Sodium Chloride and the Growth of *Desulphovibrio desulphuricans*

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SUMMARY: The effect of sodium chloride on the multiplication of four fresh-water and three salt-water strains of sulphate-reducing bacteria was examined by determining the numbers of organisms in a known population which were viable in media of unfamiliar NaCl concentrations. The salt-water strains all produced variants viable at high NaCl concentrations; a population able to multiply in 1%–10% NaCl was obtained by 'training' one salt-water strain. They differed, however, in the frequency with which non-exacting variants appeared, ranging from a strain in which the whole population grew with 0.25% NaCl to one which would not grow with less than 0.45% NaCl even after repeated attempts at acclimatization. Replacement experiments indicated that the exigent strain required chloride ion; a less-exacting strain required sodium ion. The fresh-water strains produced few variants viable at NaCl concentrations above 3%, but one strain produced variants of the salt-water type after 'training' to grow with 4% NaCl; sodium ion was mainly responsible for the inhibition of this strain by high NaCl concentrations. We conclude that the differences among the salt- and fresh-water strains of *Desulphovibrio* do not at present justify their separation into the two species *aestuarii* and *desulphuricans*.

The sulphate-reducing bacteria are common inhabitants of marine, estuarine and fresh-water environments, and laboratory strains frequently show wide differences in tolerance of NaCl. Whether these differences are sufficient to justify a separate classification of marine and fresh-water types has been disputed. Van Delden (1903) tentatively distinguished between *Microspira* (now *Desulphovibrio*) *desulphuricans*, the fresh-water species, and *Microspira aestuarii*, the salt-water species, on the grounds that neither would multiply in a medium containing the salt concentration appropriate to the other. Gahl & Anderson (1928) found that the majority of strains obtained from oil-well water grew with 0–3% NaCl, but that minorities of exacting or salt-sensitive types existed. They inclined to the view that the exigent and sensitive strains were variants of *desulphuricans*, the prevalent species. Baars (1930) and Kluyver & Baars (1932) did not favour a separate species *aestuarii*, having shown that fresh-water strains could be 'trained' to multiply in a medium containing 3% NaCl, and that marine strains could undergo the reverse change; but Rittenberg (1941) was unable to repeat the latter observation with two marine strains. Miller (1949) showed that fresh-water strains were readily trained to grow in media containing 4% NaCl, and grew more rapidly after one passage in such a medium. As a result of tests with many strains originating from sulphur domes he recommended 1% NaCl as the salt concentration permitting fastest growth and most sulphide formation. Baars (1930) noted that NaCl at 0.6–0.9% stimulated sulphide production by fresh-water strains,

a phenomenon which he believed partly accounted for an increased H_2S production that occurred in Dutch canals when sea water was admitted. Sulphate-reducing bacteria tolerating 5% NaCl or more are well known: ZoBell & Rittenberg (1948) mentioned several instances of high salt tolerance in their review of the earlier literature; further examples are strain Canet 41 which multiplied in presence of 0–12% NaCl (Senez, 1951), and Iya & Sreenivasaya's (1945) strain which multiplied in presence of 0–13% NaCl, most rapid multiplication occurring with 6% NaCl.

Negative results in growth experiments with sulphate-reducing bacteria can sometimes be attributed to inadequate control of the redox potential of the media, particularly when small inocula are used (Grossman & Postgate, 1953*a*); difficulties of this kind might account for some of the discrepant results reported in the earlier literature. The present paper reports an examination of the salt tolerance of seven strains of sulphate-reducing bacteria (four fresh-water strains and three strains of salt-water origin) in media in which the redox potential was poised with cysteine. In order to judge whether salt tolerance resembled an 'adaptive' or 'mutational' phenomenon, the numbers of organisms of each strain which were viable at different salt concentrations were determined. The nature of the ions concerned in the salt relationships of some of these strains has been studied in greater detail by replacement studies such as have been used with the 'strong' halophiles (review by Flannery, 1956).

METHODS

Organisms. Seven strains called *Desulphovibrio desulphuricans* were used: Beckton 1, a fresh-water strain isolated from sewage sludge (National Collection of Industrial Bacteria [NCIB] 8319); Hildenborough (fresh water) from clay (NCIB 8303); Teddington R (fresh water) from Thames mud (NCIB 8312); Wandle (fresh water) from the River Wandle at Wandsworth (NCIB 8305); British Guiana (of salt-water origin) from 'sling mud' (NCIB 8403); Canet 41 (salt-water origin) from Etang de Canet, Perpignan, France (kindly given by Dr J. Senez; NCIB 8393); El Agheila Z (salt-water origin) from a sulphur-producing lake in Libya (NCIB 8380) (Adams, Butlin, Hollands & Postgate, 1951). The strains British Guiana, El Agheila Z, Hildenborough and Wandle were originally isolated by the method of Butlin, Adams & Thomas (1949) using $Na_2SO_3 \cdot 7H_2O$ (3%) to hinder the growth of contaminants; Beckton 1, Canet 41 and Teddington R were isolated without this.

Cultivation. Stocks were kept in a freeze-dried condition, but during work on a particular strain it was subcultured weekly in the lactate + yeast extract + sulphate medium C of Butlin *et al.* (1949) made up with analytical grade reagents where available. This medium contained no added NaCl, but small amounts of sodium and chloride ions were present (0.014% (w/v) Na^+ , 0.065% (w/v) Cl^- ; all percentages quoted in this paper are w/v except where otherwise stated). Cultures (usually 5 ml. in $6 \times \frac{5}{8}$ in. test tubes) were incubated at 30° in McIntosh & Fildes anaerobic jars containing $H_2 + CO_2$ (1%, v/v). For experiments requiring large quantities of organisms, medium C

was supplemented with tryptone (Oxoid, 0.5 %) and the yeast extract brought up to 0.4 %. Such cultures were grown in 2 l. flasks with a plug containing 1:1 (v/v) pyrogallol (sat.) and Na_2CO_3 (sat.) or in 400 ml. flasks in anaerobic jars; the bacteria were harvested by centrifugation after 4 days of incubation. All media were supplemented with cysteine hydrochloride (5 $\mu\text{mole/ml.}$) and a trace of ferrous ammonium sulphate (4 $\mu\text{g./ml.}$). Media for natural or induced salt-tolerant strains were supplemented with the requisite amounts of NaCl. All media and solutions to be added thereto were sterilized by autoclaving except the cysteine hydrochloride solution which was sterilized by Seitz filtration. The pH value was finally adjusted to 7.4 ± 0.2 with sterile N-NaOH.

Counting procedures. Viable counts were carried out by the method of Grossman & Postgate (1953*b*). Serial decimal dilutions (0.5 ml. $\rightarrow$ 5 ml.) of a fully grown culture were made in a mineral solution consisting of medium C without lactate, yeast extract or sulphate (supplemented with 2.5 % NaCl for salt-water strains) and 0.5 ml. samples of the dilutions were tested in quintuplicate in 4.5 ml. portions of medium C supplemented with the appropriate quantity of NaCl, cysteine hydrochloride and sufficient ferrous ammonium sulphate to indicate growth by blackening (0.05 %). In certain instances MgCl_2 or Na_2SO_4 replaced some or all of the NaCl in the test medium. By this procedure the test media contained, when finally inoculated, extra NaCl equivalent to one-in-ten dilution of the mineral solution in which the culture being tested was diluted. With fresh-water strains this error was ignored; with salt-water strains carry-over amounted to 0.25 % NaCl, and allowance was made for this in preparing the test media. Because of this carry-over, 0.25 % NaCl was the lowest salt concentration in which salt-water organisms were usually tested. The tubes were incubated until no further blackening was observed. The most probable numbers (M.P.N.) of viable bacteria in the original sample were then obtained from statistical tables (Min. of Health, 1939); counts recorded in the figures as $\log \text{M.P.N./ml.} = -0.6$ represent experiments in which no growth occurred after quintuplicate decimal (0.5 ml. $\rightarrow$ 5 ml.) dilution of the parent culture directly into the test medium; they thus signify an absolute M.P.N. of less than 2 viable organisms/5 ml. culture, the usual volume of the cultures examined during this work. In certain tests with strain British Guiana the procedure was scaled up in an attempt to detect viable salt-tolerant variants of even lower M.P.N./ml.; the decimal dilutions from the parent culture were 5 ml. $\rightarrow$ 50 ml. as well as the higher dilutions mentioned already.

Total counts were determined by diluting a culture in the appropriate mineral solution, heating to abolish motility and counting microscopically in a Thoma haemocytometer (Hawksley Ltd., London) of 0.02 mm. depth.

Lysis experiments. Organisms of 'strong' halophiles sometimes burst on transfer to distilled water, owing to entry of water by osmosis (Christian, 1956). Attempts to demonstrate osmotic bursting with certain salt-tolerant strains of *Desulphovibrio desulphuricans* were made by adding small volumes of a thick suspension of organisms to saline and to distilled water and comparing the turbidities of the suspensions at intervals. The procedure was

checked by using the halophilic organism NCIB 8668 (kindly given by Professor F. Egami) which showed an immediate decrease in turbidity in distilled water to give a final optical density 16 % the original value in 1 min.

Optical density measurements. These were carried out in the E.E.L. colorimeter (Evans Electroselenium Ltd., Harlow, Essex) calibrated with the Hildenborough strain of *Desulphovibrio desulphuricans*.

Irradiation with ultraviolet light. Samples from a fully grown culture were irradiated from a 7 W. jacketed 12 in. Hanovia UV lamp (Hanovia Ltd., Slough) emitting maximally at 253.7μ . The culture fluid was 2 mm. deep, and treated in a Petri dish 35 cm. from the radiation source.

RESULTS

Distribution of NaCl-tolerance in 'normal' populations

The numbers of bacteria viable in media of different NaCl concentrations were determined using fully grown cultures of the 7 strains of *Desulphovibrio desulphuricans*. The distribution curves obtained (Figs. 1, 2) can be divided into two main types.

Fig. 1

Fig. 2

Fig. 1. Numbers of four fresh-water strains of *Desulphovibrio desulphuricans* viable in media of various NaCl concentrations. ●, Hildenborough; ●, Wandle; ●, Teddington R; ○, Beckton 1.

Fig. 2. Numbers of three salt water strains of *Desulphovibrio desulphuricans* viable in media of various NaCl concentrations. ○, British Guiana; ●, Canet 41; ●, El Agheila Z.

Fresh-water organisms

Strains Beckton 1, Teddington R, Hildenborough and Wandle, normally grown in medium C without extra NaCl, showed a relatively small range of salt tolerance; salt-tolerant variants did not appear above 3–4 % NaCl. Strains Hildenborough and Wandle showed a wider range of salt tolerance than the other two strains, possibly because of the procedure used during their isolation (see Discussion). Strain Hildenborough showed atypical growth

in higher salt concentration (3–4 % NaCl); the bacteria were associated with the precipitate and blackening did not extend throughout the supernatant medium.

Salt-water organisms

The three organisms isolated from saline environments were normally grown in medium C + 2.5 % extra NaCl. They differed markedly from the fresh-water types in that variants tolerating high NaCl concentrations were present in normal populations. These three organisms differed on the basis of the population distributions in subnormal NaCl concentrations.

Strain British Guiana. This strain showed true halophily in the sense that none of the bacteria was viable with a low (0.4 %) NaCl concentration. When a large inoculum was used (0.5 ml. or 5 ml. parent culture) a black precipitate of ferrous sulphide often developed in the subculture, but when this was examined microscopically no bacteria could be seen adhering to the particles, and none was removed by washing the precipitate. A maximum viable count was obtained in 0.6 % NaCl, but in 0.5 % NaCl the results varied considerably from experiment to experiment and were irregular in that tubes from higher dilutions sometimes gave blackening when tubes from lower dilutions did not.

Strain Canet 41. The whole population of this strain was viable in 0.25 % NaCl. This strain showed the widest range of salt tolerance, confirming and extending the original observation of Senéz that cultures of Canet 41 grew with 0–12 % NaCl (Senéz, 1951).

Strain El Agheila Z. This strain showed an intermediate behaviour in which most of the organisms were not viable with 0.25 % NaCl, but some non-exacting variants were present; the proportion of the latter was higher in young cultures (Table 1) than in older ones.

Table 1. *Effect of age of parent culture on the viability of Desulphovibrio desulphuricans strain El Agheila Z in media of low salt concentration*

The viable populations in cultures of different ages were counted in media containing: (a) 1 % NaCl (in which a maximum viable count was obtained); (b) 0.25 % NaCl; (c) 0.25 % NaCl + MgCl_2 to Cl' concentration equivalent to (a). The figures quoted are numbers of viable bacteria/ml. original culture.

Age of parent culture (days)	1 % NaCl (0.6 % Cl')	0.25 % NaCl	0.25 % NaCl + 0.61 % MgCl_2 (total [Cl'] = 0.6 %)
3	5×10^8	3.4×10^6	1×10^8
6	5×10^8	5×10^6	7×10^7
7	5×10^8	1.4×10^4	5×10^6

Distribution of NaCl tolerance in 'trained' populations

Strain Wandle was 'trained' to tolerate NaCl by subculturing eleven times in medium C containing 4 % NaCl. The distribution of salt-tolerant variants in a population grown in this medium was then comparable with that of Canet 41, the most salt-tolerant organism examined (Fig. 3).

Strain Canet 41 was trained to high salt resistance by twelve subcultures in medium C containing 10 % NaCl. The whole population of such a culture was

then viable in NaCl concentrations ranging from 1 to 10 % and variants tolerating up to 16 % NaCl were present (Fig. 4).

Three separate attempts to train British Guiana to grow with 0.4 % NaCl were not successful. Cultures able to grow with 0.5 % or 0.45 % NaCl were obtained without much difficulty, but even after several subcultures at these salt concentrations the trained cultures would not grow with 0.4 % or less NaCl. The substrain trained to grow with 0.45 % NaCl was irradiated with u.v.-radiation for 40 sec., which gave about 1 % survival of the parent strain, but no variants able to grow with 0.3 % or less NaCl were obtained.

Fig. 3

Fig. 4

Fig. 3. Numbers of *Desulphovibrio desulphuricans* strain Wandle and of a sub-strain trained to 4 % NaCl viable in media of various NaCl concentrations. O, normal strain; ●, adapted strain.

Fig. 4. Numbers of *Desulphovibrio desulphuricans* strain Canet 41 and of a sub-strain trained to 10 % NaCl viable in media of various NaCl concentrations. O, normal strain; ●, adapted strain.

During training the cultures were morphologically unusual and multiplication tended to be restricted to the precipitate in the medium. Few typical vibrios were visible under the microscope and many sluggishly motile fat spirilloid organisms, some 50 μ . long, were seen. Morphologically aberrant forms in unfamiliar salt concentration have been reported with sulphate-reducing bacteria (Baars, 1930; Rittenberg, 1941) as with other bacteria (Flannery, 1956). The substrain acclimatized to 0.45 % NaCl became morphologically more normal, however, after five or more subcultures in this salt concentration and typical vibrios then appeared throughout the medium, though a few spirilloid forms persisted.

Ions responsible for the effects of NaCl on growth

Flannery, Doetsch & Hansen (1952) showed by replacement experiments with other salts that the 'strong' halophile *Vibrio costicola* required sodium rather than chloride ion for multiplication; Robinson & Gibbons (1952) observed that *Micrococcus denitrificans* required sodium chloride specifically

for multiplication. Replacement experiments were used to study the nature of the effects of NaCl on the growth of various strains of *Desulphovibrio desulphuricans*.

Strain Wandle. This strain was tested in media in which Na_2SO_4 or MgCl_2 replaced NaCl (Fig. 5). The distribution of salt-tolerant variants in Na_2SO_4 was similar to that in NaCl provided the concentrations were expressed in % Na^+ ; the organisms tolerated a much higher concentration of Cl^- when the cation was Mg^{++} . Hence, subject to certain provisos about the dissociation of these salts (see Discussion), the inhibitory effect of NaCl is attributable to the sodium ion; it is neither a non-specific osmotic effect nor due to chloride ion.

Fig. 5. Numbers of *Desulphovibrio desulphuricans* strain Wandle viable in media containing various concentrations of NaCl, Na_2SO_4 or MgCl_2 . ○, NaCl; ●, Na_2SO_4 ; ◐, MgCl_2 .

Strain El Agheila Z. The requirement of the majority of organisms in a 'normal' population of strain El Agheila Z was shown by a similar procedure to be for sodium rather than for chloride ion (Table 1). Na_2SO_4 , added to the medium so that the final concentration of Na^+ was equivalent to 1% NaCl, permitted the whole population to multiply; MgCl_2 , added to give an equivalent Cl^- concentration, did not do this. MgCl_2 had a partial synergistic effect with NaCl (Table 1) which was most pronounced with young cultures; it was not studied further.

The inhibitory effect of high concentrations of NaCl on strain El Agheila Z could not be attributed solely to sodium ion. A 'normal' culture contained about 26 organisms/ml. which were viable in 9% NaCl, but when media were made up with 1% NaCl + Na_2SO_4 together equivalent in Na^+ to 9% NaCl, or with 1% NaCl + MgCl_2 together equivalent in Cl^- to 9% NaCl, no viable bacteria were detected.

Strain British Guiana. The requirement of this strain for NaCl was examined in a similar manner to that used with strain El Agheila Z. A maximum

viable count was obtained with 1 % NaCl or with MgCl_2 added to the medium so that the final Cl^- concentration was equivalent to 1 % NaCl. With Na_2SO_4 added to give a final Na^+ concentration equivalent to 1 % NaCl, however, a maximum count was not obtained; the results of tests with Na_2SO_4 were very irregular and recalled the dilution counts in 0.5 % NaCl referred to earlier. Hence, in contrast to strain El Agheila Z, strain British Guiana required chloride ion rather than sodium ion for multiplication.

The inhibition of strain British Guiana by high salt concentrations could not be attributed solely either to Na^+ or Cl^- ; 2.2×10^4 organisms/ml. were viable with 6 % NaCl but only 7×10^2 /ml. grew in a medium containing 1 % NaCl + Na_2SO_4 together equivalent in Na^+ to 6 % NaCl, and only 5×10^2 /ml. grew in a medium containing 1 % NaCl + MgCl_2 together equivalent in Cl^- to 6 % NaCl. As with strain El Agheila Z, the mixed salts were more inhibitory than NaCl alone.

Attempts to observe osmotic lysis. No lysis was detected turbidimetrically when strain British Guiana, strain Wandle trained to multiply in 4 % NaCl or strain Canet 41 trained to multiply in 10 % NaCl were exposed to distilled water (0.27–0.42 mg. dry wt. bacteria/ml.) for $2\frac{1}{2}$ –3 hr. at room temperature.

Ratio of viable to total count. The procedure used in this work for estimating viable bacteria was that of Grossman & Postgate (1953*b*). These authors obtained viable counts amounting to between 20 and 70 % of the total count, the error being mainly attributable to losses during manipulation. The percentage recoveries of viable bacteria obtained in the present work were of similar orders (Table 2) with the exception of strain British Guiana, suggesting that high manipulative losses occurred with this strain.

Table 2. *Relationship between viable counts and total counts of seven strains of Desulphovibrio desulphuricans*

Strain	Viable organisms in original culture (no./ml.)	Total number of organisms visible in haemocytometer (no./ml.)	Viability (% total count)
Beckton 1	1.0×10^9	1.4×10^9	71.4
Hildenborough	1.0×10^9	1.4×10^9	71.4
Teddington R	1.0×10^9	1.6×10^9	62.5
Wandle	7.0×10^8	1.14×10^9	61.4
British Guiana	3.4×10^7	4.4×10^8	7.7
Canet 41	7.0×10^8	1.3×10^9	53.8
El Agheila Z	2.6×10^8	9.25×10^8	28.1

DISCUSSION

Nature of the effect of NaCl on growth. The experiments in which NaCl was replaced by Na_2SO_4 or MgCl_2 indicated that the requirement of strain El Agheila Z was primarily for sodium ion, and that this ion was also primarily responsible for the inhibitory effects of NaCl on the viability of strain Wandle. These strains thus resemble *Vibro costicolus* (Flannery, 1952) in that sodium ion is of primary importance in their growth. On the other hand, the require-

ment of strain British Guiana was primarily for chloride ion and not sodium ion; thus it resembled neither *V. costicolus* nor *Micrococcus denitrificans* (Robinson & Gibbons, 1952).

The significance of replacement experiments on inhibition of multiplication by strong NaCl solutions can be criticized on the grounds that, if MgCl_2 were imperfectly dissociated like CdCl_2 (strong solutions of which contain much of the ionic species CdCl^+ and hence have a chloride ion concentration lower than theoretical), then the actual chloride ion concentration in the tests reported here might be much lower than the total added chloride would indicate. This possibility was examined by plotting the curves relating the equivalent conductivity to the concentration of NaCl and MgCl_2 derived from International Critical Tables and interpolating the values giving comparable recoveries of viable organisms of strain Wandle. Even if the whole of the deviation from theoretical of MgCl_2 were attributed to an association of the type $\text{Mg}^{++} + \text{Cl}' \rightarrow \text{MgCl}^+$, the chloride ion concentration would still have been *c.* 35 % greater than in the equivalent NaCl solution. The effect of sodium ion on the growth of strains Wandle and El Agheila Z emphasizes the fact, mentioned by Butlin *et al.* (1949), that 3 % $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ should not be used to suppress contaminants if obligate non-halophilic strains of *Desulphovibrio desulphuricans* are being isolated, because a 3 % solution of $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ has a Na^+ concentration equivalent to 1.4 % NaCl. Strains Hildenborough and Wandle were isolated by this procedure and this may account for their higher salt tolerance compared with strains Teddington R and Beckton 1; strain El Agheila Z was also treated with 3 % $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ (thus passing through a medium of Na^+ concentration equivalent to 3.9 % NaCl) which may have accentuated its tendency to halophily. All media used in the present work, even when not supplemented with NaCl, contained low concentrations of Na^+ and Cl' ; for this reason experiments similar to those of Ware, Childs & Smith (1955) on faecal organisms, to determine the effects of very low concentrations of NaCl on growth, were not carried out.

Most of the strains examined in this work, whether of salt- or fresh-water origin, gave maximum viabilities in 1 % NaCl, supporting Miller's (1949) contention that 1 % NaCl is most satisfactory for growth of both types of sulphate-reducing bacteria. However, strain Beckton 1 was an exception and only a small proportion of the population was viable in 1 % NaCl. In spite of Gahl & Anderson's (1928) demonstration of salt-sensitive strains in oil-well waters, media supplemented with 1 % NaCl have been recommended as 'general purpose' media for the growth of sulphate-reducing bacteria. The existence of very salt-sensitive strains such as strain Beckton 1 confirms that such media should be used cautiously.

Halophily and nomenclature in Desulphovibrio. The distribution patterns of salt tolerance in the strains studied here ranged from the strict NaCl requirements of strain British Guiana, through the partial NaCl requirement of strain El Agheila Z and the wide salt tolerance of strain Canet 41, to the comparative salt-sensitivity of the fresh-water strains. Thus there seems to be little difference of kind between the salt-water and fresh-water types: they

differ mainly in the number of variants produced which are able to tolerate particularly high or low salt concentrations. Whether these variants should properly be called mutants depends more on one's definition of the term mutant than on the experimental data presented here, for there is no obvious qualitative difference between the few variants of strain Canet 41 which are able to grow in 10 % NaCl (c. 1 organism in 10^8), which one would normally call mutants and the many able to grow with 6 % NaCl (c. 1 organism in 100) which are far more plentiful than classical mutants. The procedure used in this work would not have detected variants if their proportion was below c. 1 in 10^9 ordinary organisms, and it may well be that strain British Guiana is an extreme form of the El Agheila Z type, capable in principle of producing non-exacting variants, but at so low a frequency that the methods used here did not detect them. These strains differed in the character of their sodium chloride requirements, one requiring primarily sodium ion and the other requiring primarily chloride ion, and it is not unreasonable that the frequency with which 'chloride-less' variants arise should differ from the frequency with which 'sodium-less' variants occur.

For similar reasons the question which of these strains should properly be termed 'halophilic' has, like the mutant question, a semantic flavour. None of them, not even strain Canet 41 after training to multiply in 10 % NaCl, showed the osmotic lysis in distilled water characteristic of the more exacting halophiles of other genera studied by Christian (1956). Yet because it required some NaCl for growth, strain British Guiana might reasonably be called a halophile, but strain Canet 41 should then be termed a facultative halophile in spite of its marine origin. Strain El Agheila Z is intermediate in behaviour but is still a facultative halophile since a proportion of the population grew without added NaCl.

ZoBell, in *Bergey's Manual* (1948), decided to retain the specific name *aestuarii* for salt-requiring members of the genus *Desulphovibrio* because of Rittenberg's exception to Baars's report that marine strains could be trained to grow without NaCl. In the present work strains Canet 41 and El Agheila Z conformed to Baars's observations, but we were unable to 'train' strain British Guiana to grow without extra NaCl; the behaviour of this strain is thus comparable with the two studied by Rittenberg (1941) and reported by ZoBell & Rittenberg (1948). The apparent divergence between the results of Rittenberg and those of Baars can be reconciled if Rittenberg be regarded as having selected accidentally two strains of British Guiana type. Baars regularly added H_2S water to his cultures at inoculation, but Rittenberg's media did not include a chemical reducing agent, and it is therefore possible that, even had his strains been of the El Agheila Z type (c. 0.1 % of the population viable without extra NaCl) he would have failed to 'train' them. In our laboratory, strain El Agheila Z was believed to be an exacting halophile until the present work was undertaken; it now seems clear that the failure of this strain to grow in unsupplemented media, a failure which contrasted with the behaviour of strain Canet 41, was because the customary 1/100 inoculum was effectively 1/ 10^5 in terms of organisms viable without added salt. An

inoculum of $1/10^5$ is unlikely to grow without added reducing agent (Grossman & Postgate, 1953a).

The present work has shown a complete gradation of behaviour towards NaCl within the genus *Desulphovibrio*, ranging from very salt-sensitive types to salt-requiring types; at no point in this gradation is the difference from the neighbouring type large. The question arises whether the use of the specific name *aestuarii* for salt-requiring strains is justified at all. Certainly the name cannot be applied automatically to strains of marine origin since these may be of the Canet 41 or El Agheila Z types, which are clearly facultatively halophilic strains of *D. desulphuricans*, resembling the acclimatized sub-strain of Wandle. It could be argued that the name *D. aestuarii* might be retained, but be restricted to salt-water strains of the British Guiana type. The present authors incline to the opinion that the differences so far established between this strain and the others, i.e. requirement for Cl^- rather than Na^+ , and low rate of production of non-exacting variants, are hardly sufficient to justify separate species status unless supported by further morphological and biochemical differences. These are not yet apparent.

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