SARCINA VENTRICULI IN HUMAN FAECES

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SARCINA VENTRICULI was first observed by Goodsir (1842) in the stomach contents of a patient with gastric fermentation. The organism was subsequently reported in similar cases, but it was not isolated in pure culture from the human stomach until 1911, when Beijerinck showed that *S. ventriculi* could be grown only by using strictly anaerobic techniques; he had previously shown that the same organism occurred widely in the soil (Beijerinck, 1905).

Although *S. ventriculi* has been found frequently in the gastric contents and faeces of patients with gastro-intestinal disorders (Smit, 1933), it has never been reported as present in the faeces of healthy human adults. However, during a survey in which faeces from healthy adults in Britain, the United States, Uganda and South India were examined (Aries *et al.*, 1969), *S. ventriculi* has been found in the faeces of some persons.

This paper describes the techniques used to isolate sarcinae and reports the distribution of the organism in human faeces.

MATERIALS AND METHODS

Source of faeces. Specimens of freshly voided faeces were collected from 106 people living on principally vegetarian diets and from 123 people living on mixed diets containing animal and vegetable foods. Each group contained men and women and all except one 8-yr-old Sudanese girl were adults.

Transport of faeces. Specimens of freshly voided faeces were transported to the Wright-Fleming Institute frozen in glycerol broth (Drasar, Shiner and McLeod, 1969) from all sources except the London vegetarians in which case the faeces were sent by post and frozen in glycerol broth immediately on arrival.

Isolation of sarcinae. *Sarcina ventriculi* is difficult to maintain alive in pure culture. However, it is known to form heat-resistant spores (Knöll, 1965; Canale-Parola, 1970) and on primary isolation from soil and mud it is relatively resistant to acid and to heat at 60°C (Smit); these properties provided the basis for selective isolation methods.

The faecal suspensions were thawed, serially diluted in Brain Heart Infusion (Oxoid) and heated for 10 min. in a waterbath at 70°C; 0.1 ml of each dilution was spread on freshly poured meat infusion agar containing lactose, neutral red and egg-yolk (Willis and Hobbs' medium without neomycin), as described by Willis and Hobbs (1958), and incubated overnight at 37°C in an atmosphere of 90 per cent. hydrogen and 10 per cent. carbon dioxide. Stainless steel milking-machine pails with vacuum-tight lids (Fullwood Bland and Co.) fitted with vacuum taps and cold catalysts ("D" catalyst, Engelhard Industries) as described by Schaedler, Dubos and Costello (1965) were used in place of conventional anaerobic jars. The heating destroyed all non-sporing organisms. Colonies of sarcinae could readily be distinguished from those of clostridia and aerobic spore-bearing bacilli. Sarcinae formed

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pale yellow colonies 2–4 mm in diameter, and were usually surrounded by a yellow halo in the medium. Colonies were examined microscopically and subcultured aerobically and anaerobically.

Sarcinae were also isolated by plating either unheated or heated dilutions on freshly poured Tomato Juice Agar (Oxoid) adjusted to pH 7.0 and incubated anaerobically (Crecelius and Rettger, 1943).

Malt extract enrichment cultures designed to isolate *S. ventriculi* from the soil (Canale-Parola and Wolfe, 1960) were adjusted to several pH values over the range 2.3–6.0, inoculated with faeces and incubated anaerobically. Cultures were examined daily for sarcinae by plating on Willis and Hobbs' medium and incubating anaerobically.

Maintenance of strains for biochemical tests. Cultures of sarcinae isolated from faeces were maintained at +3°C in Robertson's cooked meat broth (Southern Group Laboratories) to which 1 per cent. of glucose had been added before they were autoclaved at 115°C for 20 min. The medium did not require deoxygenation before inoculation. Cultures were grown overnight at 37°C and stored at +3°C. With this technique, strains have remained viable for 6 mth without subculture.

A culture of *S. ventriculi* (HU1) originally isolated from soil (Stephenson and Dawes, 1970) was obtained from Professor E. A. Dawes, Department of Biochemistry, University of Hull, and maintained as described above.

Biochemical tests

Detection of cellulose. Two methods were used to detect cellulose in overnight cultures in basal medium containing 2 per cent. glucose. The bacterial cells were boiled in 1 per cent. sodium hydroxide, washed with water and suspended in Schultze's stain for cellulose (Canale-Parola, Borasky and Wolfe, 1961). The cellulose stained reddish-purple. Cells from each overnight culture were suspended in buffer at pH 4.0 containing cellulase (British Drug Houses) and incubated overnight at 37°C. The cultures were examined microscopically for disruption of the double tetrad packets into smaller clusters and single cells. Suspensions without cellulase were used as controls.

Fermentation tests. The fermentation tests were modifications of those described by Cato *et al.* (1969).

2.0 g of the test carbohydrate was added to a 100-ml volume of basal medium containing: 2.0 g peptone, 1.0 g yeast extract, 0.05 g cysteine hydrochloride, 0.001 g CaCl₂, 0.001 g MgSO₄, 0.005 g K₂HPO₄, 0.005 g KH₂PO₄, 0.05 g NaHCO₃ and 0.01 g NaCl. The media were adjusted to pH 7.1 ± 0.1 with 8N-NaOH or 5N-HCl and autoclaved at 115°C for 20 min. They did not require deoxygenation before inoculation. Two drops of an overnight culture in Robertson's cooked meat broth containing 1 per cent. glucose were used as the inoculum. All test cultures were incubated anaerobically.

Gas-liquid chromatography. Volatile products of glucose metabolism (ethanol and acetic and butyric acids) were detected by modifications of the methods described by Cato *et al.*

4 ml of an overnight culture in basal medium containing 2.0 per cent. glucose were acidified with 0.5 ml 50 per cent. aqueous sulphuric acid and extracted with 4 ml diethyl ether. The mixture was centrifuged to break the emulsion and the ether layer was pipetted off and dried over magnesium sulphate. 1.0 µl was injected on to a column containing 10 per cent. polyethylene glycol on phosphoric acid-treated diatomite contained in a "104" Series chromatograph (Pye Instruments Ltd, Cambridge). A flame-ionisation detector was used. Reference solutions of ethanol and volatile fatty acids were prepared as described by Cato *et al.*

Nitrate reduction. 2.0 per cent. glucose was added to Nitrate Broth (Difco) and autoclaved at 115°C for 20 min. Cultures were incubated anaerobically at 37°C. Nitrate and nitrite were detected by conventional procedures (Cowan and Steel, 1965).

Gelatin liquefaction. Charcoal gelatin disks (Oxoid) were added to Robertson's cooked meat broth containing 1 per cent. glucose; the test organism was inoculated and the culture

was incubated at 37°C for 14 days. Liquefaction was indicated by the appearance of free charcoal particles in the medium.

RESULTS

Sarcinae were readily isolated on either tomato juice agar or Willis and Hobbs' medium from faeces of people living on vegetarian diets. Tomato juice agar was the more selective for unheated specimens, but the heating of

FIG. 1.—Comparison of methods of enumeration of viable *Sarcina ventriculi* in seven specimens of human faeces.

suspensions before they were plated on Willis and Hobbs' medium avoided overgrowth of the sarcinae by non-sporing organisms without consistently decreasing the viable counts of sarcinae (fig. 1).

It was not found possible to increase the yield of sarcinae from faeces by the use of the acid enrichment media described by Canale-Parola and Wolfe for the isolation of *S. ventriculi* from soil. Although cultivation at pH 4.0 was highly selective, many strains of sarcinae were inhibited. At higher pH values the sarcinae were often outgrown by other organisms.

Distribution of sarcinae in human faeces

The simple heating method with subculture on Willis and Hobbs' medium was used to count sarcinae in faeces from healthy adults living on different

TABLE I
The occurrence of anaerobic sarcinae in faeces from healthy adults on vegetarian and on mixed diets

Population sampled	Diet of population sampled	Number of specimens	
		cultivated	found to contain viable sarcinae
Ugandans living in and around Kampala	Vegetarian	37	31
Indians living in and around Vellore	Vegetarian	51	38
Strict vegetarians living in London	Strict vegetarian	18	6
London students and laboratory staff	Mixed	55	0
Edinburgh students and laboratory staff		23	0
English immigrants in Kampala		16	0
Caucasian laboratory staff in Atlanta		16	1
Hospital staff in Khartoum		13	1*

* Eight-year-old girl.

FIG. 2.—Numbers of sarcinae found in faeces of carriers. One dot represents the $\log_{10}$ viable count of sarcinae in the faeces of one person. The people lived in: (a) Kampala; (b) Vellore, South India; (c) London (these people ate strictly vegetarian diets); (d) Atlanta; (e) Khartoum. The sole carrier from Khartoum was an 8-yr-old girl.

diets in various parts of the world. Sarcinae were isolated from 75 out of 106 people living on principally vegetarian or strict vegetarian diets, but from only 2 out of 123 people eating mixed diets containing animal and vegetable foods

TABLE II

Comparison of six faecal strains of Sarcina ventriculi with S. ventriculi strain HU1 isolated from soil

Test	Result of test* with strains of <i>S. ventriculi</i> derived from				Results with strain HU1 derived from soil
	Uganda (1) India (1)	Uganda (1) India (1)	Uganda (1)	England (1)	
Aerobic growth	—	—	—	—	—
Growth without carbo- hydrate	—	—	—	—	—
Fermentations at 30 days:					
glucose	+	+	+	+	+
fructose	+	+	+	+	+
sucrose	+	+	+	+	+
maltose	+	+	+	+	+
lactose	+	+	+	+	+
galactose	+	+	+	+	+
raffinose	+	+	+	+	+
arabinose	—	—	—	—	—
dulcitol	—	—	—	—	—
glycerol	—	—	—	—	—
starch	—	—	—	—	—
salicin	—	+	(+)	(+)	—
mannitol	—	—	—	(+)	—
inulin	—	—	+	—	(+)
xylose	—	—	—	(+)	—
Production of:					
ethanol from glucose	+	+	+	+	+
butyric acid from glucose	—	—	—	—	—
extracellular cellulose	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Litmus milk	(AC)	(AC)	(AC)	(AC)	AC
Gelatin liquefaction	—	—	—	—	—

* + = Positive; — = negative; (+) = positive after 7 days; AC = acid clot; (AC) = acid clot after 7 days.

(table I). In the carriers, the counts of sarcinae ranged from 10^2 to 10^8 per g faeces (fig. 2). Counts of 10^6 and more were frequently found and this indicates that sarcinae were often as numerous as coliform organisms, streptococci and lactobacilli in faeces.

Identification of sarcinae

Sarcinae are now defined as obligately anaerobic, Gram-positive cocci forming cubical packets of eight (Kocur and Martinec, 1965; Canale-Parola, Mandel and Kupfer, 1967). Only two species capable of utilising sugars are recorded, *Sarcina maxima* and *S. ventriculi* (Breed and Smit, 1957). The main differences between them are that *S. maxima* has no extracellular cellulose and

produces butyric acid from glucose, whilst *S. ventriculi* has extracellular cellulose and produces ethanol and not butyric acid from glucose.

Six strains of sarcinae, three from Ugandan, two from Indian and one from English faeces, were compared with a known strain of *S. ventriculi*. No type culture of *S. maxima* was available. All the strains were obligate anaerobes, formed cellulose and required a carbohydrate for growth; all produced ethanol but not butyric acid from glucose (table II). All strains produced acid and gas from glucose, fructose, sucrose, maltose, lactose, galactose and raffinose; none could utilise arabinose, dulcitol, glycerol or starch; salicin, mannitol, inulin and xylose were utilised by some strains, but growth was usually late. All strains, including the known culture of *S. ventriculi*, reduced nitrate to nitrite, which was then further reduced.

Although some strain-to-strain variations in biochemical reactions were found, the results show that the sarcinae isolated from human faeces were *S. ventriculi*.

DISCUSSION

The results of the present study indicate that *Sarcina ventriculi* occurs frequently in the faeces of healthy human adults living in the tropics, but only rarely in faeces of people in temperate countries. However, none of the English immigrants in the Kampala series carried sarcinae whereas these organisms were carried by many of the Ugandans. The diet of the English immigrants was mixed and included animal meat whereas the principal item of the Ugandan diet was boiled bananas; furthermore, in London, sarcinae were found only in those English persons eating strictly vegetarian diets. This suggests, therefore, that diet and not environment is a major factor controlling the distribution of *S. ventriculi* in man.

S. ventriculi is widespread in the soil (Smit, 1933) and it seems inevitable that it is ingested as a contaminant on food. Since it occurs in faeces in numbers equal to those reported for coliforms, it seems likely that it multiplies, presumably in the large intestine, as the stomach and small intestine are rarely colonised by bacteria (Drasar *et al.*, 1969). It is not known whether vegetarian foods stimulate the growth or whether animal foods inhibit the growth of the sarcinae. However, it seems likely that the addition of animal foods to the diet leads to an increase in the numbers and possibly a change in the types of the non-sporing anaerobes such as *Bacteroides* species and bifidobacteria (Aries *et al.*, 1969). It is possible that some metabolites of these anaerobes, particularly organic acids, could inhibit the sarcinae. The short-chain fatty acids are known to inhibit *S. ventriculi* (Smit).

It is interesting that the known culture of *S. ventriculi* and the six strains isolated from faeces all reduced nitrate to nitrite which was then further reduced. Previous workers have not reported this reduction of nitrate, perhaps because, having shown that nitrite was absent, they omitted to test for residual nitrate.

Although *S. ventriculi* was often observed during the last century in the stomach contents of patients with gastric disorders, similar cases have been

reported only rarely since. It is therefore interesting that Professor E. A. Dawes (personal communication), during the period 1959–60, found *S. ventriculi* in the stomach contents of patients in Glasgow with symptoms similar to those of the patient reported by Goodsir (1842).

It is suggested that *S. ventriculi* should now be considered to be part of the intestinal bacterial flora of man, but its significance in the intestine is not known.

SUMMARY

Anaerobic sarcinae were quantitatively isolated from suspensions of faeces heated at 70°C for 10 min. by plating on meat infusion agar containing lactose, neutral red and egg-yolk and incubating anaerobically at 37°C. *Sarcina ventriculi* was found in numbers up to 10⁸ per g in faeces from 75 out of 106 healthy human adults living on vegetarian diets, but the organism occurred in the faeces of only 2 out of 123 people living on diets containing animal products. The identification and distribution of the organism are discussed and it is concluded that diet influences the colonisation of sarcinae in the human intestine.

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