Use of *Salmonellae* Antagonists in Fermenting Egg White

II. Microbiological Methods for the Elimination of *Salmonellae* from Egg White[1]

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Pasteurization at 60 C cannot be used to eliminate *Salmonellae* from liquid egg white, since this process coagulates the proteins and destroys the functional properties of egg white. Therefore, other means of destroying these organisms have been investigated. These include storage of the dried egg white at 50 to 70 C (Banwart and Ayres, 1956) and irradiation with high voltage cathode rays (Nickerson *et al.*, 1957). The industrial efficacy and economy of these methods have not yet been fully evaluated.

In addition to the problem of eliminating *Salmonellae* there is also the problem of removal of glucose from the egg white in order to prepare a successful dried product (Stewart and Kline, 1941). Several types of bacterial fermentations (Ayres, 1958) and a process employing glucose oxidase (Scott, 1953) are available for sugar removal from egg white.

In the present investigations a new method for processing egg white has been explored; namely, fermentation with a nonpathogenic strain of *Escherichia coli*, which is antagonistic to the growth and survival of *Salmonellae*. This procedure overcomes the disadvantage of the multiplication of *Salmonellae* during the fermentative desugaring of egg albumen (Ayres, 1958). In addition, the prospect of eliminating both glucose and *Salmonellae* in a single procedure is attractive. That coliform bacteria of the genera *Escherichia* and *Aerobacter* are common fermenting organisms in the "natural" fermentation of egg white has been demonstrated (Stuart and Goresline, 1942a, 1942b).

Experimental Methods

Difco nutrient broth and agar, Difco tryptone glucose extract broth and agar,[2] and the brilliant green selenite broth described by Stokes and Osborne (1955) were the culture media used in most of this work. Agar was added to the selenite broth when it was used as a plating medium. Incubation temperature, except in special cases, was 37 C.

Egg white, for cultural purposes, was prepared from commercial shell eggs that were obtained from a local grocery. Shell surfaces were treated by scrubbing in a 0.2 per cent Hyamine 1622[3] solution, soaking in 70 per cent ethyl alcohol, and flaming until dry. The yolks and whites were separated under aseptic conditions and the albumen was blended in a sterilized Waring Blendor. The pH was adjusted to from 6.5 to 7.0 by the addition of 20 per cent sterile citric acid solution during the blending. One-milliliter portions of this egg white were cultured on tryptone glucose extract agar, and any sample which showed bacterial growth was discarded. Sterile samples were stored at 4 C until used.

Glucose was determined in egg white with the Somogyi reagent (1945). The deproteinization procedure of Somogyi (1930) was used.

Peroxide addition to egg white was made from a 3 per cent commercial solution, the strength of which was determined by the iodometric method described by Kolthoff and Sandell (1936).

Measurement of pH was made with a Leeds and Northrup glass electrode pH meter.

In egg white fermentations with coliform bacteria, 0.05 ml of an 18- to 24-hr culture (50,000 to 500,000 cells) per 5 ml was routinely used as inoculum. The 0 hr sampling indicates the number of *Salmonella* added.

Plate cultures for *Salmonella* from egg white which had been fermented with *E. coli* for 72 hr were often negative. However, for final detection, replicate subcultures of 1-ml samples into brilliant green selenite broth were made routinely.

Three cultures of *Salmonellae* were used, *Salmonella senftenberg* strain 2623, *Salmonella oranienburg* strain 200E and *Salmonella typhimurium* strain TM-1, all of which were obtained from the collection of the Western Regional Research Laboratory, Albany, California.

Results and Discussion

Certain strains of *E. coli* are known to produce "colicines" which exert an antagonistic effect against

[1] A report of work done under contract with the United States Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Western Utilization Research and Development Division of the Agricultural Research Service.

[2] Difco Laboratories, Inc., Detroit, Michigan. This does not constitute an endorsement by the United States Department of Agriculture of these products over other similar products.

[3] Rohm and Haas Co., Philadelphia, Pennsylvania.

other enteric bacteria (Fredericq and Levine, 1947; Levine and Tanimoto, 1954). After a survey of the effect of a number of bacteria, yeasts, and several fungi on the growth of *Salmonellae* in egg white media, *E. coli* was chosen for further study. Many strains of *E. coli* were isolated from stool samples, water, and sewage, but the only cultures which gave demonstrable inhibitory effects by the agar plate testing procedure of Fredericq and Levine (1947) were four strainss of *E. coli* which were obtained through the courtey of Dr. Max Levine.[4] These were designated as *E. coli* strains 6-204-55, 1673A, H-10, and H-23. Among the four strains, *E. coli* 6-204-55 gave more consistent results than the other three, and was used in this work.

Escherichia coli 6-204-55, which was isolated by Dr. Levine from canal water, has the cultural characteristics of a typical *E. coli*; *i.e.*, it is indole and methyl red positive and Voges-Proskauer and citrate negative. It grows at 46 C in Eijkmann's medium. Serologically it falls in O group 6, K group 13, and although its H antigen has not been determined, it does not fall in groups H 1 to H 46.[5] According to Edwards and Ewing (1955), this organism is not an enteropathogenic *Escherichia* type.

[4] Territorial Department of Health, Honolulu, Hawaii.

Some Growth Characteristics of Coliform Bacteria and Salmonellae Species in Egg White

Effect of 26 strains of coliform bacteria on the growth of Salmonella in egg white. The effects of a number of coliform strains on the growth of *Salmonella* in egg white have been compared in table 1. The counts reported refer only to *Salmonella*. These organisms, some freshly isolated and some laboratory strains, were characterized by lactose fermentation, morphology,

[5] Appreciation is gratefully acknowledged to Dr. W. H. Ewing of Communicable Disease Center for Enteric Diseases, Chamblee, Georgia, for serological typing of this culture.

TABLE 1

*Survival of Salmonella senftenberg in presence of various coliform cultures in egg white at pH 6.5**

Inoculations	No. *Salmonella*/ml Egg White				BGSB† Subcultures: 72 hr
	0 hr	24 hr	48 hr	72 hr	
Control (uninoculated)	0	0	0	0	−
Salmonella senftenberg 2623	212	12T‡	1M‡	134M	+
Escherichia coli					
Salmonella 2623 + *E. coli* 6-204-55	254	0	38	0	+
Salmonella 2623 + *E. coli* 1673A	244	0	33	20	+
Salmonella 2623 + *E. coli* H-10	240	0	1528	782	+
Salmonella 2623 + *E. coli* H-23	215	0	520	308	+
Salmonella 2623 + *E. coli* 26	7	111	632	500	+
Salmonella 2623 + mouse stool 1	454	1000	216	512	+
Salmonella 2623 + mouse stool 2	370	47	522	768	+
Salmonella 2623 + mouse stool 3	486	—	768	832	+
Salmonella 2623 + mouse stool 4	380	—	640	586	+
Salmonella 2623 + mouse stool 5	404	—	—	448	+
Salmonella 2623 + human stool A_1	362	—	192	768	+
Salmonella 2623 + human stool A_2	512	—	—	448	+
Salmonella 2623 + human stool A_3	334	—	—	512	+
Salmonella 2623 + human stool A_4	400	—	—	768	+
Salmonella 2623 + human stool B_1	570	—	13T	1900	+
Salmonella 2623 + human stool B_2	528	—	13T	1M	+
Salmonella 2623 + human stool B_3	500	—	28T	300	+
Salmonella 2623 + human stool B_4	500	—	13T	1300	+
Intermediates					
Salmonella 2623 + *Escherichia* 26 WR	—	508	2900	1900	+
Salmonella 2623 + *Escherichia* RV 38	N‡	N	N	1.4M	+
Salmonella 2623 + *Escherichia* RV 86	N	N	N	N	+
Salmonella 2623 + *Escherichia* VN 8	N	5400	5M	35T	+
Salmonella 2623 + *Escherichia* VN 6	N	3600	8.2M	117M	+
Salmonella 2623 + *Escherichia* 67 B	0	22T	308T	1.3M	+
Aerobacter					
Salmonella 2623 + *A. aerogenes* 199	N	N	1.9M	51M	+
Salmonella 2623 + *A. aerogenes* 8308	N	N	19M	200M	+

* Brilliant green selenite agar; incubation 37 C.

† Brilliant green selenite broth.

‡ Symbols: T = thousand; M = million; N = too numerous to count.

and IMViC tests as *Escherichia*, intermediates,[6] or *Aerobacter*.

During simultaneous growth of the coliform culture with *S. senftenberg* 2623 in egg white at 37 C, a 1-ml portion was plated on brilliant green selenite agar, after 0, 24, 48, and 72 hr of incubation, for enumeration of the *Salmonella* cells. Also 1 ml was transplanted to 10 ml of the same selective broth medium after 72 hr of incubation as a final test for the detection of *Salmonella*. Viable *Salmonella* cells were present in all instances except in the control.

There were varying effects on *Salmonella* growth by this group of 26 coliforms. All *E. coli* except the culture designated B_2 displayed some degree of inhibition. The two cultures of *Aerobacter* showed no inhibition nor did the intermediates, with the possible exception of *Escherichia* 26 WR.

Among the strains of *E. coli* received from Dr. Max Levine, all showed clear zones when tested for their ability to inhibit *Salmonellae* by the agar plate method. No other coliform cultures tested exhibited this effect. However, only strains 6-204-55 and 1673A consistently inhibited *Salmonellae* in egg white media. Both of the strains were efficient also in removing glucose from egg white.

Comparison of growth rates of three Salmonella species and E. coli 6-204-55 in egg white. Preliminary experiments indicated that *E. coli* 6-204-55 gave the most consistent results in inhibiting the growth of *Salmonellae* in egg white. The data in table 2 resulted from experiments to compare the growth of *E. coli* with three *Salmonellae* species in egg white media. The pH of egg white was always adjusted to from 6.5 to 7.0 in order to provide more uniform and optimal growth conditions. Most laboratory prepared samples of egg white had a pH of 8.5, a range unsuitable for the growth of *Salmonellae* or *E. coli*.

[6] Intermediate refers to citrate fermenting coliforms; *i.e.*, organisms which ferment lactose, are methyl red positive, and Voges-Proskauer negative.

Different batches of fresh egg albumen, adjusted to the same pH value, vary widely in their ability to support the growth of *S. senftenberg*. Table 2 shows two different batches of egg white, one of which supported good growth of the organism and the other only moderate growth. The egg white was prepared from two brands of shell eggs, indicated in table 2 as retail Brands A and B. Egg white cultures inoculated singly with *E. coli* 6-204-55, *S. senftenberg* 2623, *S. oranienburg* 200E, and *S. typhimurium* TM-1 were plated on tryptone glucose extract agar at various intervals during a 66-hr total incubation period. Though the size of inoculum varied somewhat, a comparison of the extent of growth of the four cultures in the fresh liquid egg white is possible.

With either sample of egg white, *E. coli* showed a low growth potential. All three species of *Salmonellae* grew better in both samples of egg white than did *E. coli*. *Salmonella senftenberg* produced a greater cell population in the Brand A than in the Brand B egg white. The remaining two *Salmonellae* species show a low growth potential with either egg white. Since *S. senftenberg* grew most vigorously, it was chosen for further inhibition studies with *E. coli* 6-204-55.

Comparison of a large vs. small inoculum of E. coli on the simultaneous growth of S. senftenberg in egg white. Because of the variation in growth characteristics between the two organisms in egg white there was some concern about the ability of *E. coli* to successfully inhibit the growth of *S. senftenberg* since the latter grew most vigorously in egg white. An experiment was designed to enumerate both *E. coli* and *S. senftenberg* when they were growing in mixed culture in egg white. The objective was to determine the fate of *S. senftenberg* when the *Salmonella* inoculum was held constant and the *E. coli* inoculum was varied.

For enumeration of both organisms in mixed culture, two different culture media were employed. It will be noted in table 3 (first line, 0 hr column) that on tryptone glucose extract agar twice as many *Salmonella* colonies

TABLE 2

*Comparative growth of Escherichia coli 6-204-55 and three Salmonellae species in two samples of egg white**

Inoculum	Retail Brand A							Retail Brand B						
	No. bacteria/ml egg white							No. bacteria/ml egg white						
	0 hr	4 hr	18 hr	27 hr	42 hr	51 hr	66 hr	0 hr	6 hr	12 hr	24 hr	32 hr	48 hr	56 hr
Uninoculated control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. coli 6-204-55	75	120	80	100	2	0	1	249	540	1,080	700	1T†	100	10
Salmonella senftenberg 2623	1,600	4,200	40T	950T	5.2M†	5.6M	212M	175	1,600	7T	44T	40T	27,400	18,700
S. oranienburg 200E	250	420	800	1T	336	350	160	274	6,300	8T	11T	15T	11,400	2,300
S. typhimurium TM-1	2,200	5T	3,400	24T	26T	24T	30T	73	181	1T	1,300	1T	800	1T

* Egg white, pH 6.5; incubation 37 C; tryptone glucose extract agar.

† M = million; T = thousand.

develop (column labeled "Total Bacteria") as on the selective brilliant green selenite agar (column labeled "*Salmonella*"). Thus by doubling the value obtained on the latter medium and subtracting it from the total count as determined on tryptone glucose extract agar, a relative count of the number of *E. coli* cells was obtained. Previous experiments had demonstrated that *E. coli* does not grow on the brilliant green selenite medium.

Quantities of 10 ml of sterile egg white were inoculated separately with dilutions of cultures of *E. coli* 6-204-55 and *S. senftenberg* grown in tryptone glucose extract broth. At 0, 24, 48, and 72 hr of incubation, samples were removed and both organisms were enumerated according to the procedure described. The size of the inoculum is shown in the 0 hr column of table 3.

When a small inoculum of *E. coli* (±500 cells per ml) was used with an equivalent inoculum of *Salmonella* (2 times 196), *E. coli* attained a 10-fold increase in 24 hr and then declined. There appeared to be a slight multiplication of *S. senftenberg* during the first 24 hr; however, the final sampling revealed no *Salmonella*.

When a large inoculum of *E. coli* (several million cells per ml) and a small inoculum of *Salmonella* (2 times 184) were used, there was a 100-fold increase in *E. coli* during the first 48 hr. It appeared that *Salmonella* cells began to die almost immediately.

Salmonella senftenberg grew to a population of seven million in 72 hr in the absence of *E. coli*. When several million *E. coli* cells were introduced, the peak of the population was at 48 hr, in the presence or absence of *Salmonella*.

The unexpected result was that the *E. coli* inoculum may have been several hundred or several million cells per ml, but *S. senftenberg* was equally well inhibited after a period of 72 hr. This inhibition may be related to the more rapid attainment of maximum growth by *E. coli* and early formation of an inhibitory principle.

Effect of the Fermentation of Egg White by E. coli on the Survival of S. senftenberg

When *S. senftenberg* 2623 was cultured simultaneously with *E. coli* 6-204-55 in nutrient broth containing glucose, there was a sharp decrease in the *Salmonella* population (Flippin and Mickelson, 1960). The effect was strong enough to result in the complete elimination of *Salmonella* from the medium in 72 hr. This did not occur in nutrient broth without added glucose.

When egg white was the culture medium the *E. coli* fermentation drastically reduced *Salmonella* growth (table 4). Multiplication of *Salmonella* in the egg white was inhibited to the extent that plates prepared with 1-ml samples on brilliant green selenite agar were frequently negative. However, replicate subcultures in brilliant green selenite broth medium were positive for *Salmonella*, indicating that total elimination did not occur. There are several explanations for the difference in results found in the nutrient broth (Flippin and Mickelson, 1960) and egg white cultures. The nutrient broth with glucose may provide a more ideal medium for the antagonism of *Salmonellae* by *E. coli*. In support of this is the fact that, though antagonistic substances against *Salmonellae* are easily demonstrated on tryptone glucose extract agar plates, establishment of their presence in egg white cultures was not accomplished. The maximum pH drop in the nutrient broth cultures was approximately a whole pH unit below that shown for the egg white cultures (4.5 *vs.* 5.5). Though lowering of pH may be a contributing factor, it does not account for the fact that in nutrient glucose broth cultures in which *S. senftenberg* was grown alone and in which the pH drop was practically identical, the *Salmonella*

TABLE 3

*Effect of size of Escherichia coli inoculum on antagonistic effect against Salmonella senftenberg in egg white medium**

Inoculum	Total No. Bacteria/ml Egg White											
	0 hr			24 hr			48 hr			72 hr		
	Total bacteria†	*Salmonella*†	*E. coli*	Total bacteria	*Salmonella*	*E. coli*	Total bacteria	*Salmonella*	*E. coli*	Total bacteria	*Salmonella*	*E. coli*
Control (uninoculated)	0			0			0			0		
S. senftenberg 2623	330	153‡	0	140T§	65T‡	0	150T	70T‡	0	14M§	7.18M‡	0
E. coli 6-204-55 Lg§	2.34M	0‡	2.34M	140M	0‡	140M	380M	0‡	380M	170M	0‡	170M
Salmonella 2623 + *E. coli* Lg	2.98M	184‡	2.98M	130M	0‡	130M	320M	1‡	320M	2.58B§	38‡	2.58B
Salmonella 2623 + *E. coli* Sm§	880	196‡	488	5.4T	600‡	4.2T	1.0T	120‡	760	10	0‡	10

* Egg white, pH 6.5; incubation 37 C.

† Total counts on tryptose glucose extract agar; *Salmonella* counts on brilliant green selenite agar.

‡ One half total *Salmonella* count.

§ Symbols: Sm = small inoculum; Lg = large inoculum; T = thousand; M = million; B = billion.

culture survived. Only in the presence of *E. coli* 6-204-55 was the *Salmonella* eliminated from the cultures. This is evidence for the action of an antagonistic principle in those cultures in which *E. coli* was present. Extra glucose was added to egg white, and though it resulted in a lowering of pH from 5.4 to 5.1, it did not cause the destruction of *Salmonella*.

The buffer capacity of egg white is considerable. To reduce the pH of 10 ml of egg white from pH 6.4 to 5.4 required 0.87 ml of 0.05 N HCl. This buffer capacity

TABLE 4

*Effect of fermentation of egg white by Escherichia coli 6-204-55 on the survival of Salmonella senftenberg 2623**

Expt. No.	Inoculum	*Salmonella*/ml Egg White after Incubation			
		0 hr	24 hr	48 hr	72 hr
I	*S. senftenberg*	144	36T†	31M†	640M
	S. senftenberg + *E. coli*	140	0	0	0
II	*S. senftenberg*	116	145T	64M	102M
	S. senftenberg + *E. coli*	111	0	55	53
III	*S. senftenberg*	89	1860	14T	210T
	S. senftenberg + *E. coli*	71	0	82	0

* Brilliant green selenite agar; incubation 37 C; pH of egg white 6.5.

† T = thousand; M = million.

TABLE 5

*Effect of the fermentation of egg white by Escherichia coli 6-204-55, followed by addition of hydrogen peroxide, on the survival of Salmonella**

Culture	*Salmonella*/ml Egg White after Incubation				BGSB† Subcultures: 72 hr
	0 hr	24 hr	48 hr	72 hr	
Control	0	0	0	0	− − −
Salmonella senftenberg 2623	23	1400T‡	7700T	280M‡	+ + +
S. senftenberg + H_2O_2§	13	1400T	70M	70M	+ + +
S. senftenberg + H_2O_2¶	20	1400T	100M	24,600T	+ + +
Salmonella + *E. coli*	195	0	0	0	+ + + + +
Salmonella + *E. coli* + H_2O_2§	64	3	2	0	− − − − −
Salmonella + *E. coli* + H_2O_2¶	19	0	0	0	+ − − + −

* pH of egg white, 6.5; incubation 37 C; brilliant green selenite agar.

† Brilliant green selenite broth.

‡ T = thousand; M = million.

§ Five-tenths per cent concentration in a single addition after removal of the 48-hr sample.

¶ Five-tenths per cent concentration, addition in three equal portions at hourly intervals after removal of the 48-hr sample.

may be part of the protective effect offered the *Salmonellae* in egg white media, as contrasted to nutrient broth, when fermented with *E. coli*.

Supplementation of the E. coli fermentation of egg white with mild heat or dilute hydrogen peroxide treatment. Since *E. coli* by itself was incapable of completely eliminating viable *Salmonella* cells from the egg white, the use of a supplementary treatment to the fermentation was considered. The addition of hydrogen peroxide following the fermentation was suggested by the results of Lloyd and Harriman (1957). The authors described a method for inactivation of the indigenous catalase by heating the egg white at 120 F for 3 min followed by treatment with 0.075 to 0.30 per cent hydrogen peroxide for 10 to 15 min. They described the product obtained by their process as "commercially sterile," with a destruction of 99.99 per cent of the bacteria present. Contrary to the statement given in the patent, we have found that exposure of egg white at pH 6.5, to 48 C for 2 min was inadequate for inactivation of the indigenous catalase. In one sample of fresh egg white there was a trace of catalase activity after 15 hr of exposure at 48 C.

Egg white containing *S. senftenberg* was fermented 48 hr with *E. coli* 6-204-55 and then treated with 0.5 per cent hydrogen peroxide (table 5). In the one case, 0.5 per cent peroxide was added in a single addition after 48 hr incubation. In the other case the same amount of hydrogen peroxide was added in three equal quantities at hourly intervals after 48 hr of fermentation time. Results with *S. oranienburg* and *S. typhimurium*, though not shown here, were identical to those with *S. senftenberg*.

The results show that with egg white cultures in which *S. senftenberg* was the only organism, elimination of the organism was not achieved by peroxide addition, irrespective of the manner of addition. In the mixed *Salmonella-Escherichia* cultures, the single addition of peroxide eliminated *Salmonella*. When the hydrogen peroxide was added in three equal portions at hourly intervals, *S. senftenberg* was detected in two out of five of the brilliant green selenite broth subcultures. These data suggest the possibility of total elimination of the *Salmonella* organisms by addition of hydrogen peroxide following the fermentation by an antagonistic strain of *E. coli*.

No attempt was made to inactivate the catalase in the egg white. Egg white samples vary greatly in catalase activity, and our experience indicates that rather drastic heat treatment is necessary to eliminate this enzyme. Hydrogen peroxide was added with gentle mixing in order to avoid excessive frothing as the oxygen was evolved. Hydrogen peroxide is considered to be a weak antiseptic, especially in the presence of organic matter. However, the foregoing evidence demonstrates its usefulness in this case. An additional

advantage is that it will be completely dissipated either through the action of indigenous or added catalase, leaving no residual foreign chemical in the final product.

In the following experiment the effect of mild temperature treatment was compared with peroxide as a means of eliminating *S. senftenberg* following fermentation of egg white with *E. coli* 6-204-55. After 48 hr fermentations of egg white, 0.5 per cent hydrogen peroxide was added to one culture. Instead of the peroxide addition, each of four other similar cultures was heated for 5, 10, 20, and 30 min, respectively, at 47.5 C, the recorded internal temperature of the egg white (table 6).

TABLE 6

*Comparison of temperature and hydrogen peroxide for elimination of Salmonella from egg white following fermentation with E. coli 6-204-55**

Culture and Conditions	*Salmonella*/ml Egg White after Incubation				BGSB† Subcultures: 72 hr
	0 hr	24 hr	48 hr	72 hr	
Control	0	0	0	0	-----
Salmonella senftenberg 2623	3	710T‡	2350T	300M‡	+++++
Salmonella + *E. coli* 6-204-55	6	0	200	0	+++++
Salmonella + *E. coli* + 0.5% H_2O_2	15	4	180	0	-----
Salmonella + *E. coli* at 47.5 C, 5 min	11	12	280	2	+++++
Salmonella + *E. coli* at 47.5 C, 10 min	3	11	150	2	+++++
Salmonella + *E. coli* at 47.5 C, 20 min	2	10	150	0	+++++
Salmonella + *E. coli* at 47.5 C, 30 min	2	1	200	3	+++++

* pH of egg white, 6.5; incubation 37 C; brilliant green selenite agar.

† Brilliant green selenite broth.

‡ T = thousand; M = million.

TABLE 7

*Influence of the numbers of Salmonella present on the effectiveness of the fermentation-peroxide treatment for elimination of Salmonella from egg white**

Culture and Conditions	*Salmonella*/ml Egg White after Incubation				BGSB† Subcultures: 72 hr
	0 hr	24 hr	48 hr	72 hr	
Control	0	0	0	0	----
Salmonella senftenberg 2623	53	6200T‡	3100M‡	100M	++++
S. senftenberg + *E. coli* 6-204-55	50	0	31	5	++++
S. senftenberg + *E. coli*	2800	570	130	42	++++
S. senftenberg + *E. coli* + 0.3% H_2O_2	60	0	20	0	----
S. senftenberg + *E. coli* + 0.3% H_2O_2	1500	550	380	1	---+
S. senftenberg + *E. coli* + 0.5% H_2O_2	48	0	103	0	----
S. senftenberg + *E. coli* + 0.5% H_2O_2	1800	110	140	0	----

* pH of egg white, 6.5; incubation 37 C; brilliant green selenite agar.

† Brilliant green selenite broth.

‡ T = thousand; M = million.

It is evident that neither the 5, 10, 20, nor 30 min exposure at 47.5 C caused a destruction of the few *Salmonella* cells which survived the *E. coli* fermentation. Hydrogen peroxide treatment of the fermented egg white, however, resulted in *Salmonella*-free subcultures.

The influence of the size of inoculum of *S. senftenberg* on the effectiveness of combined fermentation and peroxide treatment for eliminating *Salmonella* has been explored (table 7). The numbers of *Salmonella* added are noted in the 0 hr column. The peroxide additions were made after withdrawal of the 48-hr sample. In those tubes to which the larger numbers of *Salmonella* cells were introduced, a gradual reduction in viable cells occurred during the incubation period. A 30- to 40-fold increase in initial *Salmonella* inoculum was equally well destroyed by fermentation, when followed with 0.5 per cent peroxide. One positive subculture out of four resulted when 0.3 per cent peroxide was used.

The Removal of Glucose from Liquid Egg White by Fermentation with E. coli 6-204-55

Numerous analyses of egg white samples which had been fermented with *E. coli* 6-204-55 have demonstrated that the major portion of the glucose was removed. The rate of glucose removal from egg white was studied, comparing centrifuged cells and a whole broth culture of *E. coli*. Frozen egg white[7] was used.

[7] Swift and Co., Chicago, Illinois.

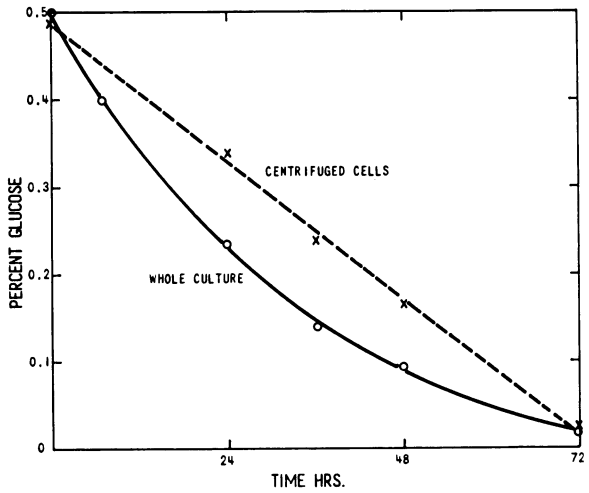

Fig. 1. Rate of glucose removal from egg white by fermentation with *Escherichia coli* strain 6-204-55.

The pH was adjusted to 6.5 with citric acid and 160-ml quantities were dispensed into each of four 250-ml Erlenmeyer flasks. *E. coli* 6-204-55 was cultured for 18 hr in tryptone glucose extract broth and used for inoculation of the egg white. One pair of flasks was inoculated with 10 per cent by volume of the whole broth culture (approximately 10^7 cells per ml), the other with the centrifuged cells from an equivalent portion of the culture. The incubation temperature was 37 C. Samples were removed at 0, 7, 24, 36, 48, and 72 hr intervals for glucose analysis. The rate of glucose removal with these two types of inoculum is shown in figure 1. It was constant with the centrifuged cells but less rapid than when the whole culture was used. Either resting cells or growing cells were equally effective in removing about 95 per cent of the glucose from egg white in 72 hr.

Summary

Certain strains of *Escherichia coli*, notably 6-204-55 and 1673A, have been found to exert a strong inhibition on the growth of *Salmonella senftenberg*, *Salmonella oranienburg* and *Salmonella typhimurium* in egg white. Numerous other strains of *E. coli*, intermediate coliform types, and several strains of *Aerobacter* were devoid of this property. Of the cultures studied, *E. coli* 6-204-55 was the most efficient antagonist of *Salmonellae*. Serological typing has indicated that this strain of *E. coli* is not an enteropathogen. The *E. coli* culture also successfully desugared egg white.

Though the *E. coli* culture showed a lower growth potential in egg white than did the *Salmonellae*, the numbers of *E. coli* present seemed to be of little significance in the inhibition of *Salmonellae*.

A laboratory procedure has been described to eliminate *Salmonellae* from fresh egg white; it consists of fermentation of the egg white with *E. coli* 6-204-55 followed by the addition of 0.5 per cent hydrogen peroxide.

In nutrient broth media with added glucose, in which *E. coli* 6-204-55 and *S. senftenberg* were simultaneously cultured, the *Salmonella* cells were destroyed. In egg white media, though drastic reduction of the number of *Salmonella* cells resulted, complete destruction of these cells was not attained. The addition of hydrogen peroxide following a 48-hr fermentation period with *E. coli* resulted in *Salmonella*-free egg white.

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