THE RELATION OF THE HYDROGEN ION CONCENTRATION OF EGG WHITE TO ITS GERMICIDAL ACTION[1]

PAUL FRANCIS SHARP AND RANDALL WHITAKER

From the Chemical and Bacteriological Laboratories of the Department of Dairy Industry, New York State College of Agriculture at Cornell University, Ithaca

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INTRODUCTION

The so-called bactericidal or germicidal property of the white of hens' eggs has attracted but little attention in the past ten years. Before that time, various investigators from 1890 onward have recorded studying this subject, some reporting a positive, others a negative action. Wurtz (1890) seems to have been the first to suggest that egg white possessed a germicidal power; others who confirmed his results and carried their investigations further were Scholl (1893) Horowitz (1902) Turró (1902) Laschtschenko (1909) Rettger and Sperry (1912) and Sperry (1913). On the other hand, Parascandole (1893) and others state that egg white does not possess such a property, but that bacterial growth can take place in it. Some investigators found a difference in the germicidal action of the egg white depending upon the organism used, the rate of seeding, treatment of the albumin, etc. Hadley and Caldwell (1916) in their extensive review of the literature conclude "in summary, perhaps all that can be said at the present time is that certain species of bacteria, when placed in albumin mixtures, survive but a short time."

[1] This work was presented in June, 1926, by Mr. Randall Whitaker as a thesis for the degree of Master of Science.

This paper is a report on a part of the coöperative investigation on the factors influencing the keeping quality of eggs in storage which is being carried out by C. K. Powell of the Department of Poultry Husbandry and P. F. Sharp of the Department of Dairy Industry. We wish to express our appreciation to the Department of Poultry Husbandry for furnishing us with the eggs used in this investigation and for aiding us in other ways.

Of the theories advanced to explain the bactericidal property of egg white, probably the first ones were those which were based on the assumption of a high osmotic pressure or lack of food materials. Laschtschenko (1909) believed that he disproved both of these theories when he showed that the white still possessed its germicidal action toward *Bacillus subtilis* when diluted with bouillon, water, or physiological salt solution. He advanced the theory that the bactericidal action was due to the presence of an enzyme of a proteolytic nature. As proof of this he found that egg white which had been heated to 55° to 60°C. for thirty minutes still possessed its germicidal action, while egg white which had been heated for the same length of time to 65° to 70°C. failed to show such an action. Other investigators have confirmed the results of Laschtschenko, and Rettger and Sperry (1912) have even suggested egg white heated to 65° to 70°C. as a good culture medium.

Bainbridge (1911), working with pure egg albumin solutions, found that when bacteria were grown in such solutions no multiplication took place when the seeding was large; small seedings did increase slightly, however, and he assumed from this that there was present an insufficient amount of non-protein nitrogenous material in egg albumin for marked bacterial growth, but that the small trace that was present permitted the slight growth observed when small seedings were used. By adding a few drops of broth or an impure albuminose solution to the pure egg albumin, he found that rapid multiplication resulted, even with the large seedings; no pure albumin, however, was broken down. Rettger and Sperry (1912) were inclined to explain Bainbridge's results on the ground that pure and unchanged proteins may play the part of antiseptics or germicides. They report in another paper, Sperry and Rettger (1915), that certain obligate aerobes, facultative anaerobes, and obligate anaerobes were unable to develop in a medium which contained pure egg albumin as the only source of carbon and nitrogen. When a small amount of peptone was added to the medium, the organisms developed and decomposed the egg albumin. They state, "The egg white even appeared to have an antiseptic, and, in a measure, bactericidal action on aerobes. This property was undoubtedly not due to the egg albumin, but to other agencies in the egg."

EXPERIMENTAL

It is unfortunate that most of the investigators who have worked on the germicidal property of egg white failed to record in their published work the exact age of the eggs when examined. Eggs are spoken of as "fresh," "reasonably fresh," "newly laid," etc., and in the majority of the contributions no adjective at all describes the egg white that was used for testing out the bactericidal property. It has been found by Healy and Peter (1925) and, independently, through extensive experiments carried out in this laboratory by Dr. Powell and one of us (Sharp), that the whites of eggs decrease in hydrogen ion concentration, under some conditions, very rapidly after being laid, approaching as a limit a pH of approximately 9.5. This phenomenon is due to diffusion of carbon dioxide from the egg white.

The results obtained in this laboratory show that the change in the hydrogen ion concentration of the white of eggs is very rapid if the eggs are kept at room temperature freely exposed to the air. The change is less rapid at lower temperatures but still takes place. The white of the egg may change as much as 1.0 pH units in twenty-four hours at room temperature and in six days the change may be as much as 1.8 pH units. Egg white obtained from eggs within an hour or two after they were laid and thoroughly beaten to mix the thin and thick white was found in our experiments to have a pH of about 8.2. The actual pH of the white of fresh hens' eggs is much lower than 8.2, probably about pH 7.6, but special precautions must be taken in determining the hydrogen ion concentration of the white in order to obtain the lower values. Eggs after aging for one week in a well ventilated place at room temperature will usually have a white with a pH of about 9.4. This is a considerable change in hydrogen ion concentration and should be great enough to affect the growth of bacteria. In table 1 will be found the limiting maximum pH for the growth of a few organisms as reported in the literature. McClendon and Medes (1925) give a list of 24 different bacteria and the range of hydrogen ion concentration through which they will grow. The values for the limiting maximum pH for growth range from pH 7.6 to 10.0 with an average of 8.1.

Eggs which have been kept at room temperature for two days in air have whites with a pH a little above 9.0. A glance at table 1 will indicate that most of the bacteria would be killed if placed in the white of such a two-day-old egg, while they would grow in the white of an egg one hour old. It should be remembered, however, that the limiting hydroxyl ion concentration for the growth of bacteria varies with the other components of the medium.

TABLE 1

The limiting maximum pH for the growth of various organisms as reported in the literature

ORGANISM	MAXIMUM HYDROXYL ION CONCENTRATION	OBSERVER
	pH	
Bacillus subtilis	9.4	Itano (1916)
Diplococcus pneumoniae	8.6	Avery and Cullen (1919)
Bacterium typhosum	8.6	Schoenholz and Myer (1921)
Bacterium aerogenes	8.4	Cohen and Clark (1919)
Bacterium dysenteriae	8.5	Cohen and Clark (1919)
Bacterium alkaligines	9.7	Cohen and Clark (1919)
Proteus vulgaris	8.8	Cohen and Clark (1919)
Lactobacillus bulgaricus	8.0	Cohen and Clark (1919)
Corynebacterium diphtheriae	8.2	Bunker (1917)

It may be safely stated that the eggs used by most previous workers had reached a pH of 8.8 to 9.0, as an egg a day or so old is considered "fresh" by the average person. In many cases they may have been several days' old; for example, Hadley and Caldwell (1916) in their investigation of the bacterial content of fresh eggs say that "During one year of the work, the eggs were never more than eight days old at the time of the examination." Such eight-day-old eggs would probably have a white with a pH of approximately 9.4, which is above the maximum pH range for most bacterial growth as indicated by the literature.

In the light of this knowledge of the change in the hydrogen ion concentration of egg white with age and in view of the really high pH value which may be reached, it is possible to explain much of the apparently conflicting evidence of previous investigators on the assumption that they were studying egg whites of different pH values. It is possible that those investigators who reported that egg white had no germicidal properties used as their experimental material relatively fresh egg white (pH 7.6 to 8.4), while those investigators who reported a germicidal action used more alkaline egg white (pH 9.2 to 9.5).

From Parascandole's (1893) description of his experiments in which he found that 9 different organisms grew in egg white, we are led to suspect that he used egg white of low pH value. He took freshly laid eggs, washed them with alcohol, immersed them in mercuric chloride solution for 10 minutes, and covered them with paraffine until he was ready to remove the egg white. Experiments in this laboratory have shown that if eggs, the white of which has a low pH, are coated with paraffine the increase in pH on aging in air is inhibited. From the account of this investigator's procedure it seems highly probable that he actually did work with egg white of a low pH value approximating that of the egg as laid, and this accounts for his obtaining growth of bacteria in egg white.

Maurer (1911) also probably investigated the germicidal action of egg white toward *Bacterium coli* before the pH of the egg white had increased appreciably for he obtained growth. We have found that this organism is able to grow in egg white with a pH corresponding to that of the fresh egg white, but is killed at pH values corresponding to that of egg white from eggs which have been aged in air for a few days.

Rettger and Sperry (1912) used eggs which were "reasonably fresh" in studying the germicidal action of the egg white, and it is therefore, highly probable that the egg white had attained a high pH value. That this was the case is also indicated in our experimental work, for we investigated two of the organisms—*Bacillus cereus* and *Bacillus megatherium*, which Rettger and Sperry found were sensitive to the germicidal action of egg white.

We found these organisms sensitive to the germicidal action of alkaline egg white corresponding to the pH of aged eggs while these same organisms exhibited growth in egg white having a pH approaching that of eggs one hour old. Turró (1902) made the (to him) surprising observation that the white of the fresh egg had a weaker germicidal power than the white of aged eggs. This observation is exactly in agreement with the postulate which led us to start this investigation.

When we call our experimental material fresh egg white, we mean egg white obtained from eggs which had been laid less than two hours. The actual pH of this material varies somewhat, depending on its age and treatment.

The literature also shows conclusively that different bacteria may vary in their sensitiveness to the germicidal action of egg white.

Sherman and Curran (1924) have shown the desirability of using young cultures of organisms in the period of rapid growth when determining gemicidal properties, in order to remove any influence of the "lag period." Therefore, in this work three-hour-old cultures were used unless otherwise stated.

In order to determine the effect of the hydrogen ion concentration on the rate of growth of bacteria in egg white, a series of test tubes was arranged containing equal volumes of fresh, well mixed egg white, ranging in pH from 5.0 to 10.0 The pH was adjusted by adding varying amounts of normal hydrochloric acid and sodium hydroxide. The tubes were all made up to the same volume with sterile distilled water. All hydrogen ion concentration determinations reported in this paper were made at 25°C. using the hydrogen electrode. In no case did the dilution due to acid or alkali exceed ten per cent of the volume of the egg white used. One cubic centimeter of a culture of *Pseudomonas pyocyaneus* (an organism which seems adapted to egg white, having been isolated from an infected egg white in this laboratory, and having been reported as present in eggs by several workers) was added to the tubes, mixed well, and incubated for six hours at 37°C. The check on the original seeding and the count after the six-hour period were made by the plate method.

In all cases the original count was determined by adding the same volume of the inoculating culture to a water blank and plating at once. This procedure was necessary because some organisms were killed so rapidly by the egg white that a substantial decrease in numbers took place before the organisms could be distributed in the egg white and a plate poured. The results are shown in tabular form in table 2 and graphically in figure 1.

From this table we see that egg white of pH 9.4 showed a marked decrease, while egg white of pH 8.7 showed a substantial

TABLE 2

The growth of Pseudomonas pyocyaneus in egg white of various hydroxyl ion concentrations. Incubation, after inoculation, at 37°C. for six hours, with an initial count of 1,410,000 bacteria per cubic centimeter

HYDROGEN ION CONCENTRATION		COUNT AFTER 6 HOURS (BACTERIA PER CUBIC CENTIMETER)
At start	After 6 hours	
pH	*pH*	
4.94	4.97	32,000,000
5.53	5.68	49,000,000
6.09	6.46	28,000,000
6.42	7.02	22,000,000
6.66	7.30	24,000,000
7.05	7.61	21,000,000
7.39	7.91	14,000,000
7.99	8.32	11,000,000
8.74	8.76	12,000,000
9.37	9.33	409,000
9.45	9.37	119,000
9.77	9.76	0
10.11	9.99	0
10.31	10.19	0
10.62	10.52	0

increase in numbers of bacteria over the original seeding. At a pH of 9.7 there is a decided germicidal effect, all the organisms being killed. This experiment was repeated with cultures of *Bacillus subtilis, Pseudomonas fluorescens, Serratia marcescens, Proteus vulgaris, and Bacterium coli* in the same manner and incubation continued for six hours, the initial count per cc. and the count after incubation being determined by the plate method. The results are given in table 3.

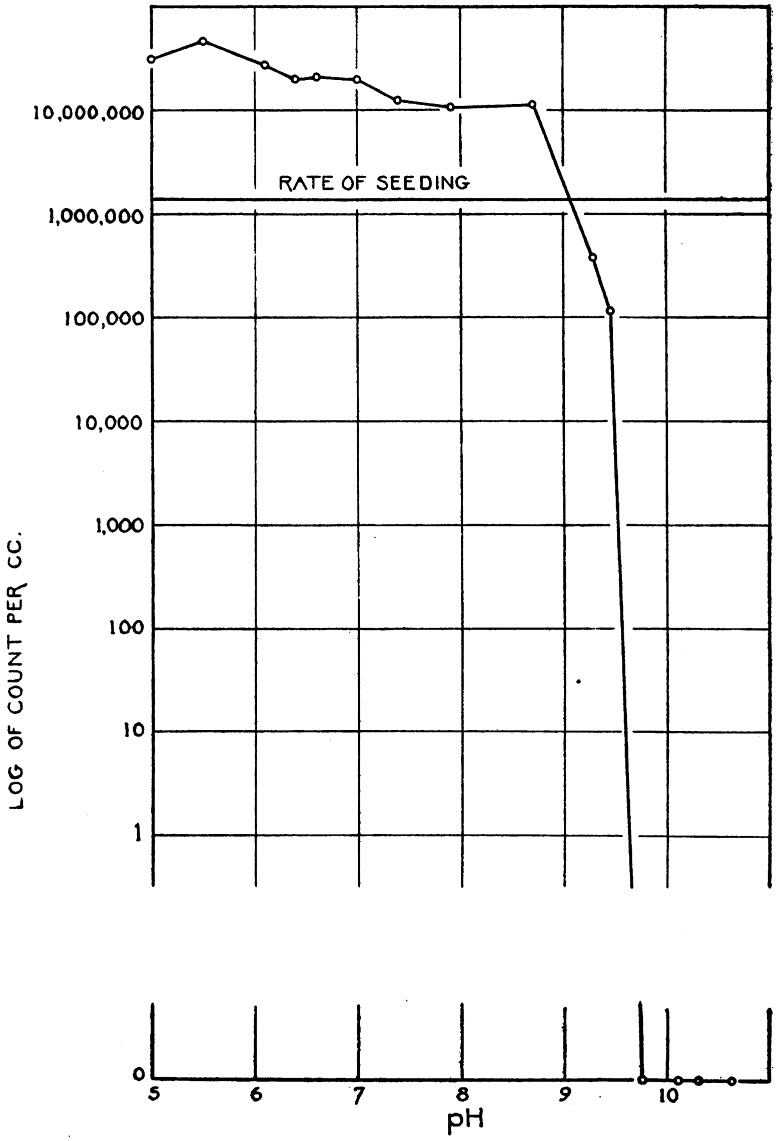

FIG. 1. THE RELATION OF THE HYDROGEN ION CONCENTRATION OF EGG WHITE TO ITS GERMICIDAL ACTION TO PSEUDOMONAS PYOCYANEUS

Plate counts were made after incubating the inoculated egg white at 37°C. for six hours.

As *Bacillus subtilis* in table 3 was the only spore producer, and as it apparently was killed very rapidly, other spore producing

TABLE 3

Egg white adjusted to various hydrogen ion concentrations and inoculated with Bacillus subtilis, Pseudomonas fluorescens, Serratia marcescens, Proteus vulgaris and Bacterium coli, incubated for six hours at 37°C. and the number of bacteria per cubic centimeter determined by the plate method before and after incubation

HYDROGEN ION CONCENTRATION	BACILLUS SUBTILIS PER CUBIC CENTIMETER	PSEUDOMONAS FLUORESCENS PER CUBIC CENTIMETER	SERRATIA MARCESCENS PER CUBIC CENTIMETER	PROTEUS VULGARIS PER CUBIC CENTIMETER	BACTERIUM COLI PER CUBIC CENTIMETER
pH					
5.34	<1,000	C	C	C	C
6.75	<1,000	C	C	C	C
8.28	<100	C	C	C	C
9.47	<100	13,000	<100	<100	19,000
10.65	<10	<10	<10	<10	<10
Original count	1,210,000	23,200,000	10,200,000	8,100,000	8,400,000

C = countless; < = less than.

TABLE 4

Egg white adjusted to various hydrogen ion concentrations and inoculated with the following spore-producing organisms, incubated for six hours at 37°C. and the number of bacteria per cubic centimeter determined by the plate method before and after incubation

HYDROGEN ION CONCENTRATION	BACILLUS MEGATHERIUM PER CUBIC CENTIMETER	BACILLUS CEREUS PER CUBIC CENTIMETER	BACILLUS MYCOIDES PER CUBIC CENTIMETER
pH			
6.76		20,200,000	230,000
7.73	10,000	5,900,000	330,000
8.52	11,700	69,000	<100
9.57	670	25,000	<10
9.88	<10	<10	<10
Original count	5,000*	1,300,000	43,000

< = less than.

* Approximated.

organisms were subjected to the same treatment. The data are given in table 4.

Tables 2, 3 and 4 all indicate the validity of our original assumption, that the pH of the natural egg white may be an important factor in determining whether or not it will permit bacterial growth. It will be noted that the egg white with a pH of approximately 9.5 was germicidal to all of the organisms which we investigated. Tables 3 and 4 indicate that egg white at the higher pH values is slightly more germicidal to the spore forming than to the non-spore forming organisms. *Bacillus subtilis* was the only organism studied which was killed at all pH values.

Pseudomonas pyocyaneus is a suitable organism for use in determining the effect of pH on the germicidal properties of egg white, having the following advantages: (1) it forms round and distinct colonies which are easy to count, (2) it does not produce "spreaders," (3) it has an average hydroxyl ion concentration death point well within the pH range which may be attained by natural egg white, and (4) it has been shown to be present in egg white by many investigators, evidently growing where the pH is suitable. It was chosen, therefore, to study the relationship of the age of the eggs and the alkalinity of the egg white to the rate of bacterial growth. Eggs were placed in storage and at various intervals some were withdrawn and their germicidal property tested by the same method as that used in table 2, except that no adjustment of the pH was made.

The eggs were brought to the laboratory and divided into three lots. The first lot was stored in air; the second lot was given the "oil-dipped" treatment, which is practiced commercially at the present time. This "oil-dipped" treatment consisted in dipping the eggs for a specified length of time in a hot oil, especially prepared for the purpose of preserving eggs by a commercial concern. The third lot of eggs was stored in water glass, made up in the ratio of one part of water glass to twelve parts of water. The eggs were laid the same day and were in storage within two hours' time. The storage temperature was 30°C. Four eggs from each lot were withdrawn from time to time, the whites removed as aseptically as possible, and separately inoculated with a culture of *Pseudomonas pyocyaneus*, incubated at 37°C. for six hours and then plated. In table 5 will be found the results of this experiment. The pH values given are for the egg white of

TABLE 5

The changes in hydrogen ion concentration and germicidal power of the white of storage eggs, stored for various lengths of time at 30°C.

The eggs were divided into three lots, each lot being subjected to a different storage treatment, as (1) storage in air, (2) previously oil-dipped and then stored in air, (3) storage in water glass solution. On being removed from storage the eggs were opened and the whites separately inoculated at a known rate with *Pseudomonas pyocyaneus*, incubated for 6 hours and the count per cc. again determined. The hydrogen ion concentration was also determined on the whites of the eggs as they came from storage.

TREATMENT	EGG NUMBER	ORIGINAL EGGS RATE OF SEEDING 3,380,000		FIRST DAY—RATE OF SEEDING 7,200,000		SECOND DAY—RATE OF SEEDING 2,100,000		FOURTH DAY—RATE OF SEEDING 1,500,000		EIGHTH DAY—RATE OF SEEDING 140,000		EIGHTEENTH DAY—RATE OF SEEDING 200,000		THIRTY-FIRST DAY—RATE OF SEEDING 3,090,000	
		Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter	Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter	Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter	Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter	Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter	Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter	Hydrogen ion concentration	Plate count after 6 hours per cubic centimeter
		pH		*pH*		*pH*		*pH*		*pH*		*pH*		*pH*	
Stored in air	1	8.45	3,470,000	8.72	2,600,000	9.01	2,230,000	9.25	660,000	9.45	1,500	9.42	880	9.37	1,680,000
	2	8.42	3,100,000	8.64	3,360,000	9.13	24,200,000	9.42	88,000	9.45	6,600	9.48	340	9.37	568,000
	3	8.53	5,620,000	8.83	4,180,000	9.23	1,350,000	9.33	97,000	9.48	0	9.42	860	9.40	680,000
	4	8.57	5,900,000	8.79	5,180,000	9.11	3,900,000	9.37	93,000	9.52	400			9.26	28,100,000
Stored as oil-dipped	1			8.57	14,500,000	8.40	6,600,000	8.34	24,200,000	8.34	23,000,000	8.59	18,500,000	8.57	77,200,000
	2			8.52	11,500,000	8.52	6,200,000	8.32	5,200,000	8.45	31,500,000	8.69	32,700,000	8.72	54,800,000
	3			8.42	11,900,000	8.50	5,100,000	8.34	1,790,000	8.54	1,900,000	8.63	18,600,000		
	4			8.50	7,700,000	8.59	3,940,000	8.34	2,200,000	8.42	20,100,000	8.72	38,500,000	8.58	50,100,000
Stored in water glass	1			8.49	6,500,000	8.44	27,900,000	8.34	16,400,000	8.36	7,100,000	8.67	7,400,000	8.60	82,400,000
	2			8.54	33,500,000	8.49	4,700,000	8.42	2,500,000	8.36	1,070,000	8.67	10,200,000	8.67	62,400,000
	3			8.47	3,280,000	8.47	7,300,000	8.39	2,590,000	8.45	1,840,000	8.57	31,100,000	8.66	71,400,000
	4			8.49	2,280,000	8.50	7,400,000	8.40	2,080,000	8.39	3,200,000	8.56	9,000,000	8.63	62,000,000

each egg as it came from storage, not after the six-hour incubation period.

A glance at table 5 shows that the whites from the eggs stored in air rapidly became more alkaline, so that at the end of one week the pH had risen to 9.4. Closely related to this is the steadily increasing germicidal effect on the organism, the first day even showing slight evidence of inhibited growth, and a decided germicidal effect later. From table 5 it can be seen that after the high alkalinity has developed a slight decrease in the pH is noted in the eggs stored for the longer lengths of time. Tice (1911) followed the fall of the alkalinity of egg white during storage, by titration with twentieth normal sulphuric acid, and found that after seven months in storage there was a gradual decrease, approaching "a common locus" with the yolk, the acidity of which steadily decreased during storage at 32°F. At this point it is interesting to note that Sperry (1913) says in writing about the germicidal properties of eggs, "The whites of eggs which are 11 months old or more showed a tendency to lose these properties." This is completely in accord with the theory that the variation in the so-called germicidal action is really due to the hydroxyl ion concentration of the natural egg white.

Table 5 also shows that eggs stored in water glass and those which were oil-dipped did not show such a rapid rise in pH. The bacteria were shown to grow in the eggs from both of these treatments indicating the absence of the bactericidal property.

It was next deemed advisable to determine if "aged" egg white of naturally high pH, to which acid had been added to bring the pH down to that of fresh egg white, would support growth the same as the fresh egg white. This was done in the following manner: whites from eight eggs were taken, numbers 1, 2, 3, and 4 having a pH of 8.4 to 8.5, and numbers 5, 6, 7, and 8 from eggs which had been stored for five days at 30°C. The hydroxyl ion concentration of the whites from the last three eggs was decreased by adding varying amounts of normal hydrochloric acid until the pH approximated that found in the first four eggs. Egg white number 5 had no acid added to it. Each egg white was inoculated with a culture of *Pseudomonas pyocyaneus*, the rate of seeding being 3,380,000 per cubic centimeter then incubated for six hours at 37°C. and the count per cubic centimeter again determined.

The results of this experiment show again the influence of the variation in the hydroxyl ion concentration on the so-called germicidal action. Egg whites nos. 1 to 4 with pH values between 8.42 and 8.57 showed 3 to 6 million bacteria per cubic centimeter after incubation as did egg whites nos. 6 to 8 (with pH value readjusted to 8.16 to 8.27. Egg white number 5, to which no

TABLE 6

Growth of various organisms in egg yolk to which alkali had been added to give a pH such as found in aged egg white

After inoculation the yolks were incubated at 37°C. for six hours. The bacteria count per cubic centimeter was determined by the plate method before and after incubation.

HYDROXYL ION CONCENTRATION	PSEUDOMONAS PYOCYANEUS PER CUBIC CENTIMETER	BACILLUS SUBTILIS PER CUBIC CENTIMETER	BACTERIUM COLI PER CUBIC CENTIMETER	SERRATIA MARCESCENS PER CUBIC CENTIMETER	BACILLUS MEGATHERIUM PER CUBIC CENTIMETER
pH					
5.88*	C	<1,000	C	C	5,100,000
8.97	15,400,000	72,000	18,000	C	<1,000
9.48	2,160,000	<1,000	<1,000	75,000	<100
9.90	<100	1,200†	<100	<100	<100
10.32	<10	1,400†	<10	<10	<10
Original count	1,280,000	491,000	2,820,000	1,440,000	157,000

* Original pH.

† It was believed this was due to contamination.

C = countless; < = less than.

acid had been added (pH 9.34), showed a marked decrease in the number of bacteria (460,000).

Experiments were carried out to see whether or not egg yolks and mixtures of whites and yolks could be made to exhibit bactericidal properties by the simple adjustment of the hydrogen ion concentration to pH 9.5 or 9.6; i.e., the pH found in the whites of aged eggs. In the first experiment various amounts of alkali were added to the yolks of fresh eggs in order to give such a pH. Into these adjusted mixtures were placed 1-cc. portions of cultures of the following organisms: *Pseudomonas pyocyaneus*, *Bacillus subtilis*, *Bacterium coli*, *Serratia marcescens*, and *Bacillus megartherium*, the count before and after six hours of incubation at 37°C. being made by the plate method. The results are given in table 6.

This experiment was repeated using the yolk and white mixed together in the same proportions as found in eggs. The results are shown in table 7.

TABLE 7

Growth of various organisms in a mixture of egg white and yolk, in the proportion found in eggs, to which alkali had been added to give a pH such as found in aged egg white

After inoculation the mixtures were incubated at 37°C. for six hours. The bacteria count per cubic centimeter was determined by the plate method before and after incubation.

HYDROXYL ION CONCENTRATION	PSEUDOMONAS PYOCYANEUS PER CUBIC CENTIMETER	BACILLUS SUBTILIS PER CUBIC CENTIMETER	BACTERIUM COLI PER CUBIC CENTIMETER	SERRATIA MARCESCENS PER CUBIC CENTIMETER	BACILLUS MEGATHERIUM PER CUBIC CENTIMETER
pH					
7.15*	C	<1,000	C	C	30,000
9.11	1,260,000	<1,000	2,000	C	6,000
9.29	290,000	<1,000	5,500	6,100	<1,000
9.56	14,000	<100	600	<100	<100
10.59	130	<10	<10	<10	<10
Original count	10,120,000	799,000	9,080,000	8,080,000	131,000

* Original pH.
C = countless; < = less than.

The data in table 6 and 7 confirm the experiment of table 2, which indicates that the germicidal action of the egg white from normal aged eggs is due in part to the high hydroxyl ion concentration. The yolk, which previous investigators have indicated possessed no germicidal property, was shown to possess such a property when brought to the same hydroxyl ion concentration as is found in the aged egg white. This indicates that the germicidal property of normal egg white which has frequently been found by others can be largely explained on the basis that the limiting factor is the concentration of the hydroxyl ions.

Laschtschenko (1909) brought out some interesting facts in regard to the germicidal action of egg white. He found that egg white diluted 1:1 with water, physiological salt solution, or bouillon, did not lose its germicidal action to *Bacillus subtilis*. He found that dilutions of 1 part of egg white to 10 parts of water or physiological salt solution were germicidal to this organism,

TABLE 8

The effect on the hydrogen ion concentration of various dilutions of fresh and aged egg white, with water, physiological salt solution and bouillon

DILUTION		HYDROGEN ION CONCENTRATION	
Parts egg white	Parts water	Fresh egg white	Aged egg white
		pH	*pH*
1	0	7.82	9.46
1	1	7.98	9.48
1	5	7.91	9.69
1	10	7.82	9.72
Parts egg white	Parts salt solution		
1	0	7.82	9.46
1	1	8.04	9.33
1	5	8.05	9.37
1	10	7.96	9.34
Parts egg white	Parts bouillon		
1	0	7.82	9.46
1	1	7.87	8.17
1	5	7.48	7.28
1	10	7.36	7.00
0	1	6.84	6.84

while he obtained growth in one experiment and not in the other when he diluted 1:2 with bouillon.

The question of what effect dilution has upon the pH of egg white was next studied, and the results obtained are found in table 8.

Dilutions were made with fresh egg white having a pH of 7.82 and of egg white having a pH of 9.46, the latter obtained from eggs which had aged in air for some time. Diluting egg white of pH 7.82 with water apparently caused a slight decrease in the hydrogen ion concentration, for dilutions up to 1 part of egg white to 10 parts of distilled water; on the other hand diluting egg white with an original pH of 9.46 with water, gave solutions which were distinctly more alkaline than the original egg white before dilution. Upon dilution of the egg white of pH 7.82 with physiological salt solution, there was a slight increase in the pH and then a decrease at the higher dilutions. Diluting the more alkaline egg white with physiological salt solution apparently caused a very slight decrease in the pH. Diluting with bouillon caused both samples of egg white to decrease in pH, the more

TABLE 9

Effect of heating egg white with a pH of 9.41 to 68°C. for thirty minutes on its germicidal properties

ORGANISM USED	COUNT PER CUBIC CENTIMETER		
	Initial	Unheated after six hours	Heated after six hours
Bacillus subtilis	1,700,000	<100	Countless
Bacterium coli	7,200,000	328,000	Countless
Serratia marcescens	10,000,000*	71,000	Countless
Pseudomonas pyocyaneus	2,670,000	808,000	Countless

* Estimated.
< = less than.

alkaline egg white decreasing the more, and at a dilution of 1 part of egg white to 10 parts of bouillon, this sample of alkaline egg white actually had become the more acid. Laschtschenko (1909) found that egg white diluted with water was still germicidal. Our results show that dilution of alkaline egg white of pH 9.46 with water actually produces solutions which are more alkaline than the original egg white. The dilution of egg white with water up to 1 part of egg white and 10 parts of water does not produce a medium more suitable for bacterial growth, than the undiluted egg white, in so far as the hydrogen ion concentration is concerned. Laschtschenko (1909) did report a germicidal action toward *Bacillus Zopfii* and *Proteus Zenkeri* in egg white diluted to fifty volumes with nutrient bouillon, but he noted that these two organisms were more sensitive to the germicidal action of egg white than was *Bacillus subtilis*. In this case the germicidal action cannot be explained on the basis of the hydroxyl ion concentration.

Laschtschenko, as was mentioned before, believed that an enzyme of a proteolytic nature was responsible for the germicidal action. He proved this by heating the egg white to a temperature of 65° to 70°C. for thirty minutes and finding the action destroyed. That heating under these conditions destroyed the germicidal effect was found to be correct by Horowitz (1902) and Rettger and Sperry (1912), and was also confirmed by our experiments. Egg white from eggs which had been stored at 30°C. for five days to allow the pH to rise, was beaten together and the pH found to be 9.410. Half of the egg white was heated to 68°C. for thirty minutes and rapidly cooled. The pH was again determined and found to be 9.406. The unheated and heated egg white was then seeded with cultures of *Bacillus subtilis*, *Bacterium coli*, *Serratia marcescens*, and *Pseudomonas pyocyaneus*. Counts were made in each case before and after incubating for six hours at 37°C. The results of this experiment are found in table 9.

Since heating destroyed the germicidal action, yet did not affect the pH, it was thought that the surface tension might be responsible for the difference in behavior. A microscopic slide was suspended from the beam of a chainomatic analytical balance so that the end just touched the surface of the liquid in a silica dish supported over the pan on a bridge, and the weight necessary to pull the slide from the surface was determined. Distilled water was used as a calibrating liquid, its surface tension being taken as 71.78 dynes. The surface tension determinations were made at 25°C. The value found for the surface tension of unheated egg white was 52.9 dynes and that for heated egg white 52.2 dynes. The value which we found for the surface tension of egg white is in good agreement with the value (52.69) reported by Quincke (1877). Apparently the loss of the germicidal action of egg white caused by heat is not due to a change in surface tension. As heating also precipitates the protein, it was thought that the disappearance of the germicidal action might possibly be due to this precipitation rather than to the destruction of a proteolytic enzyme. To test this hypothesis it was necessary to produce precipitation by a method which would not destroy an enzyme. Precipitation by ethyl alcohol meets these requirements.

An equal volume of 95 per cent ethyl alcohol was added to fresh egg white and the alcohol removed by evaporation under reduced pressure. The temperature was never raised above 40°C. during the evaporation. The dried egg white was then mixed with water so as to produce a volume of 20 cc. containing about the same total solids as fresh egg white. No bacteria were found on plating the reconstituted egg white. This reconstituted egg white was then inoculated with *Bacillus subtilis*, and plates were poured every two minutes using a loopful to each plate. The plates showed no dimunition in the numbers of bacteria, and a plate made eighteen hours later was extremely thick, showing abundant growth had taken place. The reconstituted egg white had a pH of 9.65.

This experiment indicated that the germicidal action is not due to a proteolytic enzyme. Together with the experiments on heated egg white, it shows however, that hydroxyl ion concentration is not the sole factor which prevents the growth of bacteria.

Scholl (1893) treated egg white with potassium hydroxide and then dialized the solution against 0.75 per cent sodium chloride for twenty-four hour, and tested for the removal of free alkali with indigo calcium disulphonate. He found this solution to be germicidal to *Bacterium typhosum*, and that heating this solution did not destroy the action. He offered as explanation for this, his belief that egg white contained certain atomic groups which possessed a germicidal power. He postulated that heating produced a rearrangement after which the germicidal groups no longer functioned. If the egg white was then treated with alkali (potassium hydroxide) the germicidal groups were supposed to be regenerated and were not again changed by heating. Indigo disulphonic acid changes color at a pH of about 12, so that all of the solutions with which Scholl worked may have had a high pH (even though they were acid to that indicator) and would in this case have been germicidal, due to their high hydroxyl ion concentration. Heating, naturally, would not have caused a loss of the action in this case. These experiments cannot therefore be taken as showing that treatment of heated egg white with alkali and then removing the alkali, produces a germicidal effect in the egg white.

It was found that by dissolving coagulated egg white, formed by heating to 68°C. for thirty minutes, in sodium hydroxide, and then neutralizing with hydrochloric acid until a pH of between 7 and 8 was obtained, the solution possessed no germicidal action, for *Bacillus subtilis* was able to grow. Thus the germicidal action which Scholl found was probably due to the high hydroxyl ion concentration of his solutions, and not to the rearrangement which he postulated.

It appears from these experiments that there must be some toxic substance present in egg white which is bactericidal (in presence of alkali) and which is either destroyed or rendered inactive by coagulating the albumin by heat or by alcohol. It was thought that perhaps the surface presented by the coagulated albumin might adsorb the toxic substance, and thus allow the bacteria to grow, and that if such were the case, the addition of a strong absorbtive agent, like activated charcoal, might also adsorb the toxic substance and allow growth. Experiments of this nature were performed, but the germicidal action was not impaired.

Laschtschenko (1909), and Rettger and Sperry (1912) have shown that *Bacillus subtilis* seems very susceptible to the bactericidal action of egg white. It will be seen from tables 3 and 7 that in no case did *Bacillus subtilis* grow in egg white, even when the pH was favorable for the growth of other organisms. Rettger and Sperry found that this organism was frequently killed by the egg white before the first plates could be poured. Since it appears that *Bacillus subtilis* is unique among those which we studied in its susceptibility, some experiments were undertaken to study this organism and the action of egg white on it.

To determine the rate at which *Bacillus subtilis* was killed, a quantitative attempt was made to plate every two minutes, but owing to the amount of work which had to be done in the two minute interval it was found to be impossible. A plate poured at the end of six minutes showed that there were no living organisms present, although a seeding of 160,000 per cubic centimeter was made. A cruder qualitative method was then tried, with good results.

The whites from eggs were beaten together and to 20 cc. was added 1 cc. of a *Bacillus subtilis* culture. At two minute intervals a loopful (regular laboratory inoculating loop) was removed and a plate poured from it. By doing the experiment twice, first

TABLE 10

The influence of the age of the Bacillus subtilis culture on the germicidal action of egg white having a pH of 8.38 and 9.24

AGE OF CULTURE	HYDROGEN ION CONCENTRATION	TIME ORGANISM WAS EXPOSED				
		2 minutes	4 minutes	6 minutes	10 minutes	15 minutes
hours	*pH*					
5	8.38	S	S	S	S	S
5	9.24	S	S	S	S	S
8	8.38	S	S	S	S	S
8	9.24	Sp	S	S	S	S
18	8.38	Sp	8C	Sp	S	1C
18	9.24	6C	10C	6C	8C	11C
26	8.38	80C	33C	45C	47C	48C
26	9.24	56C	Sp	31C	45C	43C, Sp

S = sterile plate; C = colonies; Sp = spreader.

using even numbers of minutes and then odd numbers, a plate was poured every minute. The pH of the egg white was 8.20.

One hundred and forty-one colonies appeared after one minute, 89 colonies after two minutes, 17 after three minutes and 6 after four minutes. The plating was continued for one hour, but all of the plates were found to be sterile after exposure to the egg white for five minutes.

Laschtschenko (1909) states that *Bacillus subtilis* was killed as readily in the spore stage as in the vegetative state. His experiment was repeated by us, and it appeared that the spores were more resistant than the vegetative cells; therefore, a more careful and systematic experiment was undertaken. Cultures of *Bacillus subtilis* of various ages were grown, and using egg white with a pH of 8.38 and 9.24 the same procedure was followed as that described above. The results of this experiment are presented in table 10.

It would appear then that between eight and eighteen hours the organisms became more resistant, and at twenty-six hours had developed so much resistance that the fifteen minutes exposure to the egg white showed almost as many colonies as the two minute interval. A stained microscopic preparation of each culture showed the five- and eight-hour cultures to be all vegetative cells. About 25 per cent of the eighteen hour culture was in the spore stage, while 80 per cent or more of the twenty-six-hour-old culture was spores. From this it would appear that in the case of *Bacillus subtilis* the vegetative cells are subject to the bactericidal action of the egg white, but that the spores are not so readily destroyed, while the pH and age of the egg are not limiting factors.

As there was some question as to the effect of the dilution of egg white on the germicidal action on *Bacillus subtilis*, egg white was diluted with varying amounts of water and with bouillon, up to dilutions of 1:10, and its germicidal effect on this organism determined. The germicidal action was found to persist throughout all the dilutions studied.

Some investigators have shown that the rate of seeding is a factor which must be considered. To show the effect of the rate of seeding of *Bacillus subtilis* on the germicidal action, 20 cc. of fresh egg white was placed in each of five sterile test tubes, various amounts of a culture of *Bacillus subtilis* were added (from 1 to 25 cc.) and the usual qualitative method of testing the germicidal action after ten minutes was followed. No growth occurred on any plates although control cultures made in sterile water at the same time showed upon plating a vast number of colonies.

This shows very strikingly the germicidal action of natural egg white toward *Bacillus subtilis* for after inoculating 20 cc. of egg white of pH 8.2 with 25 cc. of a culture of *Bacillus subtilis* containing about 10,000,000 per cubic centimeter, all organisms were killed in less than ten minutes.

In another attempt to study the toxic substance which gives to egg white its bactericidal action toward *Bacillus subtilis*, the whites of fresh eggs were mixed together and 20 cc. portions were placed in collodion bags, the tops of which were securely closed, and the bags immersed in 20 cc. of sterile distilled water. These

TABLE 11

Fresh egg white was dialyzed in collodian bags for various lengths of time at a temperature of 1 to 5°C. The dialyzed white and the dialyzate were then tested for germicidal action toward *Bacillus subtilis*, exposing the organism to it for ten minutes and then plating out a loopful. A part of the dialyzate was heated to 68°C. for 30 minutes and also tested in the same manner. The pH of the egg white used was 7.87.

TIME DIALYZED	EGG NUMBER	EGG WHITE		DIALYZATE			
				Not heated		Heated	
		pH	Growth	pH	Growth	pH	Growth
hours							
1	1	7.96	–	7.89	+	–	–
1	2	7.92	–	7.89	++	–	–
3	3	7.82	–	8.06	–	7.86	++
3	4	7.92	–	7.89	–	7.89	+++
6	5	7.81	–	7.87	+(?)	7.94	+
6	6	7.88	–	7.84	–	7.90	+++
12	7	7.98	–	7.84	+	7.97	+++
12	8	7.90	–	7.94	+	7.89	+++
24	9	8.00	–	7.89	+	7.97	+++
24	10	7.91	–	7.99	+	7.90	+++
48	11	7.87	–	7.92	++	7.89	+++
48	12	7.90	–	7.64	++	7.69	+++

– = no colonies; + = small number of colonies; ++ = medium number of colonies; +++ = large number of colonies.

were kept for varying lengths of time in a cold room having a temperature between 1° to 5°C. At the end of the various time periods, duplicate bags were removed and the contents placed in sterile dry test tubes. The diffusate from each bag was divided into two parts. One part was heated to 68° to 70°C.

for thirty minutes, the other left unheated. All six tubes were then inoculated with a three hour old culture of *Bacillus subtilis* and a loopful plated out at the end of ten minutes. The hydrogen ion concentration was also determined at the same time. The results are given in table 11, from which we see that in every case the egg white in the collodion bag retained its germicidal action. In the diffusate no germicidal action was detected in the one hour tubes, the rate of diffusion probably being so slow that it was not until the third hour that enough of the toxic substance came through to kill the cells of *Bacillus subtilis*. After six hours of dialysis, growth was again found to take place in the diffusate. It will also be seen that just as egg white itself, when heated to 68° to 70°C. for thirty minutes, was found to lose its germcidal action, so the diffusate when heated produced in every case no diminution in numbers of bacteria. These results were confirmed by repeating the experiment.

In another experiment some fresh egg white was dialyzed for four hours, this length of time producing a diffusate exhibiting the most marked germicidal action. The bags containing the egg white were then removed, and the diffusate was kept at room temperature and its bactericidal action tested at various lengths of time. The diffusate showed germicidal action at the end of two and six hours but at the end of twelve hours slight growth took place and at the end of twenty-four and forty-eight hours distinct growth occurred. These results show that the toxic material which has been separated from egg white by dialysis slowly disappears when the diffusate is kept at room temperature.

Egg white was frozen solid for several days, thawed, and the germicidal action for *Bacillus subtilis* found to be unimpared. Eggs which had been frozen or which had been submerged under water for over two weeks were also found to exhibit the same bactericidal property for this organism. The pH of the white from the frozen and submerged eggs was relatively low and about the same as the egg white of the eggs before giving them these treatments.

The organisms used in this work were obtained from the following sources:

Bacterium coli, stock culture of the laboratory of bacteriology.
Bacillus subtilis, originally obtained from H. J. Conn, Geneva, N. Y.
Serratia marcescens, originally obtained from R. S. Breed, Geneva, N. Y.
Bacillus megatherium and *Bacillus mycoides*, laboratory cultures originally obtained from W. W. Ford, Baltimore, Md.
Bacillus cereus, *Pseudomonas fluorescens*, and *Proteus vulgaris*, stock culture of the laboratory, originally obtained from the American Type Culture Collection.
Pseudomonas pyocyaneus, isolated in this laboratory from infected egg white by R. Whitaker.

DISCUSSION

Heretofore, the explanation offered to account for the comparative freedom of the normal egg white from bacterial invasion, has been that it possesses germicidal properties. We have shown that this action may be due in part to the hydroxyl ion concentration of the egg white, which rapidly increases from the time the egg is laid until under some conditions a pH of 9.5 may be reached, which does not permit the growth of most bacteria as reported in the literature. Our experiments were designed to show the effect of the hydrogen ion concentration of the egg white on bacterial growth, and do not indicate whether or not bacteria inoculated into egg white of suitable pH multiply as rapidly as they do in broth, bouillon, sugar solutions, etc. In our experiments we have not shown that egg white, at a hydrogen ion concentration at which growth occurred in our standard incubation period of 6 hours, shows no transient germicidal action for shorter periods such as has been demonstrated so clearly for freshly drawn milk by Sherman and Curran (1924). On the other hand, the fact that the bacteria in some instances were not all killed but decreased in numbers during this six hour incubation period does not preclude the possibility that if the incubation were allowed to go for longer periods of time some of the bacteria might survive and might even eventually increase in numbers. There is also the possibility that bacteria subjected to a hydroxyl ion concentration near the lethal concentration might gain a start on the surface or on the walls of the container above the liquid and gradually neutralize their way and later develop in the whole tube, although all of the bacteria which were completely immersed in the egg white were killed.

From a consideration of the literature on the subject, it seems that the infection of the so-called "fresh" eggs which have been used to determine whether or not bacteria are found in fresh eggs, could occur in any one of three ways; first, the yolk may be infected by infected ovaries before it starts down the oviduct; second, infection may occur as the yolk passes down through the oviduct; and third, infection may be produced after the egg is laid, by contact with wet and soiled nesting material, etc.

The literature is contradictory in regard to the infection of eggs by possibilities one and two, although it does indicate that the yolks of a small percentage of freshly laid eggs are infected. If we consider it possible that the yolk may be infected before it starts down the oviduct, or perhaps in passing down the oviduct, we can see that the hydroxyl ion concentration of the egg white, which is later increased due to the aging of these infected eggs in air for a few days before testing may have a considerable bearing on whether or not bacteria are found in the white. If the yolk is infected before it starts down the oviduct, the organisms will be in a medium which is not germicidal. The yolk does not develop the high hydroxyl ion concentration which limits growth, as is the case with the white. Eggs so infected would, after aging in air for a few days, probably show the presence of organisms in the yolk and not in the white. Mauer (1911), Rettger (1913), Bushnell and Mauer (1914), Hadley and Caldwell (1916), and others, have called attention to the fact that infected eggs usually contain the bacteria in the yolks while the whites are sterile. This has also been found in many instances in our own laboratory.

Laschtschenko (1909) injected *Bacillus subtilis* into the egg. He found that if this organism was injected into the white, it was killed; but if it was injected into the yolk, it grew. He also obtained growth of *Bacillus subtilis* in a mixture of the white and the yolk but not in the white alone. This organism was killed by a mixture of yolk and white in our experiment. Laschtschenko probably used a strain of *Bacillus subtilis* which was more resistant then the strain which we used.

Rettger and Sperry (1912) found that under anaerobic conditions *Bacillus putrificus* and *Bacillus edematis* grew in egg yolk but were killed in egg white, as shown by appearance of the tubes and by microscopic examination.

If the yolks are sterile as they start down the oviduct, and the oviduct is not sterile, organisms may be present in the white and may reach the yolk in this passage, or before the white develops the high alkalinity after the egg is laid. In this case also it is probable that if the eggs were examined a few days after being laid the yolks would be found to be infected while the whites would be found to be sterile even though the infection actually came from infected white in the oviduct. The investigators of fresh eggs (eggs a few days old) found the organisms chiefly in the yolk and not in the white. If the examinations had been made by a proper method within an hour after the eggs were laid, then it is possible that both white and yolk would have been found to be contaminated. In the light of this discussion it is seen that a reinvestigation of the bacterial content of fresh eggs and the germicidal properties of the secretions in the oviduct would be desirable.

If a sterile egg is actually laid, then infection might occur by the passage of the organisms from the shell to the yolk through the white before the white has had time to develop the high hydroxyl ion concentration which would prevent the organisms from reaching the yolk later. At room temperatures the organisms must pass through the shell, and shell membranes, and the white, in a period of not more than three or four days, in order to infect the yolk. Whether such rapid passage is possible is rather doubtful. Progress through these structures must necessarily be slow. It should also be remembered that the yolk of the fresh egg is surrounded with an egg white gel, the viscosity of which would tend to retard the penetration of the bacteria.

Some of the investigators of the question as to whether or not eggs are contaminated with bacteria when laid, apparently did not believe it possible that bacteria could penetrate into the egg within the first few days after being laid, when stored under fairly dry conditions, for they frequently did not examine them for the presence of bacteria until several days after the eggs were laid. If it is impossible for the bacteria to penetrate the shell, the membranes, and the thin and thick egg white within a few days, then the only explanation for the presence of bacteria in the yolk of the so-called fresh eggs is that they were actually present when the eggs were laid.

We have pointed out the importance of the hydroxyl ion concentration of the white in resisting bacterial invasion. We now must account for the spoilage of eggs and decomposition of the white due to bacterial growth. Some points in this discussion may also apply to the invasion by molds but we are considering in this paper mainly invasion by bacteria. Many factors may play a part in regulating this invasion and some of them will be considered in a later paper, but in so far as the hydroxyl ion concentration is concerned we may mention a few possibilities which seem to fit in with our present knowledge.

If eggs with infected yolks are kept at a temperature which permits bacterial growth, the organisms may increase in numbers and gradually work their way out into the white neutralizing their way with their metabolic products as they go.

If a sterile egg is laid, it may be infected with bacteria in several ways. If moisture condenses on the shell, or if moist nesting material adheres to the shell for some time and the temperature is suitable, bacteria will slowly invade the egg. The organisms probably first develop in the shell membranes where they are not exposed to the high alkalinity of the white and then gradually invade the white as their metabolic products reduce its alkalinity. It has been found that egg white which is highly infected with organisms is usually considerably more acid than the uninfected egg white, the pH frequently dropping to near the neutral point.

Another point of invasion of bacteria into eggs is by way of the air space. It is probable that bacteria can grow on the membrane forming the surface of the air space without very many of them coming in contact with the alkaline albumin. The alkalinity of the egg white would be gradually neutralized by their metabolic products, as mentioned above, thus permitting the organisms to penetrate further and further into the white. Candling evidence seems to bear out this idea.

Stored eggs are never turned, and the yolks may move through the white until they touch the shell. Bacteria then are able to grow where this contact is made, entering from the shell and its membranes and growing in the yolk. As was mentioned before, acid by-products, liberated by bacterial growth, gradually neutralize the alkalinity of the white surrounding this point of contact. Candling evidence also seems to bear out this idea.

This study has all been carried out on a relatively few organisms, but as egg white which had a hydroxyl ion concentration corresponding to pH 9.5 was germicidal to all of them, we have concluded that the hydroxyl ion concentration is the most important variable factor in limiting the growth of organisms in natural egg white. We have not eliminated the possibility of there being organisms, or even strains of the organisms which we have investigated, that can withstand this high alkalinity of the natural egg white, but such organisms are probably not very numerous.

There is one point which we have not taken into consideration in this work, that is the variation in the germicidal action of egg white of various pH values with change in temperature. All of our tests of the germicidal action were made by inoculating egg white at room temperature and then placing the tubes in an air thermostat at 37°C. and determining the count at the end of six hours. One would expect that the death rate would be slower at the lower temperatures, but that the death of the bacteria would still occur.

Rettger and Sperry (1912) state "The last two series of results seem to indicate that the bactericidal action of the egg-white is accelerated with increased temperature."

It is readily seen from the investigation reported here that the hydroxyl ion concentration of the raw egg white may assume a very important rôle in regard to the storage of eggs. It is also apparent that a marked difference exists between the effect of the hydroxyl ion on the germicidal action of raw and heated egg white. The bacteria are able to grow in egg white which has been heated, even though it has a hydroxyl ion concentration which would limit growth in the unheated material. There is apparently some other factor in raw egg white, in addition to the hydroxyl ion concentration, which affects the growth of bacteria. This other factor does not, however, minimize the practical importance of the hydroxyl ion concentration in regulating the growth of bacteria in raw egg white.

SUMMARY

1. The so-called germicidal action of raw egg white is markedly influenced by the hydroxyl ion concentration, which increases rapidly during the first few days of storage of untreated eggs in a ventilated room. It was found that the hydroxyl ion concentration of the white corresponding to that at the time the egg was laid would permit growth, while the hydroxyl ion concentration corresponding to the whites of eggs aged a few days in air was germicidal to the following organisms: *Bacterium coli*, *Pseudomonas pyocyaneus*, *Serratia marcescens*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Bacillus megatherium*, and *Bacillus mycoides*.

2. The conflicting results of previous workers may be partially explained on the basis that they did not take into account the age of the egg and, consequently, the hydroxyl ion concentration, when testing for the germicidal action of the egg white.

3. For vegetative cells of the strain of *Bacillus subtilis* which we used, normal egg white at all hydrogen ion concentrations was decidedly bactericidal, while the spores were more resistant.

4. Heat and alcohol coagulation of the egg white destroyed its germicidal action on the vegetative cells of *Bacillus subtilis*.

5. The toxic substance which kills the vegetative cells of *Bacillus subtilis* can be separated from egg white by dialysis, the diffusate gradually becoming non-germicidal on standing.

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