WATER RELATIONS OF *STAPHYLOCOCCUS AUREUS* AT 30°C

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Summary

Fourteen food-poisoning strains of *Staphylococcus aureus* have been grown in various media of known water activity at 30°C. Aerobic growth was observed at water activities between 0.999 and 0.86. The rate of growth and the yield of cells were both reduced substantially when the water activity was less than *c.* 0.94. The lower limits for growth in dried meat, dried milk, and dried soup were similar to those in liquid media. Aerobic growth proceeded at slightly lower water activities than anaerobic growth. All cells were capable of forming colonies on agar media with water activities as low as 0.92. The 14 strains proved to be a homogeneous group with similar water requirements.

I. Introduction

There is general agreement that the influence of the availability of water on the growth of microorganisms is most appropriately considered in terms of the relative humidity. Most of the experimental work has been done with moulds, especially with those which are able to grow at comparatively low relative humidities. Some of the experimental requirements have been stated by Tomkins (1929). The most important of these is that the water vapour pressure of the substrate should be in equilibrium with that of the atmosphere in which it is stored. It is only by observing this condition that it is possible to ensure uniform distribution of water through the substrate and a constant water content throughout the duration of an experiment. The experiments with moulds are usually carried out on thin films of solid media in equilibrium with sulphuric acid solutions of known composition. Walter (1924), who was one of the first to employ this technique, made some observations with bacteria. He found that none of the bacteria tested could grow at a relative humidity of less than 96 per cent., whereas several moulds grew at much lower humidities. Scott (1936), working with thin slices of beef muscle equilibrated with sulphuric acid solutions, found two strains of *Achromobacter* to have a lower limit for growth of approx. 96 per cent., and two strains of *Pseudomonas* to have a lower limit greater than 98 per cent. These experiments were done at -1°C. No other studies of bacteria growing under conditions of controlled humidity appear to have been made although many workers have studied the effects of the concentration of various solutes on the growth of bacteria. Many of these observations have been made in media for which useful approximate calculations of the equilibrium humidities can be made.

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Observations on staphylococci have shown that they generally tolerate rather higher solute concentrations than most other bacteria, and this has formed the basis of media used for enrichment and isolation of staphylococci as proposed by Chapman (1946). Segalove and Dack (1951) reported the growth of various food-poisoning bacteria on dried meat at different water contents. They found that a food-poisoning strain of *S. aureus* grew at rather lower water contents than the other organisms tested. The equilibrium humidity corresponding to the limiting water contents was, however, not given. The present paper reports the results obtained when the growth of 14 food-poisoning strains of *S. aureus* was studied in several media in which the availability of the water was controlled.

II. Methods

As the methods used for controlling the availability of water are of paramount importance and are not recorded fully elsewhere, the essential features are given in some detail. Firstly, the equilibrium humidity of an aqueous solution is the relative humidity of an atmosphere which neither gains water from nor loses water to the solution. In such a system at equilibrium the water vapour pressure of the solution is the same as in the atmosphere above it. The water vapour pressure of the solution expressed as a fraction of the vapour pressure of pure water at the same temperature (or the equilibrium humidity expressed as a fraction) is numerically equal to the activity of the water (a_w) in the solution. It will be convenient henceforth to use this quantity as a measure of the availability of water in the medium; its relation to osmotic pressure and other properties of the solution are discussed in the various textbooks on physical chemistry.

In studying the growth of bacteria on a liquid medium such as nutrient broth at various levels of a_w it is convenient to construct the vapour pressure isotherm of the medium at the appropriate temperature. This curve shows the relation between water contents and a_w at the particular temperature. Although strictly applicable only at one temperature, the isotherm is not greatly affected by temperature and in practice it is reasonably safe to assume that the isotherm will be virtually unchanged at other temperatures within 5°C.

Table 1 shows the equilibrium water contents for two lots of brain-heart infusion (B.H.) medium for various round values of a_w at 25°C. Determinations were made at approximately 12 points between a_w's of 0.75 and 0.99 by the isopiestic technique of Robinson and Sinclair (1934). Solutions, in lots of about 2 ml, were equilibrated in gold-plated silver dishes made to fit D40 standard taper glass lids. Four such dishes were placed in a copper block weighing approximately 1.5 kg. The copper block was placed in an evacuated desiccator, which was submerged in a water-bath at 25 ± 0.01°C and continuously rotated in a plane about 12° from horizontal at 40 r.p.m. The reference solution was sodium chloride (Robinson 1945). After equilibrium was reached the composition of the solutions was obtained from the dry weight after 20-24 hr at 105°C.

Determinations made by this method fell on a smooth curve, and it will be seen from Table 1 that there is close agreement between the two batches prepared according to the same formula. At a_w's greater than 0.99 the solutions are dilute and departures from Raoult's law are small. If, therefore, the equilibrium water contents are known at an a_w close to 0.99 it is permissible to calculate the water contents corresponding to any a_w above 0.99 by assuming that a_w is proportional to the mole fraction of water. The assumption is valid for un-ionized and completely ionized solutes, and also for partially ionized solutes provided that the degree of ionization does not change appreciably with further dilution. It is not necessary to know the molecular weights of the solutes concerned, as for any given mixture of solutes the mole fraction of the solvent is

TABLE 1

EQUILIBRIUM WATER CONTENTS OF TWO LOTS OF BRAIN-HEART INFUSION MEDIUM AT VARIOUS ROUND VALUES OF a_w AT 25°C

a_w	Dehydrated Infusion (Difco) (H_2O % dry wt.)	Infusion Prepared in Laboratory and 40 l. Dried from Frozen State (H_2O % dry wt.)
0·99	1990	2080
0·98	950	1030
0·97	620	650
0·96	472	490
0·94	312	320
0·92	245	230
0·90	200	185
0·88	165	150
0·86	135	125
0·84	—	102
0·80	—	82
0·75	—	67

inversely proportional to solute concentration on a weight basis. It is convenient to express water contents in terms of the dry weight of solute, as values so expressed are directly proportional to the amount of water in any given system. There is the additional advantage that such values are inversely proportional to concentration per unit weight of water and molality (m). When the vapour pressure isotherm for any particular medium has been determined, it is possible to make media with water contents corresponding to any desired value of a_w. It is to be noted, however, that any reduction of water contents inevitably introduces an associated increase in the concentration of nutrients. If therefore it is desired to ensure that the reactions of the bacteria are related to the availability of water it is necessary to extend the observations to other media with a different relation between nutrient concentration and a_w. By constructing isotherms for other media it is possible to compare the reactions of the organisms at the same a_w in the presence of different types of nutrients.

It is also possible to vary a_w by adding non-nutrient solutes in varying concentration to a basal medium in which the concentration of nutrients is maintained at a constant level. This may be done very simply by adding solutes such as sodium chloride or sucrose to nutrient broth. Such experiments are frequently performed, but the results are usually not reported in terms of a_w but only in terms of solute concentration. Osmotic coefficients for a number of electrolytes and some non-electrolytes have been published in recent years (Stokes 1948; Robinson and Stokes 1949) and it is possible to calculate the a_w of solutions over a wide range of concentrations at 25°C. It is also possible to deduce with considerable precision the a_w of solutions containing mixtures of various solutes (Robinson and Stokes 1945) when these do not react to form insoluble compounds or complex salts. This procedure has been used in these experiments using a basal medium of mineral salts, casamino acids, yeast extract, and casitone (C.Y.C.), which contains all the nutrients required by *Staphylococcus aureus*. The composition of this medium is given in Table 2 together with the contribution of each ingredient to the vapour pressure lowering. The contributions of the complex ingredients are deduced from freezing-point determinations made on solutions of known composition. In dilute solutions of low ionic strength simple addition of the various values of $1-a_w$ is permissible, and it is concluded that the medium has an a_w close to 0.999. The ionic strength (μ) is of the order of 0.04-0.05, the actual value being dependent on the composition of the complex ingredients and, as phosphate is a major constituent, on the pH.

TABLE 2

COMPOSITION OF BASAL C.Y.C. MEDIUM WITH AN a_w OF 0.999 AT 25°C

	m	g/1000 g H_2O	Calculated $(1-a_w)$
Na_2HPO_4	0·01	1·42	0·00033
KH_2PO_4	0·002	0·27	0·00007
$MgSO_4$	0·001	0·12	0·00002
NH_4NO_3	0·005	0·40	0·00016
Glucose	0·01	1·80	0·00018
Casamino acids		0·60	0·00013
Yeast extract		0·10	0·00002
Casitone		0·20	0·00002
Total		4·91	0·00093

Calculated a_w of medium is therefore 0·99907, or *c.* 0·999.

This C.Y.C. medium was adjusted to lower levels of a_w with various solutes. Most of the experiments were made when the adjustment was made with a mixture of NaCl, KCl, and Na_2SO_4 in the ratio of 5:3:2. The method for calculating the a_w of the mixture was adapted from the "second approximation" given by Robinson and Stokes (1945). This method depends on the assumption

that the relative molal lowering of the vapour pressure, $(1-a_w)/m$, for each salt is a function of the total ionic strength of the mixture. The calculations are made in two stages. Firstly, the value of $(1-a_w)/m$ is plotted against μ for each salt separately. The a_w of the mixture is then calculated for several round values of μ and a second curve is constructed relating a_w and μ for the particular salt mixture. From this curve, or by linear interpolation, the value of μ corresponding to any desired value of a_w may be obtained, and from the ionic strength so obtained the molalities of the individual salts are determined.

In determining the quantities of salt to be added to a given amount of C.Y.C. basal medium the calculation was simplified by neglecting the small contribution to the final ionic strength made by the basal medium. The final adjustment is then made by simple addition of $1-a_w$ for the basal medium and $1-a_w$ for the electrolyte mixture. For the particular mixture specified the concentrations of salts required to give various round values of a_w in C.Y.C. medium are given in Table 3. The composition of other mixtures used has been calculated in a similar way. Robinson and Stokes (1945) found that the calculated vapour pressure lowering of mixtures was within about 1 per cent. of the actual value, for water activities down to about 0.8. A check by the isopiestic technique showed agreement within this limit for the mixture in Table 3 with a calculated a_w of 0.920.

TABLE 3

MOLAL CONCENTRATIONS OF SALTS REQUIRED FOR ADJUSTING C.Y.C. MEDIUM OF a_w 0.999 TO VARIOUS VALUES OF a_w AT 25°C WITH A 5 : 3 : 2 MIXTURE OF NaCl, KCl, AND Na_2SO_4

Final a_w in Medium	$(1-a_w)$ of Salt Mixture	NaCl (m)	KCl (m)	Na_2SO_4 (m)
0·995	0·004	0·0575	0·0345	0·0230
0·990	0·009	0·1293	0·0776	0·0517
0·980	0·019	0·2789	0·1673	0·1116
0·960	0·039	0·5805	0·3483	0·2322
0·940	0·059	0·869	0·521	0·348
0·920	0·079	1·149	0·690	0·460
0·900	0·099	1·418	0·851	0·567
0·880	0·119	1·663	0·998	0·665
0·860	0·139	1·921	1·153	0·768

Media were made by adding the appropriate quantities of anhydrous salts to aliquots of basal medium containing a known weight of water. After the salts were dissolved the mixture was sterilized by autoclaving, with precautions against evaporation. Any loss of weight was restored with sterile distilled water. All media were made on the basis of dry ingredients, and corrections for water in substances such as meat extract were applied when anhydrous materials were not used. The a_w of a particular mixture was checked by determination of the dry weight before commencing an experiment. During an

experiment precautions were taken to ensure that the a_w did not change by more than 0.001. The precautions necessary depended on the duration of the experiment, the weight of water in the system, and the a_w. The latter is important because, at low values of a_w, a given quantity of water causes a greater change in a_w than in more dilute solutions.

TABLE 4

SOURCES AND PHAGE PATTERNS OF THE 14 STRAINS OF *STAPHYLOCOCCUS AUREUS*

Source	Strain No.	Phage Pattern
Central Public Health Laboratory, Colindale, London England (Dr. R. E. O. Williams)	49/1255	6/7/42B/47/47B/47C/53/54
	49/1284	6/47
	49/1974	42D
	50/225	6/7/42B/47/47B/47C/53/54
	50/3002	7
	50/3147	6/42B/47/53/54
	50/3185	6/42B/47
American Meat Institute, Chicago, U.S.A. (Dr. C. F. Niven, Jr.)	F2B	47/53
	K57	47
	S6	47/54
	S12	3A/3B/51
	S209	47
	210	47
Fairfax Institute of Pathology, Sydney, Australia (Dr. Phyllis Rountree)	R4730	7/42B/47B/53

Aerobic growth at water activities of 0.90 and above was measured nephelometrically in 15-mm Pyrex T tubes containing 10 ml of medium and rocked continuously in a water-bath at 30°C ± 0.05. Anaerobic growth rates were measured in sealed Pyrex tubes at the same temperature. The method of sealing the tubes and the characteristics of the nephelometer have been described by Ohye and Scott (1953). Each μA of the nephelometer was equivalent to approx. 3.0×10^5 cells/ml in C.Y.C. medium at several a_w's between 0.90 and 0.999. The corresponding dry weight of cells was 4.3×10^{-2} μg at 0.999 a_w. The correct determination of dry weight of cells at low values of a_w is being studied further. Growth in opaque media was followed by viable counts using the technique of Miles and Misra (1938) and counting colonies after 48 hr at 30°C on brain-heart agar. Dilutions were made in 0.9 per cent. saline. The use of isotonic diluents was not necessary. Growth at water activities of 0.88 and below was studied visually or by viable or direct counts. The observations were continued over 30 days at 30 ± 0.5°C. Cultures of about 20 ml were placed in 100-ml screw-capped bottles sealed with paraffin to reduce evaporation.

The lower limits of a_w permitting growth were studied also in three foods. For these the vapour pressure isotherms were determined at 25°C using the isopiestic technique. Aqueous suspensions were spread uniformly as a thin film on the bottom of the silver dishes and sorption isotherms determined

between 0.75 and 0.98 a_w. For this purpose it was convenient to use as reference solutes the saturated solutions listed by Stokes and Robinson (1949). When being prepared for growth studies aliquots of approx. 10 g of dry food were brought to the appropriate water contents by the cautious addition of the required weight of distilled water, the mass being continuously agitated with approx. 20 g of glass beads in a 100-ml screw-capped bottle. This ensured the even distribution of water through the food. Sterilization for 30 min at 100°C was adequate. After inoculation the bottles were shaken to distribute the inoculum, and initial samples taken for viable counts and estimate of water contents. The bottles were incubated at 30 ± 0.5C within desiccators over sodium chloride solutions of the same a_w. This precaution was found to be necessary as the total amount of water in some of the bottles was only about 1 g and evaporation of only 5 mg/day would have been serious over a period of 30 days. Viable counts and water contents were determined at intervals.

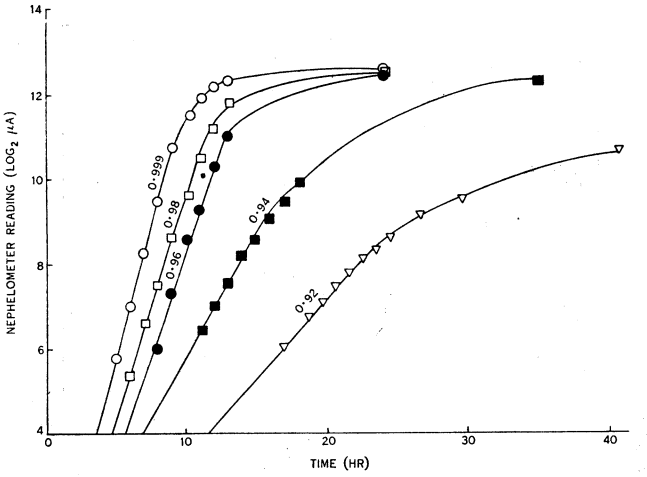

Fig. 1.—Growth curves for *S. aureus* strain 50/3002 on C.Y.C. medium adjusted to various levels of a_w with salts mixture listed in Table 3.

All cultures were inoculated with 0.02 ml of a saline suspension prepared from an overnight culture in nutrient broth. For the liquid media the inoculum gave an initial count of about 10^5 cells/ml and for the foods about 10^6 cells/g.

Experiments have been made with 14 cultures of *Staphylococcus aureus*, the organisms being typical of the species as described by Shaw, Stitt, and Cowan (1951). All strains had either been implicated in outbreaks of food poisoning or had been shown to produce enterotoxin. The phage patterns of the different strains were kindly determined by Dr. Phyllis Rountree using the method described by Williams and Rippon (1952). The phage patterns and the origins of the various cultures are shown in Table 4. With the exception of strain S.12 all strains belong to the 6/47 group of Williams and Rippon, this group including almost all the food-poisoning strains isolated in England.

III. Results

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

Some typical results for one strain grown in C.Y.C. medium are shown in Figure 1. The various levels of a_w were obtained by adding the appropriate quantities of the salt mixture given in Table 3. Reduction of the a_w below 0.99 caused a progressive decrease in both the rate of growth and the maximum density of the culture. The results with other strains were very similar and when the results for the different strains were compared no consistent differences between strains were detected. Figure 2 shows the average results for all strains on C.Y.C. medium. The greatest rates of growth were found at a_w's of 0.995 and 0.99, the rates at these a_w's being significantly greater ($P < 0.01$) than the rate at an a_w of 0.999. As the a_w decreased from 0.99 to 0.90 there was a progressive reduction in the rate of growth to a level less than 10 per cent. of the maximum rate. The yield of cells also decreased progressively over this range.

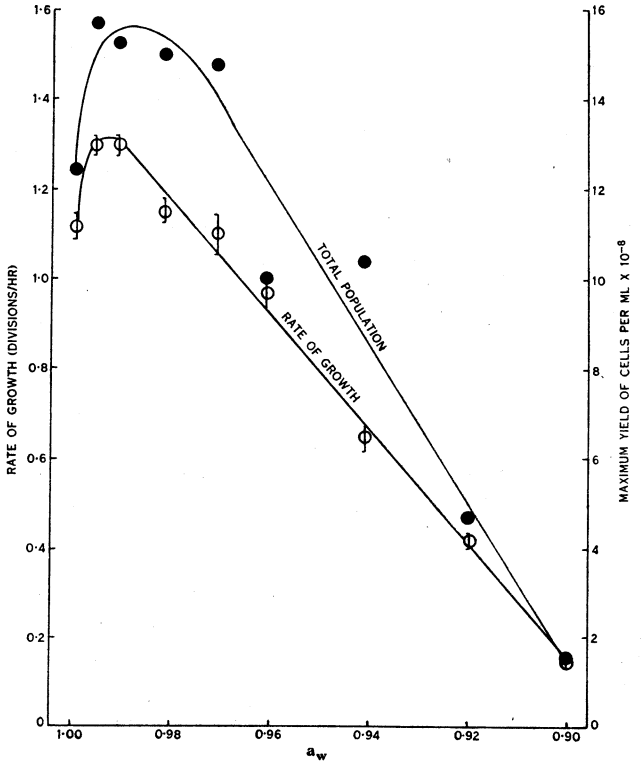

Fig. 2.—Relation between mean rate of growth, maximum yield of cells, and a_w for 14 strains of S. *aureus* grown in C.Y.C. medium with added salts.

(*b*) *Growth in B.H. Medium*

When the B.H. medium dried from the frozen state was adjusted to various a_w's by preparing media with the water contents shown in Table 1, the results

shown in Figure 3 were obtained. At the higher a_w's the medium was sufficiently transparent for rates of growth to be determined nephelometrically and by viable counts, but in the more concentrated media light-scattering measurements were not possible. In this medium somewhat greater rates of growth were realized at high a_w's, but as the a_w fell below 0.94 the rates were very similar to those shown in Figure 2 for the C.Y.C. medium with added electrolytes. It may be noted that the yield of cells/ml increased with increasing concentration of nutrients as the a_w was decreased from 0.995 to 0.98 and 0.96, but at lower a_w's the yield of cells tended to decline. The lag period in B.H. medium (not shown in Fig. 3) increased from *c.* 1 hr at 0.995 a_w to 3-6 hr at 0.96 a_w, and at lower a_w's further increased to over 3 days at 0.90 a_w. In this medium the lag periods at the lower a_w's were much longer than on C.Y.C. medium, in which the deduced lag was less than 24 hr at 0.90 a_w. Owing to these considerable differences between media it is not possible to make a useful quantitative statement about the length of the lag period in relation to a_w.

TABLE 5

MINIMUM a_w FOR GROWTH OF *S. AUREUS* IN VARIOUS LIQUID MEDIA AT 30°C

\+ = Growth and − = no growth, within 30 days. All cultures tested reacted similarly except where fraction in brackets indicates a smaller number

Basal Medium	Solutes Added	Number of Strains Tested	a_w 0·88	a_w 0·86	a_w 0·84
C.Y.C.	Salts mixture*	14	+(12/14)	−	−§
Nutrient broth	Salts mixture*	13	+	+(9/13)	−§
Nutrient broth	Sucrose	6	+	−	−
Nutrient broth	3·44m Sucrose + salts mixture*	6	+	+(5/6)	−
Nutrient broth	4·07m Glucose + salts mixture*	6	+	−	−
Nutrient broth	0·11m Glucose + salts mixture*	1	+	−	−
Brain-heart infusion	None†	1	−‡	−	−

* Details in Table 3. † Water contents in Table 1.

‡ Growth at 0·90a_w. § Saturated solution, therefore a_w approximate only.

(*c*) *Minimum* a_w*'s for Growth*

The lower limits for growth have been studied in several liquid media adjusted to different a_w's with various solutes. The results are summarized in Table 5. Slow growth has been observed down to 0.86 a_w, but not at 0.84 a_w. There is little evidence of variation between strains and, in the media used, the results are similar for the various solutes used to control a_w.

(*d*) *Growth in Foods*

The lower limits of a_w at which growth is possible have also been studied in three foods, dried milk, dried mutton, and a commercial dried soup mixture.

The equilibrium water contents for these foods at several round values of a_w are shown in Table 6. The figures have been taken from the respective vapour pressure isotherms at 25°C. When these foods were inoculated and incubated at 30°C the results shown in Figure 4 were obtained. Slow growth occurred in all foods at 0.88 a_w and in the milk and soup at 0.86 a_w. The results show satisfactory agreement with those obtained in liquid media (Table 5), and demonstrate that it is a_w rather than water content which determines whether or not a substrate is sufficiently moist to support growth of *S. aureus*.

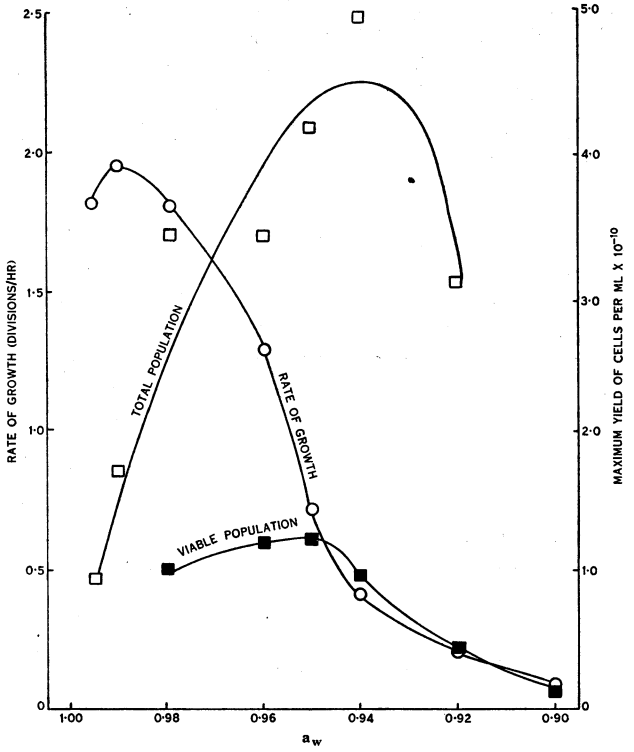

Fig. 3.—Relation between rate of growth, total and viable populations, and a_w for *S. aureus* strain 49/1974 growing on brain heart infusion.

(*e*) *Comparison of Aerobic and Anaerobic Growth*

All the foregoing experiments were carried out under aerobic conditions, and some comparisons have therefore been made of aerobic and anaerobic growth at various a_w's. The results are shown in Figure 5. The aerobic rates were similar to those found in B.H. medium, being 1.85, 1.18, and 0.45 divisions/hr at a_w's of 0.995, 0.96, and 0.92 respectively. The corresponding rates under anaerobic conditions were 1.37, 0.68, and 0.09. At 0.88 a_w, growth occurred aerobically but not anaerobically within 4 wk at 30°C. Plots of the anaerobic rate and of the ratio of the anaerobic to the aerobic rates against

a_w both indicate that anaerobic growth is unlikely at an a_w of 0.90 in this medium. The yield of cells from anaerobic growth was less than 10 per cent. of the aerobic yield at all a_w's.

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

The relation between the plate count and a_w was studied in nutrient agar adjusted with mixed electrolytes to various desired levels of a_w down to 0.90. The basal medium was obtained by adding 1 per cent. of agar to nutrient broth. The water activity was reduced by less than 0.001 a_w by the addition

TABLE 6

EQUILIBRIUM WATER CONTENTS OF THREE FOODS AT VARIOUS a_w's AT 25°C

a_w	Water (% dry wt.)		
	Dried Mutton*	Dried Milk	Dried Soup
0·97	72·6	127	305
0·96	56·8	95·5	225
0·94	39·2	54·0	156
0·92	30·6	30·5	115
0·90	26·4	21·9	89·5
0·88	23·3	18·4	74·0
0·86	21·1	15·7	62·5
0·84	19·1	14·2	55·0
0·82	17·2	13·0	49·0
0·80	15·8	12·2	45·0

*Dry matter contains 32·4 per cent fat.

of this amount of agar. The results are shown in Table 7. χ^2 tests on the counts of the triplicate plates showed that all the counts down to 0.92 a_w were homogeneous for the cells grown in nutrient broth. For the cells grown in C.Y.C. medium with mixed electrolytes at 0.96 a_w, there was a significant decrease in the plate count at a_w's of 0.94 and 0.92. With both types of inoculum no colonies were detected at 0.90 a_w after 11 days at 30°C. Two conclusions of importance emerge. The first is that all the cells in the population grown in nutrient broth were able to grow at a_w's at least as low as 0.92. The second is that cells grown at 0.96 a_w had no greater capacity to form colonies at low a_w's than cells grown at 0.999 a_w.

Although Table 7 records the plate counts after 5 days it may be noted that counts at 48 hr were not significantly less at a_w's of 0.96 and above, and after 24 hr only slightly less. At 0.94 a_w colonies reached diameters of 0.5 mm in 24-48 hr, and at 0.92 a_w in 48-96 hr. Colonies developing on agar at 0.999 a_w were observed to have a fuller development of the characteristic golden pigment than those appearing at lower a_w's.

IV. Discussion

The results provide clear evidence that the range of water activities permitting growth of food-poisoning strains of *S. aureus* was virtually independent of the nature of the solutes predominant in the medium. These solutes included

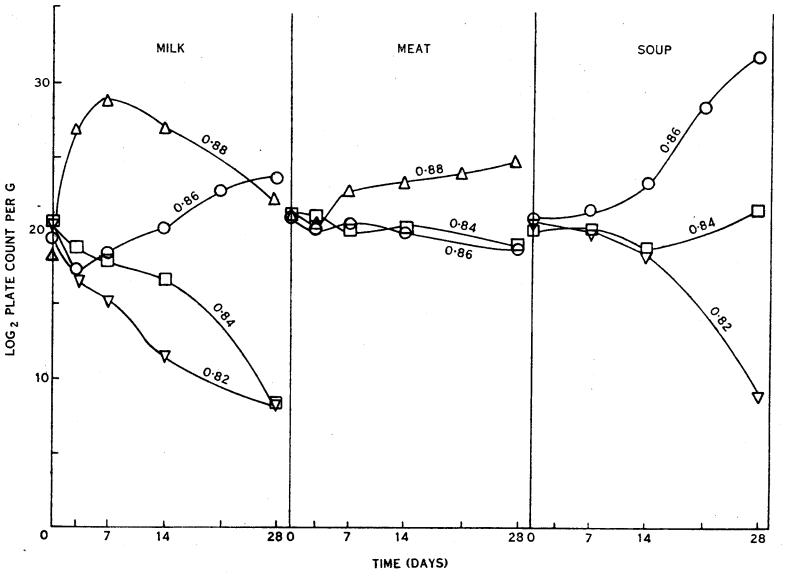

Fig. 4.—Growth curves for *S. aureus* inoculated into foods at different water activities. (Points are logarithmic means for separate experiments with strains F2B and 49/1974, both strains showing similar results.)

Table 7

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

Counts are means for triplicate plates inoculated with 10^{-6} ml of the broth culture, and incubated for 5 days at 30°C. Inoculum was strain 50/3002 grown for 18 hr at 30°C

a_w of Nutrient Agar used for Plate Count	Plate Count per ml × 10^{-6}	
	Nutrient Broth 0·999 a_w	C.Y.C. + Salts 0·960 a_w
0·999	435	111
0·990	426	117
0·980	426	114
0·960	414	116
0·940	421	81
0·920	405	38
0·900	0	0

sugars, some non-toxic salts, and the miscellaneous substances in bacteriological media and foods. Knowledge of the water activity in a particular food should

therefore enable one to predict whether or not the substrate is sufficiently moist to support the growth of these bacteria.

The lower limit of a_w for growth at 30°C has been found to be about 0.86. This result was obtained at a temperature not far below the optimum for *S. aureus*, in the presence of an adequate supply of nutrients, and at approximately neutral pH levels. It is unlikely therefore that *S. aureus* will grow over a greater range of a_w under other environmental conditions. It is in fact more likely that factors such as unfavourable temperatures will restrict the range of a_w's over which growth occurs. Tomkins (1929), for instance, found that the range of a_w for germination of fungal spores was greatest at the optimum temperature.

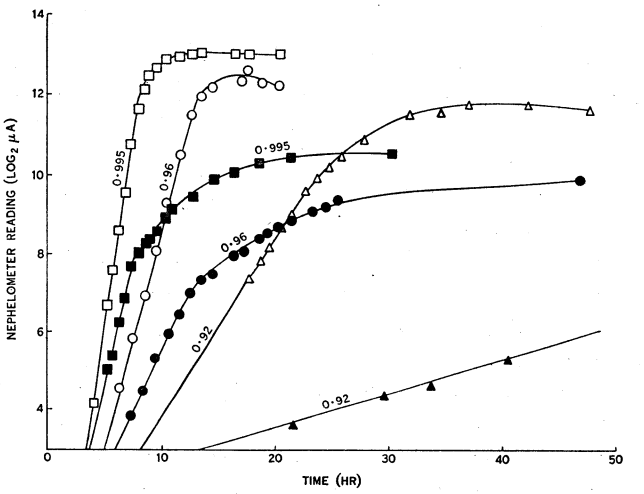

Fig. 5.—Growth curves of *S. aureus* strain 49/1974 under aerobic and anaerobic conditions at three water activities. Medium nutrient broth + 0.11m glucose + salts mixture to give desired a_w. Open symbols, aerobic; solid symbols, anaerobic.

An important feature of the experimental results was the uniformity between the strains. The 14 cultures were isolated from a wide range of sources and localities, but each has shown a very similar response under the various conditions of testing. The relative homogeneity of the group in water requirements indicates that other food-poisoning strains of *S. aureus* are likely to conform to the results obtained in the present experiments.

The expectation that other strains will behave similarly is strengthened by considering the results obtained by Segalove and Dack (1951). These authors studied the growth of another enterotoxin-producing strain (No. 161) on dried meat adjusted to water contents of 10, 20, 30, 40, 50, and 60 per cent. of the wet weight. They observed growth within 8 hr at 37°C at 40, 50, and 60 per cent. water and sometimes growth within 24 hr at 30 per cent. water. No growth was observed within 2 wk at 10 or 20 per cent. water contents. Although Segalove and Dack used meat samples varying appreciably in fat content a number of their samples had fat contents close to 30 per cent. If

one assumes that 30 per cent. of the dry matter was fat and that the water vapour pressure isotherms are not dissimilar for the non-fat solids of beef, mutton, and pork, it is easy to deduce approximate values of a_w corresponding to the various water contents used by Segalove and Dack. Two such comparisons have been made, the first with the vapour pressure isotherm of dried mutton reported in this paper, and the second with an isotherm for beef muscle at $-1°C$ (Scott 1936). On the basis of these isotherms the a_w of the Segalove and Dack samples with 10 per cent. water was *c.* 0.70 or 0.71, and for the 20 per cent. water contents 0.885 or 0.872. Clearly *S. aureus* would not be expected to grow at 0.70-0.71 a_w, and the conditions would be marginal at 0.87-0.88 a_w. Segalove and Dack reported no growth within 2 wk. At 30 per cent. water the deduced a_w's are 0.945 or 0.935, at which levels the retarded growth observed by Segalove and Dack would be expected. At 40, 50, and 60 per cent. water contents the deduced a_w's are 0.967, 0.979, and 0.985 respectively, the values being virtually the same for both the isotherms. At these water contents Segalove and Dack observed growth within 8 hr, a result in complete harmony with those recorded in the present paper.

Hucker and Haynes (1937) failed to inhibit the growth of two food-poisoning strains by adding up to 50 per cent. of sucrose, and up to 12 per cent. of sodium chloride, to veal broth. This result is in accord with the present experiments, as the a_w's of these media would be about 0.93 and 0.92 respectively.

Nunheimer and Fabian (1940) studied nine food-poisoning strains of *S. aureus* in broth with various added solutes. After incubation for a week at room temperature they observed inhibition by 15-17.5 per cent. (w/v) NaCl, 50-60 per cent. (w/v) sucrose, and 35-45 per cent. (w/v) glucose. The corresponding a_w's are approximately 0.905 and 0.885 for the NaCl solutions, in close agreement with the present results. On the other hand the limiting concentrations with both sugars correspond to a_w's of the order of 0.95-0.93. Nunheimer and Fabian's results with the sugars are, therefore, not consistent with the limiting a_w's they found with NaCl. Their results also disagree with the present experiments, in which growth was consistently observed at a_w's down to 0.88 when sucrose was added to nutrient broth (Table 5).

The a_w of the selective medium of Chapman (1946) has been calculated and found to be close to 0.95, about 90 per cent. of the osmotic effect being due to the sodium chloride. It is clear from the present results that selective media with an even lower a_w could safely be devised for the enrichment and isolation of food-poisoning strains of *S. aureus*. At its present concentration, however, Chapman's medium has the advantage that it may be used under conditions where considerable evaporation from petri dishes occurs in incubators at 37°C. Even after the evaporation of one-third of its water content the medium would still have an average a_w of about 0.925, a value still acceptable for *S. aureus*.

In the present experiments the logarithmic growth phase has been well defined even at a_w's down to 0.90, and there has been no suggestion that more rapidly growing mutants have appeared in any of the cultures. The results obtained when plate counts were made at various a_w's (Table 7) also indicate

that there is no need to invoke suggestions of training or selection of mutants as an explanation of growth at the lower levels of a_w. In some circumstances it appears that all cells grown at 0.999 a_w have the inherent ability to grow at a_w's at least as low as 0.92. At even lower levels of a_w there may simply be a reduced probability that a cell can reproduce successfully, such probability being affected by the endogenous resources of the cell as well as by its inherited capacity to reproduce in the environment concerned.

A feature of some interest is the occurrence of an optimum a_w close to 0.99. While the results do not explain the occurrence of this optimum it is clear that the effect is primarily osmotic, as both nutrient and non-nutrient solutes effect an increase in the growth rate when the a_w of the medium is decreased from 0.999 to 0.990. It is possible that *S. aureus* cells growing at 0.999 a_w have greater difficulty in maintaining a suitable intracellular osmotic pressure. In this connection it is pertinent to note that freezing points of unwashed cells centrifuged from nutrient broth of a_w 0.999 were of the order of $-1.7°C$. The cells therefore had an osmotic pressure some 20 times greater than the medium in which they were grown. The exact value cannot be stated as the deposited cells on which the freezing points were determined included an undetermined amount of the nutrient broth with a freezing point of $-0.1°C$. The correct determination of the osmotic pressure of the cells, the nature of the intracellular solutes in relation to a_w, and the amount of osmotic work performed by the cells in media of different a_w's are all matters which invite further study.

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