STUDIES ON EGG WASHING AND PRESERVATION

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STUDIES ON EGG WASHING AND PRESERVATION

By

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ABSTRACT

Present-day methods of egg washing have been more or less condemned because of increased susceptibility to microbiological invasion. This increased susceptibility of eggs to spoilage presents a major problem in cold storage, especially with regard to increasing egg production.

It is the purpose, then, of this work to develop a method of egg washing which will properly clean the egg, prevent microbiological invasion, and at the same time retain the physiochemical properties.

During the course of this work, two artificial soils were developed as a means of determining the relative efficiency of various surface-active detergents.

Through experimentation it was determined that Pseudomonas aeruginosa proved to be the most suitable organism available for this type of study. This was determined by three testing procedures devised by the author. These testing methods may also have application in future work of this nature.

As a consequence of experimentation concerning the original problems, an oil-in-water emulsion was successfully prepared. This

oil-in-water emulsion can be used to achieve a one-step operation for washing, sanitizing, and preserving eggs. By treating eggs with this emulsion, their microbiological sterility and physiochemical properties can be adequately preserved.

It is hoped that this oil-in-water emulsion will find application and will be of value to the egg industry and its various ramifications.

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INTRODUCTION

The egg loss due to breakage, blood spots, and spoilage in 1947 amounted to $651 million. Inasmuch as the total egg crop was worth $2,500 million, the percentage loss would be 26 per cent (22). There are no reliable figures on the amount of spoilage due to increased bacterial invasion caused by washing of the eggs, but losses due to this cause are large.

The washing of eggs has become an established practice so that any attempts to discourage the practice would be futile. There is a distinct need for proper methods of washing.

Although extensive study has been made, no dependable method of washing has been developed that is satisfactory. The purpose of this study, therefore, is to investigate the possibility of using chemical sanitizers in egg washing and preservation as well as to find a simple procedure whereby the producer can wash, sanitize, and preserve eggs in one operation.

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REVIEW OF LITERATURE

The egg's particular structure and the unequal distribution of its chemical constituents make it unstable. In addition, the interior of the egg is imperfectly protected and is, therefore, exposed to environmental conditions. For these reasons, the egg is in a continual state of readjustment which takes place at a rate controlled largely by external factors.

Physical disintegration within the egg is normal. In contrast, the type of decomposition that results from microbial growth, in a broad sense, may be considered pathological.

Physiochemical

From the time the egg is laid, physiochemical changes start, and the rate and degree of these changes determine the keeping quality of the egg. The measurement of these changes, caused by the methods of handling the egg, can be used to assay the quality of the egg at the time of the assay and determine the ultimate storage life of the egg.

The escape of moisture contained in the egg is the most important physiochemical process which gives rise to egg weight loss

and increased air cell height. Evaporation of water from the egg is a continuous process. It begins the moment the egg is laid, and it does not cease until the egg is completely dehydrated. At first, the weight loss is principally from the albumen, and later from the yolk as well.

Loss of egg weight under constant environmental conditions is almost a linear relationship with respect to time (108). In terms of the egg's original weight, however, the daily loss diminishes throughout the holding period (128).

The rate of weight loss is accelerated at higher temperatures, but can be lessened by maintenance of high relative humidities (108). Air movement tends to increase weight loss by the removal of higher humidities adjacent to the egg (127).

Physical properties of the egg also affect the rate of egg weight loss; the less porous the shell or the larger the egg, the less the weight loss.

Immediately after the egg is laid, its temperature is about 41°C (80), whereas the surrounding temperature is usually lower. When the egg cools, the contents shrink, forming the air cell. Thereafter, the air cell becomes larger as the egg's contents shrink due to loss of moisture. The size or height of the air cell can be

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revealed by candling. Since the United States standards for grading eggs take the height of the air cell into consideration, this characteristic is of significant value. The rate of air cell height increase is proportionate to the rate of egg weight loss.

The albumen quality is one of the most important methods by which egg quality is determined. The process of albumen deterioration, like all physio-chemical changes, may occur rapidly or slowly, depending upon environmental conditions. In the broken-out newly laid egg, the middle dense layer of albumen (the albuminous sac) stands up in an oval shape around the yolk and has a thick gelatinous consistency. As time passes, the albuminous sac decreases in height at first rapidly and later slowly (66); it also loses its firmness, so that it spreads out thinly over a wide area when the egg is opened. Eventually, the dense albumen can no longer be distinguished, the yolk becomes large and flattened, and if the egg has deteriorated sufficiently the vitelline membrane may rupture, and both albumen and yolk coalesce.

These changes in albumen may be expressed numerically according to a set of photographic standards published by the United States Department of Agriculture (11). The thinning of albumen increases (i.e., the numerical ~~number~~ value increases) as the egg becomes older, at a rate determined by the holding temperature.

Microbiological

The interior of the newly laid egg is usually free from microorganisms (123, 63, 64, 148, 156), chiefly because of the natural protection provided by the egg's physical and chemical structure. Contamination of the contents of the egg rarely occurs before the egg is laid. It is well known that Salmonella pullorum, the causative agent of pullorum disease, may enter the yolk in the ovary. Mallmann and Moore (94) found that the incidence of infected eggs from diseased birds may run as high as 56 per cent. The bird becomes infected by way of the digestive tract (124), and the organism is transported to the ovary by the blood stream (100). Experiments have not shown conclusively that ovarian infection follows the feeding or inoculation of certain bacteria (100, 103), but many organisms apparently arrive at the ovary by the same avenues traveled by S. pullorum. Among those found in both the ovary and the eggs are Salmonella anatum, Salmonella typhimurium, Salmonella enteritidis (20), and Salmonella gallinarum (124). The transitory presence of harmless bacteria in the ovary is also likely (117), for the latter tend to occur occasionally in eggs laid successively by an individual bird (99).

It is also possible that eggs sometimes acquire bacteria as they pass through the oviduct. Lawson (78) stated that bacteria were

often present in the oviduct. On the other hand, Rettger (123) maintained that the oviduct is sterile.

Haines and Moran (65) measured the diameter of the eggshell pores. They found that they may be as large as fifteen microns. This is large enough to permit bacterial penetration through the shell. The membranes that line the inside of the eggshell are composed of a matrix containing pores with an average diameter of one micron (157). Accordingly, bacterial penetration is mechanically possible.

Wilm (152) was probably the first to demonstrate the ability of bacteria to enter the egg through the shell. By smearing the eggshell with Vibrio comma or by immersing the egg into a liquid suspension of the culture, he proved that the shell is pervious to bacteria. This has been repeatedly demonstrated with organisms such as Pseudomonas species (81, 126, 148, 88, 17), Escherichia coli (152, 159, 17), Proteus vulgaris (159), Micrococcus pyogenes var. aureus (117, 17), and several others (63, 103, 64, 65, 159, 17, 6).

The most prevalent bacterial type found in eggs is the gram-negative short-rod group. Hadely and Caldwell (63) reported that 64 per cent of the bacterial flora of fresh eggs was very short rods. Haines (64) stated that only 32 per cent of the bacterial flora was nonspore-forming rods. By washing the eggs or holding them under

cold-storage conditions, the gram-negative short rods constitute overwhelmingly the major portion of the bacterial flora (1, 87, 145, 105).

It is remarkable that the interior of the egg rarely contains microorganisms, for bacteria and molds are usually present in great numbers on the surface of the eggshell. Rosser (133) reported that the surface of the shell of a fresh egg has an average of approximately 1,600 mold spores and 70,000 bacteria. The maximum bacterial number reported was 8 million (64).

Molds, as well as bacteria, have been demonstrated in the interior of eggs (123, 93, 159), although they may not constitute more than 4 per cent of the total microbial flora (123). Among the various mold genera that have been found inside the egg are Penicillium, Mucor, and Aspergillus (93, 159).

Experimental evidence suggests that enlargement of the pores by the mold hyphae may facilitate the entrance of bacteria (159). Eggs were smeared externally with a culture of Serratia marcescens, P. aeruginosa, P. vulgaris, or E. coli, and were kept four months at 18°C. The incidence of contamination of the contents was 70, 50, 30, and 20 per cent, respectively. When the inoculum consisted of the same organisms mixed with Aspergillus niger, the percentage of the internal contamination increased to 100, 100, 70, and 50 per cent, respectively.

The egg is exposed to contamination as soon as it is laid. As previously stated, the newly laid egg has a temperature of approximately 41°C, whereas the outside temperature is lower. As the egg cools, bacteria and molds are possibly drawn in with the air that forms the air cell. Using the same principle of differential pressures, Haines and Moran (65) were able to force yeast cells through the shell pores.

Regardless of all these factors, the internal contamination of fresh eggs is infrequent. The physical organization, and chemical constitution of the egg, together form a defensive system to combat invading organisms.

The shell obviously provides the egg with its greatest physical protection. Normally, the eggshell pores are filled with organic substances which hinder microbial penetration. It was believed that these organic substances, when dry, did not permit microorganisms to enter, and if they were dissolved or partially removed by abrasion, the pores were opened, and microbial invasion immediately became possible.

By an ingenious experimentation Rievel (126) was able to disprove this hypothesis. He spread a dry inoculum (a loopful from a slant culture) on the shells of twenty-three eggs. Thirty-five per cent of these eggs contained the test organism after 9 to 12 days.

The shell membranes, formed of interlacing fibers, may act as a filter for the removal of many organisms that might succeed in penetrating through the shell. It has also been shown that the shell membranes possess some bactericidal activity (148, 159).

The most important chemical defense mechanism is offered by the albumen. Firstly, although the pH of the white of a fresh egg is about 7.6, it rapidly changes to about 9.5, and therefore becomes unfavorable for bacterial growth. Secondly, the egg white contains lysozyme, which inhibits the growth of and may inactivate certain organisms (38). Thirdly, the natural proteins of the egg white are very resistant to bacterial attack. Bacterial decomposition is only possible when the resistant albumen proteins have been already partially decomposed or when other simple nitrogen sources may be available to the organisms within the egg.

Washing

Pennington and Pierce (113) examined a large number of eggs in a New York market. In one series of 258,496 dozen eggs they found 12.58 per cent were dirty, and in another series of 238,446 dozen eggs they found 13.4 per cent dirty eggs. These figures do not necessarily represent the situation on the farm at that time since

some dirty eggs which may have been washed at the source were classified as clean, hence the number of dirty eggs may have actually been higher than reported.

According to Brooks (15), Huttar (1928), in an investigation covering three years, found that, among the eggs produced by the Cornell poultry flock, the percentage of dirty eggs ranged from 9.8 per cent in July to 24.6 per cent in March, with an over-all average of 17.7 per cent for the year. This flock was maintained in the same manner comparable to that of the average commercial poultry man in the 1920's.

Funk (41) studied the factors influencing the production of clean eggs. He found that by gathering eggs four times daily rather than at the end of each day, the total number of dirty eggs for the day's production was reduced by as much as 50 per cent; however, even under the most ideal conditions dirty eggs could not be entirely eliminated. At the present time, under field conditions, about 10 per cent of the eggs produced are dirty (43, 118, 74).

The amount of soil carried on each dirty egg is quite variable. Soiled eggs are generally divided into three groups: "slightly dirty," "dirty," and "heavy dirty." Dawson and Watts (29) classified eggs more critically in six groups according to the amount of

soil on the shell. For each group, they gave a numerical value; the clean eggs (no soil) are given the numerical value of 1, and the extremely dirty eggs a numerical value of 6.

Each year more eggs are being marketed on a graded basis. Soiled eggs, even though they may be of the highest interior quality (AA), generally are sold as C grade, and at 10 to 15 cents less per dozen than received for the highest grades (155). This fact leads to increased egg cleaning on the farm.

The most generally recommended procedure for cleaning eggs has been to dry clean the slightly dirty eggs and to wash only the badly soiled eggs. Funk (45) showed that wet cleaning is more advisable than dry cleaning. Winter et al. (155) confirmed Funk's results. They used four different methods of dry cleaning. They found that all of them had the following undesirable features:

1. The abrasive removed the shell color as well as the dirt, in case of brown eggs. This left the eggs with noticeable scratches and light spots or bands on them.
2. The handling and rubbing action, necessary to remove stain and dirt by means of the abrasive, resulted in a higher percentage of cracked and broken eggs than when wet methods were used.
3. Final traces of stain were not removed as effectively by dry methods of cleaning.
4. It required considerably longer time to clean by dry methods than by wet ones.

Some large producers now wash all the eggs gathered. According to Black (7) and Pino (118), the eggs are washed in the wire egg baskets in which they are gathered. The baskets of eggs are immersed repeatedly in a hot (60°-70°C) detergent solution and then hosed with water of the same temperature. The procedure is said to save time and reduce breakage over that of sorting the dirty eggs and washing them separately.

Several investigators (143, 84, 145, 86, 87) showed that washed eggs do not keep as well as corresponding clean or soiled untreated eggs. On the other hand, some investigators (62, 118, 106, 54, 101) found little or no harm from cleaning eggs.

As a general rule most of the workers do not condemn washing in itself, but claim that by washing, eggs are rendered more susceptible to microbial invasion and quality decline. In other words, eggs lose part or all of their defensive mechanism when washed. If eggs were provided with a similar or better mechanism during and after washing, washed eggs should keep at least as well if not better than the untreated eggs.

Preservation

The egg has its maximum value as an article of food at the time it is laid. The start of change within the egg marks the beginning of its deterioration as a dietary commodity. The somewhat seasonal production, and the many phases of handling that the egg must endure before it reaches the consumer have long made its preservation desirable.

Although many methods of preserving the intact egg have been attempted, none has yet been devised that can entirely prevent changes from taking place. Essentially, all that the preservation can accomplish is to avert microbial invasion and to retard physiochemical deterioration for a reasonable length of time. These results could be achieved either by controlling the environment in which the egg is placed, or by treating the egg so that it is less easily affected by external conditions, or by both. Due to the apparent wide scope of the aforementioned points, only the treating of eggs will be discussed here.

It was noticed previously that, by treating the egg in such a fashion that the shell pores were completely or partially sealed, physiochemical deteriorations could be retarded.

Spamer (144) reported that, as early as 1807, the Dutch preserved eggs by placing them in linseed oil for half a day, then drying them on racks. Such eggs were kept for four to five months. This process continued to be used until about 1914, when it was replaced by other processes, one of which was pickling in lime water, a process first used in Holland about 1875.

Almy et al. (3) recommended the use of mineral oils as a substitute for organic oils, their reason being that mineral oils were not subject to rancidity.

Swenson and James (150) introduced their process in which oiling was done under vacuum. This method consists of evacuating the chamber in which eggs are immersed in oil, and then raising the eggs above the oil and releasing the vacuum in an atmosphere of carbon dioxide.

McIntosh et al. (89) determined the best time to oil-process eggs efficiently. He used physical and culinary methods as criteria. Eggs processed the day after gathering were superior to those processed immediately after laying. His results were confirmed by Evans and Carver (34). Eggs oiled after periods longer than one day did not keep as well as treated day-old eggs (9).

Evans (33), being aware of an excessive use of oil in oiling eggs, studied the effect of diluting oils with solvents derived from the distillation of petroleum. Results were measured by changes in the albumen index and air cell diameter of eggs held for two weeks at 15.5° to 26.7°C. He concluded that oil diluted up to 50 per cent with a solvent was as effective as undiluted oil. He noted, however, that the danger of the flammable fumes from such solvents more than offset the advantages of using the solvents. A few years later, Stewart and Bose (147) studied the efficiency of different solvent-oil mixtures. They found that a 10 per cent oil and 90 per cent solvent (pentane) mixture was as effective as undiluted oil.

Hearst and Hearst (68), in 1948, recommended the use of an emulsion for egg preservation. Their process, "stabilization of shell eggs," consists of using an oil-in-water emulsion of waxes having different melting points, to which is added a preservative (low molecular esters of parahydroxy benzoic acid). This emulsion is irradiated with ultraviolet light before using. The patentees claim irradiation of the emulsion imparts unusual preservative power to the emulsion.

Romanoff and Yushok (131) recommended the use of an emulsion of stearic and lactic acids in mineral oil to treat eggs. Such

treatment would seal the pores and reduce the egg weight loss and pH change of albumen. The effect of this treatment on the microbial flora was not determined.

Many other substances were used in sealing eggs. Funk (47) and Romanoff and Romanoff (130) presented excellent reviews on the use of these substances.

Since most of the sealing substances, including oil, do not prevent microbial invasion, control of bacterial and fungal activities became of great importance. Generally eggs are treated during preservation to inactivate the contaminating microbes. After treatment, however, eggs would not be resistant against subsequent contamination. All the physical and most of the chemical methods of combating microorganisms give rise to such conditions.

On the other hand, some chemical disinfectants exert their germicidal power over a long period of time. By applying such chemicals in treatment, eggs can be protected for a long period starting from the time of treatment until the germicidal activity of the disinfectant is exhausted or until the time when the eggs are consumed. Unless there is a great deal of contamination, the latter condition prevails.

The most universal physical method to combat microorganisms is heating. Although it is an old method, Funk (44), by introducing his thermostabilization process, renewed the interest of many investigators. By this method fertile eggs were devitalized, the thick albumen was stabilized, and a pasteurization effect was obtained. These results were secured by immersing eggs in hot water or oil long enough to heat them throughout. Eggs were immersed for 10 minutes in oil held at 60°C. This process was modified later to 15 minutes at 54.4°C. Several reports have been published (9, 137, 49) confirming these results.

Thermostabilization, however, has several disadvantages, Funk (47) stated:

> 1. The albumen of the thermostabilized eggs required more time for whipping and the volume of foam was reduced.
> 2. The incidence of stuck yolks was increased by the process.

The adverse effect of pasteurization on egg white was confirmed later by Goresline et al. (60), Clinger et al. (25), Salton et al. (137), and Carlin and Foth (24). Scott and Vickery (138), however, were not able to confirm Funk's results concerning the effect of pasteurization on the incidence of stuck yolk.

Mallmann and Davidson (91) recommended the incorporation of pentachorophenol in oil. They found that 0.25 per cent completely inhibited mold growth when incorporated in oil. They also used orthophenylphenol, but found it less effective.

Miller et al. (106) subjected Roccal and sodium pentachlorophenol to a severe test. They broke black rot eggs in the washing water to increase the incidence of rot development. They found that sodium pentachlorophenol in concentrations up to 0.5 per cent and Roccal up to 1 per cent did not prevent rot formation.

Gillespie et al. (54) claimed the spoilage of washed eggs is due to contamination from the egg-washing machines. By using 1 per cent calcium hypochlorite spoilage was reduced due to inactivation of rot-producing bacteria.

Winter et al. (155) studied several egg-washing procedures. Eggs washed with warm (49°C) water containing a detergent sanitizer (Emulsept) were the only ones found to keep as well or better than the soiled untreated eggs.

Forsythe et al. (40) recommended the use of a detergent sanitizer in washing eggs. They showed that the detergent Vel reduced the bacterial load of the shell approximately 82.2 per cent. This reduction was due to the mechanical removal of bacteria from the surface of the egg shell.

Gorseline et al. (59) noted that egg breakage during thermostabilization depends on temperature; the higher the temperature, the greater the breakage, especially in day-old U. S. Grade "AA" eggs.

Romanoff and Romanoff (129) tried a flash heat treatment using boiling water. By exposing eggs to such treatment for 5 seconds, a thin, protective film of coagulated albumen was found to adhere to the shell membrane. Feeny et al. (36), although using temperature up to 1000°C, recommended the use of boiling water.

Other physical methods of combating microbial contaminants such as X-ray radiations (9, 47) were found to be ineffective.

Chemical disinfectants have been applied to eggs since egg washing became a practice. Funk (42) advocated the use of 1 per cent sodium hydroxide (NaOH). Due to the danger of handling and the question of effectiveness, this method was not successful.

Penniston and Hedrick (114, 115, 116) recommended the use of a quaternary ammonium compound. They found fewer rots when they added the germicide to the wash water.

Rosser et al. (134) examined hydrogen peroxide, dimethylol urea, borax, and urea for their effect on molds. He found that dimethylol urea in a concentration of 2.8 per cent inhibited the molds completely. The rest of the compounds were less effective.

Conner et al. (26) washed eggs at 74°C before oiling. They incorporated 0.25 per cent pentachlorophenol in the oil as a germicide. By this method the number of bacteria in egg contents was kept low.

Winter (154) examined six different commercial preparations containing quaternary ammonium salts. The active ingredients in washing water varied from 100 to 1000 p.p.m. When dirty eggs were washed with such solutions, the percentage of rots increased to 15 to 30 per cent, compared to 4 per cent in the unwashed eggs.

Funk et al. (49) studied the value of sanitizers in egg washing. When soiled eggs were washed in water containing 162 p.p.m. of the sanitizer, before oiling, they found 8.12 per cent loss in treated eggs. By adding approximately 1800 p.p.m. of the sanitizer in oil, the percentage of loss was decreased to 7.69 per cent. The untreated soiled eggs had only 2.99 per cent loss.

Miller (104) studied the effect of some treatments on the incidence of gram-negative (spoilage) bacteria in significant numbers (more than 1,000 organisms per egg) in egg contents. When eggs were washed and rinsed in 2000 p.p.m. Roccal before being oiled, 23 per cent of the eggs contained spoilage bacteria. When 1 per cent NaOH was used as a disinfectant solution, 19 per cent were

found to contain spoilage organisms, whereas only 8 per cent of the untreated eggs contained spoilage bacteria.

In reviewing the work that has been done up to this time, the lack of adequate methods for washing and preserving eggs under field conditions is indicated. It would seem that further investigations under carefully controlled laboratory conditions would yield a better evaluation of the various procedures available as well as new methods and reagents. Then once a method of egg washing, disinfecting, and preserving measures up to the requirements of the laboratory ~~study~~ study and the limitations of each process have been determined, it may then be used under field conditions with a better chance for success.

EXPERIMENTAL PROCEDURES AND RESULTS

In egg washing and oiling two procedures are generally followed. Either eggs are washed in a detergent solution, rinsed in a disinfectant, then oiled, or eggs are washed in a detergent-sanitizer and then oiled. After washing and before oiling egg shells should be dry. Any water droplets on the shell would form pockets which prevent oil from reaching the shell and sealing the pores.

It was thought to combine all these steps (cleaning, disinfecting, and oiling) in one operation through the use of an oil-in-water emulsion containing a detergent and a disinfectant. To select each of the ingredients for such an emulsion the forthcoming experiments were performed.

Detergent Study

Experimental

On naturally dirty eggs soil is generally a heterogeneous mixture of many substances with different physical and chemical characteristics. The natural soil may be chicken manure, especially in open nest laying houses, or it may be mud encountered frequently in

rainy seasons, or it may be egg contents whenever methods of handling are inadequate, et cetera. It may also be a combination of two or more of these types; for example, the soil may be chicken feces mixed with feathers or egg meat covering a blood stain. The amount of soil on the naturally dirty eggs is variable. Sometimes only a small spot is found on the eggshell, and sometimes the entire surface is soiled. Dawson and Watts (29) classified dirty eggs into five groups according to the amount of dirt on the shell. Generally speaking, however, eggs are classified as "slightly dirty," "dirty," and "heavy dirty." It should be understood that there is no clear-cut line separating these divisions, and the extent of the overlapping is left to human judgment. Although there has not been any work done on the incidence of dirt on each surface area of the eggshell, there is no reason to believe that one location would be more frequently soiled than another. This should not, however, subordinate the fact that individual eggs differ greatly according to location of soil on the shell. For reasons mentioned above, it is a poor practice to use naturally dirty eggs in laboratory studies unless a large number are used to make up for these great variations. In such a case a standard soil would be desirable.

It is obvious that the ideal standard soil should be a natural one; therefore, soils containing chicken manure alone or manure diluted with water to 25, 29, 33, 40, 50, and 67 per cent by weight were tested. It was found that suspensions of 33 and 40 per cent gave the best results. More-concentrated soils gave a thick, lumpy, uneven film, whereas more-diluted suspensions were so light that complete coverage of the eggshell was never attained. Eggs were dipped momentarily in 33 or 40 per cent soil suspensions and dried either slowly, at room temperature for periods of 12, 24, and 36 hours, or quickly, at 65.5°C in an oven for 30, 60, and 90 minutes, or by a combination of both. It was found, however, that when these eggs were passed twice through a G. L. F. washer[1] using tap water at 55°C (in the pail), soil film was removed from all the treated eggs. This indicates that the soil was not attached strongly enough to warrant the use of a detergent.

In chicken manure soil, the mucous material present acts as an adhesive agent. When it was diluted the mucous material was diluted

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[1] G. L. F. egg washer, Model 210, is a rotary-type machine in which eggs are cleaned by the action of nylon brushes, wet with water supplied from a pail placed on top of the machine. It takes 15 seconds for an egg to run through the machine once. Water is used only once.

accordingly. The lack of sufficient concentration of the mucous material might help to explain why the films made with 33 and 40 per cent suspensions were easily removed.

An attempt to develop an artificial soil using egg albumen was made. Egg-albumen powder and rhodamine B (a red fluorescent water-soluble dye) in concentrations of 1 and 0.025 per cent by weight, respectively, were mixed well in a Waring Blendor for two minutes, then sprayed onto the eggs. Sprayed eggs were left at room temperature for five to six hours until dry, then heated at 65.5°C for 30 minutes. By washing soiled eggs with water as before, the soil was not removed to a great extent.

Anionic detergents are inactivated slowly in the presence of cations such as calcium and magnesium which constitutes the major portion of the eggshell. For this reason it is not advisable to use anionic detergents in egg-washing solutions. Cationic detergents, on the other hand, lose some of their activity and/or interfere with the activity of several germicides (79). Since the germicide was not yet chosen, several nonionic detergents and one cationic detergent were tested.

Detergents are affected to varying degrees by water hardness; some are susceptible while others are resistant. To prevent the

interference of water hardness, 0.1 per cent sodium tripolyphosphate was dissolved in the water solution before the detergents were added.

A series of egg-washing tests was made using eggs treated with artificial soil. Detergents which were used are presented in Table I. Each detergent was used in a concentration of 0.1 per cent by weight in tap water at 55°C. Washing was done by the aid of a G. L. F. egg washer through which eggs were passed twice. The results of one of these tests are presented in Figures 1 to 3. As indicated by these figures, Sterox AJ and Sterox CD (nonionic detergents) were the most efficient in removing this artificial soil. Although this soil gave a measured success, it was not critical enough to show the difference in the activities between Sterox AJ and Sterox CD.

A new approach was attempted to overcome the difficulty of attaching soil containing chicken manure to the eggshell. In this second artificial soil, albumen was incorporated as an adhesive agent. The soil containing 33 per cent chicken feces and one per cent albumen powder were mixed well in a Waring Blendor for three minutes. Eggs were immersed in the suspension momentarily and left to dry slowly for 15 hours at room temperature before heating at 65.5°C for 90 minutes. Using the same washing procedure, several tests were

TABLE I

DETERGENTS TESTED

Trade Name	Chemical Name	Manufacturer
Alro amine 0*	Heterocyclic tertiary amine	Alrose Chemical Company
Ethofat 242/25	Mono-fatty-and-resin-acid esters of polyethylene glycols	Armour and Company
Igepal CO - 633	Alkyl phenoxy poloxy-ethylene ethanol	Antara Chemical Division of General Dye Stuff Co.
Neutronyx 600	Alkyl phenol polyglycol ether	Onyx Oil and Chemical Company
Sterox AJ	Alifalic nonionic poly-ethylene ester	Monsanto Chemical Company
Sterox CD	Polyethylene ester	Monsanto Chemical Company
Synthetics D37	An ethylene oxide condensate based on hydro-alsiethyl alcohol	Hercules Powder Company
Synthetics E80	A rosen ethylene oxide condensate mixture	Hercules Powder Company
Triton X100	Alkyl aryl polyether alcohol	Rohm and Haas Company

* Also amino 0 is the only cationic detergent; the rest are nonionic.

THE EFFECT OF VARIOUS DETERGENTS ON EGGS SPRAYED WITH ALBUMEN POWDER-RHODAMINE B SOIL

Figure 1

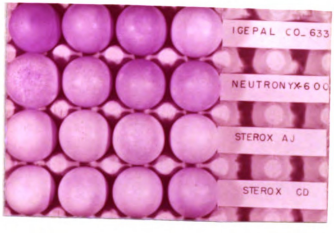

Figure 2

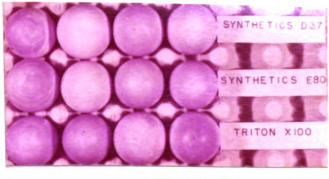

Figure 3

performed to compare the activities of three nonionic detergent solutions: Sterox AJ, Sterox CD, and Triton X-100. Figures 4 and 5 show the results of a typical run where the three detergent solutions were compared with each other, and with unwashed eggs and with eggs washed in water only. From these results it was noticed that Sterox CD was superior to the rest, followed closely by Sterox AJ.

In the production of albumen powder its physiochemical properties may have been changed. To determine if this was the case, 1 per cent albumen powder was substituted with 8.2 per cent fresh egg white (containing approximately 12.2 per cent total solids) in the second soil. By testing soil containing fresh egg white no particular advantage was noticed in its use over the soil containing albumen powder.

Discussion

The selection of an artificial soil by which detergents could be evaluated according to their efficiency in cleaning eggs was found to be difficult. Natural soils such as chicken manure alone, or diluted to various degrees with water, were tested. None of these soils was satisfactory; in the more highly concentrated soils the films obtained were uneven and lumpy, whereas in lower concentrations

THE EFFECT OF VARIOUS DETERGENTS ON EGGS
TREATED WITH CHICKEN MANURE SOIL

Figure 4

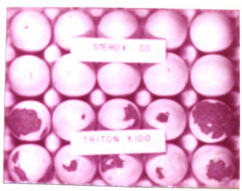

Figure 5

they were too light to give an even coating. Although suspensions of 33 and 40 per cent gave even films, they were easily removed due to insufficient adhesive power.

The addition of albumen either as a dried powder or as a fresh egg white to the 33 per cent suspension chicken manure improved the adhesive power of the soil. The retention of the soil when washed was greater, thus allowing a wider range of soil removal for comparative purposes.

A soil composed of 1 per cent albumen powder plus a dye showed some possibilities of being a good soil for the general screening of detergents. This soil, beside being easy to prepare, gave more-consistent results in repeated experiments.

With most of the commercial detergent mixtures presoaking is a must before an effective cleaning can be achieved (74). The mixtures which do not require presoaking need a long washing period. The longer the period of either soaking or washing the greater the chance for microorganisms to penetrate the shell (85), and thus a more prolonged activity is required of the bactericidal agent. Any contribution by which this period can be shortened has a value in reducing the extent of bacterial invasion. This increases the importance of the choice of detergents since they are the governing

factors in determining the length of time required for the effective washing period.

When the albumen-dye soil was used, the two Sterox detergents showed the best activity. Both detergents are nonionic types. The reason for their superiority over the cationic and the other nonionic detergents is not known.

Test Procedures

Experimental

Although "clean" eggs may look clean, they actually carry a large number of bacteria on the shell. According to Forsythe et al. (40), the clean eggshell has at least 10,000 microorganisms. For this reason it is very difficult to open the egg aseptically, without contamination by microorganisms. Early workers depended on disinfectants to sterilize the eggshell before they opened it (111, 99). Due to incomplete understanding of chemical disinfection, investigators modified these procedures by flaming after disinfection. Mallmann and Moore (94), for example, submerged the eggs in 1:1000 $HgCl_2$ solution for 10 to 30 minutes. Then they placed them on small beakers, and while the eggs were still moist a sharp-pointed gas flame was applied to the shell covering the air sac. By this method

the shell was forcibly removed. Wolk et al. (156) immersed eggs in $HgCl_2$ for 30 minutes, dipped them in 95 per cent ethanol, burned it off, and then carefully flamed them. They found, however, that this method was inadequate. Later investigators returned to chemical disinfectants without flaming. Bigland and Papas (6) washed eggs in 450 p.p.m. Roccal for one hour, followed by drying and painting with 1:1000 tincture of merthiolate. Connor et al. (26) washed eggs in distilled water, followed by dipping in alcohol, drying, and then painting both ends with Churchill's iodine.

In this study calcium hypochlorite solution, in a concentration of at least 1:1000 of active chlorine, was used to disinfect the eggshell. Eggs were immersed in this solution for one to two minutes. A circle of approximately one inch in diameter was drilled with the aid of an electric hand drill on the blunt end of the egg. The shell section was removed with a sterile forceps and the egg contents were examined. When twelve 24-hour-old clean eggs were examined, as above, no bacteria were recovered from the egg contents.

Four of the bacteria generally present in spoiled eggs were investigated. The speed by which each organism penetrates the egg was considered as a criterion for choosing the test organism. In testing the bacterial penetration into eggs, experiments using 24 or 36

eggs were carried out. All eggs were obtained from a White Leghorn flock at Michigan State College. Clean, white, sound-shell eggs were used when they were 24±6 hours old. Each batch was divided into four groups of six or nine eggs each. Each group was immersed into a 24-hour F.D.A. broth culture of one of the four test organisms (M. pyogenes var. aureus, E. coli, P. vulgaris, and P. aeruginosa). All cultures were obtained from the stock cultures of the Department of Microbiology and Public Health, Michigan State College, with the exception of P. aeruginosa,[1] which was recently isolated from a spoiled egg. Cultures were transferred daily in F.D.A. broth. Eggs were submerged for about two minutes in the culture at room temperature, dried quickly in front of a fan, then held in moisture-saturated atmosphere following the technique used by Mallmann and Davidson (91). Eggs were put in sterile 2-quart, Mason-type, wide-mouth jars. A block of wood was placed in the bottom of each jar and surrounded by water so that a saturated atmosphere would be obtained without direct contact between the water and the eggs. A square portion of an egg flat with receptacles for four eggs was placed on the wooden

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[1] Classified according to the key presented by Haynes (67), which will be used in the forthcoming seventh edition of Bergy's Manual.

block. Four eggs or less were put in each jar. The jars were incubated at 36°C for periods of 4, 6, 8, 10, 14, and 18 hours.

Eggs were opened as above and three examinations were performed:

1. Egg-white test. By the aid of a sterile graduated 2-ml pipette the membrane was punctured, and 0.1 ml of the egg white was transferred to a 10-ml F.D.A. broth tube. These tubes were incubated at 36°C for 48 hours.

2. Rinse test. Egg contents were removed and a 2-ml portion of a saline solution was pipetted inside the shell. Saline was mixed well with the albumen adhering to the membrane by reducing and increasing the pressure through the pipette. A 1-ml portion of this mixture was delivered into a 9-ml F.D.A. broth tube which was incubated as before.

3. Swab test. Eggs were emptied again and, with the narrow end up, the inner shell membrane was swabbed over an area of about one inch in diameter at the narrow end of the egg. Then the swab was broken into a 10-ml F.D.A. broth tube which was incubated as above.

Aseptic techniques were followed as closely as possible throughout all these tests. In any case of doubt that the recovered organism

was not the one originally tested, microscopic examination and physiological tests were carried out.

Results of these tests are compiled in Table II. No penetration of the eggshell by any of the test organisms was detected after an incubation period of four hours. After six hours, P. aeruginosa was recovered from two of the twenty-four eggs tested and E. coli was recovered from only one egg.

The highest number of recoveries was obtained with P. aeruginosa, followed by E. coli and P. vulgaris (close together).

The efficiency of each of the tests was calculated as follows:

$$\text{Efficiency of the test} = \frac{\text{the number of infected eggs detected by the test}}{\text{total number of infected eggs from the three tests}} \times 100$$

Table III shows the calculated efficiency of each test for each period and test organism.

Discussion

According to results obtained, the swab test gave the earliest and highest recovery in this particular study. This agrees with the results of Stuart and McNally (148) and Mallmann and Davidson (91), who found better recovery from the membranes than from either the whites or yolks. This can be explained by the fact that by swabbing,

TABLE II

SPEED OF PENETRATION OF FOUR ORGANISMS AS DETERMINED BY THREE METHODS

Test Organism	Period (hours)	Number of Positive Eggs			
		Egg White Test	Rinse Test	Swab Test	Total*
Micrococcus pyogenes var. aureus	4	0	0	0	0
	6	0	0	0	0
	8	0	0	0	0
	10	0	1	3	4
	14	5	6	6	8
	18	13	15	16	20
Escherichia coli	4	0	0	0	0
	6	0	0	1	1
	8	0	3	5	5
	10	1	4	5	7
	14	3	13	12	15
	18	16	19	20	21
Proteus vulgaris	4	0	0	0	0
	6	0	0	0	0
	8	0	4	6	6
	10	0	7	9	10
	14	4	12	14	16
	18	10	16	17	20
Pseudomonas aeruginosa	4	0	0	0	0
	6	0	0	2	2
	8	1	4	9	10
	10	3	12	13	16
	14	9	15	17	19
	18	18	20	21	22

* The total of all eggs positive by one or more tests.

NOTE: Twenty-four eggs were examined at the end of each period for each test organism.

TABLE III

EFFICIENCY OF EACH OF THE THREE METHODS OF TESTING IN DETERMINING BACTERIAL PENETRATION

Test Organism	Period (hours)	Total Positive Eggs	Per Cent Efficiency of		
			Egg White Test	Rinse Test	Swab Test
Micrococcus pyogenes var. aureus	4	0	0	0	0
	6	0	0	0	0
	8	0	0	0	0
	10	4	0	25	75
	14	8	63	75	75
	18	20	65	75	80
Escherichia coli	4	0	0	0	0
	6	1	0	0	100
	8	5	0	60	100
	10	7	14	57	71
	14	15	20	87	80
	18	21	70	90	95
Proteus vulgaris	4	0	0	0	0
	6	0	0	0	0
	8	6	0	67	100
	10	10	0	70	90
	14	16	25	75	88
	18	20	50	80	85
Pseudomonas aeruginosa	4	0	0	0	0
	6	2	0	0	100
	8	10	10	40	90
	10	16	19	75	81
	14	19	47	79	89
	18	22	82	91	95

the inner membrane was ruptured permitting the organisms between the membranes to be recovered giving rise to greater numbers and earlier recovery.

The rinse test was less accurate than the swab test but much better than the egg-white test. This result might be expected for two reasons. The first is that the thin film of egg white adhering to the membrane was mixed with the saline. This film is the first part after the membrane that comes in contact with the invading bacteria. The probability that this egg-white film contained more bacteria than any other part of the white is quite understandable. The second reason, although the writer has no proof to back it up, is that rinsing or moistening the membrane changes its physiochemical characteristics in such a manner as to cause an increase in permeability. Stewart (146) mentioned that Nathusius in 1868 reported that the membrane fibers swell in water or glycerol and shrink in alcohol. When Moran and Pique (108) used a saturated lime solution containing 5 per cent sodium chloride as a medium in which to store eggs, they found that the air sac, which was originally present, was filled after storage. Since the osmotic pressure in the lime solution was higher than that inside the egg, they postulated that the lime solution attacked the egg membranes and destroyed their permeability.

These two reports may help to strengthen the idea that the permeability of the membrane may be affected when eggs are moistened.

Brownwell (17) studied the bacterial penetration into eggs by testing egg whites. When he used M. pyogenes var. aureus and stored eggs for 8, 12, 14, 16, 18, and 20 hours, he recovered the bacteria from 0, 33, 17, 83, 92, and 83 per cent of the respective periods of exposure. With E. coli after 12, 14, 16, 18, and 20 hours' storage his recovery was 0, 25, 58, 92, and 100 per cent. He failed, however, to mention the amount of white used in sampling, which might have been larger than that used by the author and consequently could be responsible for the higher recovery.

Rievel (126) opened eggs aseptically, removed the contents, and covered the interior surface with a layer of agar. The outside surface of the shell was immersed in a broth culture of a fluorescent organism and stored at 18°C. One hundred per cent pentration was noticed in two to four days. Although he examined the eggs daily he failed to mention the progressive percentage of penetration which occurred.

Stuart and McNally (148) swabbed one-half of the shell of freshly laid eggs (still warm) with a 48-hour culture of P. aeruginosa. Immediately after this treatment and after storage periods of 3, 6, 18,

24, 48, 66, 96, and 192 hours eggs were opened aseptically. Yolks, whites, and membranes were tested for the presence of the organisms. The results showed that five out of the twenty control (untreated) eggs had infected membranes. Immediately after treatment twelve eggs out of twenty were positive, and an additional four were infected after three and six hours' storage at room temperature. Three more eggs were infected, giving the maximum of nineteen out of twenty eggs after eighteen hours. This recovery is much higher than that obtained by the author. These data show that, of the eggs which were tested immediately (within a few minutes) after smearing with the culture, twelve had infected membranes, two had infected whites, and one had an infected yolk. It seems improbable that bacteria can penetrate through the shell, membranes, white, and vitelline membrane to the yolk in such a short time.

Mallmann and Davidson (91), studying the penetration by P. aeruginosa, used the technique of Stuart and McNally (148), except for a few adaptations that were made to improve the method of culturing to lessen the extent of contamination. Eggs were examined after 1, 2, and 48 hours. They found the membranes, whites, and yolks to be negative.

In another experiment to increase the chance of bacterial contamination, the same authors stored eggs in a saturated atmosphere for 2, 7, and 10 days. In this case their results showed no bacterial invasion during the 2- and 7-day storage periods. After 10 days, however, one membrane from the four eggs tested was infected. These results were much lower than those obtained by the writer. This may be due to the method of applying the bacterial culture to the eggs. They swabbed the small end of the egg, whereas the author submerged the eggs in a broth culture for two minutes. By the latter method the probability of bacterial invasion is higher.

The method of applying the bacteria to the eggshell is important. This has been shown by Rievel (126), since when he immersed the eggs in a broth culture, 100 per cent invasion was obtained in two to four days. When he used a dry inoculum from an agar slant culture only 35 per cent of the eggs were infected in 10 to 11 days. Mallmann and Davidson (91) also, when they swabbed the narrow end of the eggs, observed no penetration even after 36 hours, but when they immersed eggs into a bacterial suspension they found that, of the twelve eggs tested, one egg yielded organisms on the membrane and in the white, and one yielded organisms on the membrane when tested immediately after treatment.

Brownwell (17) also studied the speed of penetration of P. aeruginosa. Eggs were stored at 37°C and 100 per cent relative humidity for periods of 4, 8, 12, 14, 16, 18, and 20 hours. Immediately after storage periods, eggs were opened and whites were cultured. Eggs were still free from organisms even after 8 hours' storage. Seven eggs out of the twelve tested showed infected whites at 14 hours' storage, two more eggs were positive at 16 hours' storage, and at 18 hours' storage all eggs had bacterial invasion. His recovery was slower but the percentage of recovery was higher. This may be attributed to the stock culture strain which he used, in comparison to the freshly isolated culture used by the author. He used a small number of eggs, which was evident as in a later experiment he could not recover the organism except after 24 hours' incubation. In this case he obtained only one egg with infected white from the eighteen eggs tested.

Lorenz et al. (88), in developing the "standard infection technique," submerged prewarmed (36°C) eggs in a suspension of a Pseudomonas strain. The suspension in which eggs were kept for five minutes at 15°C contained 1×10^6 viable organisms. Eggs were allowed to dry at room temperature before being stored at 15°C. They detected the infected eggs by candling, using an ultraviolet

lamp. The first fluorescent egg was observed in less than one week. According to Buchanan and Fulmer (19), Pseudomonas excretes a leuco compound which oxides to a green pigment in the medium. To get enough pigment to be detected by the ultraviolet candler a high bacterial load is needed. The 24-hour broth culture of Pseudomonas usually contains approximately 5×10^9 viable organisms in contrast to 1×10^6 in the suspension. These two facts may explain the very low incidence of invasion recorded by them.

Recently Elliot (32) inoculated eggs in the air sac with a bacterial suspension in saline of Pseudomonas ovalis. These eggs were stored for 1, 2, 3, 6, 8, 10, and 30 days at 15°C. All the eight eggs that were tested after one day's storage showed infected membranes but only one had bacteria in the white. When he used a bacterial culture, however, the only egg tested had an infected membrane and white after one day's storage.

Pseudomonas is known to be the single species that causes the greatest bacterial spoilage in washed or cold-stored eggs (27, 85, 145, 104). It is also known for its resistance to the bactericidal properties of egg white (37). These characteristics, and the fact that it can penetrate into the egg very quickly, make this species the most ideal test organism for any egg-washing or egg-storage experimentation.

It is important to note that, when conditions of moisture and temperature favorable for bacterial invasion prevail, eggs could be penetrated within six hours. Every precaution should be taken, therefore, in handling eggs before, during, and after storage in order to prevent these conditions from occurring.

Water-Soluble Disinfectant

Experimental

As the name indicates, bisphenols are composed of two phenolic structures united by various linkages. Certain members of this chemical group not only possess high bacteriostatic or fungistatic powers, but also give rise to high residual values. For these reasons, they contribute in several important practical applications. The outstanding usefulness of these compounds at the present time is in antiseptic soaps (77, 70). They are also being used in the textile industry (96), in some pharmaceutical preparations (15, 5), in agriculture (58), and several other fields. Although various fields were investigated, the application of bisphenols in egg washing has never been reported.

Fahlberg, Swan, and Seaston (35) tested the retention of 2,2'-dihydroxy-3,3',5,5',6,6'-hexachlorphenolmethane (known as G-11)

on human skin. Subjects washed their hands for six minutes with a soap containing 1 per cent G-11 for three successive days, after which the use of the antiseptic soap was stopped. For the following four days, a skin-ether extract was analyzed chemically and bacteriologically. They recovered approximately 8.0 micrograms of G-11 from each square inch of skin immediately after the third application. On the following day they obtained 0.4 microgram; two days later, 0.08 microgram; and from the third day on it was not recoverable. The bacteriostatic activity followed the same pattern as the chemical analysis.

Since bisphenols have a very low solubility in water, it was thought that the retention of G-11 by the skin is due to the fats present on it. These workers disproved this idea by washing hands with ether acetone to remove the fats present. When antiseptic soap containing G-11 was used on the defatted skin it gave better residual values. This indicates that fats do not retain G-11, but may actually interfere with its retention.

To test the retention of bisphenols by the eggshell, 2,2'-dihydroxy-3,3',5,5'-tetrachlorphenol sulphide,[1] having the generic

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[1] Produced by Monsanto Chemical Company under the trade name of "Actamer."

name of bithionol, was available. The solubility of bithionol in water is very low, being in the order of 0.0004 per cent at 25°C. The addition of an alkali such as NaOH to form the monosodium salt increases the solubility markedly. During the course of this study a monosodium salt was used instead of the original compound. For brevity, monosodium bithionate will be mentioned in this paper as M.S.B. M.S.B. was prepared by dissolving the needed amount of bithionol in a concentrated solution of NaOH; the excess alkali was neutralized with dilute HCl which was added until one more drop would turn the clear solution cloudy. It was noticed that the solution starts to get cloudy when the pH drops below 10.3.

Six large clean eggs were sprayed with a 24-hour broth culture of M. pyogenes var. aureus. After the eggs were dry, they were immersed into a 1:1000 solution of M.S.B. Eggs were disinfected for two minutes at 50±1°C; then they were left to dry at room temperature for about thirty minutes. A disc, one inch in diameter, was drilled out from the blunt end of each egg. Each disc was put on a seeded plate. T. G. E. agar plates were inoculated with 1 ml of a 24-hour broth culture of M. pyogenes var. aureus. Plates were incubated at 36°C for 24 hours. All plates showed zones of inhibition. This indicated that M.S.B. had a residual value when eggs were treated with its solution.

A more drastic treatment to show the extent of the retention of M.S.B. was tried. Six eggs were sprayed as before; however, after disinfection the eggs were resprayed. Using the technique developed by Mallmann and Davidson (91), eggs were put in wide-mouth, Mason-type, 2-quart jars where a saturated atmosphere of moisture was attained. By this method eggs would be coated with a thin water film throughout the period during which they were in the jars. The jars were incubated at 36°C for 48 hours after which eggs were examined as before. All plates showed definite inhibition zones after this drastic treatment. From these results it is evident that M.S.B. has a high residual value when used as an egg sanitizer.

When M.S.B. showed this remarkable activity against M. pyogenes var. aureus, its activity was challenged against other bacteria encountered frequently in spoiled eggs. Twenty-four large clean eggs were immersed in 4.5 liters of 1:1000 solution of M.S.B. for two minutes at 50±1°C. When the eggs were dry, discs were drilled out and placed on seeded agar plates. Plates were inoculated with 1 ml of a 24-hour broth culture of one of the following organisms: E. coli, P. vulgaris, and P. aeruginosa. Each bacterial culture was used to inoculate eight plates. All plates were incubated at 36°C for 24 hours. No plate showed any sign of antibacterial activity. Since no

activity was found against the last three organisms the use of bithionol in egg disinfection is of questionable value.

Two more bisphenols, 2,2'-dihydroxy-3,3',5,5',6,6'-hexachlordiphenolmethane,[1] having the generic name of hexachlorophene, and 4,4'-isopropylidene diphenol[2] were used. The sodium salt of each compound was prepared as before. Eggs were sprayed, disinfected, and tested following the first procedure described above except that the test organism instead of being M. pyogenes var. aureus was P. aeruginosa. Neither of these two compounds showed any activity against the test organism which hinders their use as egg sanitizers.

Since bisphenols did not possess the desired activities against P. aeruginosa, other chemical disinfectant groups were examined. Seven compounds representing three chemical groups were investigated.

The quaternary ammonium surface-active compounds have attained a prominent and important place in the fields of medical and general disinfection during the past twenty years. These compounds are of particular interest in that, unlike most disinfectants,

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[1] Produced by Sindar Corporation, under the name G-11.

[2] Produced by Dow Chemical Company, under the name of bisphenol A.

they exhibit not only germicidal action but also surface-active, detergent, and wetting properties. In this study this group was represented by three compounds: Roccal, Arquad S, and Hyamine 2389.

Halogens have been used as disinfectants since the eighteenth century. The remarkable ability of chlorine to arrest putrefaction and destroy odors attracted the attention soon after its discovery in 1774, and its use met with almost immediate approval. Chlorine is now the most widely used of all chemical disinfectants, largely due to its almost universal application in the disinfection of questionable water supplies and its ability to render sewage less objectionable. Klenzade XY12 (sodium hypochlorite), Iodine suspensoid Merck (colloidal iodine), and Iosan (iodophore) were investigated from the halogen group.

Brewer (13), in developing his metal Petri dish cover with the adsorptive disc, impregnated the disc with 5 ml of 1:30 phenyl ethyl alcohol in acetone. The impregnated pad and the metal cover were placed over an agar plate that had been streaked with Proteus, P. aeruginosa, and M. pyogenes var. aureus. After incubation, the gram-negative organisms did not overgrow the surface, whereas M. pyogenes var. aureus grew abundantly.

In a later publication, Brewer et al. (14) demonstrated the high bacteriostatic activity of phenyl ethyl alcohol against P. aeruginosa when incorporated in ophthalmic solutions.

Due to these encouraging reports, phenyl ethyl alcohol was investigated for its use as an egg sanitizer. This was the only compound examined from the alcohol group.

Large, clean white eggs were sprayed with a 24-hour broth culture of P. aeruginosa. After they were dry, the eggs were immersed in the disinfectant solution at 40±2°C for two minutes. All disinfectants were tested at a concentration of 1000 p.p.m. active ingredients. Higher temperatures were not used as they may have a pasteurizing effect on the test organism.

Each group of ten eggs was immersed in 1350 ml of disinfectant solution at the same time (approximately the same ratio of 140 eggs in 5 gallons used in egg-washing machines). When eggs were dried, discs were cut from the blunt end of the shell as before, and placed individually in sterile agar plates. At the end of 72 hours' incubation at 36°C, the plates were examined with the results shown in Table IV. Phenyl ethyl alcohol was the only compound that did not show activity in the concentration of 1:1000.

TABLE IV

GERMICIDAL ACTIVITY OF DIFFERENT COMPOUNDS AGAINST PSEUDOMONAS AERUGINOSA IN A CONCENTRATION OF 1000 P.P.M. ACTIVE INGREDIENTS

Trade Name	Chemical Name	Manufacturer	No. of Eggs Tested	No. of Eggs Positive
Roccal	Alkyl dimethyl benzyl ammonium chloride 10%	Winthrop-Stearns	10	0
Arquad S	Alkyl (C_{16}-C_{18}) trimethyl ammonium chloride 50%	Armour and Company	10	0
Hyamine 2389	Alkylaled (C_9-C_{15}) tolyl methyl trimethyl ammonium chloride 10%	Rohm and Haas	10	0
Klenzade XY 12	Sodium Hypochlorite, 1% available chlorine	Klenzade Products	10	0
Iodine Suspensoid Merck	Colloidal iodine, 20% iodine	Merck and Co., Inc.	10	0
Iosan	Nonyl phenyl ether at polyethylene glycoliodine complex, 1.75% available iodine	Lazarus Labs., Inc.	10	0
----------	Phenyl ethyl alcohol	----------	10	9

Since several disinfectants exerted high activity, a screening test was needed to evaluate these disinfectants. The same test was performed as above using a concentration of 100 p.p.m. of each compound. Results are presented in Table V. The three halogen compounds were the only active disinfectants in 100-p.p.m. concentration.

For further screening, a semiexhaustion test was employed. Eggs were treated as above (100-p.p.m. concentration); however, after two minutes another batch of eggs (ten eggs) was treated in the same solution). The pH of each disinfectant solution was determined before and after immersing the first batch, and after the second batch.

From Table VI it can be seen that sodium hypochlorite (Klenzade XY12) gave the best results since all shell discs were free from viable bacteria. In both of the iodine compounds, the second batch of eggs was not well disinfected.

Since chloride was the best germicide found, the effect of Sterox CD (the chosen detergent) on the germicidal activity of chlorine would be of great importance. If it antagonizes and reduces the germicidal activity of chlorine, either the amount of chlorine should be increased or another germicide should be used. If, however, the activity of chlorine was unaffected, or enhanced, the two compounds could be used as a detergent-sanitizer.

TABLE V

GERMICIDAL ACTIVITY IN 100-P.P.M. CONCENTRATION OF ACTIVE INGREDIENTS
(test organism Pseudomonas aeruginosa)

Sanitizer	No. of Eggs Tested	No. of Eggs Positive
Roccal	10	10
Arquad S	10	10
Hyamine 2389	10	8
Klenzade XY12	10	0
Iodine Suspensoid Merck	10	0
Iosan	10	0

TABLE VI

GERMICIDAL ACTIVITY AND pH OF SOLUTIONS TESTED BY "SEMIEXHAUSTION" METHOD

Solution	No. Eggs in First Batch		No. Eggs in Second Batch		pH		
	Tested	Positive	Tested	Positive	Initial	After First Batch	After Second Batch
Klenzade XY12	10	0	10	0	8.3	9.0	9.2
Iodine Suspensoid Merck	10	0	10	8	2.2	6.8	9.1
Iosan	10	0	10	7	2.0	6.5	9.1

Twenty eggs were sprayed with P. aeruginosa. From these, ten were immersed in a detergent sanitizer solution containing 0.1 per cent Sterox CD and 100 p.p.m. active chlorine. After being submerged for two minutes, the eggs were dried and eggshell discs were tested as before. After two minutes from the time the first batch of eggs were removed from the solution, another batch was added. The second batch was tested in the same manner as that used for the first one.

All the discs tested were free from bacteria indicating that the germicidal activity of chlorine was not destroyed in the presence of 0.1 per cent Sterox CD.

Discussion

Bisphenols are compounds in which two phenolic bodies are linked together in various ways. This may involve a direct carbon-to-carbon bond, or there may be a connecting atom such as oxygen or sulphur or a connecting group such as $-CH_2-$. The two phenolic structures may be the same (symmetrical) or different (asymmetrical). The hydroxyl groups may be in the ortho, meta, or para position to the bridge. The substitutions on the phenolic ring are several in kind and position, with innumerable combinations. Due to the great

number of possibilities many compounds have been synthesized and many more could still be considered from the bisphenol group.

It is fortunate that it has been possible to establish, to a great degree, a relationship between the chemical structure and the antimicrobial activity. Marsh and co-workers (97, 98) made an extensive study of this group for its antifungal activity. They stated that:

> Activity [of bisphenols] is influenced by the following structural features:
>
> (a) The number, type, and position of halogen atoms. Two bromine or chlorine atoms per molecule increase activity above that of unhalogenated bisphenols, whereas, too high a halogen content, especially when consisting of bromine rather than chlorine and/or particularly when occupying all 4 positions ortho to the phenolic hydroxyls, leads to low activity.
>
> (b) Molecular size. Usually large molecular size leads to low activity.
>
> (c) Chemical blocking of phenolic hydroxyls. Ether formation (but not necessarily esterification) brings about a very low potency.
>
> (d) Types of bisphenolic bridge. Bridges consisting of $-CH_2-$, -S-, -C-, $-CH{\cdot}CH_3-$, $-CH{\cdot}C_6H_5-$, or -CH=CH- are compatible with high activity; -SO-, $-SO_2-$ or -C-C- are less desirable in this respect.
>
> (e) R- group substitutions on the phenolic rings. The chlorthymol type of substitution brings about a very low activity, certain other groups are not undesirable.

From what is known about the gram-negative bacteria they follow the same pattern. The gram-positive organisms, however, have a slightly different relationship. The more halogen substitutions

on the ring the more potent the compound. Moness et al. (107) found that the addition of an alkyl group on the phenolic ring increased the activity.

By using M. pyogenes var. aureus as a test organism, monosodium bithionate showed a remarkable residual value. This agrees with what is known about bisphenols as a group. M.S.B. in a concentration of 1:1000 did not exert any antibacterial activity against E. coli, P. vulgaris, and P. aeruginosa. It did show, however, a powerful activity against M. pyogenes var. aureus. The results agree partially with those obtained by Hunter et al. (71). They found that 1:1000 concentration of bithionol was not inhibitory against either E. coli or P. aeruginosa, and a concentration of 1 p.p.m. inhibited the growth of M. pyogenes var. aureus. In the case of P. vulgaris, 100 p.p.m. was enough to prevent its growth. The high activity against P. vulgaris may be due to the procedure they used in their test. They dissolved bithionol with the aid of polyethylene glycol 400 as a solvent. Serial dilutions were prepared and inoculated with 0.05 ml of an aqueous suspension of the respective test organism. Results were read after 48 hours' incubation.

The sodium salts of either hexachlorophene or bisphenol "A" in concentrations of 1:1000 failed to inhibit P. aeruginosa. It was

reported (141) that 100 p.p.m. hexachlorophene is bactericidal to P. aeruginosa. The F.D.A. procedure used in the previous report may be responsible for the difference in the results. The solution in aqueous alkali was prepared by dissolving 0.1 gram of G-11 in 1 ml of 95 per cent alcohol and 0.75 ml of 0.5 N alcoholic potassium hydroxide and adding water to 100 ml.

Since bisphenols comprise a large group there may still be a possibility of finding a sanitizer that is active against the gram-negative organisms as well as against the gram-positive ones. Until such a compound is found, the use of bisphenols as egg sanitizers would be inadvisable.

Phenyl ethyl alcohol did not show any activity against P. aeruginosa in 1.1000 concentration. This agrees with what was reported before. Lilly and Brewer (82) observed that 1:400 concentration, but not 1:800, inhibited the growth of P. aeruginosa.

The three quaternary compounds tested did not show any bacteriostatic or bacteriocidal activity in a concentration of 100 p.p.m., although all three inhibited the test organism in 1000 p.p.m. concentration.

It is very difficult to compare results obtained in this study with those reported in the literature since the method of determining

the germicidal activity of quaternary compounds has a marked effect on the results. In the method used in this study, the quaternary compounds' activities were not stopped when eggs were dried, but when the discs were placed on agar the activity was reduced to a minimum if it was not stopped. The effect of agar on the antibacterial activities of quaternary compounds was reported by Tokie and Ayres (151), Hoogenheidle (69), and several others. Quinso, Gibby, and Foter (119, 120, 121) attribute this action to the physical absorption of the compounds by the agar.

Kivela et al. (75) studied the physical action of alkyl dimethyl benzyl ammonium chloride (A.D.B.) on several organisms. They noticed that at a concentration of 1:4000 but not 1:5000 cells of P. aeruginosa clump. This may explain why in a 1:10,000 but not 1:1000 concentration the compound failed to exert effective antibacterial activity (Table V).

Several investigators used A.D.B. in egg-washing experimentations. As expected, they obtained contradicting results due to factors such as concentration, kind of eggs, method of testing, et cetera.

Funk and Forward (48), using hatchability as a criterion, reported that there was not too much difference between the hatchability of untreated clean eggs and that of those washed in 380 p.p.m. A.D.B.

In a later publication, however, Funk et al. (49) used a concentration of 150 p.p.m., and immediately after washing the eggs were oiled in a preparation containing 1750 p.p.m. of A.D.B. Even after such treatment, they could not stop excessive bacterial invasion.

Miller et al. (106) was not able to detect antimicrobial activity of A.D.B. in the water in which eggs were washed until he used a concentration of 1 per cent Roccal.

Forsythe (39) and Forsythe et al. (40) found that 200 p.p.m. A.D.B. was not harmful to the physical quality of the egg contents. At this concentration the bacterial population was reduced on the eggshell by approximately 92.8 per cent. In their study they did not differentiate between inhibition or destruction and mechanical removal of bacteria. When they used Vel (a detergent) they obtained 82.5 per cent reduction. From this, the high activity of A.D.B. obtained by them may be attributed to mechanical removal of bacteria rather than inhibition and inactivation.

Recently, Miller (104) reported on his experiments with dirty eggs. A solution containing 100 p.p.m. A.D.B. was not effective in washing eggs. By determining the bacterial load of egg contents, he found that washed eggs had more bacteria than untreated eggs.

Botwright (10) showed that Hyamine 2389 in a concentration of 130 p.p.m. was highly bactericidal against P. aeruginosa. Winter (154), however, with a concentration of 180 to 380 p.p.m., found that 15 per cent of the treated eggs had rots, whereas only 4 per cent of the untreated eggs showed rots.

Why quaternary compounds failed to give as good results as halogen preparations may be explained as follows: The test used in this study was designed to be as close as possible to the practical conditions; also, the test organism was a resistant gram-negative bacterium. Generally speaking, and especially under practical conditions, quaternary compounds are active against gram-positive organisms (31), whereas halogens are definitely more active against gram-negative bacteria (72).

Mallmann et al. (92) compared 180-p.p.m. hypochlorite with the same concentration of a quaternary compound in the disinfection of glasses in an eating establishment. Both compounds gave good comparable results. It should be realized, however, that bacteria from the gram-positive group accounted by far for the major portion of the bacterial load in their study.

The three halogen preparations showed much better activity than the rest. They were active even in a concentration of 100 p.p.m.

At a relatively high temperature (40°C) and high pH, hypochlorites exerted better activity than the two iodine compounds.

Mandel (95) tested three iodine preparations: Iodine suspensoid, Lugol's solution, and Iodine Tincture. He disinfected chicken feces with concentrations up to 0.2 per cent I_2 at a pH 5.0 for a period of five minutes. The reduction of the bacterial load was identical for the three compounds. It seems, then, that iodine preparations possess the same bactericidal value if they are tested at the same pH.

Wyss and Strandkov (158) reported that iodine preparations decompose very quickly at pH 8.0 or higher. Mandel (95), however, believed that loss of penetrating power is the resulting effect of high pH on iodine preparations. He showed that by increasing the pH from 5.0 to 9.0 the length of zone of inhibition decreased from 12.5 mm to zero when he used the penatube method in testing the iodine suspensoid.

Regardless of what actually happens, high pH is very detrimental to iodine acting under practical conditions.

Relatively high temperatures also adversely affect iodine compounds. Gershenfeld and Miller (50) reported that iodine is more effective against M. pyogenes var. aureus at 20°C than at 37°C.

Ostrolenk and Brewer (110) found that P. aeruginosa was killed within five minutes by 1:5000 concentration of iodine at 20°C. At 37°C it required 1:4000 concentration to kill the same organism, whereas 15 minutes (the maximum exposure time used) were not enough at 1:5000 concentration.

Cantor and Shelanski (23) were the first to report on the germicidal activity of the nonyl phenylether of polyethylene glycol-iodine complex. They used their capacity test to demonstrate its great germicidal power. Their test organisms were M. pyogenes var. aureus and Salmonella typhosa. They conducted all their tests at 25°C. Ostrolenk and Brewer (110) tested fourteen different organisms against iodine. They found P. aeruginosa to be the most resistant; kill occurred at a concentration of 1:5000 at 20°C, whereas M. pyogenes var. aureus and S. typhosa were killed at a concentration of 1:8000. At 37°C P. aeruginosa, M. pyogenes var. aureus, and S. typhosa required concentrations of 1:4000, 1:8000, and 1:7000, respectively.

Hypochlorites behave differently at relatively high temperatures. Rudolf and Levine (135), using Bacillus metiens, found that the killing time was reduced 60 to 65 per cent for each 10°C rise between 20°C and 50°C. At the pH of 10 and a concentration of

25 p.p.m. available chlorine, all spores were killed in 121 minutes at 20°C and 38.7 minutes at 35°C. Allen and Brooks (2) stated that Butterfield et al. (1943) showed that the bactericidal efficiency of chlorine increases with rising temperature and the effect becomes more marked as the pH value increases.

The method of testing may affect the result of evaluating chlorine and iodine compounds. Gershenfeld and Palisi (51) developed their "semi-micro" method for evaluating the germicidal activity of available chlorine and free iodine. When they used a modified F.D.A. method, they obtained a phenol coefficient of 177 with chlorine and 133 with iodine. On the other hand, when they used the "semi-micro" method, chlorine gave a phenol coefficient of 44, whereas iodine gave 89. Both tests were run using S. typhosa.

All these factors combined may help to explain the superiority of chlorine over iodine.

When Sterox CD was added with chlorine, it did not reduce the germicidal activity of the hypochlorite solution.

It was always believed that hypochlorites should be used in cool water, because in water of higher temperatures, they lose available chlorine Hadfield (122), however, recently stated:

> Solutions containing 50, 100, 200 p.p.m. available chlorine prepared from a proprietary sodium hypochlorite made alkaline with calcium hydrate were kept at 55° C for 180 minutes. At the start and at intervals of 30, 60, 120 and 180 minutes, available chlorine determinations were made. Each solution showed, at the end of the 180-minute period, the same concentration as as the start.

Moderately high temperatures (50°C) and a contact period of three minutes were chosen by the author as conditions for egg-washing experiments. Since chlorine reacts with organic matter and dirty eggs carry organic soil, the concentration of active chlorine was increased to 200 p.p.m. so that its germicidal activity would not be reduced.

In choosing chlorine as a disinfectant certain problems arise. Hypochlorites do not have a residual value; in other words, after eggs are washed and dried they are left without antimicrobial protection. For this reason it was necessary to incorporate in the oil a disinfectant which does not lose its activity before the end of the lifetime of the egg.

Oil-Soluble Disinfectant

Experimental

When eggs washed in an oil emulsion[1] containing hypochlorite as the only disinfectant, the germicidal value of chlorine would disappear on drying. The oil droplets tend to coalesce, forming a thin oil film around the eggshell. The oil does not prevent bacterial or mold penetration through the shell if eggs are exposed to contamination (132, 17). For this reason, incorporating a disinfectant in the emulsion that inhibits bacterial and mold activities would be of great value. Such a disinfectant should be oil-soluble since it would be carried in oil especially after the water evaporates from the emulsion. It should also be sparsely soluble or nonsoluble in water. If the disinfectant is water-soluble, it will dissipate through the water phase of the emulsion or be washed out when treated eggs are exposed to sweating conditions leaving eggs unprotected.

Several phenolic compounds meet such requirements as a disinfectant. The oil-soluble compounds that were tested for their antimicrobial activity are listed in Table VII.

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[1] See "Per Cent of Oil in Emulsion."

TABLE VII

PHENOL COEFFICIENT OF SEVERAL PHENOLIC SALTS
(test organism P. aeruginosa)

Trade Name	Structure	Lowest Active Concentration	Phenol Coefficient	Manufacturer
Dowicide 2	2,4,5, trichlorophenol	1:100	1.4	Dow Chemical Co.
Dowicide 6	2,3,4,6, tetrachlorophenol	>1:100	<1.4	Dow Chemical Co.
Dowicide 7	pentachlorophenol	1:400	5.7	Dow Chemical Co.
Dowicide 32	4 and 6-chloro-2-phenylphenol	>1:100	<1.4	Dow Chemical Co.
---------	Phenoxyacetic acid	>1:100	<1.4	Dow Chemical Co.
Methyl-parasept	Methyl parahydroxybenzoate	>1:100	<1.4	Heyden Chemical
Propyl-parasept	Propyl parahydroxybenzoate	>1:100	<1.4	Heyden Chemical
Ethyl-parasept	Ethyl parahydroxybenzoate	>1:100	<1.4	Heyden Chemical
Butyl-parasept	Butyl parahydroxybenzoate	>1:100	<1.4	Heyden Chemical
Benzyl-parasept	Benzyl parahydroxybenzoate	1:100	1.4	Heyden Chemical

For testing the antibacterial activity it was found to be much easier to examine the compounds when rendered water-soluble. This was found to be most conveniently done by adding a strong alkali (NaOH) to the phenolic compound.

Kojima (76) observed that the sodium salt of a phenolic was less germicidal than the original compound. These observations were confirmed by Klarmann and Wright (122). In other words, if the sodium salt of a phenol would show activity against bacteria, the original compound should be expected to exert greater germicidal potency at the same concentration and under the same conditions.

The phenol coefficient was used as a criterion for determining the antibacterial activity. The United States Food and Drug Administration method (136) was applied with one modification. P. aeruginosa was used as a test organism instead of either M. pyogenes var. aureus or S. typhosa. All tests were conducted at 20°C. Experiments were repeated until three successive determinations gave the same value. The final results are tabulated in Table VII.

A position of considerable importance is occupied by the esters of parahydroxybenzoic acid, notably in the field of preservation of carbohydrates, gums, proteins, and other organic materials of industrial, pharmaceutical, and cosmetic significance against spoilage

by air-borne microorganisms (109). A table indicating the proper concentrations of several esters required for preservation was prepared by Suess (149). His recommendations had been confirmed by several investigators (52, 30). Gershenfeld and Perlstein in 1939 (52) regarded these esters as being among the most useful preservatives for pharmaceutical and related preparations available at that time.

Since, in the above reports, P. aeruginosa was never used as a test organism, comparison of their data with those obtained in this study is impossible.

Recently, Sokol (142) subjected methyl, ethyl, propyl, and butyl para-hydroxybenzoate to an extensive investigation. Their antibacterial and antifungal properties were examined in an endeavor to find suitable test organisms and testing methods. He used twelve different test organisms, eight of which were gram-negative. It is interesting to note that in his study he found P. aeruginosa ATCC 9027 to be the most resistant of all the test organisms he used. Complete inhibition of this organism required 0.4 per cent of either methyl or ethyl esters and 0.8 per cent of either propyl or butyl esters.

The difference in results reported by the previous author and those obtained by the writer may be due to differences in the methods of testing. Sokol prepared agar plates containing the desired

concentration of the sodium salt of the ester. He inoculated these plates by dipping a loop in a 10-ml water suspension of a 24-hour slant of the test organism and streaking on one sector of the plate. Readings were made after 24-hour incubation at 37°C.

Hearst and Hearst (68), in producing their egg-preservative emulsion, incorporated low molecular esters of parahydroxybenzoic acid. The examples they gave were the methyl, ethyl, and propyl esters. They used one or more of these esters to give a final concentration of 4 per cent of the preservative (s). In the writer's study, concentrations up to 1 per cent of the same esters did not produce any inactivation of P. aeruginosa.

Phenol inactivated P. aeruginosa in a concentration of 1:70 after 10 minutes' exposure but not after 5 minutes. Sodium pentachlorophenate (sodium salt of Dowicide 7) showed the highest activity. It was effective in concentrations as low as 0.25 per cent (phenol coefficient of 5.7).

As mentioned before, in selecting an oil-soluble disinfectant, its antifungal activity should be as good as its antibacterial potency. No antifungal examinations were performed on pentachlorophenol in this investigation since it had been thoroughly studied previously (93, 90). Mallmann and Davidson (91) recommended the use of

pentachlorophenol (Dowicide 7) in a concentration of 0.25 per cent to be incorporated in oil. By using such antiseptic oil, they could prevent mold growth inside and outside the eggs.

Percentage of Oil in Emulsion

Experimental

In oil processing, more oil than the eggshell can retain is being used. This fact has been demonstrated by Evans (33) and Stewart and Bose (147). Both reports dealt with the use of volatile solvent-oil mixtures. Evans reported that 50 per cent oil was needed in a solvent-oil mixture to protect eggs as effectively as 100 per cent oil. Stewart and Bose, however, found that 10 per cent oil in a different kind of solvent-oil mixture was practically as efficient as 100 per cent oil. If the right amount of oil is used in an oil-in-water emulsion, it should give as much protection to the eggs as 100 per cent oil.

In determining how much oil should be incorporated in the emulsion, the efficiency in reducing the weight loss of eggs held at incubation temperatures was chosen as a criterion.

A mineral oil used commercially for oiling eggs was used in all the investigations. This oil had the following physical characteristics (manufacturer's specifications):

Viscosity at 37.8°C (100°F)	180-190 Saybolt
Specific gravity at 15.6°C (60°F)	0.8816 maximum
Open flash	190.6°C (375°F)
Pour point	-26.1°C (-15°F) max.
Saybolt color	+30

The emulsions consisted of:

Oil	5.00 or 10.00 grams
Sterox CD	0.1 gram
Sodium tri polyphosphate	0.15 gram
Active chlorine (as determined by ortho tolidine flash test)	200 p.p.m.
Tap water to total of	100 grams

In the first experiment 5 per cent emulsion was tested. Three dozen clean, white, sound-shell eggs, 24±4 hours old, were used. One dozen was left as controls, and the other two dozen were treated by immersion in either 5 per cent emulsion or 100 per cent oil (one dozen each) at 50±1°C for three minutes. After treatment the eggs were left at room temperature for 60 minutes to drain and dry. Eggs were weighed individually, put in trays, and held at 36°C for three weeks with 30±3 per cent relative humidity (as determined by dry and wet bulbs). At the end of 7, 14, and 21 days the eggs were reweighed individually. Portion cups (souffles) $5\frac{1}{2}$ ounces capacity were very useful in handling eggs.

Inasmuch as the difference between the control eggs and eggs treated with 5 per cent emulsion was negligible, it was decided to try a 10 per cent emulsion. Results are summarized in Table VIII.

No significant difference was found between any of the treatments and the control at zero days. Taking the control as standard, there was no definite advantage in using 5 per cent emulsion, whereas there was a decided advantage in using either the 10 per cent emulsion or the 100 per cent oil. Oiling was better than the treatment with 10 per cent emulsion as shown after the third week of holding. In other words, 10 per cent emulsion was as good as 100 per cent oil when eggs were held for two weeks at 36°C.

Discussion

One of the major problems confronting the poultry industry is that of supplying consumers with eggs of high quality. The egg is a highly perishable food product, and may lose quality rapidly after being laid. Just before being laid, a hen's egg is in equilibrium with an atmosphere having a relative humidity of 99.5 per cent (140), and containing 10 per cent CO_2 at 25°C (139). If an egg is kept at room temperature or even under ordinary cold-storage conditions, losses of moisture and carbon dioxide from the egg

TABLE VIII

EFFECT OF TREATMENTS ON EGG WEIGHT LOSS
(each number represents an average of twelve eggs)

Treatment	Average Weight After the Following Number of Days			
	0	7	14	21
Control	59.65	56.77	54.12	51.02
5 per cent emulsion	59.77	57.51	53.29	51.44
10 per cent emulsion	59.35	58.36	57.35	55.95
100 per cent oil	58.83	58.45	58.06	57.60

Least significant difference: 1.80 at 1 per cent level; 1.36 at 5 per cent level.

through the shell are to be expected. Accompanying these losses are the various physical and chemical changes which contribute to the appearance of a stale egg.

The shell in which the hen encases her product, although seemingly an acceptable package for commercial shipment, actually was not designed for this purpose. The eggshell was planned by nature to protect the egg against destruction during incubation of the embryo. The shell was made more porous so that respiration by the embryo could take place. The shell is actually a poor package material not only because it is fragile, but because it allows the escape of moisture and carbon dioxide from the egg contents.

Sealing the eggshell pores reduces the escape of carbon dioxide and moisture, and also retards the physiochemical changes leading to deterioration. Oils of various kinds have been used at least since 1807 for sealing eggshell pores. Spamer (144) reported that as early as 1807 the Dutch preserved eggs by placing them in linseed oil for half a day, and then dried them on racks. At the present time, coating eggs with mineral oil prior to storage is a common commercial practice. The treatment is inexpensive and prevents a high percentage of the moisture loss that would otherwise occur during the holding period (83).

Since loss of weight of eggs depends on the kind of treatment and upon the eggs themselves, comparison of results obtained in the above experiments and those reported by other investigators could not be made intelligently. In the case of the untreated eggs, comparison could be made to a better degree.

The percentage loss of weight in the case of the controls was 9.10 per cent after 14 days. This is higher than that reported by Godfrey and Olsen (57). They obtained 8.42 per cent loss but failed to record the relative humidity which could account for the difference. As a general rule, regular egg incubators, similar to the one used in their study, operate within 60 to 70 per cent relative humidity as compared to 27 to 33 per cent used by the author.

Rhodes and Godfrey (125), using eggs from mixed breeds and a regular egg incubator, found 7.80 per cent loss after two weeks of incubation. However, eggs were held two to seven days before incubation, which could account for the difference in the results (9).

Moran and Pique (108), by storing eggs at approximately 0°C (30°-33°F) for 10 months at 72 to 91 per cent relative humidity, obtained an average loss of weight per egg of 2.36 grams (360 eggs lost 30 ounces). Accordingly, an egg would be expected to lose, theoretically, an average of 2.83 grams after one year of cold storage,

compared to 2.88 grams loss after one week incubation. In other words, it is safe to state that one week holding at incubation temperatures brought about a loss in weight at least equal to that during one year in cold storage. The same observations could be demonstrated in other reports by Michell (102), Pennington (112), et cetera.

In practice, after cold storage, eggs are withdrawn to be candled, they are packaged in cartons and shipped to the retailers where they are sold to the consumer, who stores them in home refrigerators. This period takes about 2 to 3 weeks, during which eggs are exposed to fluctuating higher temperatures and low relative humidities, resulting in an increased rate of egg weight loss. No work has come to the attention of the author indicating how much loss or even percentage of egg weight loss occurs during this period after one year of cold storage. Therefore, the loss of weight in the second week of holding was chosen, arbitrarily, to account for what would be lost during this period.

The higher the percentage of oil in the emulsion the better and longer the protection of eggs against weight loss should be expected, within certain limits. As loss in the two-week period of holding at 36°C corresponds to the expected loss under actual field

conditions for the whole life of the egg (storage and after storage until consumption), effective protection for more than two weeks would indicate excessive use of oil. This experiment showed that a significant difference in loss of egg weight between those treated with the 10 per cent emulsion and those treated with the 100 per cent oil was not noticed until the end of the third week. Consequently, 10 per cent oil in the emulsion seems to be approximately the optimum practical concentration.

Eggs which are consumed directly after being laid (no cold storage) also lose weight before consumption. The 10 per cent emulsion should therefore be of benefit to such eggs if its use is commercially feasible.

It was found that 10 per cent oil was approximately the optimum concentration for an emulsion. A lower concentration would not protect the eggs for the two-week holding period at 36°C which corresponds to the period, under field conditions, from the time the eggs are washed until they are consumed. A higher concentration of oil would be in excess as it would prolong protection more than required.

According to Moran and Pique (108), nine months were recommended as a maximum cold-storage period for commercial reasons,

since the highest egg production season lasts about three months. That was true in 1926 but certainly is not the case today. Eggs are generally cold-stored for about 90 days to keep the "fresh" quality. In the experiment, one week holding, which represents one year cold storage, was chosen to guarantee the "freshness" of eggs stored for 90 days.

Examination of the Final Emulsion

Microbiological Study

Experimental. After selecting each of the ingredients to be incorporated in the emulsion, it is of great importance to see how they will behave when combined in the final emulsion. If the final emulsion proves to be effective this will mean that three steps (cleaning, sanitizing, and sealing) can be done in one operation. At the present time these steps are performed in two or three operations. The only two steps that are sometimes combined are cleaning and sanitizing.

In this experiment, the emulsion was made by Dr. E. J. Miller of the Agricultural Experiment Station at Michigan State College. The oil droplets were approximately 0.5 micron in diameter. To

keep such an emulsion stable, the amount of Sterox CD was increased to 1 per cent instead of 0.1 per cent. The final emulsion was constituted as follows:

Oil (containing 0.25% Dowicide 7)	10.00 grams
Sterox CD	1.00 gram
Sodium tripolyphosphate	0.15 gram
Active chlorine (from sodium hypochlorite). .	200 p.p.m.
Tap water complete to	100 grams

Sodium hypochlorite was added to the rest of the ingredients just before use due to its instability.

Clean and slightly dirty[1] eggs were supplied from Carruthers Farm.[2] The eggs were about 24 hours old when used in this experiment. The clean and slightly dirty eggs were divided in four groups of 90 eggs each. The first group (washed in emulsion) was washed in the emulsion at 50±1°C for three minutes, then dried in front of a fan. The second group (washed) was washed in a detergent solution containing 1 per cent Sterox CD and 0.15 per cent sodium tripolyphosphate.[3] Immediately after washing for two minutes, the

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[1] Due to climatic conditions and farm management procedures, naturally dirty or heavy dirty eggs were not available.

[2] Local private producer.

[3] Antifoam A (Dow Chemical Company) was used to stop the excessive foam production during washing in the detergent solution.

eggs were immersed in a hypochlorite solution at 50±1°C for one minute. The hypochlorite solution contained 200 p.p.m. of active chlorine. The third group (washed and oiled) was treated as the second group except that after drying they were dipped momentarily in oil at 50°C. The fourth group (control) was kept untreated.

Washing was done with the aid of a Keenco Egg Washing Machine. This is an immersion-type machine that holds six gallons of the washing solution (or emulsion). The temperature of the washing solution was kept thermostatically controlled at 50±1°C. Air under pressure was pumped at the bottom of the tank through small holes, forming air bubbles. This was employed to help in shaking the dirt loose from the eggs without breaking the eggshells.

Wire screens were placed vertically in the middle of each basket to divide it into two halves. In one half clean eggs, and in the other dirty eggs, were exposed to the same washing solution at the same time, and still each type of egg could be separated easily after washing. When all the eggs were dried and drained, they were packed in new cartons and stacked in new half-case boxes. Eggs were stored at 1°C for two months at 75 to 85 per cent relative humidity.

This whole experiment was repeated again after five days.

Examination was performed using 24 eggs of each of the clean and slightly dirty eggs from each treatment and from the controls. Eggs were examined immediately after treatment and after storage periods of 30 and 60 days. It took two to three days for the examination of the eggs at each period.

Each egg was opened and egg contents were poured out under aseptic conditions as before. Two examinations were performed on each egg:

1. A 10-ml portion of saline solution was poured inside the eggshell. The saline was mixed well with the thin film of egg white adhering to the inner egg membrane by decreasing and increasing the pressure through a pipette. Duplicate samples of 1.0 and 0.1 ml portions were plated in T.G.E. agar.

2. The eggshell was emptied again and a one inch square area was swabbed as before (page 35).

Plates and tubes were incubated at 36°C for 48 hours.

Any egg which was shown to contain three organisms or less per milliliter of the sample of saline-egg white mixture (first examination) was considered free from viable microorganisms since such low numbers could be attributed to outside contamination. For the same reason the eggs which gave positive results in the swab tests in the first tube only (1:1 concentration) were considered negative.

On this basis there was no preference of one method of testing over the other, so eggs were considered free from viable organisms if both test methods showed negative results. If even one of the two tests gave positive results, the egg was considered to contain viable organisms (positive).

The data are summarized in Table IX. There was hardly any difference between the clean and slightly dirty eggs and also between the first and second experiments. Due to these reasons, the data from both the clean and slightly dirty eggs and also from the first and second experiments were combined in Table X. By analyzing the data statistically it was found that at zero time there was no significant difference between each treatment and the control or among the treatments themselves. After one month of storage, there was a significant increment in the number of infected eggs in the case of the washed, washed and oiled, and control eggs. In the case of the emulsion, however, the increment was not significant even after two months of storage. An increment in the number of infected eggs in every treatment and the control was shown after two months, but was not high enough to be significant when compared with one month. In all the periods there was no significant difference among the control, washed, washed and oiled eggs, but there was a significant difference between any one of the three and the emulsion-washed eggs after 30 and 60 days' storage.

It was noticed that after washing in the detergent solution or in the emulsion slightly dirty eggs came out as clean eggs.

TABLE IX

NUMBER OF EGGS CONTAINING VIABLE ORGANISMS

Treatment	No Storage		Storage One Month		Storage Two Months	
	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
Control:						
Clean	1	2	5	4	6	6
Dirty	1	2	4	4	6	7
Washed:						
Clean	1	1	4	4	6	5
Dirty	1	2	4	5	7	6
Washed and oiled:						
Clean	0	2	4	2	5	6
Dirty	2	1	4	4	6	6
Washed in emulsion:						
Clean	0	1	1	1	2	1
Dirty	2	1	2	2	2	3

Each number represents the number of infected eggs in 24 originally examined eggs.

TABLE X

NUMBER OF EGGS CONTAINING VIABLE BACTERIA

Storage Period (in months)	Control	Washed	Washed and Oiled	Washed in Emulsion
Zero	6	5	5	4
One	17	17	14	6
Two	25	24	23	8

Each number represents the number of infected eggs in 96 originally examined eggs.

Discussion. There is great disagreement in the literature as to the value of egg washing. A number of investigators (Bushnell and Maurer, 21; Funk, 42, 45, 46, 47; Winter, 153; Gunderson, 61; Johns and Berard, 73; Solowey et al., 143; Lorenz, 84; Davidson et al., 27; Gillespie et al., 55; Dawson and Davidson, 28; Kahlenberg et al., 74; Winter et al., 155; Starr et al., 145; Lorenz et al., 86, 85, 87; Miller and Crawford, 105; Funk et al., 49; and Miller, 104) have reported that cleaned eggs do not keep as well as corresponding clean or soiled control eggs.

On the other hand, a number of investigators (Bryant and Sharp, 18; Penniston and Hedrick, 114, 115, 116; Gunderson and Gunderson, 62; Black, 7; Pino, 118; Miller et al., 106; Gillespie et al., 53, 54, 56; McNally, 101; and Conner et al., 26) reported little or no harm from cleaning eggs.

A careful study of the conflicting reports reveals that many different procedures and quality measurements were used. This may explain the difference in results obtained.

Since the reports on the use of chlorine in washing eggs are of more importance for our study, such reports will be discussed briefly. Gillespie et al. (54) found in three farm experiments that eggs which were not cleaned showed 0.5 to 3.7 per cent rots. If

eggs were washed in a roller machine using water only, or water containing a mild detergent the percentage of rot increased to approximately 16.6 to 59.7 per cent. However, if the machines were cleaned and disinfected with 1 per cent hypochlorite solution containing 5000 p.p.m. available chlorine, the percentage of rots decreased on the same three farms. Compared with controls (untreated eggs), the percentage of rots was decreased on two out of three farms when chlorine was used. In their study they did not use any other chlorine concentrations, which might have been just as active. Gillespie et al. (56), in another set of experiments, found that 5000 p.p.m. was better than either 1000 p.p.m. or 200 p.p.m. available chlorine. In these experiments, however, the percentage of rots in which 5000 p.p.m. available chlorine was used was 0.6 to 5.6 on eggs from three different farms, compared to no rots in the unwashed eggs. They did not, however, explain the reason for the contradiction of these results with those obtained in their previous work.

Lorenz et al. (85) surveyed twenty-two ranches where egg washing was practiced. Only one farm used hypochlorite solution (Chlorox) in washing at 43°C. The per cent spoilage in washed eggs at this farm was 0.28 per cent compared to 0.25 per cent for the unwashed control. On the other hand, the per cent spoilage of washed eggs on the rest of the ranches gave an average of 7.39 per cent spoiled eggs.

Zagaevsky and Lutikova (159) demonstrated that dirt or soil on the eggshells is the major source of contamination in egg-breaking plants. As the eggs used in their experiment had been in storage before breaking, they found that bacteria are harbored on the shell, in the pores, and on the membranes. By using 1.5 per cent chlorinated lime for five minutes, eggshell pores and membranes were rendered sterile. Egg melange, prepared under strict sanitary measures without disinfection of the eggshell, contained an average of 200,000 bacteria per milliliter, whereas the melange prepared under identical conditions except that the eggshells were sterilized by chlorine contained only 20 to 60 bacteria per milliliter.

Penniston and Hedrick (115) compared the germicidal activity of chlorine and a quaternary ammonium compound. In their comparison they favored 400 p.p.m. quaternary ammonium compound over 100 p.p.m. chlorine.

In all these reports the ratio of amount of disinfectant washing solution to the number of eggs washed was never reported. This ratio is very important because the egg soil destroys the effectiveness of the disinfectant, hence the number of eggs washed for a given amount of disinfectant solution should be stated. The temperature in which chlorine solution is used is also very important

as in low temperatures (approximately 20°C) it is not as efficient as in a moderately high temperature (50°C).

In this study it was shown that, regardless of the kind of treatment, there was generally the same number of infected eggs at zero time. This was expected since the chlorine disinfects the egg-shell only and does not penetrate through the shell to disinfect the membranes.

Of all the eggs examined at the beginning of the experiment (zero day) only 20 eggs out of 384 (5.2 per cent) contained viable organisms. This agrees with the results of most workers who have indicated that generally fresh eggs do not contain bacteria. Table XI, presented by Wolk et al. (156), indicates that the lowest percentage of infected fresh eggs was reported by Anderson in 1932 when he obtained 5 per cent infection. The rest reported up to 87.0 per cent. At the present time, however, it is generally accepted that the percentage of infected fresh eggs is in the neighborhood of 5 per cent.

It was found (Table IX) that there was no difference between clean and slightly dirty eggs. This was expected since all eggs examined had sound shell and the amount of dirt was very small.

TABLE XI

BACTERIAL INFECTION OF FRESH EGGS
(after Wolk et al., 1950)

Author	Year	No. of Eggs	Per Cent Infected
Maurer	1911	100	82.0
Stiles and Bates	1912	613	87.0
Retteger	1913	3510	9.5
Bushnell and Maurer	1914	2759	19.3
Hadley and Caldwell	1916	2520	8.7
Anderson	1932	358	5.0
Haines	1938	112	7.0
Wolk	1948-49	416	12.5
		235	11.5
Sabet*	1954	384	5.2

* Added to table from this study.

Under field conditions, the percentage of dirty eggs produced is about 10 per cent (43, 4, 118, 74). The amount of dirt carried on these eggs would be the same if not less than that carried on slightly dirty eggs (50 per cent) washed in these experiments, so the conditions set up by the author are quite comparable to field conditions. The rise in the number of infected eggs from one month to two months storage in any of the treated eggs and the controls was not statistically significant, as expected. The reason for this is that the infected eggs after one month storage were actually contaminated primarily before and during the treatment, and the increase in infected eggs after two months storage was due to contamination after the treatment.

Since there was no significant difference between the number of spoiled eggs after 60 days of cold storage in the washed, and washed and oiled treatments, oil does not offer mechanical protection against bacterial invastion.

It is rather interesting that the emulsion-washed eggs would resist invasion of bacteria even better than the control eggs. This was due firstly to the fast action of chlorine because it exerts its germicidal activity within one minute which apparently inactivates microorganisms before they get a chance to be harbored inside the

pores and thus be protected against the germicide; secondly, to the disinfectant in oil which prevented any invasion after the treatment; and thirdly, to the fact that the eggs were not left at any time without protection from the time they were put in the emulsion until they were consumed.

When cleaning, disinfecting, and oiling were done in separate steps, they were not as effective in controlling the bacterial invasion of the eggs as when the three steps were combined. It must be remembered that in the washed and oiled treatment the oil did not contain a disinfectant. If the oil contained 0.25 per cent Dowicide 7, the amount of infected eggs after cold storage would have been lower (26).

The mode of action of the emulsion in removing the dirt from the eggshells and depositing an oil film on the shell is not fully understood at the present time. A possible hypothesis is that the emulsion has an excess of detergent which acts on the soil on the shell and suspends the soil in a peptized state in the emulsion. When the egg is removed from the emulsion it will pick up an insignificant amount of the soil particles that are not detectable by the naked eye. When the water in the emulsion evaporates (on the egg), the oil droplets on the shell coalesce, forming an oil film that seals

the shell pores. This involves, therefore, two different mechanisms; the first is the loosening, removing, and suspending of the soil from the eggshell, and the second is the depositing of oil on the shell. By reusing the emulsion, the soil suspended particles would increase. When these suspended particles reach a significant number, the soil will be noticed as a redeposit on the eggshells after they are washed.

The preferential wetting ability of the emulsion is not ruled out. It is possible that after the soil is removed by the detergent from the shell surface, the detergent may prefer to suspend such particles rather than oil droplets. In this condition, the oil would be left loose without any protection. It is highly probable that such loose droplets would adhere to an eggshell onto which they tend to be adsorbed.

Physiochemical Study

Experimental. Since, in the final emulsion, the amount of Sterox CD was increased and the oil deoplets' size was decreased, it is of value to find out the behavior of such an emulsion in preserving the physiochemical properties of the fresh egg. At the same time the last two experiments were performed two more experiments (page 81) were performed using pullet eggs instead of large eggs. Eggs were

stored under the same conditions (1°C and 75 to 85 per cent relative humidity).

The eggs were examined by the Department of Poultry Husbandry. Twelve clean eggs and twelve slightly dirty eggs from each of the treatments and the controls were examined after 30- and 60-day storage periods. After the air cell height was measured in sixteenths of an inch, the eggs were broken and the egg white score was determined according to photographs of the USDA standards. Cracked eggs or eggs having abnormal air cells were discarded.

When the results were analyzed, no appreciable difference was found between clean and slightly dirty eggs. Also, results from the first and second experiments were very similar. For these reasons data from the clean and slightly dirty eggs in both the experiments were combined and an average was calculated. The average of each treatment on air cell height and albumen score after cold storage for 30 and 60 days are presented in Table XII.

Washing eggs and then oiling offered the best protection against escape of moisture and CO_2 from shell eggs as measured by air cell height. After one month storage, the average air cell height was 1.2; after two months it was 1.8. The untreated eggs had an average of 2.2 and 2.5 after one and two months, respectively. The

TABLE XII

AVERAGE AIR CELL HEIGHT AND ALBUMEN SCORE

Treatment	Avg. Air Cell Depth (in sixteenths inch)		Avg. Albumen Score (USDA)	
	After 30 Days	After 60 Days	After 30 Days	After 60 Days
Control	2.2	2.5	3.7	4.5
Washed	2.2	2.7	4.0	4.8
Washed and oiled	1.2	1.8	4.0	4.0
Washed in emulsion	1.8	2.2	3.8	3.9

Each number represents an average of 48 eggs.

emulsion offered a protection with averages almost half way between the untreated, and the washed and oiled. The least protection was offered by the washed eggs. Even though the average height was the same as the controls after one month storage, after two months storage the average was 2.7, compared to 2.5 in the controls.

The best preservation of egg white quality was offered by the emulsion which gave an average score of 3.8 and 3.9 after one and two months storage, respectively. The least protection was offered by washing where the average score was 4.0 and 4.8 after one and two months, respectively. The untreated eggs had an average of 3.7 after one month and 4.5 after two months.

Discussion. Almost immediately after the egg is laid, the air cell is produced by contraction of the egg contents as it cools from the hen's body temperature of about 41°C (80) to the temperature of the external environment. Thereafter, the air cell grows larger as the moisture from the egg contents evaporates. Commercially, loss in weight and enlargement of the air cell together are known as "shrinkage." The air cell height is one of the points on which the USDA grading system for shell eggs is based. The height of the air cell as revealed by the candling lamp, therefore, has a certain significance when the condition of the egg is evaluated.

When the temperature and relative humidity of the environment are constant, the air cell height is a function of time. At first, it becomes larger rapidly, but the rate of increase soon diminishes and eventually becomes very slow in older eggs (130).

From Table XII it appears that oiling gave the best protection and the emulsion gave approximately half the protection given by oiling the eggs. The storage period could be doubled by treating eggs with the emulsion and still at the end the same quality could be obtained as in the untreated eggs. After two months storage the washed and oiled eggs declined as much as the emulsion-treated eggs after one month.

The egg with the best egg white quality has a score of one and the lowest has a score of twelve. In other words, by decreasing the quality, the score increases.

This study showed that eggs treated with the emulsion had the best quality, especially after a two-month storage period. These eggs had an average score of 3.9, to be compared with 4.5 in the case of the untreated eggs. This is of special importance practically from the consumer's standpoint.

CONCLUSIONS

1. Simple methods of washing and preserving eggs on the farm have been developed through the use of an oil-in-water emulsion.

2. By using the two artificial soils developed in this study, several surface-active detergents were compared. As a result of this comparison, Sterox CD proved to be the most efficient.

3. Of the four organisms examined, Pseudomonas aeruginosa was the earliest one recovered when speed of egg penetration was taken as criterion.

4. In determining the speed of penetration, the egg white, the rinse, and the swab tests were performed. Early invasion could be detected most accurately by the swab test.

5. The addition of hypochlorites to the oil-in-water emulsion was effective in destroying the organisms in the wash water and on the shell of the egg.

6. Pentachlorophenol dissolved in the oil acted as a bacteriostat and mycostat to prevent invasion of eggs after treatment.

7. The oil-in-water emulsion containing 10 per cent oil was found most effective for practical use.

8. Eggs treated with the emulsion were put under rigid bacteriological tests and their physiochemical qualities were determined. By treating eggs with this emulsion, their bacterial sterility and physiochemical properties were preserved very adequately.

9. By combining all the ingredients of the proposed emulsion, eggs could be washed, disinfected, and preserved at the same time.

BIBLIOGRAPHY

(1) Alford, L. R., Holmes, N. E., Scott, W. J., and Vickery, J. R., 1950. Studies in the preservation of shell eggs. I. The nature of wastage in Australian export eggs. Australian J. of Applied Sci., 1:208-214.

(2) Allen, L. A., and Brooks, E., 1952. Some factors affecting the bactericidal action of chlorine. Proc. Soc. App. Bact. (Brit.), 15:155-165.

(3) Almy, L. H., Macomber, H. I., and Hepburn, J. S., 1922. A study of methods of minimizing shrinkage in shell eggs during storage. J. Ind. Eng. Chem., 14:525-527.

(4) Anonymous, 1949. Changes in egg quality during marketing. North Central Regional Publication 15, Mich. Agric. Expt. Sta., Special Bull. 361.

(5) Artz, C. P., Pulaski, E. J., and Shaeffer, J. R., 1951. Clinical uses of hexachlorophene. U. S. Armed Forces Med. J., 2:819-825.

(6) Bigland, C. H., and Papas, G., 1953. Experiment in egg penetration by Salmonella. Can. J. Comparative Medicine, 17:105-109.

(7) Black, L. M., 1948. An easy way to clean eggs. New Jersey Extension Release, March 19, N. J. Extension Service (Poultry Digest, May, 1948, 295).

(8) Bless, A. A., and Romanoff, A. L., 1943. Stimulation of avian embryo by X-rays. J. Cellular and Comparative Physiology, 21:117-121.

(9) Bose, S., and Stewart, G. F., 1948. Comparative and complementary effects of heat treating and oiling shell eggs on their keeping quality. Poultry Sci., 27:228-234.

(10) Botwright, W. E., 1953. Detergent-sanitizers for cleaning eggs. Am. Egg and Poultry Rev., 15:(2)34.

(11) Brant, A. W., and Shrader, H. L., 1952. How to measure egg interior quality. USDA Agric. Res. Administration, Bureau of Animal Industry, May, 1952.

(12) Brewer, J. H., 1950. The utility of petri dish covers with absorptive discs. J. Bact., 59:237-239.

(13) Brewer, J. H., Goldstein, S. W., and McLaughlin, C. B., 1953. Phenylethyl alcohol as a bacteriostatic agent in ophthalmic solutions. J. Am. Pharm. Assoc., Sci. Ed., 42: 584-585.

(14) Brook, E. I., 1949. Treatment of canine and feline fungus infections. Vet. Med., 44:149-180.

(15) Brooks, J., 1951. The washing of eggs. Food Science Abstracts, 23:545-554.

(16) Brown, H. J., and Gibbons, N. E., 1954. Air cell mold in oiled eggs. Food Technol., 8:307-311.

(17) Brownwell, C. E., 1949. Penetration of Pseudomonas aeruginosa in eggs treated with copper vitresan. Thesis (M.S.), Michigan State College.

(18) Bryant, R. L., and Sharp, P. F., 1934. Effect of washing on the keeping quality of hen's eggs. J. Agric. Res., 48: 67-89.

(19) Buchanan, R. E., and Fulmer, E. J., 1928. Physiology and biochemistry of bacteria. The William Wilkins Co., Baltimore, Vol. 1:119.

(20) Bunyea, H., 1942. Miscellaneous diseases of poultry. USDA Yearbook, 1942, 993-1006.

(21) Bushnell, L. D., and Maurer, O., 1914. Some factors influencing the bacterial content and keeping quality of eggs. Kansas Agric. Expt. Sta. Bull. 201:751-777.

(22) Buster, M. W., 1948. Poultry and egg losses in market channels. Poultry Sci. Assoc., Ann. Meeting, 1948.

(23) Cantor, A., and Shelanski, H. A., 1951. A capacity test for germicidal action. Soap and Sanitary Chemicals, 27:133.

(24) Carlin, A. F., and Foth, J., 1952. Interior quality and functional properties of oiled and thermostabilized shell eggs before and after commercial storage. Food Technol., 6:443-450.

(25) Clinger, C., Young, A., Prudent, I., and Winter, A. R., 1951. The influence of pasteurization, freezing, and storage in the functional properties of egg white. Food Technol., 5:166-170.

(26) Conner, J. W., Snyder, E. S., and Orr, H. L., 1953. The influence of washing and oiling on grade and bacterial content of eggs stored for a nine month period. Poultry Sci., 32:227-235.

(27) Davidson, J. A., Dawson, L. E., and Sheppard, C. C., 1950. The occurrence of sour eggs in stored eggs. Michigan Agric. Exp. Sta. Quart. Bull. 32:526-534.

(28) Dawson, L. E., and Davidson, J. A., 1951. Farm practices and egg quality. Part II. Production practices affecting decline in egg quality. Mich. Agric. Expt. Sta. Quart. Bull. 34:89-104.

(29) Dawson, L. E., and Watts, J. P., 1952. Effect of nest litter on cleanliness of eggs. Poultry Sci., 31:913 (abs.).

(30) De Navarre, M. G., 1941. The Chemistry and manufacture of cosmetics. D. Van Nostrand Company, Inc., New York.

(31) Du Bois, A. S., and Dibblee, D. D., 1946. The influence of surface active cationic germicides on the bacterial population of milk. J. Milk Technol., 9:260-268.

(32) Elliot, R. P., 1954. Spoilage of shell eggs by Pseudomonas. Applied Microbiology, 2:158-164.

(33) Evans, R. J., 1942. Shell treatment of eggs by oiling. II. Effects of diluting the oil and of using oil with different physical properties on the interior quality of shell eggs. U. S. Egg and Poultry Mag., 48:596-599.

(34) Evans, R. J., and Carver, J. S., 1942. Shell treatment of eggs by oiling. I. Effect of time between production and oiling in interior quality of stored eggs. U. S. Egg and Poultry Mag., 48:546-549.

(35) Fahlberg, W. J., Swan, J. C., and Seastone, C. V., 1948. Studies on the retention of hexachlorphene (G-11) in human skin. J. Bact., 56:323-328.

(36) Feeney, R. E., Mac Donnell, L. R., and Lorenz, F. W., 1954. High temperature treatment of shell eggs. Food Technol., 8:242-245.

(37) Feeney, R. E., and Nagy, D. A., 1952. The antibacterial activity of egg white protein conalbumin. J. Bact., 64: 629-643.

(38) Fleming, A., and Allison, V. D., 1928. On the antibacterial power of egg white. Lancet, 206:1303-1306.

(39) Forsythe, R. H., 1952. The effect of cleaning on the flavor and interior quality of shell eggs. Food Technol., 6: 55-58.

(40) Forsythe, R. H., Ayres, J. C., and Radlo, J. L., 1953. Factors affecting the microbiological populations of shell eggs. Food Technol., 7:49-56.

(41) Funk, E. M., 1937. Factors influencing production of clean eggs. Mo. Agric. Expt. Sta. Bull. 384.

(42) Funk, E. M., 1938. Improving the keeping quality of eggs by cleaning with sodium hydroxide. Mo. Agric. Expt. Sta. Res. Bull. 277.

(43) Funk, E. M., 1940. The profitable utilization of soiled shell eggs. Am. Egg and Poul. Rev., 1:72.

(44) Funk, E. M., 1943. Pasteurization of shell eggs. Mo. Agric. Expt. Sta. Res. Bull., 364.

(45) Funk, E. M., 1948. Experiments in cleaning soiled eggs for storage. Mo. Agric. Expt. Sta. Res. Bull. 426.

(46) Funk, E. M., 1948. Wash eggs the right way. U. S. Egg and Poultry Mag., 54:(5)10.

(47) Funk, E. M., 1950. Maintenance of quality in shell eggs by thermostabilization. Mo. Agric. Expt. Sta. Res. Bull. 467.

(48) Funk, E. M., and Forward, J. F., 1949. Effect of washing soiled eggs on hatchability. Poultry Sci., 28:155-157.

(49) Funk, E. M., Forward, J., and Lorah, M., 1954. Minimizing spoilage in shell eggs by thermostabilization. Poultry Sci., 33:532-538.

(50) Gershenfeld, L., and Miller, R. E., 1932. The bactericidal efficiency of iodine solution. J. Am. Pharm. Assoc., Sci. Ed., 21:894-903.

(51) Gershenfeld, L., and Palisi, J. A., 1949. Evaluating the bactericidal efficiencies of free iodine and available chlorine by the "semi-micro" method. Am. J. Pharm., 121: 337-342.

(52) Gershenfeld, L., and Perlstein, D., 1939. Preservatives for preparations containing gelatin. Am. J. Pharm., 111: 277-287.

(53) Gillespie, J. M., Scott, W. J., and Vickery, J. R., 1950. Studies in the preservation of shell eggs. II. The incidence of bacterial rotting in unwashed eggs and in eggs washed by hand. Australian J. Applied Sci., 1:215-223.

(54) Gillespie, J. M., Scott, W. J., and Vickery, J. R., 1950. Studies in the preservation of shell eggs. III. The storage of machine-washed eggs. Australian J. Applied Sci., 1:313-329.

(55) Gillespie, J. M., and Scott, W. J., 1950. Studies in the preservation of shell eggs. IV. Experiments in the mode of infection by bacteria. Australian J. Applied Sci., 1: 514-530.

(56) Gillespie, J. M., Salton, M. R. J., and Scott, W. J., 1950. Studies in the preservation of shell eggs. V. The use of chemical disinfectants in cleaning machines. Australian J. Applied Sci., 1:531-538.

(57) Godfrey, A. B., and Olsen, M. W., 1937. Individual hen and breed differences in egg weight losses during incubation. Poultry Sci., 16:216-218.

(58) Goldsworthy, M. C., and Gerther, S. J., 1949. Fungicidal and phytotoxic properties of 506 synthetic compounds. Plant Disease Repts., Supplement 182:89-109.

(59) Goresline, H. E., Hayes, K. M., and Otte, A. W., 1952. Thermostabilization of shell eggs, quality retention in storage. USDA Circular No. 898, Jan., 1952.

(60) Goresline, H. E., Moser, R. E., Jr., and Hayes, K. M., 1950. A pilot scale study of shell egg thermostabilization. Food Technol., 4:426-430.

(61) Gunderson, M. F., 1946. A washed egg can be good. U. S. Egg and Poultry Mag., 52:111.

(62) Gunderson, M. F., and Gunderson, S. D., 1945. Eggs can be washed clean. U. S. Egg and Poultry Mag., 51:533.

(63) Hadely, P. B., and Caldwell, D. W., 1916. The bacterial infection of fresh eggs. Rhode Island Agric. Expt. Sta. Bull., 164.

(64) Haines, R. B., 1938. Observations on the bacterial flora of the hen's egg, with special description of new species of Proteus and Pseudomonas causing rots in eggs. J. Hyg., 38:338-355

(65) Haines, R. B., and Moran, T., 1940. Porosity of, and bacterial invasion through the shell of the hen's egg. J. Hyg., 40:453-461.

(66) Haugh, R. P., 1939. Unit for measuring egg storage conditions. Proc. World's Poultry Congr. (Cleveland), 7: 525-528.

(67) Haynes, W. C., 1953. Key to the species of genus Pseudomonas. Mimeo., 7 pp., presented at Symposium on Taxonomy, Aug. 13, 53rd general meeting of the Society of American Bacteriologists, San Francisco.

(68) Hearst, W. R., and Hearst, G. M., 1948. Stabilization of shell eggs. U. S. Patent Office, No. 2,438,168, March 23.

(69) Hoagerheide, J. C., 1945. The germicidal properties of certain quaternary ammonium salts with special reference to cetyltrimethyl-ammonium bromide. J. Bact., 49: 277-284.

(70) Hunter, M. C., Roman, D. P., and Shumard, R. S., 1952. Cutaneous antiseptic activity of 2,2'-thiobis (4,6-dichlorphenol). . . . actamar. Pro. Sci. Sect. Tiolet Goods Assoc., No. 18, 18-26.

(71) Hunter, M. C., Roman, D. P., and Shumard, R. S., 1953. Cutaneous antiseptic activity of 2,2'-thiobis (4,6 dichlorophenol). Amer. Perfume Essent. Oil. Rev., 61:122-127.

(72) Johns, C. K., 1947. A method for assessing the sanitizing efficiency of quaternary ammonium and hypochlorite products. Am. J. Public Health, 37:1322-1327.

(73) Johns, C. K., and Berard, H. L., 1946. Effect of certain methods of handling upon the bacterial content of dirty eggs. Sci. Agric., 26:11-15.

(74) Kahlenberg, O. J., Gorman, J. M., Goresline, H. E., Howe, M. A., Jr., and Baush, E. R., 1952. A study of the washing and storage of dirty shell eggs. USDA Circular No. 911, October, 1952.

(75) Kivela, E. W., Mallmann, W. L., and Churchill, E. S., 1948. Physical action of surface-active cations upon bacteria. J. Bact., 55:565-572.

(76) Kojima, S., 1931. Studies on peroxidase. II. The effect of peroxidase on the bactericidal action of phenols. J. Biochem. (Japan), 14:95-109.

(77) Kunz, E. C., Luthy, M., and Gump, W. S., 1944. Germicidal soap. U. S. Pat. 2,353,735, July 18, 1944.

(78) Lamson, G. H., Jr., 1908. Infection and preservation of eggs. Storrs (Connecticut) Agric. Expt. Sta. Bull., 55:203-214.

(79) Lawrence, C. A., 1950. Surface-active quaternary ammonium germicides. Academic Press Inc., New York.

(80) Lee, D. H. K., Robinson, K. W., Yeates, N. T. M., and Scott, M. I. R., 1945. Poultry husbandry in hot climates experimental inquiries. Poultry Sci., 24:195-207.

(81) Levine, M., and Anderson, D. Q., 1932. Two new species of bacteria causing mustiness in eggs. J. Bact., 23:337-347.

(82) Lilly, B. D., and Brewer, J. H., 1953. The selective antibacterial action of phenylethyl alcohol. J. Am. Pharm. Assoc., Sci. Ed., 42:6-8.

(83) Lorenz, F. W., 1949. On the efficiency of processing oils. Poultry Sci., 28:119-127.

(84) Lorenz, F. W., 1950. Egg washing field trials. Nulaid News, 27:(10)9-10.

(85) Lorenz, F. W., Ogasawara, F. X., and Starr, P. B., 1952. Spoilage of washed eggs. 3. A survey of ranch practices and results. Poul. Sci., 31:221-226.

(86) Lorenz, F. W., and Starr, P. B., 1949. What can be done about sour eggs? Nulaid News, 26:(1)3-4.

(87) Lorenz, F. W., and Starr, P. B., 1952. Spoilage of washed eggs. I. Effect of sprayed versus static water under different washing temperatures. Poultry Sci., 31:204-214.

(88) Lorenz, F. W., Starr, P. B., Starr, M. P., and Ogasawara, F. X., 1952. The development of Pseudomonas spoilage in shell eggs. I. Penetration through the shell. Food Research, 17:351-360.

(89) MacIntosh, J. A., Tanner, R., Evans, R. J., and Carver, J. S., 1942. Cooking properties of eggs processed in oil. U. S. Egg and Poultry Mag., 48:345.

(90) Mallmann, W. L., and Carr, R. E., 1941. The use of egg containers treated with a mycostat in commercial cold storage. U. S. Egg and Poultry Mag., 47:344-347.

(91) Mallmann, W. L., and Davidson, J. A., 1944. Oil-protected shell eggs. U. S. Egg and Poultry Mag., 50:113.

(92) Mallmann, W. L., Kivela, E. W., and Turney, G., 1946. Sanitizing dishes. Soap and Sanitary Chemicals, 8:130.

(93) Mallmann, W. L., and Michael, C. E., 1940. The development of mold on cold storage eggs and methods of control. Mich. Agric. Expt. Sta. Tech. Bull. 174.

(94) Mallmann, W. L., and Moore, J. M., 1936. Studies of pullorum disease. II. The incidence of Salmonella pullorum in eggs from infected hens. J. Am. Vet. Med. Assoc., 89, n.s. 42(1)35-52.

(95) Mandel, M., 1952. An approach to the mensuration of disinfectant penetrability. Thesis (PhD), Michigan State College.

(96) March, P. B., 1947. Mildew and rot resistance of textiles. Textile Research J., 17:597-615.

(97) March, P. B., and Butler, M. L., 1946. Fungicidal activity of bisphenols as mildew preventives on cotton fabric. Ind. Eng. Chem., 38:701-705.

(98) March, P. B., Butler, M. L., and Clark, B. S., 1949. Fungicidal activity of bisphenols. Ind. Eng. Chem., 41:2176-2184.

(99) Maurer, O., 1911. Bacteriological studies on eggs and egg products with special reference to B. coli. Kans. Agric. Exp. Sta. Bull. 180, 333-396.

(100) May, H. G., 1924. The examination of eggs from infected and immunized hens, with germicidal tests on albumen and blood serum. Rhode Island Agric. Expt. Sta. Bull. 197.

(101) McNally, E. H., 1952. Effect of drying after washing on the incidence of eggs infected by spoilage microorganisms. Poul. Sci., 31:1102-1104.

(102) Michell, L. C., 1934. Progressive changes in the composition of edible shell eggs during storage. Jour. Assoc. Offic. Agric. Chem., 17:506-511.

(103) Miles, A. A., and Halnan, E. T., 1937. A new species of micro-organism (Proteus melanovogenes) causing black rot in eggs. J. Hyg., 37:79-97.

(104) Miller, W. A., 1954. The microbiology of dirty eggs treated in various ways and stored at different temperatures and humidities. Poultry Sci., 33:735-742.

(105) Miller, W. A., and Crawford, L. B., 1953. Some factors influencing bacterial penetration of eggs. Poultry Sci., 32:303-309.

(106) Miller, M. W., Joukovsky, V., and Kraght, A., 1950. Experiments relating to the spoilage of washed eggs. Poultry Sci., 29:27-33.

(107) Moness, E., Braher, W., and Christiansen, W. G., 1932. Germicidal activity of aromatic sulphides. J. Am. Pharm. Assoc., 21:557-569.

(108) Moran, T., and Pique, J., 1926. The storage of eggs. Dept. Sci. Ind. Research (Brit.) Food Investigation Spec. Repts. 26.

(109) Neidig, C. P., and Burrell, H., 1944. The esters of para-hydroxybenzoic acid as preservatives. The Drug and Cosmetic Industry, 54:408.

(110) Ostrolenk, M., and Brewer, C. M., 1949. A bactericidal spectrum of some common organisms. J. Am. Pharm. Assoc., Sci. Ed., 38:95-106.

(111) Pennington, M. E., 1909-1910. A chemical and bacteriological study of fresh eggs. J. Biol. Chem., 7:109-132.

(112) Pennington, M. E., 1934. Loss of weight of eggs in cold storage. U. S. Egg and Poultry Mag., 40:(April)19-22.

(113) Pennington, M. E., and Pierce, H. C., 1910. The effect of the present method of handling eggs on the industry and the product. USDA Yearbook, 1910:461-476.

(114) Penniston, V., and Hedrick, L. R., 1944. Use of germicide in wash water to reduce bacterial contamination on shell. U. S. Egg and Poultry Mag., 50:26.

(115) Penniston, V. S., and Hedrick, L. R., 1945. The germicidal efficiency of emulsept and of chlorine in washing dirty eggs. Science, 101:362-363.

(116) Penniston, V., and Hedrick, L. R., 1947. The reduction of bacterial count in egg pulp by use of germicides in washing dirty eggs. Food Technol., 1:240-244.

(117) Pernot, E. F., 1909. An investigation of the mortality of incubator chicks. Oregon Agric. Coll. Expt. Sta. Bull. 103.

(118) Pino, J. A., 1950. Effect of washing with a hot detergent solution on keeping quality and hatchability of eggs. Poultry Sci., 29:888-894.

(119) Quinso, R. A., Gibby, I. W., and Foter, M. J., 1946. The effect of agar upon the germicidal efficiency of the quaternary ammonium salts. J. Am. Pharm. Assoc., Sci. Ed., 35:317-319.

(120) Quinso, R., Gibby, I. W., and Foter, M. J., 1946. The effect of agar upon the germicidal potency of the quaternary ammonium salts. J. Bact., 51:602.

(121) Quinso, R. A., and Foter, M. J., 1947. Errors in compounding and testing semi-solid products containing quaternary ammonium salts. J. Bact., 53:501-502.

(122) Reddish, G. F., 1954. Antiseptics, disinfectants, fungicides, and chemical and physical sterilization. Lea and Febiger, Philadelphia.

(123) Rettger, L. F., 1913. Bacteriology of the hen's egg, with special reference to its freedom from microbic invasion. Storrs (Connecticut) Agric. Expt. Sta. Bull. 75:191-213.

(124) Rettger, L. F., and Stoneburn, F. H., 1911. Bacillary white diarrhea of young chicks. Storrs (Connecticut) Agric. Expt. Sta. Bull., 68:278-301.

(125) Rhodes, W. F., and Godfrey, G. F., 1950. The effect of washing eggs on the cuticle as measured by moisture loss and hatchability. Poultry Sci., 29:833-836.

(126) Rievel, H., 1939. Die Durchlässigkeit Der Eischale für die Bakterien und ihre Bedentung für die Kühlhauslagerung. Proc. World's Poultry Congr. (Cleveland), 7:478-483.

(127) Romanoff, A. L., 1940. Physiochemical changes in unfertilized incubated eggs of Gallus domesticus. Food Research, 5:291-306.

(128) Romanoff, A. L., 1943. Study of various factors affecting permeability of bird's eggshell. Food Research, 8:212-223.

(129) Romanoff, A. L., and Romanoff, A. J., 1944. A study of preservation of eggs by flash-heat treatment. Food Res., 9:358-366.

(130) Romanoff, A. L., and Romanoff, A. J., 1949. The avian egg. John Wiley and Sons, Inc., New York.

(131) Romanoff, A. L., and Yushok, W. D., 1948. Preservation of intact eggs by sealing with chemical agents. Food Res., 13:331-335.

(132) Rose, D., and Gibbons, N. E., 1950. The effect of oiling and air cell mold on the oxygen and carbon dioxide contents of the air cells of eggs. Canadian J. Res., F., 28:101-106.

(133) Rosser, F. T., 1942. Preservation of eggs. II. Surface contamination on egg-shell in relation to spoilage. Can. J. Res., D, 20:291-296.

(134) Rosser, F. T., White, W. H., Woodcock, A. H., and Fletcher, D. A., 1942. Preservation of eggs. I. Treatments for maintaining quality in shell eggs at ordinary temperatures. Can. J. Research, D, 20:57-70.

(135) Rudolf, A. S., and Levine, M., 1941. Factors affecting the germicidal efficiency of hypochlorite solutions. Iowa Eng. Exp. Sta. Bull. No. 152.

(136) Ruehle, G. L. A., and Brewer, C. M., 1931. United States Food and Drug Administration methods of testing antiseptics and disinfectants. USDA Circular No. 198.

(137) Salton, M. R. J., Scott, W. J., and Vickery, J. R., 1951. Studies in the preservation of shell eggs. VI. The effect of pasteurization on bacterial rotting. Australian J. App. Sci., 2:205-222.

(138) Scott, W. J., and Vickery, J. R., 1954. Studies in the preservation of shell eggs. VII. The effect of pasteurization on the maintenance of physical quality. Australian J. App. Sci., 5:89-102.

(139) Sharp, P. F., 1937. Preservation and storage of hens' eggs. Food Res., 2:477-498.

(140) Sharp, P. F., and Stewart, G. F., 1936. Effect of relative humidity on the growth of mold on eggs in storage. Cornell University Exper. Sta. Mem. 191.

(141) Sinder Corporation, 1952. G-11 (brand of hexachlorophene) bacteriological properties. Technical Bull., H4, December, 1952.

(142) Sokol, H., 1952. Recent developments in the preservation of pharmaceuticals. Drug Standards, 20:89-106.

(143) Solowey, M., Spaulding, E. H., and Goresline, H. E., 1946. An investigation of a source and mode of entry of Salmonella organisms in spray-dried whole-egg powder. Food Res., 11:380-390.

(144) Spamer, C. O., 1931. Historic method and practice of preserving eggs in Holland. U. S. Egg and Poultry Mag., 37:(2)48-51.

(145) Starr, P. B., Lorenz, F. W., and Ogasawara, F. X., 1952. Spoilage of washed eggs. 2. Laboratory versus ranch washing. Poultry Sci., 31:215-220.

(146) Stewart, G. F., 1935. The structure of the hen's egg shell. Poultry Sci., 14:24-32.

(147) Stewart, G. F., and Bose, S., 1948. Factors influencing the efficiency of solvent-oil mixtures in the preservation of shell eggs. Poultry Sci., 27:270-276.

(148) Stuart, L. S., and McNally, E. H., 1943. Bacteriological studies on the egg shell. U. S. Egg and Poultry Mag., 49:28.

(149) Suess, A., 1936. Preservatives for cosmetics. Am. Perfumer Essent. Oil Rev., 32:55-57.

(150) Swenson, T. L., and James, L. H., 1931. Storage experiments on vacuum oil dipped eggs. U. S. Egg and Poultry Mag., 37:(5)28-31.

(151) Tobie, W. C., and Ayres, G. B., 1944. Lack of correlation between plate tests and phenol coefficient determinations on germicides and antiseptics. J. Bact., 47:109.

(152) Wilm, 1895. Ueber die ein Wanderung von Choleravibrionen ins Huhnerei. Arch. Hyg., 23:145-169.

(153) Winter, A. R., 1942. Black rot in fresh shell eggs. U. S. Egg and Poultry Mag., 48:506.

(154) Winter, A. R., 1954. Direct communication.

(155) Winter, A. R., Burhart, B., and Wettling, C., 1952. Cleaning eggs for market. Ohio Agric. Expt. Sta., Res. Bull. 710.

(156) Wolk, J., McNally, E. H., and Spicknall, N. H., 1950. The effect of temperature on the bacterial infection of shell eggs. Food Technol., 4:316-318.

(157) Wolken, J. J., and Schwertz, F. A., 1948. Physical structure of shell membranes. J. General Physiology, 32:153-161.

(158) Wyss, O., and Strandskov, F. B., 1945. Germicidal action of iodine. Arch. Biochem., 6:261-268.

(159) Zagaevsky, J. S., and Lutikova, P. O., 1944. Sanitary measures in the egg breaking plant. U. S. Egg and Poultry Mag., 50:17-20, 43-46, 75-77, 88-90, 121-123.