BACTERIUM DYSENTERIAE SONNE: A STUDY OF FORTY STRAINS WITH PARTICULAR REFERENCE TO THE APPEARANCE OF THE COLONIES

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(With 1 Figure in the Text)

Introduction

The appearance of the colonies of *B. dysenteriae* Sonne has been described by a number of authors (Thjøtta, 1919; Patterson & Williams, 1922; Johnston & Kaake, 1932; Reynolds, MacCleskey & Werkman, 1934; Glynn & Starkey, 1939), and both the last groups of workers have referred to minute daughter colonies, or papillae, which develop on the original colonies after some days' incubation. Thus Glynn & Starkey, plating their strains on MacConkey's medium, noted that, after 48 hr. incubation, certain colonies developed definite raised papillae. These papillae were convex, entire outgrowths from the original 'pale' colonies and were pink, due to lactose fermentation. They observed that the time required for the appearance of the papillae corresponded roughly with the time needed for the strain to ferment lactose in peptone water medium.

This paper records the results of an examination of forty strains of *B. dysenteriae* Sonne with particular reference to the occurrence of papillae.

Methods

Source of strains. Twenty-seven strains had been recently isolated in this laboratory from acute cases of dysentery (Table 1, nos. 1–27). Twelve strains were obtained from the National Collection of Type Cultures, London (nos. 28–39). The remaining (Kasauli) strain (no. 40) was kindly supplied by Lt.-Col. R. F. Bridges. This organism was isolated by him in India in 1928 and has been maintained ever since by plating and picking the smoothest colony at monthly intervals (Bridges, personal communication).

To ensure purity before beginning experimental work all cultures were on three occasions alternately plated on MacConkey's medium and the smoothest colonies subcultured to agar slopes.

Appearance of colonies. The structure of individual colonies was observed by examining surface growths on MacConkey's medium or ordinary agar, using a plate culture microscope. The plates of medium were incubated at 37° C. in an incubator with a moist atmosphere.

Biochemical reactions. The following 'sugars' were tested for fermentation using a peptone water basis and neutral-red indicator: glucose, lactose, saccharose, dulcite, mannite, maltose, dextrin, and glycerol. The medium was contained in screw-capped vials to prevent evaporation.

Other tests carried out were as follows: acid formation in litmus milk, indole reaction, Voges-Proskauer and methyl red tests, production of H_2S, and liquefaction of gelatin.

Serological tests. Antisera were prepared in rabbits to strain 2 (a papilla-producing culture) and strain 6 which did not form papillae. Each rabbit received five intravenous injections of a formolized antigen which had been made up at the beginning of the experiment. The animals were eventually bled and the sera found to have titres of 1/7680 for the homologous antigen.

In the conduct of agglutination tests these two sera were tested against formolized suspensions of all the strains, the range of dilutions extending from 1/120 to 1/7680. Tests were incubated in a 55° C. water-bath for 4–5 hr., and were read after standing at room temperature overnight. In Table 1 results are expressed in terms of T, T/2, etc., T indicating that complete agglutination occurred to the titre of the serum (i.e. 1/7680), and T/2 indicating that complete agglutination did not occur in dilutions higher than 1/3840.

Results

Appearance of colonies. All the strains used in this investigation, with the exception of no. 40 (Kasauli), showed the following appearance after 24 hr. incubation at 37° C. on MacConkey's medium: the colonies were 'pale', slightly cream coloured, and rather opaque, with a slightly convex surface and a crenated edge; the colonies were 2–4 mm. in diameter; no noticeably rough colonies were encountered. After 3–5 days' growth on MacConkey's or ordinary agar medium twenty-five of the strains isolated in this laboratory and ten of the N.C.T.C. strains developed definite papillae on the original 'pale' colonies (Table 1). These papillae were smooth rounded protuberances, which increased in size and by the seventh day were about 0·5–1 mm. in diameter (Fig. 1). If the strain fermented lactose in fluid culture the papillae on MacConkey's medium appeared pink, the parent colonies being 'pale'. If, however, the strain did not ferment lactose in fluid medium, both the papillae and parent colonies appeared 'pale' on MacConkey's medium.

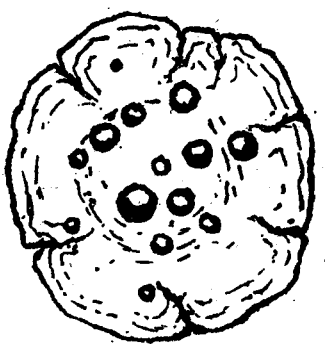

Fig. 1. Colony of *B. dysenteriae* Sonne, showing papillae (×20). Seven days' growth on MacConkey's medium.

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Four strains (two recently isolated and two N.C.T.C.) failed to produce papillae, and in these the whole colony became pink in 4–5 days, lactose also being fermented in fluid medium. On MacConkey's medium the colonies of the Kasauli strain were smooth, 'pale', and with an entire edge after 24 hr. On further incubation the colony turned pink and an occasional pink papilla appeared near the periphery.

An attempt was made to subculture those papillae which turned pink, but although numerous papillae were picked off with a fine wire, under the plate culture microscope, and inoculated on to MacConkey plates 'pale' colonies always resulted; colonies which appeared pink from the start were never obtained. These 'pale' colonies themselves later developed pink papillae. Similar inoculations of papillae on to ordinary agar were equally unsuccessful.

Biochemical tests. The results are given in Table 1, where it will be seen that all strains fermented glucose in 24 hr. with the production of acid, thirty-nine strains fermented mannite, maltose, dextrin, and glycerol in a similar time; the same thirty-nine strains fermented saccharose in 3–14 days. Thirty strains fermented lactose and acidified litmus milk in 1–9 days, while ten failed to do so. All strains proved to be methyl red positive (see also Bamforth, 1934).

In addition all strains failed to produce indole or H_2S, or to give a positive Voges-Proskauer test, or to liquefy gelatin. Reference to Table 1 will show that biochemical tests afford no means of distinguishing between those strains which produce papillae and those which do not.

Serological tests. Reference to Table 1 will at once show that straight agglutination tests with antisera to a papilla-forming and to a non-papilla-forming strain disclosed no differences between the two types of strain. Thus the four non-papilla-forming strains were agglutinated to titre by both the antisera, and correspondingly the majority of the thirty-six papilla-forming strains were also agglutinated to titre by both sera.

DISCUSSION

It will be seen that thirty-five of the forty strains of *B. dysenteriae* Sonne showed the development of definite raised papillae on their surface colonies on MacConkey's medium or ordinary agar. Twenty-five of these papilla-forming strains fermented lactose in fluid culture, and on MacConkey's medium the papillae appeared pink against the background of the 'pale' mother colony. Ten of the papilla-producing strains, however, did not ferment lactose, and in them both papillae and original colonies were 'pale'. Four strains did not show the development of papillae, but all of these fermented lactose in fluid medium, and on MacConkey's medium the whole colony turned pink. The colonies of the Kasauli strain turned pink and showed a few isolated papillae. Glynn & Starkey (1939) suggest that late fermentation of lactose by *B. dysenteriae* Sonne 'is due to the appearance in ageing cultures of variants with new

Serial no. of strain	Reference no. and source of strain	Production of papillae	Fermentation of 'sugars' in peptone water: Glucose hr.	Lactose days	Saccharose days	Dulcite	Mannite hr.	Maltose hr.	Dextrin hr.	Glycerol hr.	Methyl red test	Acidity in litmus milk days	Results of agglutination with antiserum to: Strain 2 (papilla-producing)	Strain 6 (non-papilla-producing)
	Recently isolated in Edinburgh													
1	M 52289	+	A 24	—	A 10	—	A 24	A 24	A 24	A 24	+	—	T	T
2	M 52290	+	A 24	A 9	A 12	—	A 24	A 24	A 24	A 24	+	A 11	T	T/2
3	M 52240	+	A 24	—	A 10	—	A 24	A 24	A 24	A 24	+	—	T	T
4	M 52174	+	A 24	—	A 10	—	A 24	A 24	A 24	A 24	+	—	T	T
5	M 50672	+	A 24	A 5	A 7	—	A 24	A 24	A 24	A 24	+	A 7	T	T/4
6	M 50456	–	A 24	A 7	A 5	—	A 24	A 24	A 24	A 24	+	A 6	T	T
7	M 49439	+	A 24	A 7	A 5	—	A 24	A 24	A 24	A 24	+	A 6	T	T
8	M 49160	+	A 24	A 7	A 6	—	A 24	A 24	A 24	A 24	+	A 8	T	T/2
9	M 53175	+	A 24	—	A 5	—	A 24	A 24	A 24	A 24	+	—	T	T
10	M 53485	+	A 24	A 8	A 10	—	A 24	A 24	A 24	A 24	+	A 8	T	T
11	M 53731	+	A 24	A 7	A 10	—	A 24	A 24	A 24	A 24	+	A 7	T	T
12	M 53906	+	A 24	A 5	A 5	—	A 24	A 24	A 24	A 24	+	A 5	T	T/2
13	M 53919	+	A 24	A 5	A 10	—	A 24	A 24	A 24	A 24	+	A 7	T	T
14	M 52405	+	A 24	—	A 10	—	A 24	A 24	A 24	A 24	+	—	T	T
15	M 52401	+	A 24	—	A 4	—	A 24	A 24	A 24	A 24	+	—	T	T
16	M 52382	+	A 24	A 4	A 4	—	A 24	A 24	A 24	A 24	+	A 4	T	T
17	M 52788	–	A 24	A 5	A 5	—	A 24	A 24	A 24	A 24	+	A 5	T	T
18	M 52795	+	A 24	A 2	A 3	—	A 24	A 24	A 24	A 24	+	A 4	T	T
19	M 52980	+	A 24	—	A 6	—	A 24	A 24	A 24	A 24	+	—	T	T/4
20	M 52985	+	A 24	—	A 6	—	A 24	A 24	A 24	A 24	+	—	T	T/4
21	M 56810	+	A 24	A 7	A 12	—	A 24	A 24	A 24	A 24	+	A 7	T	T
22	M 56849	+	A 24	A 5	A 8	—	A 24	A 24	A 24	A 24	+	A 7	T	T
23	M 57035	+	A 24	A 5	A 6	—	A 24	A 24	A 24	A 24	+	A 7	T	T
24	M 57036	+	A 24	A 6	A 12	—	A 24	A 24	A 24	A 24	+	A 7	T/2	T
25	M 57058	+	A 24	A 6	A 7	—	A 24	A 24	A 24	A 24	+	A 7	T	T
26	M 57087	+	A 24	A 5	A 10	—	A 24	A 24	A 24	A 24	+	A 6	T	T
27	M 57464	+	A 24	A 7	A 7	—	A 24	A 24	A 24	A 24	+	A 7	T/4	T
	National Collection of Type Cultures													
28	268	+	A 24	A 1	A 14	—	A 24	A 24	A 24	A 24	+	A 2	T	T
29	2182	+	A 24	A 6	A 6	—	A 24	A 24	A 24	A 24	+	A 6	T	T
30	2183	+	A 24	A 7	A 7	—	A 24	A 24	A 24	A 24	+	A 7	T/4	T
31	2285	+	A 24	A 6	A 6	—	A 24	A 24	A 24	A 24	+	A 7	T/2	T
32	2406	+	A 24	A 7	A 8	—	A 24	A 24	A 24	A 24	+	A 7	T	T
33	2541	+	A 24	A 6	A 3	—	A 24	A 24	A 24	A 24	+	A 7	T	T
34	2542	–	A 24	A 7	A 12	—	A 24	A 24	A 24	A 24	+	A 7	T	T
35	2941	+	A 24	—	A 6	—	A 24	A 24	A 24	A 24	+	—	T	T
36	3082	+	A 24	—	—	—	—	—	—	—		—	– ve	T/4
37	3363	–	A 24	A 7	A 13	—	A 24	A 24	A 24	A 24	+	A 7	T	T
38	4051	+	A 24	A 7	A 6	—	A 24	A 24	A 24	A 24	+	A 5	T/2	T
39	4494	+	A 24	A 6	A 6	—	A 24	A 24	A 24	A 24	+	A 7	T	T
40	Kasauli	+	A 24	A 5	A 10	—	A 24	A 24	A 24	A 24	+	A 5	T/8	T/4

fermenting powers. These variants are seen in the original colony as secondary papillae arising directly from the mother colony only after the colony has aged.' We would suggest, however, that while this explanation may hold in certain cases, it obviously cannot in all, because, as just mentioned, four strains fermented lactose yet failed to produce papillae, and, further, ten definitely papilla-producing strains did not ferment lactose at all.

With regard to this question of the organisms constituting the papillae being considered as variants of the parent strain, it is interesting to recall certain early work on variation in coliform bacilli. Thus in 1907 Massini described an organism, *B. coli mutabile*, which on first incubation on a lactose-containing medium produced 'pale' colonies, but on further incubation showed the development of lactose-fermenting papillae. He was able to obtain a pure subculture of these lactose-fermenting variants from the papillae by plating, colonies being produced which were lactose positive from the start. Then, later, Penfold (1910, 1911) showed that while *B. typhosus* normally produced neutral or alkaline colonies on dulcite agar a proportion of strains showed the development of acid-forming papillae. He found that subcultures from these papillae gave rise to colonies which fermented dulcite from the beginning.

Whether the organisms occurring in the papillae of the colonies of *B. dysenteriae* Sonne can be regarded in the same light as those variants described by Massini and by Penfold is uncertain, for all attempts to obtain pure subcultures of them have failed. It is probable, however, that in the case of *B. dysenteriae* Sonne the organisms in the papillae can be considered as variants of the parent organisms which are dependent for their growth on factors elaborated during the development of the parent colony.

SUMMARY

1. Certain appearances of the colonies and some biochemical reactions of forty strains of *B. dysenteriae* Sonne have been investigated.

2. In thirty-five strains definite raised papillae occurred on parent colonies after 3–5 days' growth on MacConkey's medium or ordinary agar.

3. Papillae were produced both by strains that fermented lactose in fluid culture and those that did not; in the former case they were pink, in the latter pale.

4. Papilla-producing and other strains could not be differentiated by various biochemical reactions or by straight agglutination tests.

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