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Green Rot in Eggs

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Summary

A laboratory study was made of the conditions favorable to the occurrence of "green rot" or "sourness" in chicken eggs. Pseudomonas fluorescens, one of the micro-organisms known to cause this condition, was used as a test organism. Groups of eggs were subjected to various treatments and then infected by warming them to 97° F. and dipping them in water containing cells of P. fluorescens. The time required for development of "green rot" and the number of eggs damaged by such treatment were then determined for the various groups of eggs.

Little relationship was found to exist between shell porosity and the incidence of fluorescent eggs. However, egg shell porosity and the number of fluorescent eggs increased when eggs were held for short periods and then inoculated. Growth as measured by the development of albumen fluorescence in test tubes also was more rapid in aged albumen than in fresh.

The length of time the eggs were held in a suspension of pseudomonads was of little consequence after the eggs were immersed 5 minutes. However, when eggs were washed in warm water (120° F.), or warm water and a detergent, they became more susceptible to infection than when washed in cold water.

Very little spoilage occurred when eggs were swabbed with suspensions of P. fluorescens or swabbed and then "sweated." Egg flats contaminated with P. fluorescens were not an important cause of green rot.

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COVER PICTURE

Two eggs photographed under ultraviolet light. Left, a normal egg. Note the fluorescent appearance of the egg on the right which was infected with Pseudomonas fluorescens about a week before this picture was taken.

Green Rot in Eggs

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Introduction

EGGS ARE AN IMPORTANT FOOD in the human diet not only because they contain essential nutrients required for human nutrition but also because of their mild delicate flavor and ready availability.

Because of their functional properties, eggs are an important ingredient in a number of cooked foods. They act as thickening agents in custards and puddings, leavening agents in cakes, binding agents in meat loaves, coatings for breaded meats and emulsifiers in salad dressings. Because of their widespread use in connection with other foods, they may become a potent source of bacterial contamination if not produced and handled under sanitary conditions.

In a survey of the eggs produced on 94 Canadian farms, Trussell *et al.* (1954) found that the incidence of spoiled eggs ranged from 0 to 25.8 percent with an average for all farms of 3.2 percent. From these spoiled eggs, 71.0 percent were classified as green rots.

During 1956, 369 eggs per person were consumed in the United States. Approximately 2,339,000,000 of the 61,339,000,000 eggs produced came from Texas hens. If a 3.19 percent loss is assumed, as indicated in the study of Trussell *et al.* (1954), about 74,614,000 eggs are lost in Texas annually, causing a loss of over $739,000 from bacterial contamination alone.

According to Winter *et al.* (1952) a number of investigators have reported that cleaned eggs do not keep as well as natural clean or soiled eggs.

Two trends in the poultry industry have made washing of eggs a common practice: (1) the strong resistance of buyers and consumers to dirty eggs and (2) the large number of slightly soiled eggs obtained from hens housed in laying cages. A few producers now wash all eggs produced on their farms; others wash dirty eggs only. Since there is no satisfactory method of testing for washed eggs now in general use, many such eggs are purchased unknowingly and spoil during storage.

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Cause of Green Rot

Eggs may spoil because of a large variety of micro-organisms. One group of bacteria, the pseudomonads, are particularly troublesome. Lorenz *et al.* (1952) state, "Egg spoilage due to bacteria of the genus *Pseudomonas* is the most common type of bacterial loss in cold storage eggs, and frequently the only type found immediately after eggs are removed from the warehouse." In individual egg cases, spoilage of as many as 80 percent of all the eggs has been reported.

Micro-organisms of the genus *Pseudomonas* are found in large numbers in soil, manure, dust and water. Some of these bacteria break down proteins and fats and unlike many micro-organisms they grow well at refrigerator temperatures.

Some species produce a pigment in the egg white of infected eggs which emits a greenish fluorescence when held under ultraviolet light. Eggs infected with this organism cannot be detected by ordinary examination in the early stages even when broken out. However, after a large portion of the egg white becomes infected, the organism breaks down the yolk and yolk membrane and the egg becomes a mixed rōt.

Elliot (1954) reported that it takes from 30 to 60 million organisms per gram of egg white for all of the albumen to fluoresce. For that reason there is as yet no practical means for detecting eggs infected with pseudomonads until pigment has been produced.

The production of fluorescent pigment by certain pseudomonads and the development of ultraviolet candlers which detect the presence of such pigment provide a method for tracing penetration and for studying the growth of these organisms in eggs. By using a standard infection technique, experiments can be duplicated and the time and rate of infection controlled. Contamination of eggs by organisms other than pseudomonads does not present a problem since fluorescence usually appears in contaminated eggs before extensive spoilage by other organisms takes place.

Lorenz *et al.* (1952) have described the odor of infected eggs as "sour," "fruity," "fishy," "cheesy" or "cabbage water." Because of their characteristic odor such eggs are sometimes referred to as sour eggs.

Elliot (1954) reported difficulty in detecting odors in eggs spoiled by pseudomonads because the odors quickly passed through the porous shell. By holding eggs in sterile bottles for several days he demonstrated that when the bacterial count of the albumen reached approximately 100 million organisms per gram, the odor was strong enough for a judging panel to reject such eggs. Soft cooking intensified the odors of the spoiled eggs.

Penetration of Bacteria Through Egg Shells

Numerous reports are available in the literature on factors affecting penetration of bacteria through the egg shell. In many instances it is difficult to interpret the results of earlier studies because mixed bacterial cultures or unknown species were used. There appears to be general agreement that (a) the shell is permeable to micro-organisms, (b) eggs are easily infected by a liquid medium, especially when the medium is cooler than the egg and (c) permeability increases with the age of the egg.

Controversy still exists about the role of the membranes and cuticle. Stuart *et al.* (1943) reported that the shell membranes slow down penetration of *P. aeruginosa*. Stokes *et al.* (1956) concluded that the membranes are a source of nutrients for the bacteria.

Shell porosity also has been considered as a factor affecting bacterial penetration. Kraft *et al.* (1956) reported that porosity as measured by the penetration of an alcoholic solution of methylene blue dye was the most promising index for classifying eggs on their susceptibility to bacterial infection.

The present study was initiated to determine (1) some of the characteristics of eggs which make them vulnerable to infection by *P. fluorescens* and (2) some of the handling practices of eggs during marketing which may increase the incidence of infection and spoilage of eggs by this organism.

Egg Treatments and Characteristics Which Influence Penetration of *P. Fluorescens*

Experimental Procedure

The present work was done in the Poultry Science Market Technology laboratory under carefully controlled conditions. To avoid using large numbers of eggs, the eggs from individual hens were classified as to shell weight, porosity and albumen quality. Only normal, sound, clean eggs were used.

After eggs were subjected to the various treatments described in the following experiments, they were inoculated by the standard infection technique developed by Lorenz *et al.* (1952). With this method eggs are infected with a suspension of *P. fluorescens* by warming the eggs to 97° F. and then dipping them in cold water containing cells of *P. fluorescens*. The test strain of *P. fluorescens* used was selected because of its ability to grow rapidly in egg white and produce a green fluorescence. The eggs were candled at weekly intervals to determine the number of green rots. Eggs showing questionable fluorescence were held an additional week and rechecked.

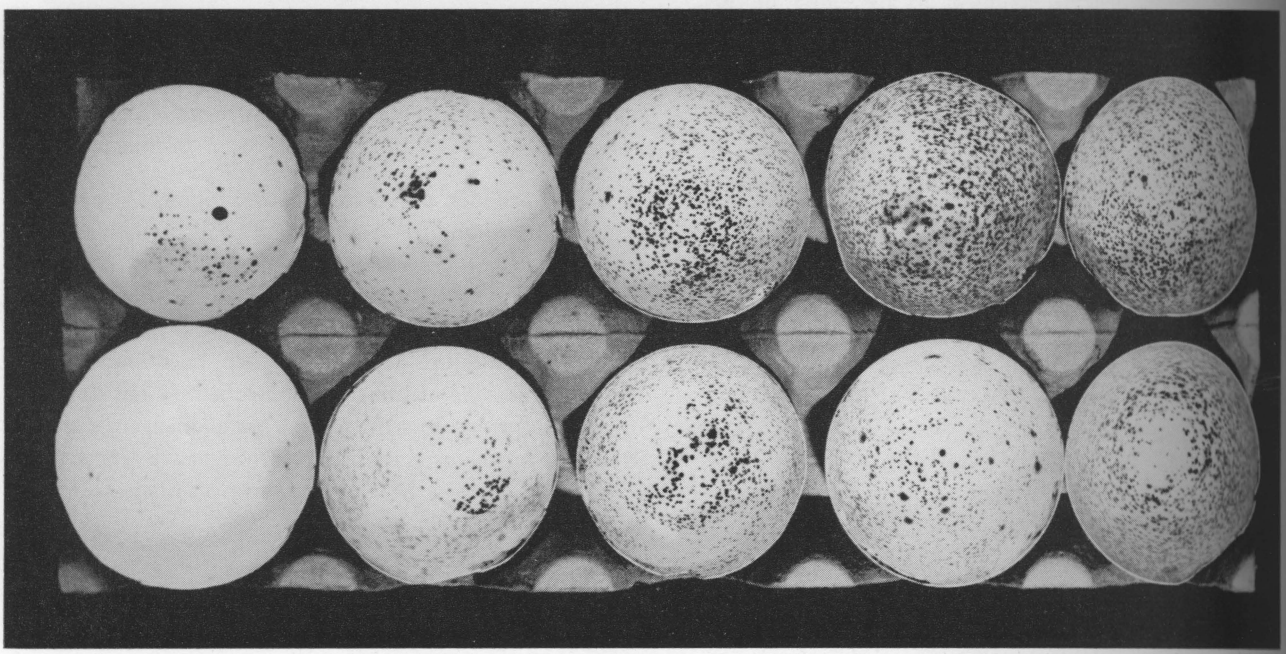

Figure 1. Egg shells with varying degrees of porosity.

Effect of Pore Size

Egg shells vary in porosity or in number and size of pores. Porosity can be measured by immersing eggs in an alcoholic dye solution, Figure 1, or by determining the weight loss of eggs incubated at 97° F. for 2 weeks. Porosity is influenced by age of birds, inheritance, management, practices used in the flock and environment. Preliminary work indicated that there is little difference in the porosity of successive eggs from the same hen within the time limits of these experiments.

To determine the influence of shell porosity on bacterial penetration, eggs were selected from hens that laid eggs previously classified according to the percent weight lost during 2 weeks' incubation. Twenty-five eggs from hens whose previous eggs had lost from 7.6 to 12 percent of their total weight after 2 weeks' incubation were classified as a high porosity group, 30 eggs from hens whose previous eggs had lost from 6.3 to 7.5 percent were classified as a medium porosity group and 28 eggs from hens whose previous eggs had lost from 1.5 to 6.2 percent were classified as a low porosity group. These eggs were inoculated with *P. fluorescens* stored at 50° F. and candled for green rots at weekly intervals. A second egg, one from each hen in each group, designated as a control egg was treated the same as the inoculated eggs except that they were immersed in sterile water instead of in water containing *P. fluorescens*.

Figure 2 shows the incidence of infection for the three porosity groups during the 5-week storage period. Almost 90 percent of all the treated eggs spoiled by the end of 5 weeks. Statistical analysis using Chi square disclosed no significant difference between any of the three porosity groups, which would indicate that shell porosity does not play an important role in bacterial penetration.

To determine whether eggs with shells containing large pores were infected easier than those with small pores, groups of eggs (40 per group) selected at random, were inoculated with *P. fluorescens* by the standard infection technique. When an egg became infected, it and another nonfluorescent egg from the same group were immersed in a 60-percent alcoholic solution of crystal violet to stain the pores. Preliminary work showed that this solution penetrated only the largest pores in the shell. After the dye solution dried on the shell the eggs were opened and the number and size of the pores on the inside of the shell observed. No relationship could be established between the number or size of pores and spoilage as determined by fluorescence. Eggs shells with large pores were found in both fluorescent and non-fluorescent eggs.

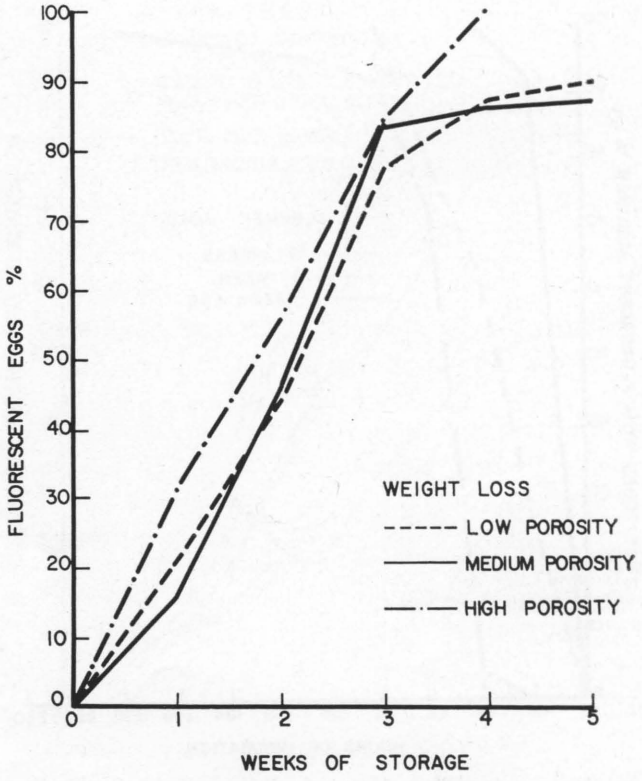

Figure 2. Relation between egg shell porosity and penetration of *P. fluorescens*.

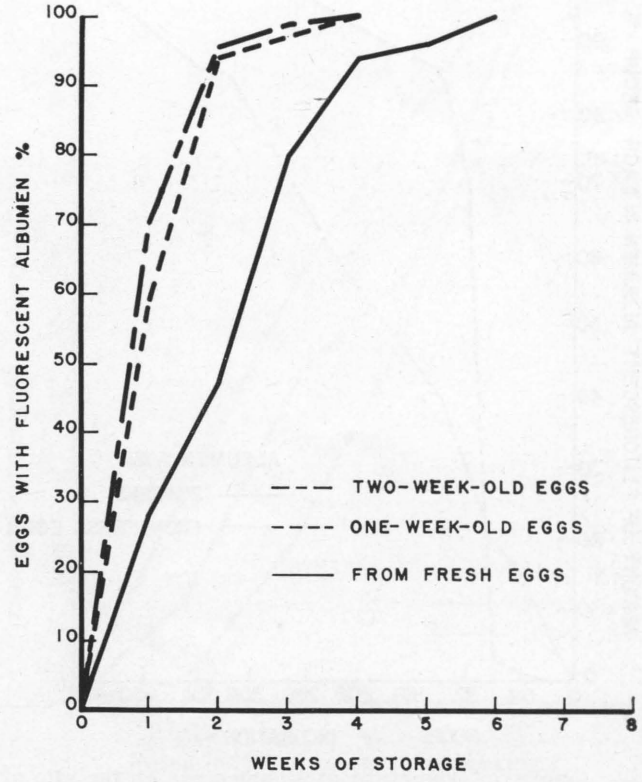

Figure 3. The effect of the age of the eggs on the penetration of *P. fluorescens*.

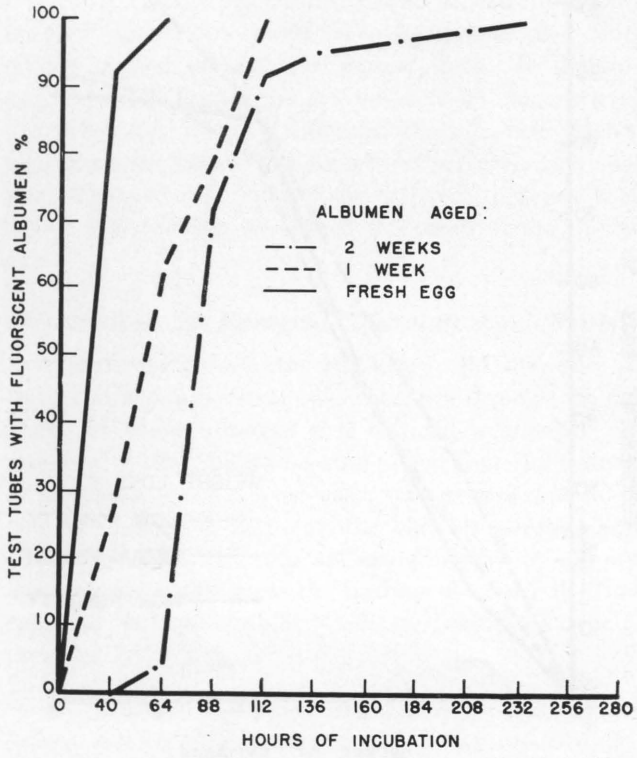

Figure 4. The effect of albumen age on the first appearance of fluorescent pigment.

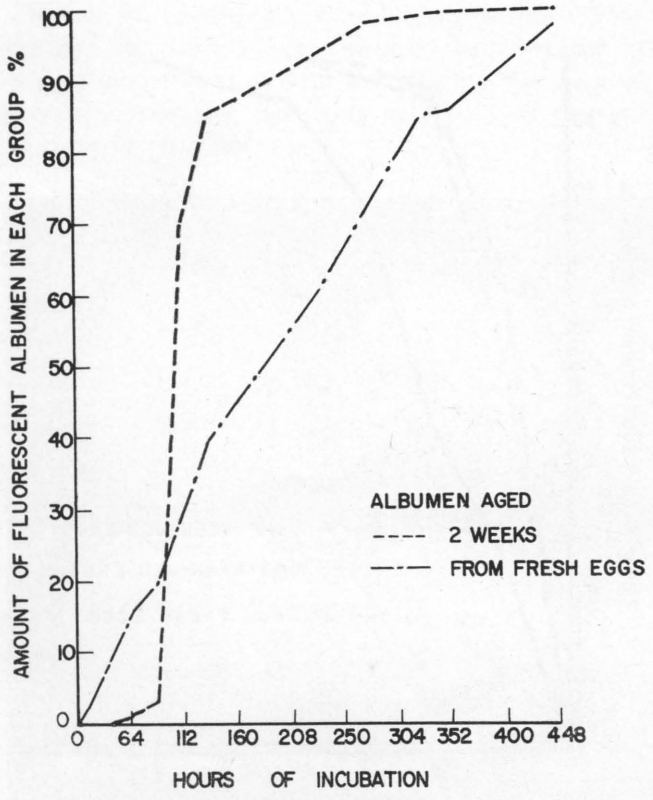

Figure 5. The effect of albumen age on the rate of development of fluorescence in albumen incubated in test tubes.

Effect of Age and Heat

After eggs are laid they begin to break down in quality. Almquist *et al.* (1931) demonstrated that shell porosity increases with age. The interior quality of eggs also breaks down, especially when exposed to high temperatures.

To determine the effect of age of the egg on rate of infection and spoilage by *P. fluorescens*, three groups of 30 eggs of known history were treated as follows. One group of eggs (one egg from each one of 30 hens) was incubated at 97° F. for 2 weeks, another group for 1 week and a third group consisted of fresh eggs. After this treatment the eggs were inoculated with *P. fluorescens*, stored at 50° F. and candled at weekly intervals.

Eggs held for 1 or 2 weeks spoiled faster than fresh eggs, Figure 3. The rate of spoilage of the 1 and 2-week-old eggs, however, was very similar.

Since old eggs apparently spoil faster after inoculation than fresh ones, the question arises whether they spoil because bacteria penetrate the more porous shells faster or because some factor initially active in fresh eggs and which inhibits growth of the organism breaks down.

To determine the influence of the age of egg albumen on the rate of growth of *P. fluorescens*, three eggs from each of 35 hens were collected. The first group (one egg from each hen) was incubated at 97° F. and 85 percent relative humidity for 2 weeks. The second group was held under the same conditions for 1 week. The third group consisted of fresh eggs. Fifteen milliliters of albumen then were removed from each egg under aseptic conditions with a hypodermic needle, placed in a sterile test tube and inoculated with *P. fluorescens*.

Fluorescence was observed in the fresh albumen earlier than in the samples held for 1 or 2 weeks, Figure 4.

The rate of pigment production in fresh and 14-day-old egg white was determined by measuring the height of the pigment produced by the organism at selected intervals as it passed down the tube. During the early period of growth, fluorescence did not proceed as fast in 14-day egg white as it did in the tubes with fresh albumen, Figure 5. However, after a short lag period fluorescence proceeded at an accelerated rate. All of the white in the 14-day group fluoresced before the fresh white did. Fresh egg white fluoresced almost completely after 14.5 days. The fact that the overall growth rate was faster in aged than in fresh albumen suggests that a change in the albumen is at least one of the factors associated with the rapid growth of pseudomonads in aged eggs. Changes in the CO_2 concentration, pH and oxidation-reduction potential might be involved.

Effect of Washing

Four groups of 30 eggs of similar history received the following treatments: (a) unwashed control group, (b) dipped in sterile distilled water for 5 minutes, (c) washed in a Kitson egg washer with 120° F. water for 3 minutes, and (d) same as (c) with a special egg washing detergent. The eggs then were dried, inoculated with *P. fluorescens*, stored at 50° F. and candled weekly.

Washing of eggs in water at 120° F. with or without a detergent prior to inoculation with *P. fluorescens* increased the number of green rots when compared to eggs washed in cold water, Figure 6.

Effect of Humidity

Three groups of 30 eggs of similar history were stored at 50° F. at 100, 80 and 55 percent relative humidity for 5 days. The eggs then were inoculated with *P. fluorescens* and stored at 50° F. and candled at weekly intervals. Eggs stored at 100 percent relative humidity spoiled faster than those stored at the lower humidities, Figure 7.

Effect of Various Methods of Contaminating Eggs

To duplicate some of the methods of contamination found under field conditions, six groups of 30 eggs of similar history were treated as follows:

(1) Warm eggs were swabbed with *P. fluorescens*.

(2) Cold eggs were swabbed with *P. fluorescens*.

(3) Warm eggs were swabbed with *P. fluorescens* and then sweated.

(4) Clean eggs were stored in flats contaminated with *P. fluorescens*.

(5) Clean eggs were stored in contaminated flats and then sweated.

(6) Clean eggs were inoculated by the standard infection technique (control group).

Except for the control group very little spoilage (0 to 5 percent) occurred. After 6½ weeks all eggs that did not show signs of spoilage were warmed up to 95° F. and immersed in cold sterile water for 5 minutes. Such treatment should force viable organisms through the shell if present. Little change was observed in the number of infected eggs that could be attributed to this treatment.

Discussion

On the basis of this work the following general conclusions may be drawn:

1. Little if any relationship exists between shell porosity and penetration of eggs by *P. fluorescens*.

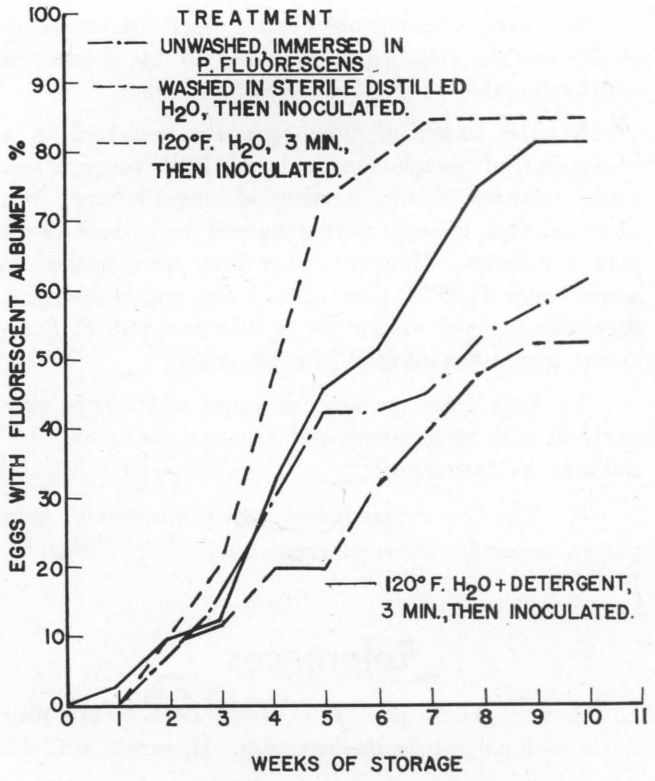

Figure 6. The effect of washing eggs on the penetration of *P. fluorescens*.

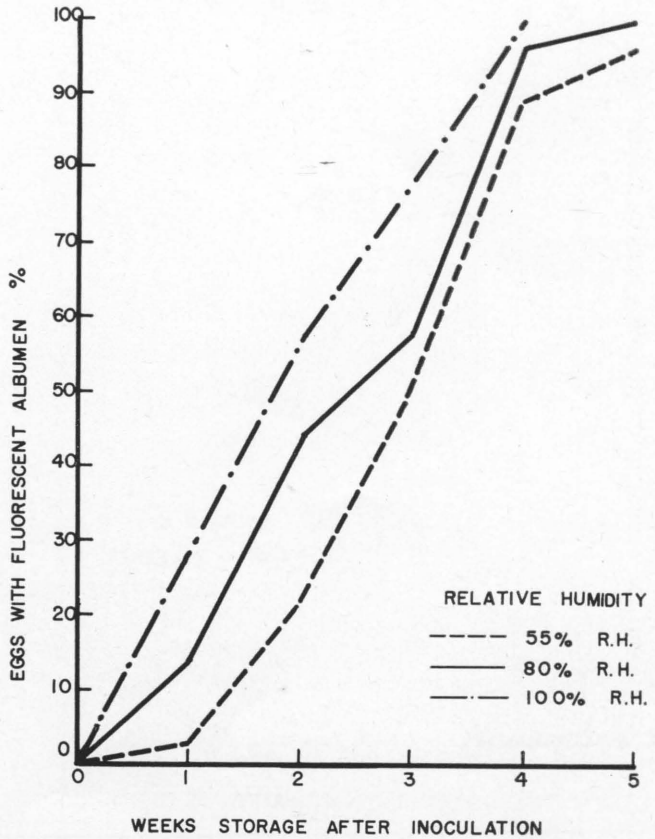

Figure 7. The effect of humidity on the penetration of *P. fluorescens* through the egg shell.

2. As eggs become older certain shell characteristics change and the albumen breaks down so that it becomes more vulnerable to attack by pseudomonads.

3. The length of time eggs are immersed in a suspension of pseudomonads is of little consequence. Little increase in the number of eggs infected was observed when the eggs were immersed for periods longer than 5 minutes. However, when eggs were washed in warm water (120° F.) or warm water and a detergent, they became more susceptible to infection with *P. fluorescens* than when washed in cold water.

4. Very little spoilage occurred when eggs were swabbed with suspensions of *P. fluorescens* or swabbed followed by "sweating."

5. Egg flats contaminated with *P. fluorescens* were not an important cause of green rot.

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