MICROBIAL PENETRATION OF EGGS

by

LESLIE BRYCE CRAWFORD

B. S., Kansas State College
of Agriculture and Applied Science, 1949

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1950

TABLE OF CONTENTS

INTRODUCTION

The avian egg has been an important article of diet since the days of primitive man. Today the type of egg which he consumes will vary with the locality in which he lives. In some parts of England and Germany, Plovers' eggs are considered a delicacy; however, the hen's egg is generally the most widely used. Preserved hen eggs, or "pinda", are considered a delicacy by the Chinese. The average American on the contrary prefers his eggs fresh; he does not care for cold storage eggs.

The trend toward larger consumption of eggs has emphasized the problems of handling and storage of eggs. The restriction of mass production of eggs, principally to rural areas, usually necessitates the handling and shipping of eggs long distances in order to reach the urban centers for distribution and storage.

The egg is a perishable product and must be handled carefully to prevent undesirable changes in quality. The greater production takes place in the spring and, because of this, eggs must be stored for winter consumption. Eggs are subject to both physical and chemical changes which result in deterioration of quality and in addition they may undergo microbial spoilage.

This study was undertaken in an effort to obtain factual information relative to the influence of various factors of treatments and subsequent storage.

REVIEW OF LITERATURE

The ways and means of bacterial penetration of the egg have been a subject

of controversy for many years. It is possible that microorganisms may gain entrance into the egg both before and after the shell is laid down.

Relative to bacteria entering the egg before the shell is formed Tanner (23) reported the following:

> In keeping with those who believe in bacterial penetration before the shell is deposited, Pernot (1909) stated that infection of the yolk even in the normal ovary is possible. Zimmerman (1878), Abel and Draer (1895), Cao (1908), McClintock (1894), Poppe (1910), and others maintained that the oviduct is not sterile. Hadley and Caldwell (1916) thought that the preponderance of yolk infections indicated that bacteria are present in the ovaries of the hen. Lamson (1908) made dissections of hens for examination of the ovary and oviduct. Bacteria were present in the oviduct of the hen, even in the upper portions, so that an egg may be infected in the earlier stage of its formation, particularly at the time when the white or albumen is secreted. Bushnell and Mauer (1914) pointed out that there are factors which lower the vitality of the hen and render her unable to resist invading bacteria. A diseased condition of the ovary of the hen may cause infection of the egg. Rettger (1912) reviewed the results obtained by earlier investigators which are not in harmony with those which he obtained. He suggested that the methods employed in making previous tests may have been at fault and it was highly improbable that normal fresh eggs contain bacteria and molds in such large proportions as various investigators have indicated. The views of Horowitz substantiated those of Rettgar. It was believed that autosterilization of the oviduct is due to the following: (1) phagocytosis, (2) mechanical action of the walls of the oviduct, and (3) bactericidal action of the secretions.

Romanoff and Romanoff (18, p.567) cite Arnold as reporting in 1929 that "raw egg albumen increases the permeability of the intestinal wall to bacteria".

The egg may be infected after it has been laid, since it is possible for microorganisms to pass through the pores of the shell.

Tanner (23) reviewed the literature on this phase of the subject in the following terms:

> The experiments of Zorkendorfens (1893) indicated that neither the outer shell nor the membrane next to the shell are impervious to bacteria. Nine species of bacteria were found in one nest, hence Lamson thought that nesting material was a great source of infection; if it has been allowed to remain unchanged for a long time it becomes foul and teems with bacteria. Mauer (1911) concluded that fecal matter is the source of many of the colon bacilli often present in egg preparations. Kossowicz (1913) said eggs, the shells of which were soiled by the contents of

> either fresh or decayed eggs, were found to be more susceptible to the invasion of microorganism. Wilm (1895) succeeded in infecting eggs with Vibrio comma. When the eggs were covered with a broth culture, the organisms passed through the shell in from 15 to 16 hours. Golokov (1896), Piorowski (1895), Lange (1907), and Popper (1910) demonstrated the same thing with other bacteria, both pathogenic and nonpathogenic. Rullman (1916) never observed the penetration of bacteria into eggs the shells of which were intact.

Romanoff and Romanoff (18, p.495) stated that although there is a wide variation in the size of the pores of the shell, some are large enough to allow microbial penetration. They believed that moisture plays an important part in egg infections. Normally the fresh egg is covered with a mucilaginous coating which acts as an efficient mechanical barrier if kept dry (p.498); however, it is water soluble and infection is not difficult when the egg is moist (p.691).

Tanner (23, p.917) states:

> Whatever the means of infection, larger percentages of the July, August, and early September eggs were infected or contained a greater number of bacteria (at a time when they were called fresh) than the eggs of the other months of the year.

Kennard (3) believed weight and shell strength of eggs are of extreme importance in connection with the production of market eggs. Weak shelled eggs are a nuisance and a loss to all concerned. Eggs were weighed and tested for egg shell strength several times between January 26 and September 9. Warm weather conditions reduced the weight and lessened the shell strength of eggs from all groups of layers. With the onset of cool weather the weight of the eggs promptly increased and the breakage from shock tests was reduced from 41 percent to 14 percent.

Perry (13), in comparing the methods of handling shell eggs today with those in general use 15 to 20 years ago, found the biggest difference in methods of collection.

Stuart and McNally (22) expressed the opinion that bacteria may penetrate

the shell almost instantaneously, and emphasize the necessity of keeping the shell clean to prevent infection. They found bacteriostatic activity on the part of the shell membrane and suggested that if penetration of the shell is accomplished only by limited numbers of bacteria the shell membrane may be able to destroy them before they succeed in passing through it into the albumen and yolk.

Miller and Crawford (7) studied the antibacterial action of egg shell membranes in numerous tests involving 450 eggs over a period of several months. When the macerated membrane was suspended in peptone water and innoculated with Pseudomonas aeruginosa (peptone water alone as a control), inhibition was obtained for about 16 hours. The degree of inhibition was increased with constant shaking or aeration of flasks. Microscopically no clumping or adsorption of organisms by the membrane was observed.

Romanoff and Romanoff (18, p.496) stated that, "the presence of moisture on the exterior of the egg is conducive to bacterial invasion".

Stuart and McNally (22) showed that penetration of the wet shell may be effected by certain bacteria as shown by a study in which eggs were swabbed externally with a liquid culture of Pseudomonas aeruginosa, (Fig. 1).

Miller and Crawford (8) endeavored to determine the approximate time, under severe conditions, necessary for spoilage bacteria to penetrate the shell and shell membranes and to initiate growth in the white or yolks. A total of 210 clean, fresh, spring and summer eggs were dipped in broth cultures of egg-spoilage organisms (Pseudomonas and other types) and incubated at 37° C. in a saturated atmosphere where shells were constantly moist. Penetration and growth were negligible up to three days as evidenced by negative plate and broth cultures.

Khoury (4) stated that in warm weather, many eggs are handled in the same way that they are in cold weather.

Romanoff and Romanoff (18, p.693) state that, "cooling of the egg at the place of production is an important step in preliminary handling. If the egg is not cooled as soon as possible after it is laid, the processes of deterioration may soon begin".

Peterson (14) stated that all eggs that do not go into storage or are not broken for freezing or drying should be eaten within three weeks after they are laid. They should reach the consumer's refrigerator within two weeks.

Methods of Preserving Eggs

Since heaviest egg production occure during a few months of the year, it is necessary to resort to methods of preservation to prevent physiochemical deterioration and microbial spoilage. The nature of the egg and its chemical constitution make it very susceptible to attack by microorganisms. Jones and DuBois (1920) classified the various methods of preservation as follows: (1) Low temperature, (2) airtight packing, (3) sealing with various agents, and (4) immersion in preserving solutions. Romanoff and Romanoff (18, p.697) also list "dry packing".

Dry Packing. Romanoff and Romanoff (18, p.697) reported that:

> Preservation of intact eggs by dry packing has been frequently attempted in the past and is occasionally still practiced today. If the packing material is loose it does not prevent evaporation or decomposition. If it is compact, it can only retard, and not prevent, the growth of microorganisms, although some anti-biotic packing substances have been suggested. Dry packing is not feasible commercially, because of the necessity of transporting the excess weight of the packing material with the eggs. The Chinese have preserved eggs by methods similar to dry packing. The egg is not retained in its original state but rather converted into an entirely different article of food (Wang, 1929), probably by bacterial action.

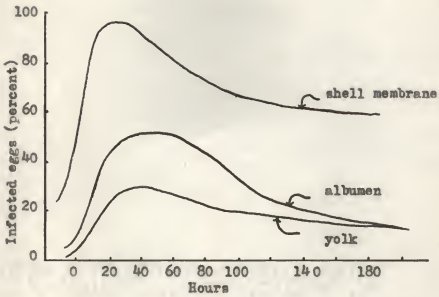

Fig. 1. Incidence of contamination in shell membrane, albumen, and yolk observed during 200 hours' incubation of eggs, the shells of which had been smeared with a culture of Pseudomonas aeruginosa. (After Stuart and McNally, 1943)

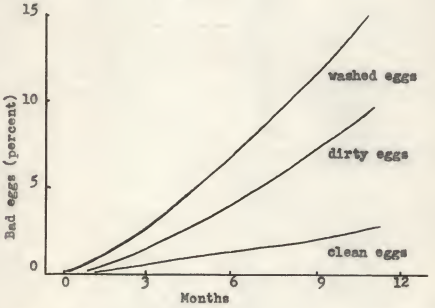

Fig. 2. Incidence of rotting in clean, dirty, and washed eggs held in cold storage. (After Jenkins, Hepburn, Swan and Sherwood, 1920)

Low Temperature. Romanoff and Romanoff (18, p.703) suggested that intact eggs be held at the lowest possible temperature without freezing. The relative humidity should be between 80 and 90 percent unless mycostatic agents are used, in which case the relative humidity may be increased to 92-98 percent.

Airtight Packing. A new process in preserving eggs (11) was described in 1912 in which carbon dioxide and nitrogen gases were employed as bacteriostatic agents. In this process eggs were candled and placed in metal containers. The containers were then placed in large tanks which were filled with a mixture of the gasses under pressure; the pressure is removed and the egg containers sealed.

Wilhelm (26) described the vacuum packing of eggs under 14 inches of vacuum (mercury) in which the vacuum is released with carbon dioxide. This process is about 40 percent better in preserving eggs than no treatment.

Tanner (23, p.936) states that "gas storage" in atmosphere of carbon dioxide was believed to offer some help. Sixty percent of carbon dioxide was required to completely inhibit mold development on eggs stored at 0° C. over a period of nine months. Ozone has been suggested as a preservative for eggs. The amount used in the atmosphere must be small and well controlled. Ten percent of ozone in the air produced eggs which were off odor. Three percent ozone did not give an unpleasant taste and inhibited mold growth.

Romanoff and Romanoff (18, p.711) state that Pennington and Horne in 1924 suggested that "eggs held in low concentrations of ozone did not acquire any trace of mustiness and were said to be indistinguishable in taste from fresh eggs, even after eight months of storage".

Sealing with various agents. Sealing the pores of egg shells with sealing agents has been practiced for many years. Some sealing agents, although highly

efficient as preservatives, are of questionable value as they impart undesirable odors.

Eggs preserved by coating with lard (15) was recommended by Campanini (Italy). No holding temperature was given, but the eggs kept very satisfactorily throughout the cold winter and hot summer.

Lorenz (5) reported that coating eggs with mineral oil or light paraffin oil containing 0.25 percent pentachlorophenol prior to storage is a common commercial practice. The treatment is inexpensive and prevents a high percentage of the moisture loss that otherwise occurs during the holding period. The major objection to this process is the oily shine. The persistence of the oil is the major characteristic determining the amount of shine remaining on the shell at the end of the storage period.

Romanoff (17) states that oil treating was ineffective unless the eggs were held at low temperature, and that the oil remaining on the surface of the egg promoted the growth of molds.

When eggs were vacuum oiled (and the vacuum replaced with carbon dioxide) (25) penetration of the oil was increased about 4½ times. After 10 months' storage these eggs lost only 0.1 percent of their weight, while eggs oiled in open vessels lost 16 times as much and untreated eggs nearly 27 times as much moisture.

Romanoff and Yushok (19) described a lactic acid treatment of eggs. This method does not seem to be so efficient in sealing the pores as the oiling method. The calcium lactate formed is soluble in hot water, but only slightly soluble in cold water. When lactic acid treated eggs are immersed in hot water the calcium lactate dissolves and the expanding gasses escape. Dipping eggs in normal lactic acid was effective in reducing their loss of water and in slowing down the change in pH of the albumen.

Heat also plays an important part in the preservation of eggs in many processes, although it usually entails extra storage expense.

A treatment has been described (21) in which the eggs are immersed for about five seconds in an oil solution heated to 121° C. The immersion is said to sterilize the egg and close the pores of the shell, but is so rapid that the yolk and white are not affected and remain in a natural condition.

Highly satisfactory results have been obtained in experiments where fresh eggs have been roasted (16) at oven heat for a short period, just long enough to seal the membrane to the inside of the shell and destroy bacteria. Eggs so treated and held several months without refrigeration when broken show an unusual degree of freshness.

"Plast-O-Treat" (12) is a new type thermoplastic resin egg preservative. The resin is a neutral, water soluble, colloid which possesses dispersing, wetting, stabilizing, and disinfecting qualities. The thickness of the film surrounding the egg may be altered thus varying the permeability.

Yushok and Romanoff (28) stated that oil treatment apparently had little effect upon losses of water and carbon dioxide from cracked eggs. Double dipping of cracked eggs with chlorinated rubber and n-butyl stearate sealed the cracks. The cracked eggs were as well preserved as eggs with sound shells. For best preservation the plastic coating should be carried out at the place of production, preferably the same day the egg is laid. This process does not require elaborate equipment and cost of materials is not prohibitive for practical use.

Immersion in Preserving Solutions. Riccardo and DiGenova (15) described a method of preserving eggs by covering them with sodium or potassium silicate solutions in combination with calcium and magnesium compounds. Insoluble silicates thus formed seal the pores of the egg shell.

Rumball (20) stated that cold storage is not always practicable. Under

such conditions eggs may be packed in salt or greased with a suitable fat. Clean fresh eggs may be stored by immersing in a solution of sodium silicate (water glass) or lime water until ready for use.

Methods of Cleaning Dirty Eggs

There are always a considerable number of dirty eggs which must be cleaned before they are sold. Many cleaning methods are detrimental to the keeping quality of eggs from a bacteriological standpoint.

Romanoff and Romanoff (18, p.690) state that dirty eggs should not be considered for storage since they are more subject to spoilage than normally clean eggs, (Fig. 2). Increased losses in storage resulting from washing dirty eggs can be reduced if the washing water contains certain chemical agents.

Johns and Berard (2) found that washing eggs with a wet cloth before storage did not increase the number of infected eggs, but the average bacterial count was appreciably higher than that of eggs washed shortly before analysis.

Winter (27) believed that when eggs cool and the contents shrink there is a tendency to pull bacteria through the shell. Eggs therefore should be washed in a solution warmer than the egg. The contents then have a tendency to expand and keep out microorganisms.

Moyer (9) described a power driven cloth buffer wheel and an abrasive compound that have been used with some success in cleaning eggs.

An egg washing machine developed at the Cornell University Research Foundation (1) consists essentially of a series of abrasive coated cloth discs. The eggs are passed under these discs by means of a series of moving fingers supplied with hot water (71.5° C.) through a perforated pipe. The eggs are dried rapidly.

EXPERIMENTAL

Analytical Procedures

Organoleptic observations and quantitative microbiological determinations (plate counts) were made on each egg in all phases of the experimental work.

Tanner (23, p.939) listed several methods of examining shell eggs. Of the methods listed none was found to be completely satisfactory. It was desired to keep the manipulation of the egg and equipment at a minimum in order to reduce to a minimum the incidence of contamination.

The eggs were taken from the storage room at definite time intervals. Each egg was washed in a detergent (Tide) solution, rinsed in warm water and allowed to drain dry on a wire rack. The eggs were then candled, principally to ascertain if the shells were intact, and then placed in the refrigerator. Each egg was then cultured individually for numbers and types of microorganisms.

All eggs were opened aseptically by heating the small end in an open flame until a film of albumen was coagulated on the inside of the shell. The end of the egg was then knocked off with a sterile case knife, and the contents emptied into a sterile "Mason" jar. The jar was then fitted with a special four bladed cutter and attached to an "Osterizer" (a high speed blender) and the white and yolk mixed for one minute. Appropriate dilutions were made and the eggs were plated out on tryptone glucose-extract agar. Colonies were counted after incubating plates three to five days at room temperature.

In view of the fact that approximately 25 percent of all fresh eggs examined contained small numbers of bacteria, in all subsequent reference to the absence of microorganism in treated eggs it should be interpreted as meaning

that there were no more microorganisms present than in the original analysis of freshly laid eggs or, in other words, that no significant increase in numbers had taken place. On the other hand, no particular significance is attached to the actual numbers recorded in Tables 1 and 2. Interest has been centered primarily on whether significant growth of microorganisms would take place in the variously treated eggs. It was impracticable to plate out all eggs in dilutions such as to make possible accurate colony counts where variations of from less than 10 bacteria per gram to more than a billion per gram might be expected. Hence the data in most instances merely indicate that the number of organism per gram in the highest dilution plated out were in excess of the number which could be estimated with any degree of accuracy. In all such instances, however, there is no question but that significant growth had occurred.

Summary of Preliminary Results Obtained

Some exploratory work was done on 500 fresh day old eggs. The eggs were treated in various ways and held under varying conditions of temperature and humidity. In Table 1 a portion of the experimental data are shown.

Washed and unwashed eggs, smeared with contaminated material, resisted penetration from 7 to 14 days when held at room temperature with the humidity approaching saturation. The shells were not noticeably wet.

Similarly treated eggs held at refrigerator and room temperatures with shells noticeably wet showed penetration and growth of bacteria and molds within 14 days.

No microorganisms were isolated from 12 out of 13 contaminated unwashed

Table 1. Periodical examination of eggs held under varying conditions of temperature and humidity.

Storage conditions	Days of storage	Washed		Unwashed	
		Total no. examined	No. containing microorganisms #	Total no. examined	No. containing microorganisms #
Refrigerator (5-7° C., over wet cotton)	14-18	4	3 >200	0	
	20-28	0		4	1 41T
	36-38	2	2 > 115M	2	1 114M
	50-52	2	2 > 1B	1	1 < 10
Constant temp. and humidity (15-18° C., 60-65 percent relative humidity	14-18	4	2 > 3T	0	
	20-28	4	3 > 28M	0	
	36-38	0		4	1 5M
	50-52	2	2 > 418M	9	9 < 10
Room temperature (25-32° C.)	14-18	8	8 > 140M	8	8 > 16M
	20-28	3	3 > 16M	3	3 > 400M

\# T thousands per gram
M millions per gram
B billions per gram

> greater than
< less than

eggs which were held 52 days at 15-18° C. and 65 percent relative humidity (constant temperature storage room).

Eight out of 10 washed and contaminated eggs stored in the above mentioned room for 28 days contained spoilage bacteria in appreciable numbers.

Twelve clean fresh eggs held at room temperature for 35 days with no treatment and kept dry showed no colonies when plated.

Methods of Procedure

From the data obtained in the preliminary results it was decided to use the constant temperature and humidity room for storage of additional eggs. The results obtained indicated that egg spoilage microorganisms would grow in eggs at this temperature making it possible to use a shorter period of storage than would be possible under commercial refrigeration (0 to -1.8° C.).

Fresh eggs for all phases of this experiment were obtained from the college poultry farm between February 21 and March 11, 1949. Extremely dirty, infertile eggs were selected in order to provide large numbers of microorganisms on the shells since this type of egg is a commercial problem of some magnitude.

Each group consisting of 108 eggs was collected over a period of not more than one week and held in the egg storage cellar at 2-8° C. during the gathering period. Six groups were collected and treated as follows:

Group # 1. Lye Treated. The eggs were placed in one percent lye (Lewis lye) solution (26-32° C.) and allowed to stand for 20 to 25 minutes. After removal from the solution any remaining dirt was wiped off with a dry clean cloth. The eggs were then redipped in clean one percent lye solution (26-32° C.),

removed and placed on a wire rack to drain dry, treated with commercial egg processing oil (25-30° C.) and stored.

Group #2, Roccal Treated. The eggs were washed in a one percent detergent (Tide) solution (26-32° C.) and drained dry, then immersed in Roccal solution (one ounce per four gallons of water, 26-32° C.) for 15 minutes, drained dry, oiled, and placed in storage.

Group #3, Water Washed Treated. This group of eggs was washed in tap water at 26-32° C., drained, oiled, and stored.

Group #4, Pasteurized Treated. These eggs were washed in water (35-40° C.) and without drying were immersed in hot water at 73-75° C. for 20 seconds, drained dry, oiled, and stored

Group #5, Sanded Treated. Large encrustations of dirty material were removed with a knife. The remaining visible dirt was removed with 1/0 sandpaper on a power buffer. The eggs were then dipped in egg processing oil and placed in storage.

Group #6, Lactic Acid Treated. The eggs were washed in a one percent "Tide" solution (26-32° C.) rinsed in tap water (26-32° C.), drained dry, immersed in two percent lactic acid solution (26-32° C., pH 2.1 to 2.32) for one minute, drained, oiled, and stored.

All groups of eggs were placed in storage at the college poultry farm in the constant temperature and humidity room (15-18° C., 60-65 percent relative humidity). Eggs were removed and cultured at definite time intervals over a period of 35 weeks.

Results Obtained

Each treatment is listed and discussed individually. In addition, the

complete data are shown in Table 2.

Group #1. The lye treated eggs showed no mold growth over the 35 week period of examination. Pseudomonas type, cocci or diphtheroid bacteria were present in a total of 6 percent of the eggs examined during the first three months.

Pseudomonas types were present in 4 percent of these eggs during the fourth, fifth, and sixth months. Cocci or diphtheroid types were present in 12.5 percent of eggs cultured in this period.

During the seven to nine-month period eggs containing Pseudomonas type organisms had increased to 13 percent. The cocci or diphtheroid types were present in 15 percent of the eggs examined.

Group #2. No microorganisms were isolated from the "Roccal" treated eggs during the first three months of the storage period.

During the fourth to sixth month period 12 percent of these eggs contained appreciable numbers of microorganisms (Pseudomonas types, 4 percent; cocci or diphtheroids, 4 percent; molds, 4 percent).

During the seven to nine-month period 12.8 percent of the eggs contained Pseudomonas, cocci or diphtheroids. No molds were isolated in the latter period.

Group #3. No molds or diphtheroids were isolated from the water washed eggs; however, Pseudomonas types were present in 5.3 percent of those eggs examined during the first three months.

During the second three-month period 16.5 percent of the eggs contained microorganisms (Pseudomonas type in 4 percent; cocci or diphtheroids in 12.5 percent).

In the third three-month period 15 percent of these eggs contained

Pseudomonas types and 2.5 percent yielded molds. The cocci or diphtheroid types were present in 17.5 percent of the eggs examined.

Group #4. In the pasteurized eggs, plate cultures revealed that only 2 out of 100 eggs contained microorganisms.

Group #5. No gram negative bacilli were isolated from the sanded eggs examined during the first six months; however, cocci or diphtheroid bacteria were found in appreciable numbers in 5 percent of the eggs.

In the third three-month period Pseudomonas types, molds, and cocci or diphtheroids were present in 17.3 percent of the remaining eggs.

Group #6. No molds were isolated from the lactic acid group of eggs. Pseudomonas types were present in 10.8 percent of the eggs examined in the first three-month period, while no cocci or diphtheroids were recovered.

During the second period Pseudomonas types and cocci or diphtheroids were present in 25 percent and 10 percent of the eggs, respectively.

Eggs examined in the third period contained the same organisms in approximately the same percentages as during the second three-month period.

The combined data obtained from all treatments of eggs are shown graphically in Fig. 3. From this chart it is evident that the percentage of eggs containing microorganisms (all types) increased monthly over the period of examination, with the exception of the last three-week period. The failure to show an increase during the last period may have been due to a smaller group of eggs being examined.

In Fig. 4 is shown the relative frequency with which the dominant types of microorganisms were encountered in variously treated eggs. It is difficult to draw definite conclusions from the data in Fig. 4 since only 100 eggs were used in each treatment; however, the Pseudomonas type was encountered with

relatively high frequency in the "lactic acid" group and very low in the group pasteurized at 75° C. for 20 seconds.

Microorganisms Isolated. In every instance the organism isolated was the predominant type appearing on the culture plate.

1. Penicillium brevicaule
2. Aspergillus sp.
3. Pseudomonas rathonis
4. Pseudomonas desmolyticum
5. Pseudomonas aeruginosa
6. Pseudomonas putrefaciens
7. Pseudomonas dacunhae
8. Pseudomonas ambigua
9. Micrococcus flavus
10. Micrococcus candidus
11. Streptococcus sp.
12. Corynebacterium holvolum
13. Escherichia freundii
14. Escherichia intermedium
15. Proteus sp.
16. Streptomyces sp.

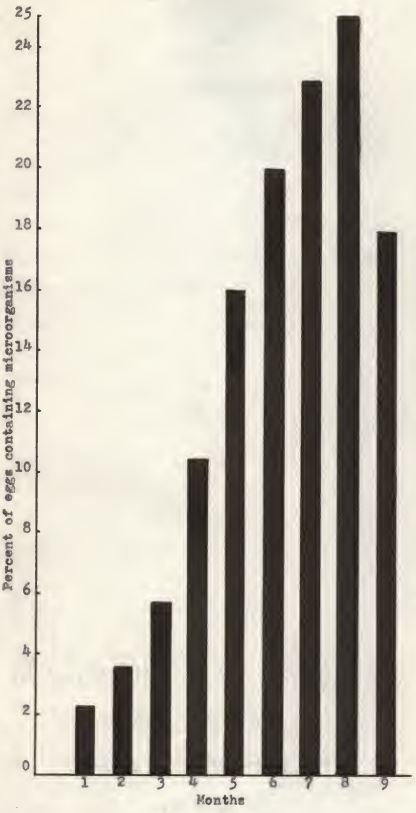

Fig. 3. Percentage of eggs per month containing microorganisms in significant numbers (all treatments). The last period of storage was only a three-week period, in which a smaller group of eggs was examined.

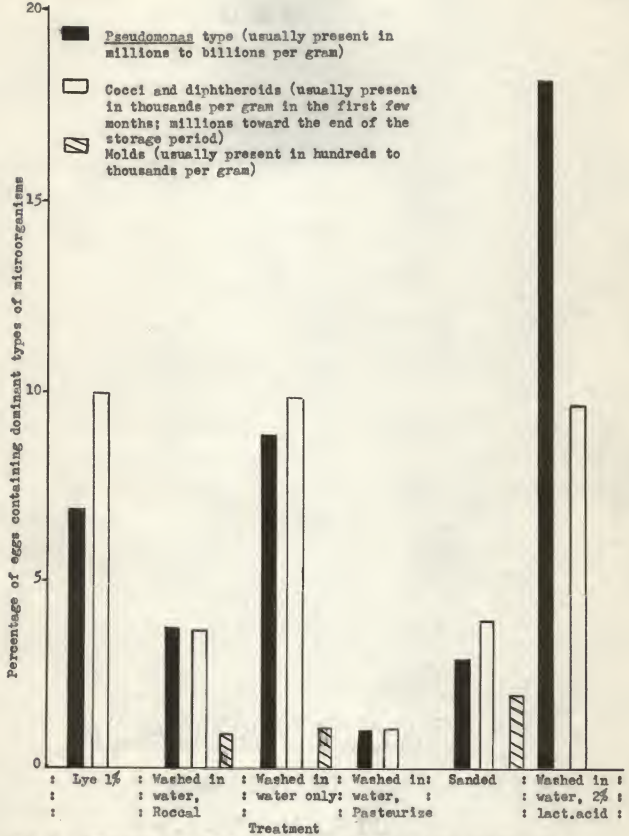

Fig. 4. Frequency with which the three major types of organisms were encountered in variously treated and stored eggs.

Table 2. Data showing weekly quantitative microbiological examinations of eggs stored at constant temperature and humidity (15-18° C., 60-65% relative humidity).

Week	# 1 Lye Group T#	P	C	M	# 2 Boccal Group T#	P	C	M	# 3 Water Washed Group T#	P	C	M
2	5	•	•	•	5	•	•	•	5	•	•	•
3	5	•	•	•	5	•	•	•	5	•	•	•
4	5	•	•	•	5	•	•	•	5	1 14M	•	•
5	5	•	•	•	5	•	•	•	5	•	•	•
6	5	•	1 140T	•	5	•	•	•	5	•	•	•
7	5	•	•	•	5	•	•	•	5	1 58M	•	•
8	0	•	•	•	0	•	•	•	0	•	•	•
9	4	1 >1M	•	•	4	•	•	•	4	•	•	•
10	0	•	•	•	0	•	•	•	0	•	•	•
11	4	•	•	•	4	•	•	•	4	•	•	•
12	0	•	•	•	0	•	•	•	0	•	•	•
13	4	•	1 17T	•	4	•	•	•	4	•	•	•
14	0	•	•	•	0	•	•	•	0	•	•	•
15	4	•	•	•	4	•	•	1 300T	4	1 >1M	•	•
16	0	•	•	•	0	•	•	•	0	•	•	•
17	4	•	•	•	4	•	•	•	4	•	•	•
18	0	•	•	•	0	•	•	•	0	•	•	•
19	4	•	1 >1M	•	4	•	•	•	4	•	•	•
20	0	•	•	•	0	•	•	•	0	•	•	•
21	4	•	1 >1M	•	4	1 >1M	1 >1M	•	4	•	1 >1M	•
22	0	•	•	•	0	•	•	•	0	•	•	•
23	0	•	•	•	0	•	•	•	0	•	•	•
24	4	1 >1M	•	•	4	•	•	•	4	•	2 >13T	•
25	0	•	•	•	0	•	•	•	0	•	•	•
26	0	•	•	•	0	•	•	•	0	•	•	•
27	4	2 >1M	•	•	4	•	•	•	4	1 >1M	1 104T	•
28	0	•	•	•	0	•	•	•	0	•	•	•
29	4	1 >1B	1 448T	•	4	•	1 715T	•	4	2 >1M	1 >1M	•
30	6	1 >1B	1 42T	•	6	•	1 42T	•	6	1 >1M	1 >46T	1
31	6	•	•	•	6	•	•	•	6	1 >1M	•	•
32	0	•	•	•	0	•	•	•	0	•	•	•
33	6	1 >1B	1 >2T	•	6	•	•	•	6	1 >1M	3 >2T	•
34	6	•	2 >600T	•	12	1 104M	•	•	5	•	1 810T	•
35	7	•	1 >6M	•	9	2 >1M	1 126M	•	9	•	1 48M	•

Table 2. (concl.)

Week	# 4 Pasteurized group				# 5 Sanded group				# 6 Lactic acid group			
	T#	P	C	M	T#	P	C	M	T#	P	C	M
2	5	*	*	*	5	*	*	*	5	1 > 3T	*	*
3	5	*	*	*	5	*	*	*	5	*	*	*
4	5	*	*	*	5	*	*	*	0	*	*	*
5	5	*	*	*	5	*	*	*	0	*	*	*
6	5	*	*	*	0	*	*	*	5	1 > 100T	*	*
7	0	*	*	*	5	*	*	*	4	*	*	*
8	5	*	*	*	4	*	*	*	0	*	*	*
9	4	*	*	*	0	*	*	*	9	2 > 1M	*	*
10	0	*	*	*	4	*	*	*	0	*	*	*
11	4	*	*	*	0	*	*	*	4	*	*	*
12	0	*	*	*	4	*	*	*	0	*	*	*
13	4	*	*	*	0	*	*	*	4	1 > 5M	1 14T	*
14	0	*	*	*	4	*	*	*	0	*	*	*
15	4	*	*	*	0	*	*	*	4	*	*	*
16	0	*	*	*	4	*	*	*	0	*	*	*
17	4	1 > 1M	*	*	0	*	*	*	4	1 286M	*	*
18	0	*	*	*	4	*	1* 35T	*	0	*	*	*
19	4	*	1 10T	*	0	*	*	*	4	? > 1M	1 > 1M	*
20	0	*	*	*	4	*	*	*	0	*	*	*
21	4	*	*	*	0	*	*	*	0	*	*	*
22	0	*	*	*	0	*	*	*	4	1 > 1M	*	*
23	0	*	*	*	4	*	*	*	0	*	*	*
24	4	*	*	*	0	*	*	*	0	*	*	*
25	0	*	*	*	0	*	*	*	4	1 > 1M	*	*
26	0	*	*	*	4	*	*	*	0	*	*	*
27	4	*	*	*	0	*	*	*	4	1 > 1B	1 720T	*
28	0	*	*	*	4	*	*	*	0	*	*	*
29	4	*	*	*	0	*	*	*	6	3 > 1B	*	*
30	0	*	*	*	6	1 > 1B	*	*	24	3 > 1M	6 > 2T	*
31	6	*	*	*	0	*	*	*	0	*	*	*
32	0	*	*	*	6	*	*	*	0	*	*	*
33	6	*	*	*	14	1 26M	2 > 60T	1 233T	0	*	*	*
34	10	*	*	*	12	1 > 1M	1 > 1M	1 16T	0	*	*	*
35	8	*	*	*	0	*	*	*	0	*	*	*

T#-total no. eggs exam.
P -Pseudomonas types
C-cocci & dipth.
M-molds
> greater than
* less than 10/gm
T-thousands per gm
M-millions per gm
B-billions per gm

DISCUSSION AND SUMMARY

All cultures isolated from eggs grew well at 37° C., but only members of the genus Pseudomonas, Escherichia and Streptomyces grew at 5° C. within two weeks. All other cultures failed to grow after incubation at 5° C. for six weeks.

In preliminary experiments to determine which types of microorganisms, if any, were present in fresh eggs, 10 ml. portions of the mixed egg were placed in broth. Appropriate dilutions were also made and plated out.

Approximately 25 percent of the broth cultures contained cocci or diphtheroid types of organisms while only about 10 percent of the corresponding culture plates with low dilutions showed the presence of these organisms.

The cocci or diphtheroid types of organisms were never present in large enough numbers to cause appreciable deterioration in fresh eggs. This might have been due to the antibacterial activity of the egg contents.

After extended storage cocci or diphtheroids were present in significant numbers (millions per gram) - - probably enough to cause quality deterioration. These organisms did not grow at temperatures below 5° C. and it is doubtful if they would grow in eggs stored under commercial conditions (0 to -1.8° C.).

In no instance were the Pseudomonas types of organisms or other gram negative bacilli isolated from the fresh eggs; however, upon storage Pseudomonas was found to be the predominant organism in the majority of eggs containing bacteria in large numbers (millions to billions per gram).

Organisms of the genus Streptomyces appeared several times, but seldom as the predominant type.

Penicillium brevicaule was encountered several times in significant

numbers. Species of Aspergillus were not encountered as frequently as were species of Penicillium.

Corynebacterium holvolum gave a musty manure odor when first grown on the egg plate, but only a slight musty odor was noticeable when the organism was grown on nutrient agar.

It is of interest to note that none of the members of the genus Escherichia was identified as Escherichia coli.

Twenty-seven percent of the eggs in the lactic acid treated group contained microorganisms in significant numbers (highest incidence recorded), as compared with two percent in the pasteurized group. In the water washed and lye treated groups the percentage of eggs containing many microorganisms was also relatively high; i.e., 19.6 and 16.8 percent, respectively.

A relatively low percentage incidence of high bacterial counts was recorded for both the sanded and "Roccal" treated eggs; i.e., only 8.7 and 8.3 percent, respectively, contained large numbers of microorganisms.

Compared with the other groups, the pasteurized eggs appeared to be of highest quality from the physical standpoint, the yolk standing up especially well. However, in view of the small number of eggs studied no definite conclusions can be drawn or recommendations made from this study relative to treatment of eggs for storage. In addition, treatments were not correlated with the economics of commercial or farm practice.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Wm. A. Miller, Major Advisor, for his help and encouragement in working on this problem and to express appreciation to Dr. P. L. Gainey, Head of the Department of Bacteriology, for many helpful suggestions.

LITERATURE CITED

(1) Egg washing machine. Amer. Egg and Poultry Rev. 9:54. January, 1948.

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

(3) Kennard, D. C.
Warm weather affects egg weight and shell strength. Ohio Ag. Expt. Farm and Home Res. Bul. 246:102-6. May, 1947.

(4) Khoury, M. A.
What goes on inside an egg. U. S. Egg and Poultry Mag. August, 1947.

(5) Lorenz, F. W.
On the efficiency of egg processing oils. Poultry Sci. 28:119-27. January, 1949.

(6) Mallman, W. L. and J. A. Davidson.
Oil protected shell eggs. U. S. Egg and Poultry Mag. 50:113-5, 169-71, 189-91. March, 1944.

(7) Miller, Wm. A. and L. B. Crawford.
Anti-bacterial action of egg shell membranes toward Pseudomonas aeruginosa. Reported at the annual meeting of the Mo. valley branch A.S.B., Kansas State College, Manhattan, Kansas. April, 1949.

(8) Miller, Wm. A. and L. B. Crawford.
Investigations concerning the approximate time necessary for spoilage bacteria to penetrate into fresh eggs. Unpublished, Kansas State College, Manhattan, Kansas. 1949.

(9) Moyer, D. D.
Want clean eggs. Successful Farming. p. 97. February, 1948.

(10) New Italian method of preserving eggs. Sci. Amer. supp. 98:394. May, 1908.

(11) New Process in preserving eggs, A. Sci. Amer. supp. 74:76. August, 1912.

(12) New thermoplastic resin egg preservative (Plast-O-Trete) now available. Amer. Egg and Poultry Rev. 9:12-14. May, 1948.

(13) Perry, J. L.
Preserving quality is the big job in handling shell eggs. U. S. Egg and Poultry Mag. 51:485-6. October, 1945.

(14) Peterson, G. A.
Want more for your eggs. Successful Farming. 46:86+. April, 1948.

(15) Riccardo, S. and A. Di Genova.
A method for the preservation of eggs. Ann. Tecn. Agar. 15:5-27. 1943. Original not seen. Abstract in Chem. Abs. 38-5992. July - November, 1944.

(16) Roasted eggs. Amer. Egg and Poultry Rev. 7:46. February, 1946.

(17) Romanoff, A. L.
Eggs preserved by plastic coating. Farm Res. 15:3. July, 1949.

(18) Romanoff, A. L. and A. J. Romanoff.
The avian egg. New York: John Wiley and Sons, 918 p. 1949.

(19) Romanoff, A. L. and W. D. Yushok.
Preservation of intact eggs by sealing with chemical agents. Food Res. 13:331-5. July, 1948.

(20) Rumball, P.
Home storage of eggs. Queensland Agr. Jour. (Australia) 60:107-8. February, 1945.

(21) Sterilizing eggs for storage. Sci. Amer. Monthly. 125:167. September, 1921.

(22) Stuart, L. S. and E. H. McNally.
Bacterial studies on the egg shell. U. S. Egg and Poultry Mag. 49:28-31+. January, 1943.

(23) Tanner, F. W.
Microbiology of foods, 2nd ed. Champaign: Garrard Press. 1196 p. 1944.

(24) Thayer, R. H. and R. B. Thompson.
Oklahoma farm egg sealer. Okla. Agr. Expt. Bul. B287:1-14. 1945.

(25) Vacuum-oiling eggs to preserve them. Sci. Amer. Monthly. 145:47. July, 1931.

(26) Wilhelm, L. A.
Eggs packed under 14 in. vacuum released with carbon dioxide. Poultry Sci. 17:437. October, 1938.

(27) Winter, A. R.
Microbiology of eggs. Amer. Egg and Poultry Rev. 6:8304. December, 1945.

(28) Yushok, W. D. and A. L. Romanoff.
Studies on preservation of shell eggs by coating with plastics. Food Res. 14:113-22. March-April, 1949.