FERMENTATION STUDIES ON *SHIGELLA SONNEI*

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IN studies on the epidemiology of Sonne dysentery I have used colicine production as a marker of *Shigella sonnei*. During 1959–62, strains of *Sh. sonnei* that were untypable, i.e. that did not produce colicines active against any of the 15 indicator strains, amounted to only 8·1 per cent. of those examined (Gillies, 1964). More recently, the proportion of untypable strains in the Edinburgh area has risen rapidly; in 1963, 79·5 per cent. of isolates could not be typed, and this figure has risen to 92·3 per cent. in the first 8 mth of 1964 (Gillies, 1965). Tests with many untypable strains against several hundred fresh potential indicator strains revealed no colicine production, and I have sought other means of typing strains that do not produce colicine.

Bacteriophage typing of Sonne strains has been found to be unreliable as an epidemiological marker because of evidence of type instability both *in vitro* and *in vivo* (Mayr-Harting, 1952; Tee, 1955).

An attempt to characterise *Sh. sonnei* strains by their fermentative activities was reported by Bojlén (1934, p. 30) who recognised four biochemical types in tests with maltose and xylose. However, Hammarström (1949) considered that reliance on the utilisation of maltose was not a satisfactory criterion; by employing additional substrates he found that different strains of the same phage type were, in general, identical in their biochemical reactions.

This paper records my attempts to subdivide by biochemical tests strains of *Sh. sonnei* that were identical in being unable to produce detectable colicines.

MATERIALS AND METHODS

Strains. A total of 487 strains of *Shigella sonnei* was examined. These had been isolated from cases of bacillary dysentery and identified biochemically in composite media (Gillies, 1956) and then serologically using antisera supplied by the Standards Laboratory for Serological Reagents, Colindale Avenue, London. Of this total, 422 strains (untypable) did not produce colicines and 65 strains belonged to 8 different colicine types; the colicine type and the number of strains of each type were as follows: type 7, 29 strains; type 4, 8 strains; type 11, 8 strains; type 2, 7 strains; type 3, 7 strains; types 6, 1A and 3A, each 2 strains.

Media. The substrates xylose, raffinose and melibiose were prepared as 1 per cent. solutions in peptone water according to Cruickshank *et al.* (1960, p. 200) and distributed in 5 ml. amounts in ¼ oz. (7 ml.) screw-capped bottles. Inoculation was with a standard loop made from nichrome resistance wire SWG no. 24 with an internal loop diameter of 2 mm.; this was loaded from an overnight culture grown at 37° C on nutrient agar (Cruickshank *et al.*, p. 195) and thorough emulsification of the bacterial suspension in the relevant substrate was ensured by rotating the loop in the sugar medium for 1 min. After inoculation, incubation was maintained at 37° C for 21 days and the cultures examined every 24 hr for evidence of acid production.

RESULTS

Without exception, all 487 strains of *Shigella sonnei* fermented glucose and mannitol anaerogenically within 24 hr, but did not attack sucrose or salicin in this time and did not produce urease, indole or H_2S; all of these tests were made on composite media. The activities of the strains on xylose, raffinose and melibiose are shown in table I.

I first examined the 65 strains that had been typed by their production of colicines. All of these were in groups of replicate isolates obtained from the same

patients or in groups of isolates obtained from different patients in the same epidemic foci. Typical results are shown in table II.

The fermentation reactions of strains of colicine types 3 and 7 show no difference between these types, which differ so markedly in their production of colicines.

TABLE I

Fermentation reactions of 487 strains of Shigella sonnei

Sugars fermented within 21 days			No. of strains fermenting sugars indicated by +
Xylose	Raffinose	Melibiose	
+	−	−	6
+	+	−	1
+	−	+	11
+	+	+	9
−	+	−	22
−	+	+	280
−	−	+	152
−	−	−	6

The variation in the number of days required for the production of acid was no greater between the type-7 strains from patient A and the type-3 strains from patient B than it was between the different strains isolated from the same patient.

Similarly the fermentation reactions of 28 strains from 10 epidemics were analysed and it was found that in each epidemic, regardless of the fact that there was uniformity of colicine type among the strains, the substrates attacked and the time at which acid production was first noted bore no relation to the epidemiological circumstances.

TABLE II

Colicine type and fermentation reactions of 11 strains of Sh. sonnei isolated from 3 patients

Patient	Colicine type of strains	No. of times isolated	Fermentation reactions of replicate isolates *
A	7	3	M5 R4, M3 R6, M4 R7
B	3	3	M3 R11, M3 R7, M3 R3
C	2	5	M2, M4, M3 R4, M2, R3

* M = melibiose; R = raffinose. The number following the letter indicating the substrate shows the day after inoculation on which fermentation was noted.

In spite of the above evidence, the 422 strains that were untypable by colicine production were examined for activity against the three substrates, but again, when the results were analysed in regard to replicate isolation from individuals or the grouping of strains in epidemics, whether in families or institutions, there was no relation between these circumstances and the biochemical findings.

DISCUSSION

The increasing proportion of strains of *Shigella sonnei* that could not be typed on the basis of colicine production led me to examine the possibility of typing

them by biochemical methods. In Denmark, Bojlén (1934, p. 32) identified four biochemical types in tests with maltose and xylose, and found that 12·5 per cent. of 1032 strains fermented xylose. Hammarström, in Sweden, considered that phage typing was a more satisfactory epidemiological tool than biochemical typing, but he noted that 13·8 per cent. of 1716 strains could utilise xylose.

Carter (1937) examined 120 strains of *Sh. sonnei* from the Glasgow area and grouped these according to Bojlén's method; he found only two xylose-fermenting strains and made no mention of any correlation with epidemiological facts. Cruickshank and Swyer (1940) reported that 18 of 24 strains isolated from an outbreak in a children's nursery attacked xylose and considered that xylose fermentation may be useful for tracing the source of Sonne dysentery when the incriminated strain has this ability.

In an investigation of 812 strains from 812 patients, Tee (1952) found that only 5·4 per cent. of the strains fermented xylose, a proportion similar to that noted in table I (27 of 487 or 5·5 per cent.); however, I found that out of 460 strains that failed to ferment xylose, 158 (34·3 per cent.) also failed to ferment raffinose, whereas Tee found that only 2·5 per cent. of his strains did not attack these two substrates simultaneously. Melibiose was utilised by 449 (92·3 per cent.) of my strains, and this is in agreement with Tee's experience that out of 129 strains tested for melibiose fermentation, 118 (91·4 per cent.) were active; Tee also thought that the ability to ferment xylose might assist the tracings of certain strains of *Sh. sonnei* and help in the elucidation of the epidemiology of Sonne dysentery.

None of the British papers has included as a test of validity an analysis of the degree of constancy of fermentative ability of replicate isolates from the same patient. The results of applying this test in my series, e.g. on patient C's strains (table II), showed that the biochemical typing method was unreliable. The other index of reliability, namely the degree of similarity of the biochemical reactions of strains isolated from different patients in a single epidemic focus was also found to be deficient.

Thus I have not succeeded in subdividing, by biochemical methods, the strains of *Sh. sonnei* that lack the ability to produce detectable colicines.

SUMMARY

Because an increasing proportion of strains of *Shigella sonnei* isolated in the Edinburgh area were found not to be typable by colicine production, an attempt was made to type the strains by their fermentation reactions; 422 untypable strains and 65 strains of 8 different colicine types were examined for their ability to ferment xylose, raffinose and melibiose during 21 days.

Different reactions were found both among different strains isolated from the same epidemic and among replicate isolates from the same patient. This finding indicates that biochemical reactions are not reliable as epidemiological markers of strains of *Sh. sonnei*.

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PRIMARY LEIOMYOSARCOMA OF BONE

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PLATES LXXXII–LXXXIV

STANDARD works of reference on bone tumours (including Dahlin, 1957; Jaffe, 1958; Lichtenstein, 1959; Ackerman and Spjut, 1962) do not include leiomyosarcoma as a possible primary malignant tumour of bone. Miles and Waterhouse (1962) described a case of primary leiomyosarcoma of the oral cavity, which probably arose in the mandible: its exact site of origin, however, was not established. To our knowledge, no instance of leiomyosarcoma affecting the long bones has been reported in the literature, although Professor A. P. Stout (personal communication) has seen one in the fibula of an elderly woman. We therefore consider that the following case is worthy of communication.

CASE REPORT

A retired milkman, aged 73, was admitted to hospital complaining of swelling of the left knee. This had been present for about 1 yr, had been growing larger and was causing considerable pain. There was also a history of " asthma " of 4 years' duration, dyspnoea on exertion, and swelling of the ankles. He had lost the lateral three fingers of the right hand in the 1914–18 war. Joint aspiration before his admission had revealed a haemarthrosis.

On admission, he looked ill and had orthopnoea, central cyanosis, warm extremities, finger clubbing, jugular venous engorgement, oedema of the sacrum and legs, and auricular fibrillation. There was a productive cough with no evidence of bronchospasm. The chest was emphysematous with moist sounds at the bases. Examination of the left lower limb showed wasting of the quadriceps and a bony hard swelling of the upper end of the tibia; the overlying skin was warm and had distended superficial veins. The knee was held in 30° flexion and pain prevented its movement. Radiologically there was an extensive osteolytic lesion of the upper end of the left tibia (fig. 1) with probable extension into the joint. Chest X-radiographs showed cardiac enlargement, pulmonary congestion, but no detectable tumour deposits.

Biopsy showed a fleshy tumour which was histologically reported as a leiomyosarcoma—with the caution that it should not be considered as primary unless a primary tumour was not found elsewhere. The details of the histology will be given fully in conjunction with the necropsy findings. Radical surgery was contraindicated by his poor general condition and he was therefore transferred to another hospital for radiotherapy with protracted mega-voltage cobalt 60 irradiation. He died 4 wk after his first admission and 6 days after commencement of radiotherapy.