ON THE SURVIVAL OF SALMONELLA IN EGGS AND ON THE SKIN OF HUMAN BEINGS

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ON THE SURVIVAL OF SALMONELLA IN EGGS AND ON THE SKIN OF HUMAN BEINGS

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OUTLINE

Introduction To Subject As A Whole and Literature Review

The salmonellae are becoming increasingly important as factors in or producers of "food poisoning". A great deal of research is being carried out at present to clarify their exact role in relation to food borne infection. The practical value of the determination of the role of Salmonella pullorum as a human pathogen, because of its common occurrence in eggs from infected flocks, cannot be overlooked.

Edwards and Brunner[1], in their review of the occurrence and distribution of salmonellae, show world-wide distribution of salmonella types. The frequency of occurrence of various types in man and animal is quite similar. The possibilities of direct transference of salmonella infection from animals to man are quite obvious. These authors state that they have unpublished data which include a number of instances in which infection in man was traceable directly to animals. They isolated S. pullorum from the stool of a patient suffering from gastro-enteritis. The infrequent recovery of S. pullorum from human stools, Edwards and Bruner believe, might, in the majority of cases, be due to its poor growth on artifical media.

Verder and Sutton[2] gathered experimental data suggesting that living bacilli, rather than thermostable toxic products, are the cause of salmonella food poisoning. They found that heated and filtered cultures of Salmonella enteritidis strains produced no symptoms of food poisoning when fed to human volunteers or to monkeys. The symptoms produced by eating a culture of salmonella are not unlike those resulting from the ingestion of staphylococcus toxin,

although in cases of salmonella "food poisoning" the period of incubation is longer, the onset more gradual and the illness more prolonged. This might be explained by the gradual accumulation of and gradual decrease in absorbable toxic substances in the intestine following ingestion of cultures of salmonellae.

In a survey made by Seligmann, Erich and Wassermann[3] it is stated that thirty-eight different types of salmonellae were identified from one thousand cases. The predominant type was Salmonella typhi-murium which occurred in 37% of the cases and in one-third of the outbreaks. Next in frequency ranked members of the C Group, Salmonella newport, Salmonella choleraesuis, Salmonella oranienburg, Salmonella montevideo, and Salmonella paratyphi B. These organisms caused 9% of all cases. A third group, composed of Salmonella panama, S. enteritidis, Salmonella anatum, and Salmonella derby, accounted for a total of 12%, about 3% for each, of these cases. The majority of the species were recovered from stool specimens but quite an appreciable number were found in the blood stream. S. choleraesuis was found in 60% of the blood cultures. This exceeded all the other types and is undoubtedly the most invasive organism of the group. S. paratyphi B ranked next with almost 22% in blood cultures, while all the other salmonellae fell far behind. Astonishing was the number of purulent infections from which salmonellae could be isolated, and even more surprising was the number of meningitis cases caused by one or the other of the types. A few cultures were recovered from urine, pleural chest, and peritoneal fluids, bile, kidneys, inflamed uterine tubes, spleen and mesenteric lymph nodes and in one instance from sputum (S. newport).

There appears to be a relative lull in salmonella infection of all kinds during the winter with a slight rise around Christmas. A definite increase begins in April and continues with slight remissions to a peak in October. In November the decline begins.

Of 874 salmonella infections, 59 were reported to terminate fatally. Altogether 18 different types of Salmonella were found as causes of death. S. choleraesuis produced the highest mortality rate with 16 deaths among a total of 78 patients. Of 71 S. paratyphi B infections, two were fatal, and of 329 S. typhi-murium, 19 were fatal.

These investigations of Seligmann, Erich and Wassermann further confirm the wide prevalence of these bacteria among all sorts of animals. Fowls and swine were found to be the most important sources of infection. The organisms were also found in hen's eggs, duck's eggs, cheese, ice cream, and meat products.

Mallmann[4], over a period of years, examined fresh and storage eggs for bacterial contamination. Bacteria other than S. pullorum were not found in fresh eggs. Frequently as many as 56% of the eggs of pullorum infected hens yielded S. pullorum[5].

During a bacteriological examination made by Andresen[6] of 586 eggs, secured from 14 different sources, 43 were found to contain bacteria; S. pullorum was present in 13 of them.

The dangers arising from the consumption of raw and incompletely cooked duck eggs are well known. Poisoning by eggs from ducks has occurred much more frequently than by eggs of hens or pigeons. Salmonella infection of ducks, chiefly by S. typhi-murium and S. enteritidis seems to be commonest[7].

From egg powder Solowey and McFarlane[8] isolated salmonella types which had the same morphological and cultural characteristics and gave the same biochemical and seriological reactions as types isolated from infected humans. S. pullorum has been ignored to some extent in egg products for human consumption because it had always been assumed to be non-pathogenic for man. In the last few years it has been isolated from infected human beings. In their investigations Solowey and McFarlane found that there was no evidence that any salmonella type is specific for egg powder. A total of 5,198 samples of spray-dried whole egg powder were examined. The samples had been sent in from 100 dehydration plants located in 26 states. Salmonellae were isolated from 1,810 samples (35%). Of these, 562 (11%) were positive for S. pullorum. Out of the 100 plants, 95 contributed salmonella positive samples. S. pullorum contamination was found to be high in samples taken during February (22%), March (18%) and in April (16%). The information gathered proved that the drying process of the egg powder does not destroy the salmonellae and the types that occur with greatest frequency in dried eggs are among those which commonly occur in human and animal infections.

Additional evidence of the pathogenicity of S. pullorum for mammals was offered when the organism was recovered from infections in foxes, minks[9], and rabbits. Olney[10] reported that four rabbits died of S. pullorum infection following the feeding of infertile, incubated eggs obtained from a commercial hatchery.

Mitchell[11] reported on an outbreak of gastro-enteritis at the Army Air Forces School of Aviation Medicine at Randolph Field, Texas. The outbreak involved 423 persons who required medical treatment.

This number included people from eight different squadrons. Of these, 172 required hospitalization. The febrile state and other symptoms continued for 48 hours. At the end of the third day all the patients were well enough for discharge. Since all these cases were traced to a single mess, and rice pudding, served on three successive occasions, formed the only common link among all patients, Mitchell believed that this presented strong evidence of true "food poisoning". The degree of contamination increased with each successive serving--showing that the organisms increased in numbers. There was a definite decrease in the incubation period and an increase in the morbidity rate with each successive serving of pudding. Epidemiologically and clinically the outbreak behaved as a food-borne infection and did not resemble either an intoxication or gastro-enteritis associated with a virus infection. A specimen of the pudding was not available for bacteriological examination, and, because of the method used for assembling mixed lots of eggs in unmarked crates, it was impossible to obtain specimens of the eggs used or to determine their source. Cultures were made from the stools of 171 patients. From these, 20 (11.7%) cultures of S. pullorum were isolated. Since 80% of the significant cultures were obtained at the time the patients were already recovering from the acute stage of the disease, the number of isolations of pathogens was probably smaller than what might have been obtained during an earlier phase of the outbreak. Therefore, S. pullorum was rather definitely incriminated in this outbreak.

Other cases have been reported by Judefind[12] who isolated S. pullorum from the stool of a patient ill with a diarrhea for about a

month. The same organism was isolated from six patients afflicted with a mild dysentery-like disease by Felsenfeld and Young[13]. D'Aunoy[14] reported an outbreak of food poisoning afflicting 90 persons following the ingestion of cream puffs infected with S. enteritidis. None of the food handlers showed agglutinins for this or closely related microorganisms, nor could any significant forms be isolated from their feces. S. enteritidis was isolated from rodent excreta found in the bakery as well as from the intestinal contents of mice trapped within the building. Both S. typhi-murium and S. enteritidis are commonly carried by rats and mice, and it is probable that in many cases these rodents are responsible for the infection of food.

The hands of temporary or chronic human carriers are likewise an important source from which food material may become contaminated. Salmonellae are resistant to low temperatures and repeatedly outbreaks of infection have been traced to ice cream as noted in Bornstein's[15] paper.

Members of the Salmonella group are quite frequently found in market meats, particularly in pork products. Of 250 meat samples, examined by Cherry and Bailey[16] 13 (5.2%) yielded salmonellae. Out of 170 pork products 10 (5.9%) yielded these organisms. The types of Salmonella isolated were; S. typhi-murium, Salmonella give, S. derby, S. anatum, S. newport, Salmonella bredeney, Salmonella senftenberg, and Salmonella newington.

The wide distribution of salmonellae among animals and man, their presence in foodstuffs on the market, the large number of healthy carriers and the frequent observation of salmonella-carrying food handlers demand both intensive and extensive research, aimed at

determining actual and potential hazards to the public health resulting from this source.

This study was initiated mainly for the purpose of adding to our knowledge concerning those characteristics of S. pullorum which might have special public health significance. It has been divided into four parts and will be presented in the following order: 1. The Survival of Salmonella in Eggs over a Period of Twelve Months, 2. Egg Shell Penetration by Salmonella Enteritidis, 3. The Survival Time of Salmonella Pullorum on the Human Skin, and 4. Survival of Salmonella Pullorum in Egg Albumen and Yolk After Boiling, Frying, and Poaching.

Pure cultures of all the organisms, but S. pullorum, used in this study were obtained from the Bureau of Laboratories of The Michigan Department of Health. The culture of S. pullorum was contributed by the Department of Bacteriology and Public Health, Michigan State College. All the eggs used in these experiments were obtained from the Poultry Department, Michigan State College.

Part 1

Survival of Salmonella in Eggs Over A Period of Twelve Months

Experimental Procedure

Fresh eggs were inoculated November 11, 1947 with: S. schottmülleri, S. typhi-murium, S. enteritidis, S. choleraesuis, S. paratyphi A. and S. pullorum. Six sets of eggs were inoculated, each set with one of the six organisms, half of the eggs were incubated at 25° C and half at 4° C. Two sets were examined approximately every two months.

Previous to inoculation each egg was tested for sterility by the withdrawal of approximately 1-ml samples of the yolk and albumen which were added to tetrathionate broth and incubated for a period of 12-24 hours at 37° C. They were then streaked out on S. S. and MacConkey agar plates. No organisms were encountered on these initial plates.

A 24-hour tryptose broth culture of each organism was used for the inoculations. Each egg was inoculated with approximately .01 ml of the culture and one half of the total number of eggs was incubated at 25° C and the other half at 4° C.

Every two or three months a set of these eggs, was examined by placing one egg in a flask of tetrathionate broth and incubating at 37° C for 12-24 hours. Following incubation, a loopful of broth was streaked on S. S. and MacConkey agar. Colonies characteristic of salmonellae were picked and transplanted to motility medium, triple sugar iron agar (T. S. I.), the various fermentation broths: dextrose, lactose, maltose, mannite, sucrose, inositol, arabinose, xylose, and dulcitol. They were tested for indol

production and stained with the Gram stain.

Results

Table I shows that after three months incubation at 25° C and 4° C all the organisms were recovered with the exception of S. typhi-murium which was found to survive in eggs held at 25° C but not in those held at 4° C. At the end of six months all the organisms were isolated from both lots except S. enteritidis which was recovered from the egg held at 4° C. Of the five salmonellae which were recovered after nine months, S. schottmülleri and S. enteritidis, were the only two isolated from both lots. S. paratyphi A. and S. pullorum survived at 25° C while S. choleraesuis was not recovered after having been held for nine months at that temperature but was isolated from the egg held at 4° C. At the end of twelve months S. schottmülleri, S. typhi-murium, S. enteritidis and S. paratyphi A. were found to have survived holding at 25° C while none survived from the eggs held at 4° C.

Discussion

By the end of six months the eggs which had been kept at room temperature (25° C) were completely dehydrated. This necessitated crushing the dried yolk and albumen before placing them into tetrathionate broth. The refrigerated eggs appeared to have lost little moisture content during the twelve-month period.

While none of the salmonellae were recovered from eggs held at 4° C after nine months, they were recovered up to twelve months in eggs held at 25° C. Mallmann, in 1931, encountered similar results. He

TABLE I

Survival of Six Salmonella Species in Eggs over a Period of Twelve Months

ORGANISMS	TEMPERATURE	1/8/48	2/28/48	5/5/48	8/11/48	11/16/48
S. schottmülleri	25° C	+	+	+	+	+
	4° C	+	+	+	+	-
S. typhi-murium	25° C	+	+	+	-	+
	4° C	+	-	+	-	-
S. enteritidis	25° C	+	+	+	+	+
	4° C	+	+	-	+	-
S. choleraesuis	25° C	+	+	+	-	-
	4° C	+	+	+	+	-
S. paratyphi A.	25° C	+	+	+	+	+
	4° C	+	+	+	-	-
S. pullorum	25° C	+	+	+	+	-
	4° C	+	+	+	-	-

Original inoculation 11/11/47

had refrigerated a supply of salmonella types, mostly S. pullorum, in a semi-solid medium and had sealed the tubes with paraffin. This group included a number of organisms of permanent smooth and permanent rough colony types. When the organisms were needed for further research, after about three months, it was discovered that the majority of the organisms were no longer viable.

The results of this experiment would be of greater value and significance if larger quantities of eggs could have been examined at the end of each time period rather than just the single eggs. The results obtained indicate that salmonellae are capable of survival in eggs for a period of nine months at temperatures of 4° C and 25° C and for twelve months at 25° C.

Part 11

Egg Shell Penetration By *Salmonella Enteritidis*

Literature Review

A fresh egg is covered by a thin coating consisting of protein and salts, which gives to the egg its dull, velvety appearance. Shrader[17] made the following statement: "It has been generally thought that washing removes the protective film, opening the pores, of the shell to passage of organisms, but this is not entirely true. It is the soiling of the shells, especially with fecal matter, and storage of eggs in damp places, rather than washing or otherwise cleaning the shells, which facilitate microbial invasion".

Another factor involved is the formation of the shell itself. Scientists of the Bureau of Animal Industry[18] have learned from breeding experiments that this "packaging ability" is inherited. A loss of weight in the egg during the first 14 days of incubation proved to be a good way of testing shell quality. The lime used for shell formation is secured largely from the chicken's food, but during periods of heavy egg production, the bird also draws on the calcium in it's bones. For this reason the shell is generally of good texture during the fall and winter months but, as spring approaches, the hens lay at a more rapid rate and cracked and weak-shelled eggs are more frequent. Although hens do not lay as rapidly during the summer, an increase of weak-shelled eggs often occurs and this may be the result of insufficient assimilation of calcium due to excessive heat[19].

Smyth[20] reports that no difference in absorption has been found between shells and membranes of brown eggs and those of white eggs.

Schrader[17] states that there has been no evidence that fertilized eggs undergo natural bacterial decomposition more readily than the unfertilized egg.

Practically all investigators agree that the yolk contains the greater number of bacteria. Tanner[21] states that the germicidal power of the albumen of the egg decreases rapidly with age.

Eggs are known to deteriorate because of chemical changes involving the release of carbon dioxide. By increasing the concentration of carbon dioxide within the egg, the rate of these reactions and therefore the deterioration is retarded. Fresh eggs should be cooled as rapidly as possible and kept at low temperatures. This retards enzyme activity within the egg, slows down bacterial growth and helps to preserve the eggs for longer periods of time[22].

The usual procedure used in experiments on penetration of egg shells has been to take washed, sound eggs and immerse them in a solution containing the bacteria concerned[23]. Workers have not been successful by simply smearing the organisms on the shell.

Pirokowski[24] in 1895 demonstrated penetration by Eberthella typhosa, Wilm[25] in 1895 by cholera vibrios, then in 1907 Lange[26] by Escherichia coli, S. enteritidis, S. schottmülleri, and in 1910 Poppe[27] by S. schottmülleri.

C. Brownwell[28] worked on penetration of eggs using Bacillus subtilis, E. coli, and Pseudomonas aeruginosa. The eggs were either immersed in a 24-hour culture of the organism or the culture was sprayed on the shell. Both washed and unwashed eggs were used. Greater penetration took place through washed shells. With a relative humidity of 100% at 37° C, an average time for penetration

was found to be about 14-18 hours.

Experimental Procedure

Both scrubbed and unscrubbed eggs were used to determine the penetratability of the organisms. Twenty-four hour tryptose broth cultures of S. enteritidis were used on all but three dozen eggs on which S. pullorum was used. A small area of the egg shell was at first swabbed with the broth culture, after which the eggs were incubated at 37° C. Only negative results were obtained so the eggs were immersed in the culture, likewise with negative results.

Penetration did not take place until a mixture of chicken droppings and broth culture was smeared on the egg shells. The eggs were placed in sterile mason jars and incubated at 37° C for varying periods of time.

At specified time intervals the eggs to be examined were taken out of the jars and the organic matter washed off. They were then placed in a 5% solution of colloidal iodine for approximately five minutes for the purpose of disinfecting the shell. The eggs were then opened aseptically and the contents dropped into tetrathionate broth and incubated for 12-24 hours at 37° C. From the tetrathionate broth seedings were made on S. S. or MacConkey agar plates.

Typical colonies were picked and transferred to motility medium, T. S. I. medium, indol test medium, and the sugars (dextrose, lactose, maltose, mannite, and sucrose). They were stained with the Gram stain and sent to the Bureau of Laboratories, Michigan Department of Health for serological confirmation.

Results

Approximately four dozen eggs were either swabbed or immersed in broth cultures of S. enteritidis and held for periods varying from eleven to ninety-six hours, all with negative results.

A total of 270 eggs were smeared with infected chicken droppings during the spring and summer terms, including the months March to September. Of these, 26 eggs (9.6%) showed penetration, all by S. enteritidis. Since only 36 eggs were smeared with S. pullorum the negative results are not by any means conclusive evidence that penetration is not possible.

As shown in Table II, positive results were obtained over a time range of 23 to 552 hours. During the spring term, 67 eggs were used in the experiment in which there was a total of 18 penetrations by S. enteritidis. During the summer term (Table III) a total of 203 eggs were used with only 8 penetrations.

Discussion

In this experiment an attempt was made to simulate as far as possible conditions normally found in the barnyard without creating unnatural conditions by greatly increased moisture.

The use of droppings greatly enhanced the penetrating powers of the organisms, as did the use of mason jars which helped to maintain the moisture supplied by the mixture of broth and fecal material.

The eggs examined during the months of March through June showed greater penetration (26.8% or 18 out of 67) than did those examined during June to September (3.9% or 8 out of 203). However, penetration

TABLE II

Penetration of Egg Shells* by S. enteritidis during the Months of March through June, 1948

Number of Eggs Showing Penetration	Number of Hours Necessary for Penetration	Preparation of Egg
2	23	Scrubbed
1	33	Unscrubbed
1	43	Unscrubbed
1	162	Scrubbed
1	192	Unscrubbed
1	211	Scrubbed
1	248	Unscrubbed
1	289	Unscrubbed
3	316	Scrubbed
1	412	Scrubbed
2	432	1 scrubbed 1 unscrubbed
1	453	Scrubbed
2	552	Scrubbed

*Egg shells smeared with S. enteritidis-infected chicken droppings.
Total Eggs used - 67

Total of 18 penetrations (12 scrubbed eggs and 6 unscrubbed eggs.) 26.8%

TABLE III

Penetration of Egg Shells* by S. enteritidis during the Months of June to September, 1948

Number of Eggs Showing Penetration	Number of Hours Necessary for Penetration	Preparation of Egg
1	44	Unscrubbed
1	52	"
1	68	"
1	75	"
1	94	"
1	139	"
1	151	"
1	161	"

* Egg shell smeared with S. enteritidis-infected chicken droppings.

Total Eggs Used - 203.
Total of 8 penetrations (unscrubbed eggs) 3.9%

Thirty-six eggs were smeared with S. pullorum-infected chicken droppings -- No penetration demonstrated.

took place in a shorter time during the summer than it did in the spring. Logically one might expect greater penetration during the summer months which many investigators consider as the time of more frequent infections in eggs. A possible explanation could be that the condition of the shells of these particular eggs was exceptionally good during the summer of 1948.

There appeared to be little advantage in scrubbing the egg shells as shown in Table II, so this procedure was discontinued and all the eggs used during the summer term were unscrubbed. The data presented in this experiment further substantiate the importance of moisture and fecal matter as factors in microbial penetration of egg shell.

Part 111

Survival Time of Salmonella Pullorum on the Human Skin

A Study of the so-called Self-disinfecting power of Human Skin

Literature Review

The skin, besides being a mechanical barrier to microbes, possesses a so-called "self-disinfecting power" by which it destroys most of the microorganisms which become lodged upon its surface.

So far no satisfactory explanation has been given for the fact that bacteria die rapidly when smeared upon the surface of the skin, although many theories have been offered. Arnold[29] and his co-workers showed reduction of 90-100% of organisms during a period of 10-30 minutes. They demonstrated that clean skin caused more rapid killing than dirty skin. S. enteritidis was used as the test organism, and after a period of 30 minutes it was found that only a 5% reduction occurred on dirty hands while on clean hands a 100% reduction was demonstrated after 20 minutes. Arnold believes that the sugar and nitrogen content of the sweat is able to form a substrate for bacterial growth. He also suggests that the fatty content of the sweat may play an important role in the growth of bacteria. This fatty substance prevents them from coming in contact with the stratum corneum and therefore they remain viable. Two years earlier Usher[30] had demonstrated that sweat may be a medium for the growth of bacteria.

Using yeast cells and Staphylococcus aureus as test organisms Cornbleet and Montgomery[31] found moist areas less effective in destroying the organisms than dry areas and denuded areas less effective

than normal areas.

Norton and Novy[32, 33], through their research, came to the conclusion that the skin possesses no inherent germicidal activity and that the disappearance of certain bacteria from the surface of the skin is largely dependent upon the removal of moisture. On surfaces kept moist bacteria remained viable for much longer periods than when the surfaces were permitted to dry.

Burtenshaw[34] made comparative counts of the sweat gland ducts in the skin of the finger, palm and forearm. There were nearly twice as many ducts in the palm as in the finger, and in the forearm they were sparsely scattered. He assumed that a greater concentration of glands implied a greater secretion of sweat; and if sweat is an important inhibiting agent, the more that is secreted on the surface the greater is the bactericidal power of that surface. Burtenshaw stated that the germicidal powers of the skin is due to its high acidity. Arnold agreed with this, but in the work done by Schade and Marchionini[35] it was shown that the skin becomes less acid while sweating and the tissue that sweats most, for example the palm, should then be less germicidal.

The finger-nail region appears to have considerably less self-disinfecting power, since Singer and Arnold[36] discovered that test bacteria, when applied to this region, remained viable for longer periods of time.

Daily and individual differences as well as a decided inhibition to organisms on the skin during menstrual periods is clearly brought forth in the work of Fisher[37].

In 1929 Christiansen demonstrated that menstrual blood would kill

yeasts or change them morphologically even through a quartz cover glass. The menstrual toxin is identical with or closely related to oxycholesterol as shown by Macht and Lubin[38]. Rahn and Barnes[39] found that this effect was not alone limited to menstruating women. A man was able to kill yeast by his finger tips through a quartz plate in 15 minutes in one half of all the tests performed. Even his eyes and nose at very close range had the same effect through quartz. These authors suggest that this radiation is due to pathological conditions.

Mitogentic rays emitted by the body are offered as a possible explanation for this power of the skin by research workers Reiter and Gábor[40]. These rays were first discovered by Guiwich and they have been found to be identical in action with ultra-violet rays of the wave length 300-350μμ.

Another possible factor, suggested by Arnold and Bart[41] is keratin, which appears to play a role in the removal of bacterial as well as other antigens from human skin. They found that a ketogenic diet greatly increases the rate at which bacteria disappear from the skin.

Bryan and Mallmann[42] concluded that desiccation plays an important role in the self-disinfecting power of the skin. A residual gremicidal action was supplied to the skin by irradiation with ultra-violet light. Both a local and systemic reaction was observed, but the local action appeared to be the more intense. Cornbleet and Montgomery[31] disagree with this; they report that previous exposures of the skin to ultra-violet light does not change the destructive powers against yeasts and staphylococci.

Data obtained by Mallman[43] indicate that individual variation, rather than moisture, perhaps plays the most important role in the skin's disinfecting power. E. coli was used as the test organism. It was covered and kept moist in order to keep the organisms from drying. In the case of one subject there was 100% reduction while another showed no reduction but rather an increase in the number of organisms.

It can readily be seen that there is a wide variance of opinions and theories based on contradictory results obtained by the numerous workers studying this phenomenon. Perhaps this is to be expected since no two groups of investigators used the same subjects, worked under the same experimental conditions or used the same test organisms. It is reasonable to assume that if these variations in results are mostly due to these differences, greater conformity might have been obtained if more uniform procedures had been followed.

Since S. pullorum is now known to be pathogenic for man, it is of considerable public health interest to know the longevity of the organism on the skin of human beings. This is particularly important in the case of poultrymen, butchers and cooks who handle poultry and poultry products and are, therefore, most prone to come in contact with the organism.

Experimental Procedure

Wallbank[44] employed the following procedure which was likewise used in this experiment.

A 24-hour culture of S. pullorum, grown in tryptose broth, was used. The back and the palm of the hands were marked off into fourteen areas 3-4 sq. cm., after first washing the hands thoroughly with soap and water. The organisms were swabbed upon the surface of the skin following a five minute waiting period after washing. The culture was shaken thoroughly and the back of the hand was swabbed with undiluted culture.

For controls sterile index cards of uniform size were used. These were dipped into the broth culture and at the appropriate time intervals were dropped in 99 ml saline dilution blanks.

Swabs were taken from the hand at 1/2, 1, 2, 3, 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120-minute intervals and dropped into saline dilution blanks. The controls were dropped into saline dilution blanks at the same time as the swabs and both swabs and controls were shaken 25 times immediately after being placed in blanks, and again 25 times before making the plates.

Uniformity in swabbing, shaking of dilution tubes, time intervals, etc. was observed as closely as possible.

The organisms stood no longer than 15 minutes in the dilution tubes before the plates were poured. MacConkey's agar was used as plating medium to which 1-ml of the dilutions had been added. The plates were incubated at 37° C for 24 hours and then the count was taken and recorded.

Results

An average count, taken of six experiments, using the back of the hand, which covered a period of three weeks during the summer term, showed survival of S. pullorum after 5 minutes with a 98.9% reduction (table IV). There was a 100% reduction in ten minutes. The controls showed a 99.4% reduction in organisms at 120 minutes. Three experiments, completed during winter term, showed survival of S. pullorum after 45 minutes with a 99.9% reduction (table V).

An average of 99.9% reduction in organisms was demonstrated in thirty minutes in the experiments on the palm of the hand. The controls showed a 99.3% reduction in 120 minutes. The experiments on the palm of the hand numbered twelve and covered a period of nineteen days.

Discussion

S. pullorum survived for 5 minutes on the back of the hand in the experiments performed during the summer while during the winter term it showed a survival for 45 minutes. These data would seem to indicate a variation in germicidal effect of the skin at different seasons of the year. For more conclusive results experiments would have to be performed at intervals throughout a year. Such experiments might reveal a daily variation, due to the physical condition of the individual tested, rather than a seasonal variation.

S. pullorum survived for thirty minutes on the palm of the hand in the experiments performed both summer and winter terms. Sweat on the palm of the hand perhaps accounts to some extent for this

TABLE IV

Survival On the Back of the Hand*

SWAB FROM SKIN

TIME (Min.)	COLONY COUNTS	% REDUCTION
1/2	4149	--
1	2188	47.3
2	159	96.2
3	155	96.3
5	43	98.9
10	0	100.0

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TIME (Min.)	COLONY COUNTS	% REDUCTION
1/2	5633	--
1	5037	10.6
2	4120	26.9
3	4062	27.9
5	3607	35.9
10	3109	44.8
15	1982	64.8
30	855	84.8
45	108	98.1
60	86	98.5
75	99	98.2
90	6	99.9
105	11	99.8
120	34	99.4

* An average taken from six experiments covering a three week period.

Summer term.

TABLE V

Survival On the Back of the Hand*

SWAB FROM SKIN

TIME (Min.)	COLONY COUNTS	% REDUCTION
1/2	21040	--
1	12717	39.5
2	8836	58.0
3	5617	73.3
5	1454	93.0
10	278	98.6
15	236	98.8
30	35	99.8
45	15	99.9
60	0	100.0

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TIME (Min.)	COLONY COUNTS	% REDUCTION
1/2	26073	--
1	22298	14.4
2	17310	33.6
3	17000	34.7
5	13500	48.2
10	10400	60.1
15	9666	62.5
30	1785	93.1
45	1400	94.6
60	650	97.5
75	550	97.8
90	411	98.4
105	505	98.1
120	110	99.5

* An average taken from three experiments covering eight days.

Winter term.

TABLE VI

Survival On the Palm of the Hand*

SWAB FROM SKIN

TIME (Min.)	COLONY COUNTS	% REDUCTION
1/2	13459	--
1	11297	16.1
2	8325	38.2
3	4855	63.9
5	1060	92.1
10	339	97.5
15	244	98.2
30	2	99.9
45	0	100.0

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TIME (Min.)	COLONY COUNTS	% REDUCTION
1/2	20410	--
1	19283	5.5
2	16742	17.9
3	15295	25.1
5	12870	36.9
10	10707	47.5
15	6589	67.7
30	1327	93.5
45	1357	93.4
60	353	98.3
75	82	99.6
90	117	99.4
105	174	99.2
120	136	99.3

* An average taken from twelve experiments covering 19 days.

Summer and winter terms.

survival period of S. pullorum; dessiccation would be more rapid on the back of the hand because of the lack of or small amount of sweat present.

No significant increase in germicidal effect was evident during the menstrual cycle such as that present in papers by Fisher[37] and Montgomery[31].

Part IV

Survival of Salmonella Pullorum in Egg Albumen and Yolk After Boiling, Frying, and Poaching.

Literature Review

Rettger and Hull[45] in 1916 demonstrated that soft boiling, coddling and frying eggs do not necessarily render the yolks free of viable bacteria. They used a .25 ml water suspension of S. pullorum and injected it into the yolk of an egg. The eggs were incubated for three to five days and then placed in boiling water for one to four minutes. They were chilled in cold water, opened and inoculations made from the yolk on agar slants which were incubated at 37° C. The boiling point of water was 99.2° C. S. pullorum was found in the egg yolk after heating for four minutes. This resistance, they believe, is due to the shell, the albumen and the yolk itself, which has a high percent of fat.

Funk[46, 47] announced the process of thermostabilizing shell eggs to improve keeping quality. This process improved the keeping quality of shell eggs by devitalizing the embryo and stabilized the albumen. Many bacteria, causing spoilage in shell eggs, could be destroyed if they were on or in the shell, the shell membrane or in the albumen. The process is based upon the principle of pasteurization by application of heat at temperatures from 130° F to 142° F. "The time varies depending upon the initial temperature of the eggs, the temperature of the oil and other medium used, and the heat conductivity and rate of circulation of the medium".

This experiment was carried out with the intention of collecting more data on this problem by checking with modern methods the isola-

tion of S. pullorum on differential and selective media. It is also of considerable interest and value to demonstrate that the ordinary means of preparing eggs is insufficient in the majority of the cases to kill S. pullorum which is known to be present in some eggs.

Experimental Precedure

The precedure followed in these experiments is approximately the same, except for the media, as that used by Rettger and Hull[45] in their work. Three-hundreths of one milliliter of a 24-hour tryptose broth culture of S. pullorum was injected either into the yolk or albumen of 379 eggs which were then incubated at 37° C for three days.

A total of 214 eggs were boiled for varying lengths of time ranging from 1 to 4 minutes.

Eighty-three eggs were fried 1, 1.5 or 2 minutes. The eggs were fried either on one side or on both sides.

Only two periods, 1.5 and 2 minutes, were used in scrambling 29 eggs.

The cooking time for 53 poached eggs was 1 and 1.5 minutes.

Following boiling, frying, poaching, or scrambling, the eggs were placed into tetrathionate broth and incubated at 37° C for 12-24 hours. From the tetrathionate, S. S. agar plates were streaked with the egg material and incubated at 37° C for 24 hours. Typical colonies were picked and run through the usual identification procedures.

Results

Table VII summarizes the data on boiled eggs. In the case of the infected albumen the percentage of kill was fairly consistent with 11.1% in 1 minute, 31.5% in 2 minutes, 52.6% in 2.5 minutes, 75% in 3 minutes, 88.2% in 3.5 minutes and 92.3% in 4 minutes. The infected yolks likewise showed a progressive increase in kill of S. pullorum. In 1 minute the percentage destroyed was 0%, in 2 minutes 7.1%, in 2.5 minutes 50%, in 3 minutes 64.2%, in 3.5 minutes 68.7%, and in 4 minutes a kill of 76%.

Table VIII presents the data on the fried, scrambled, and poached eggs. In the case of fried eggs, with infected albumens, 1-minute frying on one or both sides had no effect. After 1.5 minutes there was a kill of 48% in those fried on one side only and 73.3% in those that were fried on both sides. The eggs with infected yolks showed approximately the same increase in destruction of organisms upon longer exposure to heat. The single side fried eggs showed 50% kill of organisms in 2 minutes. Those fried on both sides for 1.5 minutes showed 71.4% kill.

Scrambled eggs with infected albumens had a kill of 81.8% in 1.5 minutes, in 2 minutes there was a destruction of 83.3%. Those with infected yolks showed a 100% kill in both 1.5 and 2 minutes.

The poached eggs which had infected albumens displayed a 58.8% kill in 1 minute and 100% in 1.5 minutes while those with infected yolks had 22.2% destruction of organisms in 1 minute and 44.4% in 1.5 minutes.

TABLE VII

Survival Time of S. pullorum in Egg Albumen and Yolk During Boiling.

Egg Albumen			Egg Yolk		
Time in Minutes	Number of eggs with and without growth.	Percentage of kill.	Time in Minutes	Number of eggs with and without growth.	Percentage of kill.
1	16 + 2 -	11.1	1	12 + 0 -	0.0
2	13 + 6 -	31.5	2	13 + 1 -	7.1
2.5	9 + 10 -	52.6	2.5	7 + 7 -	50.0
3	5 + 15 -	75.0	3	5 + 9 -	64.2
3.5	2 + 15 -	88.2	3.5	5 + 11 -	68.7
4	2 + 24 -	92.3	4	6 + 19 -	76.0

Key: + = Growth
- = No growth

Total eggs 214
Total percent kill of organisms in both yolk and albumen in four minutes -- 84.3

TABLE VIII

Survival Time of S. pullorum in Fried, Scrambled, and Poached Eggs.

Preparation and Time Intervals	Number of Eggs with or without growth.	Percentage of kill.
Fried (infected white) one side		
1 minute	3 +	0.0
	0 -	
1.5 minutes	13 +	48.0
	12 -	
Fried (infected white) both sides		
1 minute	3 +	0.0
	0 -	
1.5 minutes	4 +	73.3
	11 -	
Fried (infected yolk) one side		
1 minute	3 +	0.0
	0 -	
1.5 minutes	5 +	16.6
	1 -	
2 minutes	2 +	50.0
	2 -	
Fried (infected yolk) both sides		
1 minute	3 +	0.0
	0 -	
1.5 minutes	6 +	71.4
	15 -	
Scrambled (infected white)		
1.5 minutes	2 +	81.8
	9 -	
2 minutes	2 +	83.3
	10 -	
Scrambled (infected yolk)		
1.5 minutes	0 +	100.0
	3 -	
2 minutes	0 +	100.0
	3 -	
Poached (infected white)		
1 minute	7 +	58.8
	10 -	
1.5 minutes	0 +	100.0
	9 -	
Poached (infected yolk)		
1 minute	14 +	22.2
	4 -	
1.5 minutes	5 +	44.4
	4 -	

Key: + = Growth
- = No growth

Discussion

In general, as might be expected, the organisms showed a lower percentage of kill when inoculated into the yolk than when inoculated into the albumen. This is due to the yolk's fat content and the added protection offered by the albumen.

It can be seen from the data presented that S. pullorum is not readily killed by the usual amount of heating employed in the cooking of eggs. Although the percentage of infection in eggs is lower now than before the pullorum disease control program was developed, there is still the possibility that susceptible individuals, who eat foods which contain virulent strains of S. pullorum in sufficient numbers, might become ill.

From the results of the experiment it would appear that scrambling or boiling (4 minutes) would be the best method to render eggs safe for human consumption.

Summary

In trying to determine whether salmonellae can penetrate egg shells, attempts were made to simulate conditions normally found in the barnyard. Penetration of the shell by S. enteritidis was demonstrated. These data substantiate the importance of fecal matter and moisture as factors in microbial penetration of egg shells.

The survival time of four salmonellae: S. schottmülleri, S. enteritidis, S. typhi-murium, and S. paratyphi A. in eggs was shown to extend through a period of twelve months; S. choleraesuis and S. pullorum were found to survive a period of nine months. The experiment indicates that salmonellae tend to survive longer at 25° C then at 4° C.

The skin as demonstrated by many workers possesses a germicidal effect upon organisms. In these experiments, using S. pullorum, greater germicidal effect was demonstrated on the back of the hand than on the palm of the hand. Daily as well as individual differences of the skin must be considered in evaluating the data presented.

By ordinary methods of egg preparation, boiling, frying and poaching, S. pullorum, frequently an inhabitant of eggs, is not always destroyed. The organisms showed a lower percentage of kill when inoculated into the yolk than when inoculated into the albumen. It would appear from the results of these experiments that scrambled or four-minute boiled eggs would be the safest.

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