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Multiplication and Survival of Salmonellae in Raw Meat

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Introduction

The most important and most frequent source of salmonella food poisoning in man is contaminated food, among which contaminated meat- and egg-products are particularly concerned (Buxton 1957, WHO/FAO 1959, Report 1967). Contaminated food is especially dangerous when the organisms in the food can survive for long periods and can thus multiply under favourable condition of moisture and temperature.

Multiplication of salmonella organisms at various temperature has been studied in custard ham salad and "chicken a la king" (Angelotti, Foter & Lewis 1961), digest broth and sterilized milk(Johne 1961), and culture media(Petzold & Scheibner 1965). Survival of salmonellae at different temperatures has also been studied by many workers in foods other than meat (Wallace & Park 1933, Wallace 1938, McCleskey & Christopher 1941, Gunderson & Rose 1948, Hartsell 19 49, Hahn & Appleman 1952, Raj & Liston 1961, Aea & Bushnell 1962, Kampelmacher 1963).

However, no work has been carried out in meat except the observation of Georgala & Hurst (1963) who studied the suruvival of *S. typhi-murium* in comminuted beef at freezing temperatures.

In the present study, Attempts were made to observe multiplication and survival of Salmonella organisms in raw meat, at various temperatures.

Materials and Methods

Salmonellae employed in these experiments were *S. typhi-murium*, *S. anatum* and *S. meleagridis* which were isolated from apparently healthy pigs (Chung 1968).

Temperatures selected were 10, 7, 5 and -12°C.

Preparation of meat.

The raw meat used was topside meat purchased from a local butcher. The preparation of meat samples was carried out in an aseptic chamber which had been fumigated with disinfectant and in which ultra-violet light had been acting for one hour. The meat was immersed in boiling water for approximately 2-3 minutes to kill the majority of organisms on the surface. The surface of the meat, fat and connective tissue were trimmed and discarded . The muscle tissue was minced using a sterile mincer. The minced meat was well mixed and a quanity of 5g was weighed and distributed in one-ounce MacDartney bottle and stored at 0°C overnight prior to inoculation.

Inoculation of meat

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An 18-hour brothculture, obtained after the third consecutive transfer of actively growing cells, was diluted to 1 : 100 with sterile phosphate buffer solution of pH 7.2 (Anon 1958). The meat was inoculated with 0.1 ml of inoculum, which contained approximately 10^5 organisms The inoculated meat was mixed by using a sterile wood stick and stored at the appropriate temperature immediately. Eighty samples were prepared for each sero-type at each temperature.

Enumeration of Salmonella organisms in the meat sample

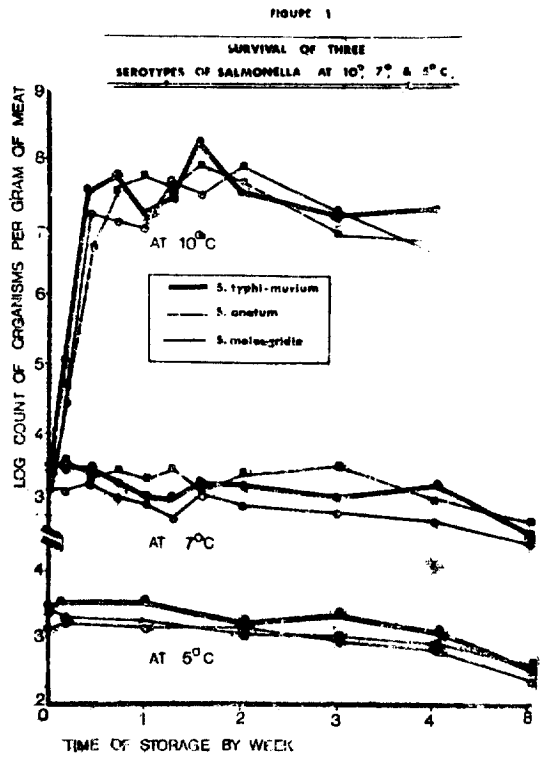
At predetermined intervals, five samples were randomly taken, representing each serotype at the appropriate temperature. Ten ml of sterile phosphate buffer solution was added to each sample, which was broken into small pieces by means of a sterile wood stick and shaked vigorously for five minutes with a mechanical shaker. The bottle was then allowed to stand for one minute and the supernatant fluid was removed and the organisms enumerated.

Enumeration of salmonella organisms was carried out by a surface viable count method(Miles & Misra 1938). Three drops (0.1 ml) from each of ten-fold dilutions were dropped on the surface of well-dried brilliant green agar using constant dropping pipettes, giving 30 drops to the milliter A separate sterile constant dropping pipette was used for each dilution. Counts were made in drop areas containing the largest number of colonies without signs of confluence or of gress diminution in colony size due to over-crowding. The maximum number of salmonella colonies obtainable without confluence in the drop area was usually 15 to 20. The number of colonies was estimated from the sum of the three drop counts. The count for each serotype was estimated from the mean of log. counts of five meat samples.

Suspicious colonies on brilliant green agar were confirmed by inocululating into TSI slant agar and by carrying out a slide agglutination test with specific antisera. Samples in which contaminating bacteria grew to the extent that adequate enumeration was impossible were encountered occasionally especially during the later periods of storage. The samples were excluded from the enumeration, and a further sample was randomly selected next day, enumerated, and included with the other four counts.

Experimental Results

Counts of S. *typhi-murinm S. anatum* and *S. meleagridis* experimentally inoculated in the raw meat were observed over a period of one month for the samples stored at 10°C, for two months at 7 and 5°C, and for six months at -12°C. The results are shown in Figures 1 and 2, and 2 and a summary in Table 1.



The growth of the three serotypes at 10°C was quite rapid, reaching the maximum number in three to five days. The number of organisms, then, remained almost constant for about three weeks but decreased slightly thereafter. There was no indication of multiplication of any of the serotypes in raw meat at 7°C or below.

The number at 7°C remained almost constant

FIGURE

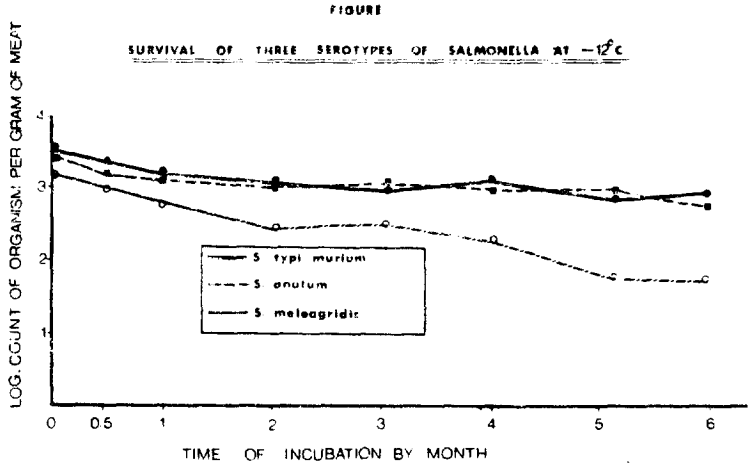


Table 1. Comparison of Number of Salmonellae in Raw Meat Stored at Different Temperatures

Time of storage	Average log. number stored at (°C)			
	10	7	5	−12
0	3.36	3.36	3.36	3.36
1 day(s)	4.64	3.39	3.36	3.29
3 〃	7.17	3.33	−*	−
5 〃	7.50	3.24	−	−
7 〃	7.32	3.11	3.30	3.55
9 〃	7.28	3.09	−	−
11 〃	7.87	3.15	−	−
14 〃	7.69	3.17	3.15	3.18
21 〃	7.10	3.11	3.07	3.17
1 month(s)	6.87	3.11	2.93	3.03
2 〃	−	2.53	2.47	2.85
3 〃	−	−	−	2.85
4 〃	−	−	−	2.78
5 〃	−	−	−	2.52
6 〃	−	−	−	2.47

* not tested

for the first four weeks but decreased slightly thereafter. The survival rate after two months was 10 per cent for S. typhi-murium, 19 per cent for *S. anatum* and 16 per cent for *S. meleagridis*. The survival rate of the three serotypes at 5°C was very much similar to that at 7°C, the survival rates for *S. typhi-mrium*, *S. anatum* and *S. meleagridis* being 10, 13 and 17 per cent respectively.

When the samples were stored at -12°C, the numbers of *S. typhi-murium* and *S. anatum* decreased very slowly and the survival rate after a six months storage period was 27 per cent for *S. typhi-murium* and 22 per cent for *S. dublin*. However, the number of S. meleagridis decreased faster than the other two serotypes, where only 4 per cent survived after six months.

Discussion

The enumration of salmonellae in a mixed flora of psychrophilic organism in meat samples was the main difficulty in these studies. Similar difficulties were also described by Georgala & Hurst (1963) as a main reason for delaying studies in this field. It was found through preliminary experiments that some precaution were essential to overcome this difficulty. Every precaution was then taken to minimize the number of contaminating organisms in the meat particularly during the preparation of the sample. The use of a relatively large number of organisms in the inoculum was also found to be important. Then salmonella organisms outnumbered the other contaminating organisms so that enumeration become easier.

Confirmation of suspicious colonies on the enumeration agar plate was another important factor in the experiments, and the method used were found to be rapid and accurate.

All the serotypes examined multiplied at 10°C

but there was no indication of multiplication at 7 °C or below. Johne (1961) observed that all of *S. typhi-murium, S. dublin* and *S. abortus equi* grew in milk and broth at 12°C and in milk at 9°C but none of the serotypes grew at 5°C. Petzold & Scheibner (1965) also confirmed that none of the forty salmonella strains of various serotypes grew in various media at 5±0.5°C. It is quite likely that salmonellae in general can multiply in highly nutritious food such as meat and milk at temperature between 7 and 10°C. Accordingly the temperature which prevents multiplication of salmonella organisms in raw meat would be 7°C or below.

The survival period for salmonellae in raw meat at 7, 5 and —12°C would be much longer than the observation period in the present study, This period was limited due to the multiplication of contaminating bacteria which made accurate enumeration impossible.

The results on the survival rate of salmonellae in the raw meat stored at −12°C were comparable with that of others who performed similar studies. Raj & Liston (1961) reported that the decimal reduction period for *S. typhi-murium* in frozen fish homogenate stored at -17.8°C was eleven months and Georgala & Hurst(1963) found that 50 per cent of *S. typhi-murium* survived in comminuted beef at -20°C for three months; the surival rate of *S. typhi-murium* at -12°C after three months was approximately 30 per cent and after six months 27 per cent in the present study.

The survival of salmonella organisms in raw meat for such a prolonged period at freezing temperature or below must be considered a serious public health problem. Thus rapid multiplication of salmonellae would result if the temperature of the meat were allowed to exceed 10°C, allowing further dissemination of the organism, more chance if it survives cooking and hence its chance of producing disease in man.

Summary

Multiplication and survival of *S. typhi-murium* *S. anatum* and *S. meleagridis* in raw meat were observ ed at different storage temperatures.

All the three serotypes grew in raw meat at 10°C reaching the maximum numbers in three to five days. However, none of the serotypes grew at 7°C or below. At both 7 and 5°C the number of organisms remained almost constant for the first few weeks but decreased slightly thereafter At -12°C the numbers of *S. typhi-murium* and *S. anatum* decreased very slowly over a period of six month but the number of *S. meleagridis* decreased faster than the other two serotypes.

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Salmonella菌의 生肉內 增殖 및 生存

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여러가지 貯藏溫度에서 *S. typhi-murium*, *S. anatum* 및 *S. meleagridis*의 生肉內 增殖 및 生存에 관하여 관찰하였다. 10°C에서는 세가지 菌種이 모두 증식하고 接種後 3日 내지 5日만에 最大菌數에 이르렀지만 7°C나 그 이하에서는 증식하지 못하였다. 7°C 및 5°C에서는 接種後 3適日까지는 菌數의 변동이 별로 없었지만 그 이후엔 약간 감소되었다. -12°C에서는 6個月間의 실험기간 동안에 *S. typhi-murium*과 *S. anatum*은 菌數가 대단히 서서히 감소되었지만 *S. meleagrids*는 다른 두菌種보다는 菌數의 減少率이 약간 더 컸다.