WATER RELATIONS OF SALMONELLAE AT 30°C

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Summary

Sixteen strains of salmonellae have been grown in various media of known water activity (a_w) at 30°C. The reactions of 15 motile strains were very similar, whereas the single non-motile strain grew more slowly and over a smaller range of a_w's.

For the motile strains aerobic growth occurred in liquid media at a_w's between 0.999 and 0.945. In foods the lower limit for growth was slightly less. Anaerobic rates of growth were only slightly less than the aerobic rate at all a_w's. A large percentage of the cells could form colonies on agar media with a_w's as low as 0.96.

I. INTRODUCTION

Although outbreaks of food poisoning caused by salmonellae are known to be the result of infections rather than intoxications (Dack 1943), there is no precise information on the numbers of organisms which need to be ingested in order to produce the characteristic clinical symptoms. It is known, however, that the disease is likely to be severe and the incubation period short when the number of organisms ingested is large, and in many outbreaks there is evidence that the salmonellae had multiplied in the food concerned. It is important therefore to know the extent to which the growth of salmonellae will be dependent on the water contents of different types of foods.

In the preceding paper (Scott 1953) the importance of the water activity (a_w) in relation to the growth of *Staphylococcus aureus* is discussed, and this paper records the results obtained when 16 strains of *Salmonella* were studied in media in which the a_w was controlled. Similar studies do not appear to have been made previously, although some authors have studied the inhibition of growth by solutes such as sodium chloride. Severens and Tanner (1945) reported that 3 per cent. NaCl was the highest concentration permitting growth of unadapted strains of *S. pullorum* and *S. schottmuelleri* (*S. paratyphi B*). After adaptation for about 10 wk, growth was observed in media with 8 per cent. NaCl. In experiments with dried meat adjusted to various water contents Segalove and Dack (1951) reported that a strain of *S. enteritidis* required greater amounts of water for growth than *Staphylococcus aureus* or an alpha-haemolytic-type *Streptococcus.*

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II. Methods

The methods and media employed were the same as those described by Scott (1953) with the following minor modifications. Growth was measured nephelometrically at a_w's of 0.96 and higher when the media were sufficiently transparent. For several strains grown in the casamino acids + yeast extract + casitone (C.Y.C.) medium each μA of the nephelometer reading was equivalent to approx. 5×10^5 cells/ml at a_w's between 0.999 and 0.96. At lower a_w's and in opaque media growth was followed by viable counts on nutrient agar incubated for 48 hr at 30°C.

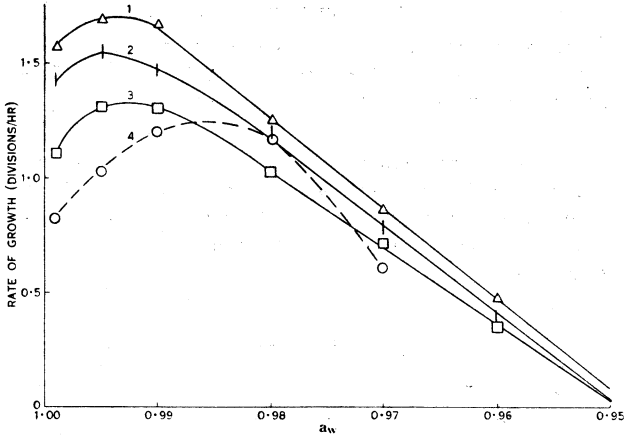

Fig. 1.—Relation between rate of growth and a_w for 16 strains of *Salmonella* in C.Y.C. medium with added salts.

Curve 1: Fastest growing motile strain S. *newport* (215).

Curve 2: Mean for 15 motile strains (vertical lines indicate mean ± its standard error).

Curve 3: Slowest growing motile strain S. *choleraesuis* (5735).

Curve 4: Aberrant non-motile strain S. *enteritidis* (3045).

Observations were made with the following 16 strains. Strains of S. *meleagris* (202), S. *typhimurium* (203), S. *oranienburg* (206), and S. *newington* (209) were isolated in this laboratory from Australian frozen eggs. The cultures were kindly identified by Miss N. Atkinson of the University of Adelaide. The authors are indebted to Miss Atkinson for also supplying a culture of S. *paratyphi B* (N.C.T.C. 3078) and the following five cultures which were recently isolated in Australia: S. *typhimurium* (210), S. *bovis-morbificans* (213), S. *anatum* (214), and S. *newport* (215). The following six N.C.T.C. cultures were kindly supplied by Dr. S. T. Cowan: S. *thompson* (5740), S. *choleraesuis* (5735), S. *dublin* (5766), S. *potsdam* (5744), S. *montevideo* (5747), and S. *enteritidis* (3045). All except the last-named culture were typical smooth motile salmonellae giving the characteristic biochemical reactions. S. *enteritidis* (N.C.T.C. 3045) was an aberrant non-motile strain which, in our hands, also failed to produce acid and gas from maltose. Although antigenically it was not dis-

tinguishable from S. *pullorum,* certain of its growth characteristics differed from those of strains of S. *pullorum* with which it was compared.

The cultures studied included the species which have most frequently been involved in outbreaks of food poisoning in the United Kingdom (Haines and Wilson 1947).

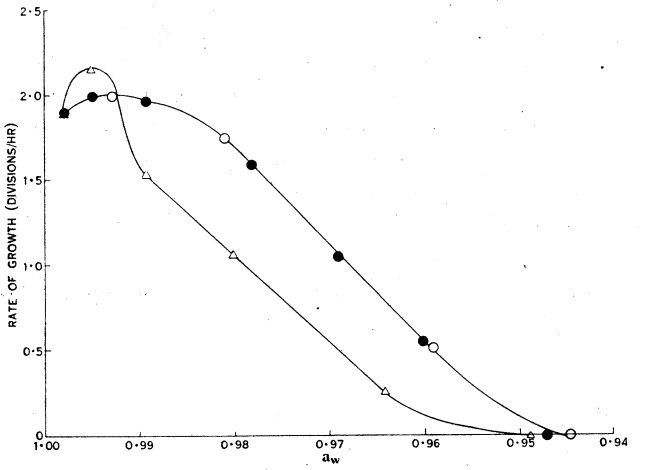

Fig. 2.—Relation between rate of growth and a_w for S. *oranienburg* (206) grown in B.H. medium using three methods for controlling a_w.

○, Water contents from vapour pressure isotherm for B.H. medium.

●, B.H. medium 9.25 g/1000 g H_2O and NaCl:KCl:Na_2SO_4 mixture in ratio of 5:3:2 moles.

△, B.H. medium 9.25 g/1000 g H_2O and sucrose.

III. Results

(*a*) *Growth in C.Y.C. Medium*

All strains were grown in C.Y.C. medium adjusted to various round values of a_w by a mixture of NaCl, KCl, and Na_2SO_4 in the molal ratio 5:3:2. The results are summarized in Figure 1. The 15 motile strains all showed a similar relation between rate of growth and a_w. Statistical analyses of the individual rates showed that these 15 strains were a homogeneous group with only a small variation between the fastest and slowest growing strains. For these 15 strains the mean rate of growth at 0.995 a_w was significantly greater than at 0.999 a_w. At a_w's below 0.99 the mean rate of growth decreased steadily with reduction of a_w, the rate at 0.96 being about 25 per cent. of the maximum rate. Linear extrapolation of the curves shows that growth is likely to be very slow at a_w's less than 0.95.

The broken curve in Figure 1 shows the results for the aberrant non-motile S. *enteritidis* N.C.T.C. 3045. For this strain the rates of growth were lower at all a_w's, the optimum was less well defined, and the rate of growth declined

much more rapidly as the a_w was reduced below 0.98. Limited observations on three strains of S. *pullorum* suggested a similar type of response to that found for strain 3045.

(*b*) *Growth in Brain-heart (B.H.) Medium*

Growth of S. *oranienburg* was studied in B.H. medium using three different methods for controlling a_w. These were: firstly, by adjusting the water contents to the desired a_w according to the vapour pressure isotherm (Scott 1953, Table 1); secondly, by adding the appropriate amounts of the NaCl:KCl:Na_2SO_4 (5:3:2) mixture to a basal B.H. medium containing 9.25 g dry matter per 1000 g water; and thirdly, by adding sucrose to the basal medium of 9.25 g per 1000 g water. This basal medium contains one-quarter of the usual concentration of nutrients in B.H. medium and has an a_w close to 0.998.

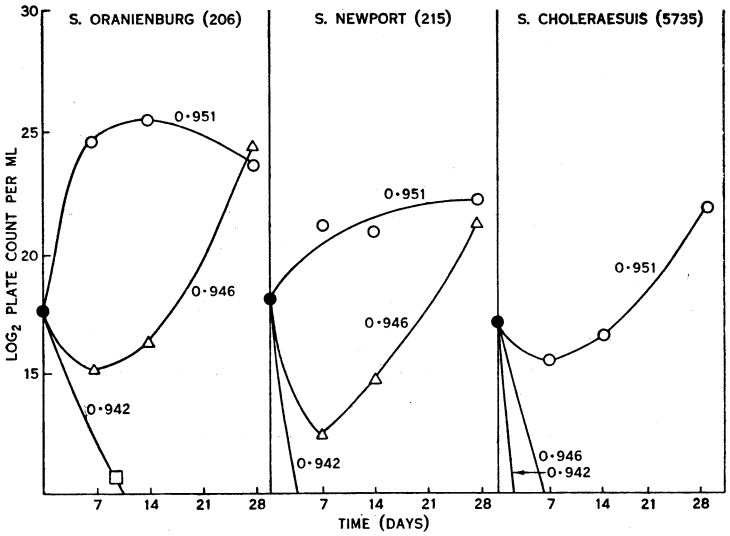

Fig. 3.—Limiting water activities for growth of three strains of *Salmonella* in B.H. medium.

The results (Fig. 2) show that the first two methods of controlling a_w gave a similar relation between the rate of growth and a_w. When sucrose was the principal solute there was some stimulation by the low concentrations of sugar at 0.995 a_w, but at a_w's less than 0.99 the rates of growth were lower than in the other two media. Growth was inhibited in all three media at a_w's between 0.95 and 0.94. The data show that the range of a_w's over which growth occurred was substantially the same when sugar, salts, or miscellaneous nutrients were the predominant solutes controlling the a_w.

(*c*) *Effect of* a_w *on the Lag and Yield of Cells*

As was shown for *Staph. aureus* (Scott 1953) reduction of the a_w below 0.99 led to an increase in the lag period and to a reduction in the total yield of

cells. Estimates of the lag period were too variable for valid general conclusions to be drawn. In two media, however, the lag was less than 1 hr at a_w's above 0.99, slightly greater at 0.98, and *c.* 10 and 24 hr at a_w's of 0.97 and 0.96 respectively. Limited data on the yield of cells in C.Y.C. medium showed a decrease of about 50 per cent. as the a_w was decreased to 0.96. Evidence obtained in other media suggested that the maximum viable count decreased more rapidly as the a_w was reduced from 0.98 to 0.96.

(*d*) *Minimum* a_w*'s for Growth in Liquid Media*

Growth of three strains was followed in B.H. medium with water contents corresponding to three levels of a_w between 0.942 and 0.951. The results in Figure 3 show that all strains grew at a_w 0.951, that two strains grew also at a_w 0.946, and that none grew within 28 days at a_w 0.942. Under similar conditions *S. oranienburg* grew in nutrient broth at 0.945 a_w, but not at 0.940. Other experiments with this strain showed that the minimum a_w for growth was between 0.94 and 0.95 in C.Y.C. and B.H. media using either salts or sucrose to control a_w.

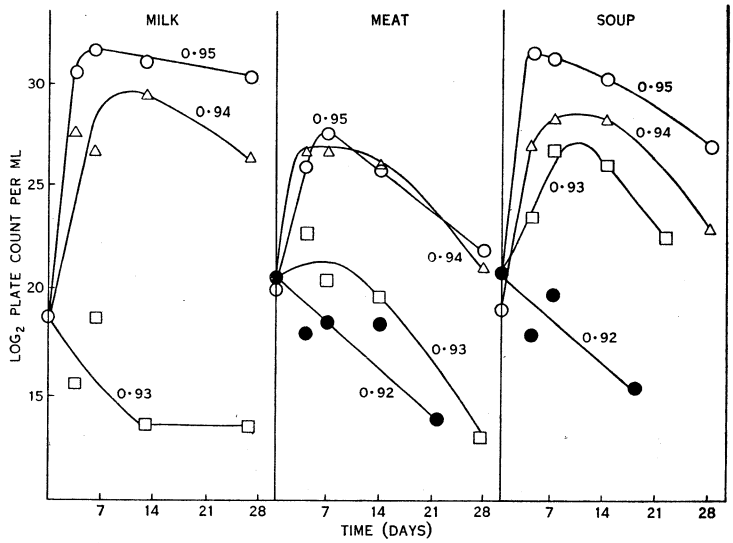

Fig. 4.—Limiting water activities for growth of *S. oranienberg* (206) in three foods.

(*e*) *Growth in Foods*

The growth of *S. oranienburg* was studied in dried meat, dried milk, and dried soup adjusted to water contents corresponding to the desired level of a_w. The results in Figure 4 show that growth occurred in all three foods at a_w's of 0.95 and 0.94. At an a_w of 0.93 the results differed according to the food. In milk the bacteria failed to grow, in meat there was perhaps a small increase, and definite growth occurred in the soup. There was no growth in either the meat or the soup at 0.92 a_w. Taken as a whole the results in foods are in fair agreement with those obtained in liquid media, although growth apparently

occurred at somewhat lower a_w's in the foods. Reference to the data on the equilibrium water contents of the three foods (Scott 1953, Table 6) shows that at the limiting a_w's of 0.93-0.94 the water contents of the soup are some three to four times as great as those of milk and mutton. It is clear therefore that it is a_w rather than the water content which determines the availability of water for the growth of these bacteria.

(*f*) *Aerobic and Anaerobic Growth*

All the foregoing experiments were made under aerobic conditions and some comparisons were therefore made of aerobic and anaerobic growth at various a_w's. The average results for three strains are given in Figure 5. It is clear that the anaerobic rates of growth were only slightly less than the aerobic rates at all a_w's tested, and that the lower limits for growth were virtually the same under both conditions.

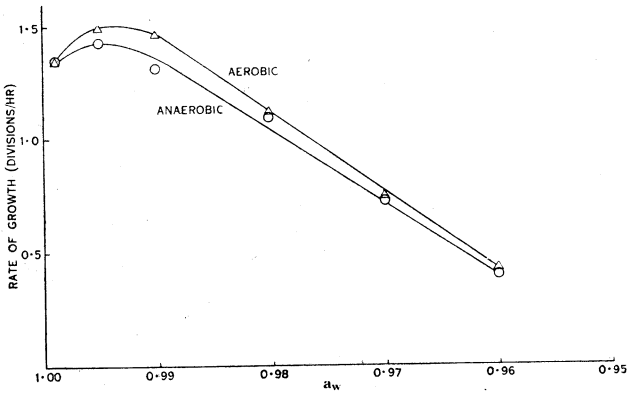

Fig. 5.—Comparison of aerobic and anaerobic rates of growth for three strains of *Salmonella* in C.Y.C. medium adjusted to various levels of a_w with added salts. The points are the means of at least two determinations for each of the three strains S. *newport* (215), S. *oranienburg* (206), and S. *choleraesuis* (5735).

(*g*) *Effect of* a_w *on the Plate Count*

Some experiments were made with one strain using nutrient agar adjusted to various a_w's with the NaCl-KCl-Na_2SO_4 mixture mentioned previously. The results are summarized in Table 1. For cells grown in nutrient broth of 0.999 a_w χ^2 tests showed that the number of cells forming colonies at 0.96 a_w was significantly less than at the higher a_w's. For cells grown at 0.96 a_w the counts were consistent with homogeneity at all a_w's. It may be concluded therefore that under some conditions virtually all the cells in a culture have the capacity to grow at a_w's as low at 0.96. Even for cells grown in nutrient broth of 0.999 a_w there were still some 40 per cent. of the cells which formed colonies at 0.96 a_w.

IV. Discussion

The results with salmonellae confirm the finding previously reported for *Staph. aureus* (Scott 1953) that the range of a_w's permitting growth is substantially independent of the type of solute predominant in the medium. For the salmonellae, however, the range of a_w's in which growth occurs is much less than for the staphylococci. In liquid media the lowest a_w at which growth was observed was close to 0.945, whereas in foods growth occurred at slightly lower a_w's. In liquid media there can be little uncertainty about the uniformity of a_w throughout the medium, but in the moist foods there is greater difficulty in ensuring and maintaining uniform distribution of the water added. It is suggested therefore that the results obtained in the liquid media may be the more reliable. It is, however, not possible to reject the idea that growth on solid media may occur at slightly lower a_w's than in liquids.

Table 1

Effect of a_w of the plating medium on the plate count of *S. oranienburg*

Counts are means for four plates incubated for 5 days at 37°C

a_w of Nutrient Agar Used for Plate Count	Plate Count	
	Nutrient Broth 0·999 a_w *	C.Y.C. + Salts 0·960 a_w †
0·999	258	56
0·995	246	69
0·990	244	75
0·980	207	70
0·970	248	73
0·960	104	63

* Plates inoculated with 2×10^{-8} ml of culture grown for 16 hr at 30°C.

† Plates inoculated with 2×10^{-6} ml of culture grown for 4 days at 30°C.

The uniformity with which the various strains have reacted is an important feature of the results and it seems reasonably safe to assume that other motile salmonellae would have similar water requirements. This supposition is further strengthened by the fact that the organisms studied have included both recent and old isolates from a variety of sources. The different results obtained with the non-motile S. *enteritidis* 3045 are of interest, although there is no evidence that the reactions of this organism are typical of non-motile salmonellae generally. From a practical point of view the important feature is that the typical motile organisms exhibit the greater tolerance as the a_w is reduced below 0.97.

It is of some interest to compare the limiting a_w of *c.* 0.945 in the present experiments with the approximate a_w's which may be deduced from the limiting conditions reported by other workers. Severens and Tanner (1945) found no growth of unadapted salmonellae above 3 per cent. NaCl, but after adaptation growth occurred in up to 8 per cent. NaCl. The latter solution has an

a_w close to 0.95. Segalove and Dack (1951) inoculated *S. enteritidis* into dried meat with various water contents for which the approximate corresponding a_w's have been deduced by Scott (1953). At 50 per cent. water contents scant growth occurred on one sample within 24 hr, but no growth within the same time at 40 per cent. water contents. The corresponding a_w's were *c.* 0.979 and 0.967 for meat samples without added salt. Some of the samples with added salt may, however, have had a_w's as much as 0.02 less and this is doubtless the explanation for Segalove and Dack's finding that the results depended on the salt content. In general the results of Segalove and Dack are consistent with those in this paper, although it might be expected that they would have observed growth in some samples with 40 per cent. moisture if observations had been continued beyond 24 hr.

There are some grounds for the belief that the water requirements of most coliform bacteria may be similar to those of the salmonellae. With *E. coli*, for example, there is general agreement (Foda and Vaughn 1950) that 5-8 per cent. NaCl is needed to inhibit growth. These concentrations correspond to a_w's of about 0.97 and 0.95 respectively. Foda and Vaughn also point out that most strains of *Aerobacter aerogenes* do not tolerate more than 6.5 per cent. (w/v) NaCl (a_w 0.96), and unpublished observations by one of us (J.H.B.C.) have shown that the Hinshelwood strain of *A. aerogenes* is able to grow at 30°C at a_w's down to about 0.94.

The results reported in this paper have been obtained with unadapted cultures and no attempt has been made to determine whether salmonellae could be adapted or selected to grow at even lower a_w's. Although Severens and Tanner (1945) reported that salmonellae acquired permanently increased tolerance to NaCl as a result of selection, the maximum concentration of salt tolerated after adaptation was 8 per cent. (a_w 0.95). Similarly the results of Doudoroff (1940) and of Foda and Vaughn (1950) show that most coliform organisms could not be trained to grow in NaCl concentrations with a_w's less than about 0.945. The only exception was a group of *Aerobacter* strains which Foda and Vaughn isolated by enrichment from olive brines, and which they found could grow in the presence of up to 14.5 per cent. (w/v) NaCl, the corresponding a_w being close to 0.91. The possibility of training or selecting salmonellae to grow at a_w's below 0.94 certainly exists, but there are no indications that any such attempts are likely to be successful. On the other hand the present experiments have always shown a well-defined logarithmic phase at 0.96 a_w and there has never been any suggestion that more rapidly growing variants had appeared in any of the cultures. A decisive answer regarding the chances of adapting salmonellae to low a_w's must await further experiments.

V. References

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