OCCURRENCE OF SULPHATE REDUCING BACTERIA IN THE HUMAN INTESTINAL FLORA AND IN THE AQUATIC ENVIRONMENT

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Abstract—The occurrence of sulphate reducing bacteria at high levels in various types of water suggests their faecal origin. This prompted an examination of faecal specimens for sulphate reducing bacteria. In a random choice of the same samples *E. coli* was also enumerated.

Sulphate reducing bacteria were found at a mean rate of $10^6/100$ g in faeces, $10^6/100$ ml in crude sewage, $10^5/100$ ml in river water and about $10^2/100$ ml in drinking water. Distribution curves of sulphate reducing bacteria and *E. coli* were found to be rather similar in some types of water. The ubiquitous character of sulphate reducing bacteria, their high resistance to extraenteric conditions and the rather complicated techniques required for their detection make them nevertheless less appropriate as indicator organisms in practical monitoring work.

INTRODUCTION

The presence of sulphate reducing bacteria is as a rule demonstrated by their capacity to reduce sulphate to sulphide. Although they belong to various groups (Butlin & Postgate, 1956) the most frequently occurring types are (Postgate & Campbell, 1966): 1. *Desulfovibrio*: vibrioform non-sporing cells, motile by polar flagellae, with a high GC% (55–60) and possessing a cytochrome C_3 and a characteristic pigment, desulfoviridine, as electron carrier. 2. *Desulfatomaculum*: rod-like sporing cells, motile by peritrichous flagellae, with a low GC% (41–45) and having a cytochrome *b*.

Desulfovibrio, was first isolated by Beijerinck (1895) and given the name *Spirillum desulfuricans*. This was later changed to *Desulfovibrio* by Kluyver & Van Niel (1936). These bacteria were subsequently isolated from many terrestrial and aquatic biotopes. Their detection in sewage and in river water samples at cfu/ml values which were similar to those of bacteria of faecal origin prompted to consider them also to be of intestinal origin (Marez, Tellier & Leclerc, 1971). *Desulfatomaculum* was originally named *Sporovibrio* (Prevot *et al.*, 1967). At present it includes three species: *D. orientis*, *D. ruminis* and *D. nigrificans*.

This study aims to assess the occurrence of sulphate reducing bacteria in water samples with varying levels of pollution and in human faeces.

MATERIALS AND METHODS

A total of 609 samples of water, sludge and human stools were examined for sulphate reducing bacteria and a random choice of 325 also for *E. coli*. The distribution of samples was as follows:

220 samples of tap water, 34 being chlorinated and 186 not;

45 samples of river water, varying in degree of pollution;

61 samples of influent, 30 samples of primary sewage effluent, i.e. after decantation and 65 samples of secondary sewage effluent, i.e. following activated sludge treatment;

45 samples of activated sludge drawn from aeration tanks;

143 stool samples of hospitalized patients not being under antibiotic therapy.

Sulphate reducing bacteria were enumerated by an MPN procedure based on Postgate's method (1969). Three tubes per dilution were used for the examination of water and one tube per dilution when examining faecal specimens. Postgate's *B* medium was used for enrichment. It was modified by omitting yeast extract because this gave falsely positive results when examining stools. Presumptive presence of sulphate reducing bacteria was concluded from blackening of the medium after anaerobic incubation. Their presence was confirmed by microscopic examination of such cultures for vibrioform bacteria and sometimes, in stools, for straight rods.

Numbers of cfu/ml of *E. coli* were determined by the elevated temperature (44°C) technique of Buttiaux (1958). A most probable number method relying on Mackenzie, Taylor & Gilbert's (1948) was used for sewage and sludge. A membrane filtration technique with subsequent culturing of the filters on Chapman's medium (1947) incubated at 44°C was used for drinking waters.

RESULTS

Human faeces

A total of 143 samples of human faeces was studied. Among these 121 (85%) contained sulphate reducing bacteria. The quantitative data varied widely, as is

Table 1. Occurrence of sulphate reducing bacteria in various materials

	% of positive samples	Geometric mean of $\log_{10}$ of $(N + 1)$ SRB*	Standard deviation	Dispersion factor	Confidence interval	
					5%	1%
Chlorinated tap water	32	0.51	0.85	1.65	0.287	0.378
Tap water	51	1.09	1.32	1.20	0.19	0.249
River water	100	5.18	1.22	0.23	0.356	0.468
Secondary sewage effluent	97	5.77	1.5	0.26	0.368	0.484
Primary sewage effluent	100	6.72	0.65	0.09	0.24	0.329
Crude sewage	100	6.49	1.1	0.17	0.278	0.366
Sludge	100	7.35	0.99	0.13	0.291	0.383
Stool	85	4.69	3.02	0.64	0.495	0.65

* SRB: Sulphate reducing bacteria per 100 ml water or 1 g of stool.

Table 2. Distribution (%) of counts of sulphate reducers

Counts of SRB*	0	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}	10^{11}
Chlorinated tap water	67	3	15	15									
Tap water	49	1	22	18	5	3.5	1	0.5					
River water				2	6	33	29	9	20				
Secondary sewage effluent	3	0	0	3	1.5	11	34	29	17	1.5			
Primary sewage effluent							20	50	26	4			
Crude sewage						5	18	54	18	5			
Sludge							13	29	22	29	6		
Stool	15	0	1.5	12	8	8.5	6	14	17	6	6	0.5	3.5

* SRB: Sulphate reducing bacteria per 100 ml water or 1 g of stool.

shown in Tables 1 and 2 and Fig. 1. The mean counts were $5 \times 10^4 \, g^{-1}$.

E. coli was always found in numbers between 10^7 and $10^8 \, g^{-1}$.
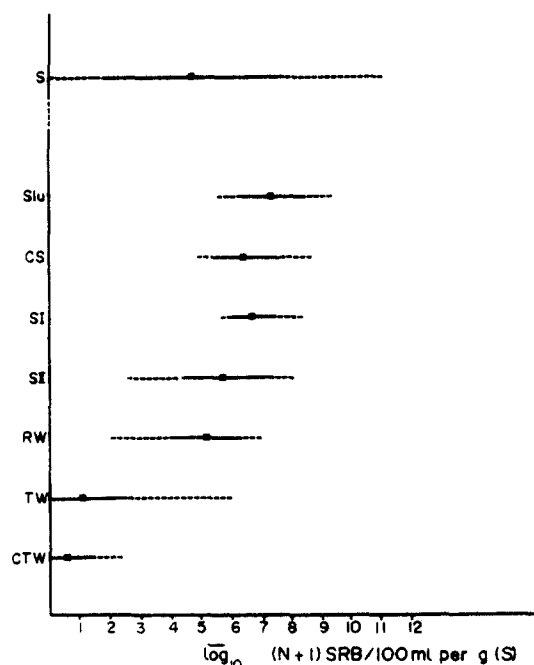

Fig. 1. Sulphate reducing bacteria in water and faeces. Counts per 100 ml water or 1 g of stool: SRB—Sulphate reducing bacteria; S—Stool; Slu—Sludge; CS—Crude sewage; SI—Primary sewage effluent (after decantation); SII Secondary sewage effluent (after activated sludge treatment); RW—River water; TW—Tap water; CTW—Chlorinated tap water.

Sewage, river water and sludge

Sulphate reducing bacteria were found to be usually associated with sewage and river water. In sewage their numbers greatly varied, i.e. from 10^3 to 10^{10}/100 ml, the mean value being 3.1×10^6/100 ml. These numbers are not greatly changed as a result of primary treatment (decantation), but a slight decrease occurs during aeration treatment. More than 50 years ago, Hotchkiss (1924) in the United States found average numbers of 7.3×10^4 sulphate reducing bacteria/ 100 ml for influent, 9×10^4 after digestive chamber treatment and 1×10^4 in final effluent. These levels are about a hundred times lower than ours. It may well be that these differences result from the use of less advanced analytical techniques by the earlier author and from differences in sewage composition and treatment.

For *E. coli* the mean count was 1.3×10^7/100 ml in crude sewage as well as after decantation, and only 1.2×10^6/100 ml in secondary sewage effluent.

In the case of river water two maxima of the distribution curve are observed: one at 10^4–10^6, the other between 10^7 and 10^8/100 ml. The first level is that of river water down stream of a waste water treatment plant. The second is characteristic of strongly polluted water of very low Eh. In this niche sulphate reducing bacteria occur in higher numbers than in sewage. Numbers of *E. Coli* are more uniformly distributed than those of sulphate reducing bacteria, the mean value being 2.8×10^4/100 ml.

The highest levels were found in sludge, which concentrates all bacteria of aquatic origin. The distribution curve of sulphate reducing bacteria shows two
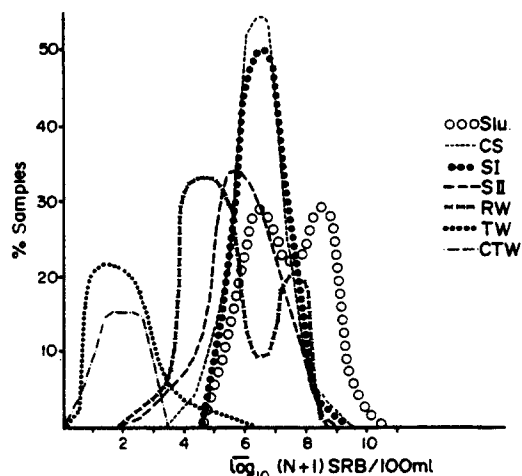
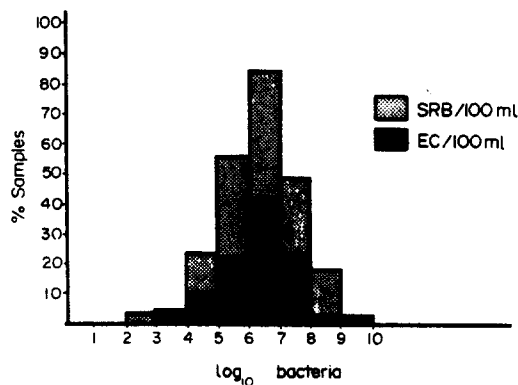Fig. 2. Sulphate reducing bacteria in water. Enumeration in 100 ml water.

Fig. 3. Sulphate reducing bacteria and *E. coli* in water (sewage and river samples). Logarithm of the number of bacteria in 100 ml of water: SRB—Sulphate reducing bacteria; EC—*E. coli*.

peaks, respectively situated between 10^6–10^7 and 10^8–10^9/100 ml. *E. coli* occurred in numbers in the bracket 10^7–10^8/100 ml.

Drinking water

Sulphate reducing bacteria occur only sporadically in drinking water. Counts are approximately 10^2/100 ml for chlorinated as well as untreated waters.

E. coli was encountered occasionally in untreated waters with a mean value of 3.7/100 ml. It was never isolated from chlorinated waters.

DISCUSSION

Since their discovery by Beijerinck (1895), sulphate reducing bacteria were isolated from various aquatic biotopes. Their occurrence in high numbers in strongly polluted river water and in sewage suggests their faecal origin. As shown by the data in Figs 2 and 3 numbers of sulphate reducing bacteria increase in the sequence drinking water to sewage, like those of *E. coli*.

However, as shown by the calculations made in Table 3, in stool sulphate reducing bacteria are greatly outnumbered by *E. coli*. This was substan-

tiated by the microscopic examination of our enrichment cultures. Even the highest dilutions of stools always revealed a mixed Gram negative and Gram positive bacterial flora as expected (Mossel, 1959). In similar cultures of contaminated waters vibrioform bacteria dominated, even to the extent of often occurring virtually in pure cultures.

These observations point to a high resistance of sulphate reducing bacteria to unfavourable physical and chemical factors in water. In strongly reduced aqueous environments they may even grow, explaining the observed variations of their fate and that of *E. coli* in some types of water. However, in drinking water sulphate reducing bacteria persist in significant quantities even after chlorination, when *E. coli* is conspicuously absent.

Despite a certain parallelism in behaviour, sulphate reducing bacteria seem yet less suitable as indicator organisms for faecal contamination. Ecological objections to their use as indicators are their ubiquity (Soimajärvi et al., 1978) the capacity to increase in numbers in extraenteric environments under anaerobic conditions and their high resistance to water purification procedures. In addition there are reasons of analytical nature which make sulphate reducing bacteria less appropriate as indicator organisms. Techniques for their detection are not only far more com-

Table 3. Sulphate reducing bacteria and *E. coli* in various materials

| | Geometric mean number of (N + 1) | | $\log_{10} \dfrac{SRB}{EC}$ |
	SRB*	EC†	
Chlorinated tap water	3.28	1	0.5158
Tap water	12.52	3.7	0.532
River water	1.5×10^5	0.28×10^5	0.7312
Secondary sewage effluent	0.6×10^6	1.2×10^6	−0.343
Primary sewage effluent	5.3×10^6	9.4×10^6	−0.248
Crude sewage	0.3×10^7	1.3×10^7	−0.625
Sludge	2.3×10^7	2.2×10^7	0.0086
Stool	5×10^4	5×10^7	−3

* SRB: Sulphate reducing bacteria.
† EC: *E. coli*.

plicated than those currently used for other indicator or index organisms (Mossel, 1978) but also much more time-consuming. Sulphate reducing bacteria share this unsuitability as indicator organisms with other obligately anaerobic bacteria of enteric origin (*Bifidobacterium* and *Bacteroïdes* spp.), although for entirely different reasons, the latter showing virtually no extraenteric persistence (Mossel, 1959).

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