

## BACTERIOLOGICAL FINDINGS IN AN EPIDEMIC OF SONNE DYSENTERY

BY A. BECK, M.D. (*Frankfurt*)

AND

D. F. BUCKLE, M.B., D.P.M.

*From the Central Pathological Laboratory of the London County Hospitals  
for Nervous and Mental Disorders and Devonport Laboratories,  
Seamen's Hospital, Greenwich*

DURING an epidemic of Sonne dysentery in Tooting Bec Hospital of which the epidemiological aspects have been described by one of us (Buckle, 1938), and during a recurrence of this epidemic, a number of strains were isolated of the causal organism (140) and various bacilli which were somewhat similar in their cultural and biochemical behaviour. It was thought that a close bacteriological and serological examination of these might be useful in giving information about the constancy of behaviour of the Sonne group, the question of possible biochemical subgroups of Sonne bacilli (Bojln, 1934), and the relationship of the dysenteroid organisms isolated to the true dysentery bacilli, their classification, and significance.

The diagnosis of Sonne dysentery was made when the isolated culture showed the following behaviour: typical pale and tender colonies with a tendency to "bursting bombshell" appearance (Braun & Weil, 1928) on MacConkey's agar, non-motile, giving early acid formation without gas in glucose and mannitol, late acid formation in lactose and sucrose, and no indole. In common with numerous previous investigators (Fraser *et al.* 1926; Wiseman, 1927; Fyfe, 1927; Kerrin, 1928; etc.) the agglutinatory diagnosis of freshly isolated strains was not found to be of great value, as such strains only produced weak agglutinations.

From the 140 Sonne strains isolated, thirty were picked at random and were retested after 6 months, during which time they had been subcultured repeatedly through agar and broth. The following carbohydrate fermentations were examined: glucose, mannitol, lactose, sucrose, xylose, arabinose, raffinose, rhamnose, sorbitol, dulcitol. The cultures were also tested for indole formation, and were agglutinated with Oxford Sonne serum and with a serum prepared from one of the strains (strain H, serum 509, titre 1:3000).

Repeated testing of the Sonne strains after this period showed no alteration of their original biochemical behaviour, except for slight changes in the time needed for the fermentation of lactose and sucrose. As these changes did not reveal any regularity no significance could be attached to them.

In the table below (Table I) the biochemical behaviour of our Sonne strains, and the times of the fermentations, are shown.

Table I

Glucose	ac	Raffinose	ac 8 (5-9)*
Mannitol	ac	Rhamnose	ac
Lactose	ac 8 (4-12)*	Sorbitol	0
Sucrose	ac 8 (2-12)*	Dulcitol	0
Xylose	0	Indole	0
Arabinose	ac		

\* Showing earliest and latest times in days, the preceding figure being the average.

Two strains from the same patient, not included in the table, which were serologically identified as Sonne's, fermented lactose and sucrose in 24 hr. The occurrence of early fermentation of lactose in some strains has been stressed by Thornton & Darmady (1938). Early sucrose fermentation was found in one other strain; and one strain differed from the group in not fermenting rhamnose. Otherwise there were no differences from the characteristic results shown in the table. None of our strains fermented xylose. It would appear that xylose-fermenting strains such as were described by Lester (1926) and by Bojlén (1934) are rare in this country, having been reported only by Carter (1937), who, among 120 Sonne strains isolated in Glasgow during 16 months, found only two which fermented xylose. In view of the varying reports of sucrose fermentation of Sonne bacilli (Channon, 1926; Fyfe, 1927; Hay, 1930) it may be pointed out that in the whole course of the two epidemics no strain was found which failed to ferment this sugar.

All strains of the sample group were agglutinated to the full titre by both the Oxford Sonne serum and the serum H (prepared from one of the cultures isolated).

Thus the Sonne group is characterized by a remarkable uniformity of biochemical and serological behaviour of its members. Therefore the examination of the fermentative reactions is a reliable and easy method for their diagnosis, the only disadvantage being the late appearance of results in lactose and sucrose, which in the first day may make it difficult to differentiate between Flexner and Sonne bacilli; but the positive indole reaction of some Flexner strains and their agglutination by homologous sera, and the typical colonial appearance of Sonne bacilli on agar, and their tendency to early sedimentation in broth, are aids to the quick differentiation of the two varieties.

It is a common experience that during an epidemic of dysentery, a great number of organisms can be isolated which in their cultural behaviour more or less resemble dysentery bacilli. These organisms have been considered by some authors (Seligmann, 1917; Hübschmann, 1925) as transitional forms between non-pathogenic *B. coli* and dysentery bacilli, without, however, convincing proof. Such strains have been isolated also in the present investigation. In those cases where the production of gas is observed in one or more sugars, their separation from the dysentery bacilli was an easy one. Thus of the sixty-three

gas-forming bacilli which failed to ferment lactose in 24 hr., five could be classified as *B. Morgan*, forty-one as *paracolon*, and seventeen as late-lactose fermenting *B. coli*. There remained a number of strains (twelve) which on the grounds of their biochemical reactions were suspected of being related to the Sonne or Flexner types. These were subdivided by their fermentative and serological behaviour into three groups, the former reactions being set out in Table II.

Table II

Group	No. in group	Glucose	Mannitol	Lactose	Sucrose	Xylose	Arabinose	Raffinose	Rhamnose	Sorbitol	Dulcitol
F	6	ac	ac	ac 2-3	0	ac	ac	0	ac	ac	ac 4-6
G	2	ac	ac	ac 4-7	{ 0 ac 2	ac	ac	0	—	{ 0 ac	0
B	4	ac	ac	0	0	ac	—	0	ac 2	ac 1-7	0

The figures refer to the time of acid production in days.

Litmus milk: acid and clot (late) in each case.

Indole: strong reaction in 24 hr. in each case.

The clinical course of the disease in all but one of these patients yielding the anomalous strains was characterized by an unusually persistent mild diarrhoea, relapsing constantly over a period of 3-5 weeks. Those of these patients which were tested with the Widal reaction for Sonne (six) gave negative results. In most of the cases, the "atypical" strains have been repeatedly isolated, even seven times in one patient (F).

Antisera were prepared in rabbits with one representative of each group (serum anti-F 505, anti-G 519, anti-B 499) and it was found that whilst each serum agglutinated all the members of its own group up to its full titre, no cross-agglutination occurred between groups.

From the biochemical behaviour described in Table II, it would appear that the members of the F and G groups are somewhat similar to the Sonne bacillus, from which they differ by their fermentation of xylose, their production of indole, lack of sucrose fermentation (in the F group and one member of the G group) and the fermentation of sorbitol and dulcitol (in the F group). Serologically they failed to reveal any relationship to the Sonne group. Neither were they agglutinated by the Sonne sera used, nor did sera representing the F and G groups agglutinate Sonne bacilli. In absorption tests they failed to bind any agglutinins of a Sonne serum. The groups were themselves serologically uniform. It follows that there is no justification in relating these strains directly to the Sonne group. At the same time the results emphasize the reliability of a strict biochemical characterization of Sonne bacilli. It would appear that these strains are similar to the Dispar strains of Andrewes (1918), which in later investigations (Forsyth, 1933; Bamforth, 1934) were shown to be quite distinct from Sonne strains, and which are probably a late-lactose fermenting variety of *B. coli* anaerogenes (Bamforth, 1934).

The fermentations shown by the members of the B group suggest the possibility of their being Flexner bacilli. Agglutination of freshly isolated cultures with a polyvalent Flexner serum were negative, and their later examination with the Oxford sera prepared against the Flexner types V, W, X, Y and Z also failed to reveal close relationship to any of these (the highest titre of agglutination being 1 : 50 with Y serum, which agglutinated its homologous type-strain to a titre of 1 : 500). Absorption of the five Flexner type sera with two representatives of the B group also failed to remove any agglutinins for the homologous type-strain. On the other hand it was remarkable that an antiserum prepared against a member of the B group (anti-B, 499) agglutinated some of the Flexner type-strains. (The type-strains X, Y and V to  $\frac{1}{4}$ ,  $\frac{1}{8}$ , and  $\frac{1}{16}$  respectively of its titre.) It is possible that this one-sided agglutination is an expression of a substrain super-strain relationship (Schütze, 1921) between the B group and some of the Flexner types. It is probable that the B strains are in fact Flexner strains which do not fall within the antigenic range of the common Flexner races. The possibility of the existence of such strains is mentioned by Gardner (1929), and their occurrence has been described by Blacklock & Guthrie (1937). It is possible that Bamforth's D group of organisms also falls under this classification. The occurrence of Flexner bacilli during a Sonne epidemic does not appear extraordinary in the special conditions of an institution population, and epidemics with dysentery bacilli of different groups occurring together have been described (Bojlén, 1934; Bloch, 1938).

#### CONCLUSION

The work reported confirms the sharp cultural and serological definition of the Sonne group of dysentery bacilli. No "subgroup" or "atypical" representatives of *B. dysenteriae* Sonne could be demonstrated. A closer examination of dysenteroid strains, serologically divided into three homogeneous groups, showed in a number of strains (F and G groups) a similarity to the indole-forming group of *B. dispar* (Andrewes), which has been shown by previous investigators to be distinct from the Sonne group.

Evidence is brought forward to indicate that four other strains, serologically uniform, which culturally resemble the Flexner group but could not be classified in one of the common Flexner races, may be Flexner bacilli of a differing antigenic structure.

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