effect on other substances. The light from a lamp will penetrate clear water a distance of 45 cm., but fails to penetrate many opaque substances. Even ordinary transparent glass is non-permeable by ultra-violet light, and the results to be reported later on the cultures show this. Milk has not been sterilized by this means because of its opacity. However, an egg-shell is translucent, and it is thought that the light can pass through the shell and penetrate the interior of an egg unless stopped by the contents. Experiments demonstrating this possibility need yet to be performed. If an egg be blown and then filled with nutrient gelatin, a growth will take place at the center of the egg if an inoculation be made through the blow-hole with a culture of a facultatively anaerobic organism. If the ultra-violet light can penetrate the egg-shell no growth will occur.

The possibility of the light's having some destructive effect on the contents of the egg was disproved. An egg was placed in the full light of the lamp for an hour and then examined. No change had taken place, and the poached egg was perfect. Egg-white in an open dish was exposed to the light, but no effect was noticed. The yolk of an egg was placed in the light, and, aside from the evaporation due to the heat from the burner, no change was produced.

10 tubes, containing 10 cc. each of the culture of Staphylococcus were used as controls. 10 tubes were placed in an almost horizontal position beneath the ultra-violet light lamp. In this way the minimum thickness of glass had to be penetrated, and the maximum surface was exposed. The tubes were turned at the end of 15 minutes and taken out in half an hour.

10 tubes were placed in a bottle provided with a stop-cock and exposed to a pressure of 1 cm. for 30 minutes. 10 tubes were given sudden evacuation. The atmospheric pressure was 740 mm., and

the pressure in the vacuum tank was 4.5 cm. From the known volume ratio the final pressure in the system can be computed easily.

279,080 cc. at 45 mm. = 279,080 x 45/740 at 740 mm.

= 16,524.4 cc.

opening the connection bewteen the small and the large volumes is equivalent to allowing the air in the small container and the air in large (reduced to atmospheric pressure) to expand to fill the combined volumes of the system.

16,524.4 + 7000 = 23,524.4 cc.

This volume at atmospheric pressure is allowed to expand to a volume of 279,080 + 7000 = 286,080 cc. The resulting pressure is

740 x 23,524.4/286,080 = 57.1 mm.

= 5.71 cm.

Ten tubes, containing 10 cc. each of a 24 hour culture of Bacillus bulgaricus in sterile litmus milk, were used as controls. 10 tubes were exposed in the inclined position to the ultra-violet light, 10 were evacuated for 45 minutes to a pressure of 1 cm., and 10 were given a sudden evacuation, the two initial pressures being 740 mm. and 90 mm.

At the end of four days the tubes were examined and titrated. In no tube had growth been stopped; in all the litmus had turned pink. The results of titration, however, indicated that both the ultra-viloet light and the evacuation treatments had reduced the growth to a small amount. In all cases the amount of acid produced by the treated cultures was slightly less than that produced by the controls.

The acidity is expressed in cubic centimetes of twentieth-normal acid determined by titration with a standard alkali solution rather than in percentage, because several acids were produced in the same culture.

The average amount of acid produced by the broth control cultures was .745 cc. of N/20 acid in 10 cc of broth. An average of .404 cc. N/20 acid was produced by the broth cultures exposed to ultra-violet light for 30 minutes, showing by this reduction of acidity that subjection to low pressure killed some of the bacteria. It must be remembered also that Staphylococcus aureus is a peculiarly resistant organism.

The results from titration of the milk tubes bore out those of the broth cultures, although in the case of the buillon tubes the ultra-violet light penetrated the tube-cultures, while it had no effect on the milk. Bacillus bulgaricus is a prolific acid-producer. This bacillus, according to the researches of Bertrand and Weissweiller.[1] produces as much as 25 grams of acid per liter of milk. For this reason the milk tubes were titrated with N/2 alkali, and all the results must be multiplied by 10 to be expressed in terms of cubic centimeters of N/20 acid. The average acidity of the control tubes was 5.03 cc. N/2, and that of the light-treated tubes even more, 5.04 cc. The evacuation for 60 minutes reduced the acidity 1.2%, or to 4.98 cc. N/2,

[1] Bertrand and Weissweiller, <u>Ann. de l'Institute Pasteur</u>, 1906.

while the sudden evacuation reduced the amount of acid 4.4%, or almost 4 times as much. These tubes produced 4.82 cc N/2 acid.

Our anticipatory opinion that sudden evacuation would have a germicidal power and that it would be more effective than gradually produced and long continued exhaustion is thus corroborated by the results of experiment. Sudden evacuation can not be applied to the sterilization of eggs because the shell, although porous, does not permit free enough escape of the air and a quick adjustment of the internal pressure. Eggs have often been broken during the work with pressure reductions. Viscous liquids also prevent the quick exhaustion of the dissolved air. When the bouillon and the milk cultures were suddenly exposed to a vacuum, the air rushed out and caused effervescence. Although sterilization by sudden evacuation, if ever developed successfully, would not be applicable to eggs, these results are not valueless. For they indicate that even a long continued evacuation may sometime be used successfully for sterilization, if the pressure be reduced sufficiently. If this method of sterilization can be made effective, our process of preserving eggs becomes complete.

The eggs, immersed in the aluminum soap, are exposed to a very low pressure maintained for an hour. The evacuation sterilizes the egg no matter how badly it is contaminated. Atmospheric pressure is suddenly restored. The tendency of the air to rush into the egg to equalize the pressures inside and out causes the soap solution to impregnate the pores. The eggs are then stored or packed for shipment. The gasoline evaporates, and an air-tight, water-proof, elastic coating is left on the egg which prevents access of air. Sterilization is followed by defertilization. As a practical process of sterilization at this time, however, continued exhaustion has not been successful, and much work remains to be done upon this problem, so important both practically and theoretically.

Attempt at internal sealing.

A great deal of work was done for the purpose of ascertaining whether it was possible to seal an egg internally. Egg albumen, on drying, becomes a water-proof solid. The failure of this substance to seal when applied by simple immersion is attributed to the cracking of the dry coating. If the egg-white were actually in the pores of the egg-shell, preservation would be likely to follow. The possibility of sucking the egg-white inside of the shell right into the hollows of the shell presented a preatical and very simple method of preservation. If exhaustion of the egg, which we have found inhibits, if it does not even in some cases actually prevent, the growth of the germ, will also produce sealing, no method ever proposed could equal this in simplicity, economy and harmlessness.

In this work it was considered that dipping in dilute hydrochloric acid would probably facilitate the process of exhaustion and sealing. The first experiment aimed to show whether it was possible actually to force the egg-white through the shell to the outside. Egg number 1 was exhausted 10 minutes in a vacuum dessicator containing calcium chloride. The filter pump was used, giving a pressure of 1.53 cm. at 18°. Number 2 was washed to remove its mucilaginous coating, dipped in 5N hydrochloric acid 5 seconds, washed, and dried. It was then exhausted 10 minutes at 18°. Number 3 was washed and dried, and then evacuated for 10 minutes. Number 4 was washed, dried, and dipped in 5 N hudrochloric acid for 20 seconds. It was then washed, dried, and evacuated 10 minutes. At the end of the treatment there was no sign of moisture on any of the shells. This result is not surprising in view of the structue of the shell. Although the shell itself is porous, just within lies the double-walled shell-membrane, forming by the separation of its layers at the broad end of the egg the air-chamber which is usually richer in oxygen than the surrounding air. This membrane is attached firmly to the shell at many separate points, and thus hinders the passage of the white through it. The white may not be able to force its way out by actually rupturing the shell-membrane, but it was considered likely that if the reduction of external pressure were sudden enough, the white might press the membrane itself firmly into the pores of the shell. This is considered a possible explanation of the peculiar success of the evacuation experiments in preserving the eggs.

Normal pressure was restored to 1, 2, 3, and 4, and these eggs were given sudden evacuation with a volume ratio of 1 : 10. One egg burst, and no moisture could be seen on the others. Sudden evacuation with a volume ratio of 1 : 400 was tried on two eggs. For number 135 the initial pressures were 9 cm. and 74 cm., and for 136 4 cm. and 76 centimeters. Both eggs were incubated for two weeks. In neither case did the germ grow.

The effect of ultra-violet light on eggs.

The question of the permeability of sealed and unsealed eggs to ultra-violet light can only be answered by experiment. Numbers 123 and 124 were old eggs used as controls. Numbers 125 and 126 were eggs from the same lot. They were exposed to the ultra-violet light for 10 minutes at a temperature of 40°. They were then sealed with aluminum soap. Numbers 127, 128, 129, and 130 (fresh) were given the same treatement. Number 131 was exposed to the ultra-violed light for 60 minutes and was turned once in order to expose all sides equally. It was then rubbed with vaseline. This treatment, being practically the same as that on 127-130, afforded opportunity for comparing the relative values of aluminum soap and vaseline. In order to eliminate the effect due to the ultra-violet light, number 132 was only rubbed with vaseline. 133 was first dipped in the aluminum soap solution and then immediately exposed to ultra-violet light for 60 minutes. 134 acted as a control.

The results were as expected, with two exceptions. Numbers 123 and 124 were infertile and spoiled. 125 and 126 were both good. These eggs showed again that aluminum soap is able to preserve any egg that has not actually commenced to spoil. No process, of course, can rejuvenate a bad egg. Numbers 127 to 130 were perfectly preserved, but no better than many other fresh eggs treated simply with aluminum soap. There was no turbidity, odor, evaporation, or sticking of the yolk to the shell. 131 and 132 were both good, but the vaseline had discolored the inside of the shell, and the eggs tasted slightly of vaseline. 133 and 134 gave anomalous results. 133 was bad. The white was turbid and stringy, and hard, gelatinous masses were formed.

The only shortcoming which the gasoline solution of aluminum soap has had is that a slight taste of gasoline is left in the egg. Such a drawback makes any process useless, and it must be removed. Numbers 127 to 130 were poached and then eaten. All tasted very slightly of gasoline. One was given to two men to taste, neither of whom knew of the previous treatment given the egg. One detected some foreign substance, but the taste was not strong enough for him to identify it. The other identified the substance producing the taste as some kind of fuel oil.

Three eggs were immersed in a gasoline solution of paraffin on a Saturdary. They were taken out Tuesday, and allowed to stand until the following Saturdary. Thus they were in intimate contact with gasoline for one week. Two were broken and poached, and one was incubated. The poached eggs tasted offensively of gasoline. The preserved egg was fairly well kept.

The method of double sealing.

This process of preservation must be made tateless or it can not be used. Two methods of solving the problem suggested themselves, and both were entirely successful. The first tried was to protect the eggs by a preliminary coating before sealing with the gasoline solution. Three substances were experimented with, sulphuric acid, paraffin, and egg-white.

Number 138 was placed in 20 N sulphuric acid. Effervescence continued for about ten seconds, and ceased as a coating of calcium sulphate was formed in the pores of the egg. The calcium sulphate acts as a ploarizer, and stops the reaction. The egg was then dipped without drying in the aluminum soap solution and placed on a special dripping rack. Number 139 was dipped quickly in melted paraffin and immediately removed. The dipping was done quickly since the heating of the egg causes the air to expand and bubble through the paraffin. In this way holes are left in the coating. This egg was then dipped in the gasoline solution and placed on the rack. Number 140 was dipped in egg-white and then plunged into boiling water to coagulate the white on the shell. The coagulated white did not stick to the shell. These three eggs were kept in the gasoline solution of aluminum soap from Monday till Friday, when they were taken out and allowed to dry on the rack. On Saturday the eggs were opened and poached.

Number 138 was tasted by two persons who did not know of the treatment, and no gasoline could be tasted. The contents were absolutely sweet, and no slightest taste of gasoline was left in the mouth after eating. Number 139 tasted noticeably of the solvent, and 140 very strongly. The method of double sealing with sulphuric acid is entirely satisfactory. The extra cost of the preliminary sealing, either in time or money, is negligible. The acid solution may be used for hundreds of eggs, and the sealing of the pores requires only an extra dipping. One of the objections to those processes requiring the use of alcohol or other strongly tasting and smelling substances is that the eggs preserved are not palatable. And, since the substance thus affecting the egg is the principal agent of preservation, no remedy is possible. On the other hand, the principal agent in our process is a tasteless, odorless solid, absolutely without effect on any food. The gasoline is merely an agent for applying the soap and may be kept from touching the egg without any sacrifice of efficacy.

Preparation of a tasteless, odorless solvent.

The necessity of using two dippings according to the method developed is, without doubt, a weakness, and a far better solution of the problem was found. An attempt was made to purify gasoline. Gasoline is essentially a mixture of hydrocarbons of the aliphatic series. All of the saturated hydrocarbons of the $C_nH_{2n} ! _2$ series are odorless and tasteless compounds. The taste and the smell of commercial gasoline, then, are due to other minor contituents, chiefly unsaturated olefines, both aliphatic and aromatic, free sulphur, mercaptan compunds, and other substances. The gasoline was agitated for two hours with concentrated sulphuric acid in order to remove unsaturated hydrocarbons. It was refluxed for three days with Gatterman copper, and finally distilled over copper oxide and filtered through animal charcoal. The odor was undiminished at the end of this treatment.

Gasoline is largely composed of pentane, which is a tasteless, odorless, and colorless liquid, boiling at 37°. Since gasoline dissolves aluminum soap well, it is certain that its chief constituent will do so, and the problem is to prepare chemically pure pentane on a large scale economically.

The best method of preparing pentane has seemed to be the reduction of amylene. A very convenient and cheap process has recently been devised by Roger Adams for making amylene, or pentene. In this laboratory his small scale method was used for the production of small amounts of pentane. 1500 cubic centimeters of amyl alcohol and 100 of concentrated sulphuric acid were placed in a 3-liter flask, and the mixture was violently refluxed on a sand-bath for 14 hours. The reflux condenser was inclined at an angle of 30° from the vertical, to insure free return of the condensed liquid, and the water in the jacket was kept at $60^{\circ} - 90^{\circ}$. Low boiling products were thus separated and condensed in a second Liebig condenser arranged to deliver downwards. At the end of the 14 hours the residue in the orginal flask was fractionated, and that fraction boiling below 100° collected, and added to the first distillate. The crude distillate was separated and washed with dilute sodium hydroxide solution. The upper layer was then fractionated, and the portion boiling below 100° collected. Since the amylene thus produced is only an incidental product in our preparation, the distillate is used without further purification. By distilling the vapors of amylene through a heated tube (300°) containing pyropheric nickel in the presence of hydrogen pentane is produce. By fractional distillation chemically pure pentane can be produced.

Experiments are being continued to confirm the prediction of the success of pentane in dissolving aluminum soap and preserving eggs. The production of pure pentane on a commercial scale is made possible by Adams' pyrogenic-catalytic method of preparing amylene. By this process, so the author declares, the $125^{\circ} - 130^{\circ}$ fraction from technical fusel oil may be introduced a liter at a time and supplied to the generator indefinitely without exhaustion of the catalyst. The cheapness with which amylene may now be made and the facility with which the product can be turned into pentane make this solution of the problem satisfactory in every way.

Summary.

1. Previous methods have affected the egg adversely or have not been practicable for commercial use.

2. Evacuation at less than 1 millimeter pressure has been found to effect partial destruction of the germ. With available vacuums 2 hour exhaustion has given maximum results.

3. Sudden evacuation has shown a bactericidal effect, the reduction of growth being proportional to the product of the pressure reduction and the time required for the change.

4. A saturated gasoline solution of aluminum soap has produced perfect preservation for long periods at 37.5° C.

5. Double sealing with sulphuric acid has prevented any adverse effect of the gasoline on the egg.

6. The problem of preparing an inactive solvent for aluminum soap has been solved by the use of chemically pure pentane.

7. A commercial process of producing pure pentane has been developed by the aid of Adams' method of preparing amylene.

8. A criterion for the accurate determination of the value of preservative processes expressible in terms of the water-glass coefficient has been developed.

Chronological Bibliography.

Year	Name	Journal	Method
1871	M. Dubrunfaut	Comptes Rendus lxxii,1871,106	Lime-water and the sinking test
1873	F. C. Calvert	Comptes Rendus lxxvii,1873,1024,	Whole or pierced in nitrogen hydrogen, or carbon dioxide Weak chlorine water. Calcium hydroxide and calcium sulphite. Calcium chloride solution. Phenol.
1880	Adam Good	U. S. Patent 225518. March 16, 1880.	Solution of borax, sugar, and lime with drying.
1880	Halvor Halvorson	U.S.P. 233750, Nov. 26. 1880.	Dessication
1882	Stead	British Patent 4910.	Water-glass
1888	Lake	British Pat.1653.	Carbon dioxide.
1890	W.P. Wheeler	Ninth Ann. Rept. of the First Ass't. 1890, 122 - 140. New York State Experiment Station.	Rubbed with oil containing an antiseptic, and packed in salt and bran. Vaseline containing boracic acid and salicylic acid.

1891	I. P. Roberts	Cornell (N.Y.) Station Bull., 1891, 376-377.	Mineral preservative, consisting of silicates of aluminum, iron, and calcium. salt.
1891	Jessen	British Patent 15580	Rubber-coated paper
1891	Mills	British Patent 17717	Gelatin
1892	Farquhar and North	British Patent 15128	Gelatin
1892	McArdle	British Patent 3592	Lime
1895	Markham	British Patent 3513	Boric acid.
1896	R. Strauch	Das Huhnerei als Nahrungsmittel u. die Conservirung der Eier, Bremen, M. Heinsius, p. 50	General methods.
1897	E. S. Ladd	N.D. Exp.Sta.Bull. 1897, also U.S. D. A. Bull. 103.	Water-glass
1897	R. Strauch	Milch Zeitg. xxvi, 1897, No.22, 342	Vaseline and lime-water Water-glass
1897	L. Bernegau	Pharm Zeitg.,xlii, 1897, 381; abs. in Vierteljahr. Chem Nahr. u. Gen.,12, 101 (Number 2)	General methods

1897	J.H. Thieriot	U.S.Consular Rpts. Dec. 1897, 563-564	Vaseline Calcium hydroxide. Water-glass
1897		Land W. Centralblatt Posen, xxv, 1897, No.34, 209.	Peat Dust
1898	L. G. Jarvis	Ontario Agri. College and Expt. Farm Rept., 1898, 193 - 196.	Lime-water Water-glass
1898	E. F. Ladd	N.D.Exp.Sta.Bull. 35, 330 - 332.	Water-glass
1898	K. Reinhardt	British Patent 18130, noted in Sc. Am. Suppl., xlix, 1900, No.1273, page 20405	Dipping in sulphuric acid to form a coating of calcium sulphate in the pores of the shell.
1899		Queensland Agri. Journal, iv, 1899, No. 3, 418-419.	Water-glass Salt and borax. Ashes, bran, salt.
1899	L.G.Jarvis	Ontario Agr. College and exper. Farm Rept., 1899, 130 - 134.	Water-glass. Calcium solutions. Coating with vaseline. Packing in salt, dry oats. Coating with water-glass

1899	F.T.Schutt	Canada Expt. Farms Repts.,1899,223.	Water-glass. Lime-water Glycerin. Distilled water.
1899	Coakley	British Pat. 18816	Gelatin
1899	Wilson and Wilson	British Pat. 15461	Lime.
1900	A.G.Gilbert	_Canada Expt. Farms_ Reports,1900,251-277	Lime-water. 2% water-glass. Gum arabic + formalin Gum arabic + salicylic acid. Dextrin and sal. acid. Sulphuric acid. Vaseline.
1900	H.Borntraeger	_Oesterr. Chem. Zeitg._, iii,1900,No.12,295.	Water-glass.
1901	F.T.Schutt	_Agr.Journ.CapeGood Hope_ xix, 1901,1,55-56.	Calcium hydroxide.
1901	A.A.Brigham	_Rhode Island State Experiment Station Report_,1901,304	Water-glass Salt Calcium hydroxide. Brine. Vaseline. Ashes,powdered sulphur, gypsum, sulphur fumes, potassium permanganate, salicylic acid.

1901	W. R. Graham	Ontario Agr. and Ext. Union Rept., 1901,31-3	Salt, with and without grease. Water-glass. Lime-water, with and without salt.
1901	Lorne	British Pat. 18439 and 9898.	oil, wax, or varnish with and without antiseptics.
1901	Rylander	British Pat.23523.	Water-glass
1902		Bull. Soc. Vaud. Agr. et Vit.,Lausanne, 1902, No.151, 29.	Immersion 5 seconds in boiling water.
1902	E Teisler	Journ. Soc. Cent.Agr. Belg.,xlix. 1902. No. 11-12, 401.	Fluosilicic acid
1902	Barlow	British Pat. 11054	Oil + an antiseptic.
1902	Stukes	U. S. Pat. 649899.	Oil + an antiseptic.
1902	Schultz	British Pat. 1328	Gelatinous starch solution
1903	Garantol Gesellschaft. Lt.	German Pat. 178343	Calcium hydroxide + calcium magnesium carbonate + magnesium phosphate + calcium phosphate.
1903	C.E.C.Landsperg	Danish Pat.27267	Casein or cheese substance finally removed by weak ammonia or vinegar

1903	E. Brown	Journ. Bd. Agr.,(London,ix, 1903,No.4, 494 - 497	Lime-water Water-glass Storage
1903	A.devillèle	Rev.Agr.Rêunion,ix,No.10	Molasses.
1903	Marks	British Pat. 12867.	Sulphuric acid.
1904	L. Irwell	Dietet. and Hyg. Gaz., xx, 1904,No.8,457.	Water-glass
1905	Ernst Utescher	German Pat.850693,ass'r to Garantol Gesellschaft.	Lime-water containing egg-shells.
1905	F. Haxton	Reliable Poultry Journal. xi, 1905,No.11, 1096.	Storage
1906	J. S. Jeffrey	North Caroline State Experiment Station Bulletin 191, 1906, 11 -17.	Waterglass, lime-water, lime + salt, lime + salt + British Egg Preservative, paraffin, collodion, gum-arabic, and cottonseed hulls.
1907	R.W.Thatcher	Wash.Stat.Bull.71,1907,14.	Water-glass
1907	De Loverdo	Comptes Ren. Ac. Sc.,Paris cxliv, 1907, 41 - 43.	Storage
1907	F. Prall	Zeitschrift Untersuch. für Nahrungsmittel und Genusemittel, xiv,1907,No.7.445; abstracted in Zentralblatt Agri. Chem.,xxxvii,1908, No.7,486-489, also in Hyg.Viande et Lait,ii No. 8, 362.	Sand, straw, storage, paraffin, hydrofluosilicic acid, shellac, potassium permanganate water-glass, hot water + alcohol, glycerin, and lime-water

1907	Garrigon	French Pat. 394455.	Plunging in boiling water, piercing and introducing an inert gas, and dipping in alum-treated plaster solution, and drying.
1908	Ballard	French Pat. 389543.	Dipping in saturated solution of propolis.
1908	Campanini	Bull. of the Italian Minister of Agr., through the Journ. Roy. Soc. Arts, lvi, 1908, 866.	Coating with lard and preserving in boxes.
1908	James Hendrick	Journal of the Agr. Soc., ii, 1908, 100.	Water-glass.
1908	J.Vosseler	Der Pflanzer, iv, 1908, 129, also Chemisches Zentralblatt, ii, 1908, 1214.	Chopped straw, salt, ashes, slaked lime, lime-water, water-glass, potassium permanganate, salicylic acid, and glycerin.
1908	P.A.Schmitt	German Pat. 233971.	Benzoic acid in alcohol.
1909	J.D.Smithers	U.S.Pat. 912909.	Treatment with solution of lime 51 + salt 21 + sodium bicarbonate 1 + potassium bitartrate 8 in water 1000, drying, and dipping in melted lard.

1909	F. Lescarde	British Pat. 7804	Evacuation, carbonation, and atmosphere of carbon dioxide + nitrogen
1909	J.G.Stephens	Daily Consular and Trade Reports of the U.S., 1909, No. 3377, 16.	evacuation in paraffin.
1910	W.H.Burns	U.S.Pat. 938965.	Water-proof cover and underground storage on sulphur.
1910	H. J. Keith	British Pat. 114499	Saccharine presevative + freezing.
1910	L.Prenzlau	British Pat. 15309	Subjection to gaseous formaldehyde and packing in material saturated with HCHO.
1910	N.P.Jacobsen	Danish Pat. 14902	Immersion 15 minutes in water at 35° and in boiling water 5 seconds, cooling in water, and drying.
1910	E. W.Fischer	Swedish Pat. 31624.	Gelatinous substance + paraffin.
1910	M.delaroquette	L'ind. beurre, 1,1910. 600-603; through Mois chim. et electro-chim.,viii,1910, 31-32	Dry sand, chalk, wrapping in paper and storing in cold cellar, hot brine, salty fat bath, lime-water, and water-glass.

1910	A. Cihlar	German Pat. 245785.	Immersion 6 hours in strong alcohol.
1910	J. M. Bartlett	Maine Agr. Expt. Sta., Orig. Comm. 8th Int. Congress App. Chem., xviii, 1910, 51-56.	Water-glass
1910	E. Jacoby	German Pat. 251281.	Paraffin containing HCHO.
1910	F. Lescarde	Berichte II Internat. Kältekong. Wien, ii, 1910 373 - 374.	Freezing.
1911	A. G. C. Sturley	U.S. Patent 995198.	Benzoic acid in alcohol.
1911	R. Berger	Journ. Ind. and Eng. Chem., iii, 1911, 493-495.	Water-glass
1911	H. Jerne	British Patent 2145.	Coating with gelatin and then with camphor + nitrocellulose.
1911	A. Duboux and C. H. Rapin	German Patent 262064.	Rubbing with vaseline to which have been added talcum 10% ! aluminum tannate 1% ! pulverized tragacanth 40%
1912	A. Duboux and C. H. Rapin	U. S. Patent 1019614.	The same.
1913		U.S. Patent 1043600	Coating with gelatin and a celluloid-like compound.

1913	L. Prenzlau	U. S. Patent 1045178.	Packing in excelsior saturated with HCHO.
1913	A. Evequoz and E.P.Haussler	Zeit. Unter. Nahr.-Genussmittel,xxv,1913,96.	Water-glass
1913	M.deKeghel	Rev. Chim. Ind.,xxiv,1913, 12 - 18.	General mehtods.
1916	Georg Eichelbaum	Biochem. Zeit.,lxxiv, 1916, 176 - 184; also in Physiolog. Abst.,1,1916, 306.	Toluene, chloroform, hydrochloric acid acetic acid, oil of mustard, sodium benzoate, boric acid, and salicylic acid.
1916	M.J.Davis and H.A.Metz	British Patent 105840.	Coating with tung oil containing dissolved resin and with a flattener.
1917	H.D.Bostock	U.S. Patent 1212614.	Solution of Desmodium tortuosum meibomium, 1 lb. in 1 gal. water.
1917	I.Subirana	Swiss Patent 74124.	Coating with a liquid containing a dry oil.
1918	F.E. De la Mata	U.S. Patent 1229592.	Coating with a fatty substance and then with a colloidal material.

Bibliography

A. Changes in eggs.

B. Composition of eggs.

C. Egg industry.

D. Infection of Eggs.

E. Preservation of eggs.

F. Sterilization

A.Changes in eggs.

Poultry experiments)Loss of weight in incubation), J. H. Stewart and H. Atwood, West Va. Sta. Bull. 73, 1902, 35-47.

The production of oxygen in hens' eggs, K. A. Hasselbalch, Skand. Arch. Physiolog., xiii, 1902, Numbers 1-2, 170-192.

The spoiling of eggs, H. Schlegel, Ber. Untersuch. Aust. Nürnberg, 1904, 9-10; abstracted in Zeit. Unter. Nahr.-Genuss., x, 1905, No.4, 255.

The analysis of the cleavage products of egg albumin, P. A. Levene and W. A. Beatty, Biochem. Zeit., iv, 1907, No. 4-6, 305-311.

Changes which take place in eggs, A. Chrétien, Hyg. Viande et Lait, i, 1907, No. 5, 193-201.

The rate of growth of the egg yolk in the chick, and the significance of white and yellow yolk in thé ova of vertebrates, O. Ridde, Science, new series, xxvii, 1908, No. 703, 945.

Changes in the purine, pentose, and cholesterol content of the developing egg, C. S. Leavenworth, Am. Journ. Physiol., xxi, 1908, No. 1, 77-84.

Experiments on the metabolism of matter and energy during incubation of hens' eggs, F. Taugh and A. von Mituch, Arch. Physiol. (Pflüger), cxxi, 1908, No 8-10, 437-458.

The transformations in the phosphorus compunds in the hen's egg during incubation, R. H. A. Plimmer anf F. H. Scott, Journ. Physiol., xxxviii, 1909, No. 4, 247-253.

The osmotic pressure of the egg of the common fowl and the changes during incubation, W. R. G. Atkins, Bio-chem. Journ., iv, 1909, No. 10, 480-484.

The transformation in the fats in the hen's egg during development, Elizabeth C. Eaves, Journ. Physiol., xl, 1910, No. 6, 451-453.

Oxidation in the egg, Otto Warburg, Zeit. Physiol. Chem., lx, 1909, 443-452.

The part played by gases in the coagulation of albumin, E. Mathieu and V. Urbain, Comptes Rendus, lxxvii, 706-709.

On the decomposition of eggs, W. Thompson, Chemical News, xxx, 159.

Deportment of the shells of hens' egg during incubation. C. Voit, Zeitschrift für Biologie, xiii, 518-526.

On certain modifications of the substance of eggs determined by fungoid growths from without, a. Bechamp and G. Eustache, Comptes Rendus, lxxxv, 854-857.

Researches on the chemical changes occurring in the fowl's egg during incubation, R. Pott, Landw. Versuchs. Stat., xxiii, 203-247.

Investigation of the processes of decomposition occurring during the rotting of eggs, C. O. Cech, Journ. pr. Chem. (2) xxii, 338-344.

Occurrence of peptone in hens' egg during incubation. Zeit. Physiol. Chem., x, 11 - 13.

Decomposition of proteids by means of bacteria, A. E. Taylor, Zeit. Physiol. Chem., xxxvi, 1902, 487 - 492.

Decomposition of proteids by means of bacteria, Oskar Emmerling, Zeit. Physiol Chem., xxxvii, 1902, 180.

Osmotic phenomena of the yolk of egg, William Alexander and Hilda E. Kincaid, Biochem. Journ., viii, 1914, 28-29.

The coagulation of albumin by pressure, P. W. Bridgman, Journ. Biol. Chem., xix, 1914, 511-512.

Changes which occur in eggs, and the sanitary control of the egg business, Lindet and Hussen, Ann. fals., x, 1907, 106-111.

B. Composition of eggs.

Concerning the iron content of hens' eggs and experiments on the increase of iron in the egg when haemogallol and ferrohaemol are fed, P. Hoffman, Zeit. Analyt. Chem., xl, 1901, No.7, 450-459.

The coagulable material in the white of an egg, L. Langstein, Beitr. Chem Physiol. und Pathol. Zeit. Biochem., i, 1901, 83; abstracted in Hyg. Rundschau, xii, 1902, No. 5, 239.

The iron content of hens' eggs. C. Hatung, Zeit. Bilog. xliii, 1902, No. 2, 195-212.

Hens' Eggs, E. Carpiaux, Bul Agr. (Brussels), xix, 1903, No. 2, 200-212; also noted in Bul. Inst. Chim. et Bact. Gembloux, 1903, No. 73, 39-51.

The existence of arsenic in hen's eggs, G. Bertrand, Ann. Inst. Pasteur, xvii, 1903, No. 7, 516 - 520

The coloring matter and fat of egg yolk and the detection of egg yolk in foods, Laves, Versamml. Deut. Naturf, und Afzte Cassel, 1903; abstracted in Zeit. Unter. Nahr. - Genuss., vii, 1912, 754 - 755.

The globulin of egg yolk, L. Morochowetz, Physiol. Russe, iv. 1905, No. 61 - 67,- 53 - 59.

Analysis of egg yolk, N. A. Barbieri, Comptes Rendus Acad. Science. (Paris), cxlv, 1907, No. 2, 133 - 135.

The quantity and distribution of phosphorus in certain kinds of food, W. Heubner and M. Reeb, Arch. Expt. Path. und Pharmakol., 1908, sup., 265 - 272.

The proteins of egg yolk, R. H. A. Plimmer, Journ. Chem. Soc., xciii, 1908, No. 550, I, 1500 - 1506.

Spectuuscopic properties of yolk of egg, L. Lewin, A. Miethe, and E. Stenger, Arch. Physiol. (Pfluger), cxxiv, 1908, 585 - 590; abstracted in Journ. Chem. Soc., xciv, 1908, 554, II, P.1054.

Concerning the fat of hens' eggs, R. Paladino, Zeit. Biochem., xvii, 1909, No. 4, 356 - 360.

Analyses of eggs, J. T. Willard and R. H. Shaw, Kans. Sta. Bul. 159, 1909, 143 - 177.

Lecithin and other components of egg yolk, E. Tornani, Bol. Chim. Farm., xlviii, 1909, 520 - 521.

The vitellin membrane in eggs of birds, A. Lecaillon. Comptes Rendus Soc. Biol., lxviii, 1910, No. 5, 218 - 219; Comptes Rendus Acad. Science, xl, 1910, No. 4, 240 -242; also in Journ Royal Micros. soc. (London), 1910, No. 3, 293-294.

Composition of eggs with special reference to biological problems, V. Diamare, Sienna, 1909, pp. 14; reviewed in Chem. Zentral., 1910, I, no. 19, 1732.

The dextrose of the egg and its biological significance. V. Diamare, Rend. Accad. Sci. Fis. e Mat. Napoli, iii, ser., xvi, 1910, 242-244; abstracted in Journ Chem Soc., c, 1911, No. 580, II, 129.

Biology of the egg; a chemico-anatomical coordination, V. Diamare, Anat. Anzeiger, xl, 1911, 205-207.

Coagulation of albumin, E. Varenne, Comptes Rendus, cii, 129-131.

Coagulation of albumin, E. Varenne, Comptes Rendus, cii, 129-131.

Coagulation of albumin, V. Michailoff, Chem Zentral., 1887, 108

Proteids of white of egg, G. Corin and E. Berard, Archiv. de Biol., ix, 1-16.

Coagulable components of white of egg, Leo Langstein, Beitr. Chem. Physiol. Path., i, 1901, 83-104.

Ovumucoid and sugar in egg white; a systematic investigation, Darl Th. Morner, Zeit. Physiol. Chem., lxxx, 430-473.

Carbohydrates of hens' eggs, H. Bierry, Hazard, and Albert Ranc, Comptes Rendus Soc. Biol., lxxiii, 93-94.

C. Egg Industry.

On the determination of the soundness of eggs by their specific gravity, C. Cogg, Giov. R. Soc. Ital. Ig., April 30, 1901; abstracted in Public Health, xiv, 1902, No. 7, 423.

Coopefative records of the cost of producing eggs (in winter), H. H. Wing, New York (Cornell) Station Bul. 212, 1904.

The poultry and egg industry of leading European countries, A. Fossum, U. S. D. A. Bur. An. Ind. Bull. 65, 1904,79

Poultry and eggs, F. R. Mowrer and R. P. Skinner, Daily Consular and Trade Reports of the U. S., 1909, No. 3400, 8-12.

Preliminary Report of the Dairy and Food Commissioner for the year 1908, J. Foust, Penna. Dept. Agr. Bull. 183, 1908,57.

The marketing of eggs, A. G. Philips, Kansas Sta. Bull. 162,1910, 243-258.

The egg trade of the U. S., M. M. Hastings, U. S. D. A. Bur. An. Ind. Circ. 140, 1908.

Deterioration in eggs as shown in the moisture content, A. D. Greenlee, Science, new series, xxxiv, 1911, No. 868, 223-224.

The improvement of the farm egg, H. M. Lamon and C. L. Apperman U. S. D. A. Bur. An. Ind. Buol. 141, 1911, 43.

D. Infection of eggs.

About the transfer of disease organisms by fowl eggs, with a contribution to the bacteriology of the normal egg, K. Poppe, Arb. K. Gandht-samt., xxxiv, 1910, No. 2, 186-221.

Animal life without bacteria in the alimentary canal (Bacteria in eggs), George H. F. Nuttall and Hans Thierfelder, Zeit. Physiol. Chem., xxiii, 1897, 231-235.

Permeability of membranes for putrefaction processes, Hans Hensen, Zeit. Biol., xxxv, 1897, 101-115.

A bacteriological study of shell, frozen, and dessicated eggs, G. W. Stiles, Jr. and Carleton Bates, U.S.D.A.Bur.Chem. Bull.148.

The bacteria and catalase content of hen eggs, W. Rulliman, Centralblatt fur Bakteriologie.

The Growth of molds in eggs. August Postolka, Central. Bakt. Parasitenk, Abt. II, xlvi, 1916, 320-330.

A bacillus found in eggs, L. Palmans, Bul. Agr. (Brussels), xx, 1904, No.3, 447-452.

<u>Prosthogonimus cuneatus</u> in the hen's egg, K. Wolffhugel, Zeit. Infektionskrank. u. Hyg. Haustiere, i. 1905, No. 1,21-25.

The penetration of bacteria through the shell into the interior of eggs, R. Lange, Arch. Hyg., lxii, 1907, No. 3,201-215

Can dysentery bacillus pass through the shell of fresh eggs? Sachs-Muke, Arch. Hyg., lxii, 1907, No. 3, 229-238.

Contribution to the study of the bacterial flora of eggs, A. Chretien, Hyg. Viande et Lait, ii, 1908, No. 6, 247-254.

On the penetration of eggs by bacteria, G. Cao, Ann. Ist. Ig.Sper. Univ. Roma, xviii, 1908, No.1,39-63; abstracted in Bull. Inst. Pasteur, vi, 1908, No. 10, 472.

Studies on the bacteria in hens' eggs and suggestions for keeping eggs, Zorkendorfer, Arch. Hyg., xvi, 1893, 368-401.

Infection and preservation of eggs, G.H.Lamson,Jr., Conn. Storrs Sta. Bull. 55, 2030214.

Chemical and bacteriological study of fresh eggs, Mary E. Pennington, Journ. Biol. Chem., xii, 1910, 109-132 (65-74)

E. Preservation of eggs.

Eggs in cold storage, M. Cooper, Chicago, H. S. Rich ane Co.,1899.

Cold storage of eggs, H. V. Jackson, Agr. Gaz. New So. Wales, xiv, 1903, No. 8, 113-115.

Preservation of foods on a commercial scale (Les industries de la conservation des aliments). X. Rocques, Paris, Gauthiervillars, 1906.

See also under Chronological Bibliography, ante pages 68 ff.

F. Sterilization.

Duration of life of typhoid bacilli from different sources, in ice, and the effect of intense cold on bacteria, W.H.Park, abstracted in Science, new series, xiii, 1901, No. 322,323

Action of light upon bacteria. A. Bournaret, Thesis, Toulouse,1900.

Effect of hens' eggs of the rays emitted by radium, G. Schwarz, Arch. Physiol. (Pfluger), c, 1903, No. 9-10, 532-546.

A preliminary note on the action of the radiations from radium bromide on some organisms, H. H. Dixon and J.T. Wigham, Sci. Proc. Roy. Dublin Soc., n. ser., x, 1904,II, No. 19, 178-192.

A note on the action of radium on micro-organisms, A.B.Green, Proc. Roy. Soc., lxxiii, 1904, No. 474, 375-381.

Do Roentgen rays exert an influence upon bacteria. J. Wittlin, Central. Bakt. und. Par. Allg., ii,1896,No.21,676-677.

Action of certain bacteria under high pressure, H. Rogers, Comptes Rendus, cxix, 1894, No. 23, 963-965.

Influence of the electric current on bacteria, H. Friedenthal. Central. Bakt. und Bar. Med., xix, 1896, No.9-10, 319-324.

Sterilization by ultra-violet rays; application to butter, Dornie and Daire, Comptes Rendus, 1909, cxlix, 354-356.

Effect of high pressure on micro-organisms, G. W. Chlopin and G. Tamman, Zeit. Hyg. und Infektionskrank.,xlv,1903, No. 2, 171.

The effect of freezing on bacteria, E.F. Smith and D.B.Swingle, abstracted in Science, n. ser., xxi, 1905, No. 535,481-483.

The destruction of bacteria by high pressure, E.W. Duckwall, Canner and Dried Fruit Packêr, xxv, 1908, No. 25,21-25

Influence of electricity upon micro-organisms, G.E.Stone, Bot. Gaz., xlviii, 1909, No. 5, 359-379.

The behaviour of bacteria in a nitrous oxide atmosphere, A.Maasen and Schonewald, Mitt. K. Biol. Anst. Land und Forstw., 1910, No. 10, 32-34.

Influence of light on the development of bacteria, J. Jamieson, Bied. Centr., 1883, 857.

Destruction of bacteria by light, Hermann Thiele and Kurt Wolf, Arch. Hygiene, lvii, 1906, 29-55.

Action of carbon dioxide, oxygen, and hydrogen at various pressures, Berghaus, Arch. Hygiene, lxii, 1907, 172-200.

Chemical effect of quartz-mercury vapor lamps on water, Th. Nogier and A. Rochaix, Comptes Rendus, cxlix, 1909,160-161.

New observations on disinfection with gas and gas-forming substances U. Friedemann, Berl. klin. wochschr., xliv, 1907,695-698.

The effect of egg albumin in killing spores and retarding development, P. Lashchenko, Zeit. Hyg., lxiv, 419-427.

Sterilization of large quantities of water by the ultra-violet rays, G. Vallet, Comptes Rendus, cl, 1076.

Sterilization of milk by ultra-violet rays, R. Masse, Milch Zeitg., xxxviii, 566.

Sterilization of water by ultra-violet rays, E. Urbain, C. Seal, and A. Feiges, Comptes Rendus, cl, 548.

The action of ultra-violet rays upon bacteria, O. Bujwid, Gasbel., liv, 853-863; through Chem. Central., 1911, II, 1542.

The antiseptic and bactericidal properties of egg white, Leo F. Rettger, and Joel A. Sperry, Journ. Med. Res., xxvi, 55-64.

Osmotic Activity in the egg of the common fowl, A.D. Greenlee, Journ. Am. Chem. Soc., xxxiv, 1912, 539-545.

List of Journals Referred to.

Agricultural Experiment Stations (of the various states), Bulletins, Circulars, and Technical Papers.

Agricultural Gazette of New South Wales, Sydney,N.S.W.

Anatomischer Anzeiger, Jena.

Annales des falsifications, Paris.

Annales de l'institut Pasteur, Paris.

Anzeiger der Kaiserlichen Akademie der Wissenschaften, Vienna.

Archiv für Anatomie und Physiologie, Leipzig.

Archives de biologie.

Archiv für experimentelle Pathologie und Pharmakologie, Leipzig.

Archiv für Hygiene, Munich.

Archiv für Physiologie, Leipzig.

Beiträge zur klinik der Tuberculose, Wurtzburg.

Berichte der deutschen chemischen Gesellschaft, Berlin.

Berliner klinische Wochenschrift, Berlin.

Biochemical Journal, London.

Biochemische Zeitschrift, Berlin.

Botanical Gazette, Chicago.

Bulletin de la Societé française de mineralogie, Paris.

Bulletin de la Societé chimique de France, Paris.

Bureau of Chemistry, Bulletins and Technical Papers.

Centralblatt für allgemeine Pathologie und pathologische Anatomie, Jena.

Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Jena.

Chemical News and Journal of Physical Science, London.

Chemisch Weekblad, Amsterdam.

Chemisches Centralblatt, Berlin.

Chemiker-Zeitung, Cöthen.

Comptes rendus hebdomadaires des séances de l'académie des sciences, Paris.

Comptes rendus hebdomadaires des séances de la societé de biologie, Paris.

Hygienische Rundschau, Berlin.

Hygiene de la Viande du lait, Le.

Journal of the American Chemical Society, Washington, D.C.

Journal de l'anatomie et de la physiologie normales et pathologiques de l'homme et des animaux, Paris.

Journal of Biological Chemistry, Baltimore.

Journal of the Chemical Society, London.

Journal of Medical Research, Boston.

Landwirtschaftliche Zeitung, Stuttgart.

Landwirtschaftliche Jahrbücher, Berlin.

Oesterreichische Chemiker-Zeitung, Vienna.

Proceedings of the Royal Society, London.

Revue de chimie industrielle, Paris.

Science, Garrison-on-Hudson, N.Y.

Scientific Proceedings of the Royal Dublin Society, Dublin.

Skandinavisches Archiv für Physiologie, Helsingfors.

United States Department of Agriculture Bulletins.

Zeitschrift für allgemeinen Physiologie, Jena.

Zentralblatt für Physiologie, Vienna.

Zeitschrift für Fleisch und Milchhygiene, Berlin.

Zeitschrift für Hygiene,

Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, sowie Gebrauchsgegenstände, Berlin.

Zeitschrift für physiologische Chemie, Strassburg.