AN ANAEROGENIC STRAIN OF *BACTERIUM PARATYPHOSUM* C

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Introduction

In 1939 I described fully the characteristics of about twelve strains of *Bact. paratyphosum* C causing enteric fever in Egypt (Nabih, 1939). These strains, which were isolated in the summer of 1937 during routine work on samples of urine and faeces sent to the Laboratories for the bacteriological diagnosis of enteric fevers, were shown to be identical bacteriologically, biochemically and serologically with each other as well as with Hirschfeld's *Bact. paratyphosum* C in all respects.

In August 1937 another strain was isolated from the urine of a patient suffering from enteric-like fever, which possessed the same bacteriological, biochemical and serological characteristics except that it did not produce gas from ordinary "sugars"; this was isolated together with another typical strain from the urine of this patient.

The division of the Salmonella group into aerogenic and anaerogenic strains is recognized, and anaerogenic variants of *Bact. paratyphosum* A and *Bact. paratyphosum* B have long been noticed and reported with varying frequency.

An anaerogenic strain of *Bact. paratyphosum* C is new in my experience, and this paper draws attention to its existence in Egypt.

Bacteriological investigations

This anaerogenic strain shows the following characteristics.

Morphological characteristics. It is a short, motile, non-capsulated, Gram-negative coliform bacillus.

Cultural characteristics. Young cultures on agar give rise to small, circular, moist, translucent colonies of low lenticular form with a smooth surface and entire edge.

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Old cultures give rise to similar colonies, slightly opaque, with a serrated edge, flat with a slight convexity.

Broth cultures are homogeneously turbid, without characteristic odour.

Biochemical reactions. Table 1 gives the biochemical reactions of the anaerogenic strain, of the aerogenic strain isolated from the urine of the same patient, and of a strain of Hirschfeld's *Bact. paratyphosum* C from the N.C.T.C. of the Lister Institute.

Table 1. *Biochemical reactions*

	Aerogenic strain	Anaerogenic strain	Hirschfeld's *Bact. paratyphosum* C, N.C.T.C. of Lister Institute
Lactose	–	–	–
Sucrose	–	–	–
Inositol	–	–	–
Xylose	AG	A	AG
Arabinose	AG	A	AG
Dulcitol	AG	A	AG
Glucose	AG	A	AG
Mannitol	AG	A	AG
Sorbitol	AG	A	AG
Laevulose	AG	A	AG
Maltose	AG	A	AG
Galactose	AG	A	AG
Raffinose	–	–	–
Dextrin	–	–	–
Inulin	–	–	–
Adontil	–	–	–
Salicin	–	–	–
Rhamnose	AG	A	AG
Lead acetate agar	Blackened	Blackened	Blackened
Indol	–	–	–

AG = acid and gas; A = acid only; – = no acid, no gas. The action of the organisms on maltose and rhamnose was somewhat delayed. The cultures were kept under observation for 14 days. All three strains turned milk acid and failed to liquefy gelatine.

Agglutination tests with various specific sera. The agglutination tests were carried out with sera supplied by the Standard Laboratories of the M.R.C., Oxford. The "H" emulsions were prepared by the addition of 0·2 % formalin to veal broth cultures. The results are shown in Table 2.

Table 2. *Agglutination tests with various specific sera of the Standard Laboratories of the M.R.C., Oxford*

Organism	Aerogenic strain	Anaerogenic strain	Hirschfeld's *Bact. paratyphosum* C
Bact. typhosum, O serum	0	0	0
Bact. typhosum, H specific serum	0	0	0
Bact. paratyphosum, A serum	0	0	0
Bact. paratyphosum B, H specific serum	0	0	0
Hirschfeld's *Bact. paratyphosum* C, H specific serum	1/250	1/250	1/250
Bact. aertrykum, O serum	0	0	0
Bact. aertrykum, H specific serum	0	0	0
Bact. enteritidis serum	0	0	0
Bact. Flenneri serum	0	0	0
Ty Vi of the N.C.T.C. serum	1/320	1/320	1/160
Saline (control)	0	0	0

The *Ty* Vi of the N.C.T.C. of the Lister Institute serum was prepared locally and diluted to a titre 1/320.

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Agglutination tests with sera locally prepared against "H" emulsions of the organisms isolated. Two sera were prepared by inoculating rabbits intravenously with "H" emulsions of the aerogenic and anaerogenic strains and tests carried out as is shown in Table 3.

Table 3. *Agglutination tests with sera locally prepared*

Sera prepared locally	Aerogenic strain	Anaerogenic strain	Hirschfeld's *Bact. paratyphosum* C
Hirschfeld's *Bact. paratyphosum* C, H diphasic emulsion	1/250	1/250	1/250
Bact. typhosum, O emulsion	0	0	0
Bact. typhosum, H emulsion	0	0	0
Bact. paratyphosum, A emulsion	0	0	0
Bact. paratyphosum B, H diphasic emulsion	1/125	1/125	1/250
Bact. aertrykum, O emulsion	0	0	0
Bact. aertrykum, H diphasic emulsion	1/125	1/125	1/250
Bact. enteritidis emulsion	0	0	0
Ty Vi of the N.C.T.C. murcolated emulsion	1/160	1/160	1/80
Saline (control)	0	0	0

The non-specific "H" agglutinins for *Bact. paratyphosum* B and *Bact. aertrykum* were then absorbed from the prepared sera by incubating with corresponding emulsions of fairly high density for 24 hr. in a water-bath at 54° C.

The sera thus treated were then tested and proved to be free from agglutinins except the "H" specific agglutinins of the organisms, as is shown in Table 4.

Table 4. *Agglutination test to prove that the prepared antisera are free from all agglutinins except only the "H" specific agglutinins of the organisms*

Antisera against organisms	Aerogenic strain	Anaerogenic strain	Hirschfeld's *Bact. paratyphosum* C
Hirschfeld's *Bact. paratyphosum* C emulsion	1/250	1/250	1/250
Typhoid, O emulsion	0	0	0
Typhoid, H emulsion	0	0	0
Bact. paratyphosum A emulsion	0	0	0
Bact. paratyphosum B emulsion	0	0	0
Bact. aertrykum, O emulsion	0	0	0
Bact. aertrykum, H emulsion	0	0	0
Bact. enteritidis emulsion	0	0	0
Control (saline)	0	0	0

With sera thus treated the final tests for identification were carried out, and the cross-agglutination (Table 5) and cross-absorption (Table 6) tests defined more precisely the antigenic relationships of the two strains with one another and with Hirschfeld's *Bact. paratyphosum* C. These tests show that the anaerogenic strain is serologically identical with the aerogenic strain and with the Hirschfeld's *Bact. paratyphosum* C strain of the N.C.T.C. of the Lister Institute. They are all diphasic, containing (C) specific "H" antigen and (1, 4, 5) non-specific "H" antigen.

Table 5. *Cross-agglutination test*

Antisera	Aerogenic strain, H emulsion	Anaerogenic strain, H emulsion	Hirschfeld's *Bact. paratyphosum* C, H emulsion	Control (saline)
Aerogenic strain	1/250	1/250	1/250	0
Anaerogenic strain	1/250	1/250	1/250	0
Hirschfeld's *Bact. paratyphosum* C	1/250	1/250	1/250	0

Table 6. *Cross-absorption test*

Antisera from which agglutinins were absorbed	Hirschfeld's *Bact. paratyphosum* C emulsion	Aerogenic strain emulsion	Anaerogenic strain emulsion
Hirschfeld's *Bact. paratyphosum* C antiserum, absorbed by Hirschfeld's *Bact. paratyphosum* C emulsion	0	0	0
Hirschfeld's *Bact. paratyphosum* C antiserum, absorbed by aerogenic strain emulsion	0	0	0
Hirschfeld's *Bact. paratyphosum* C antiserum, absorbed by anaerogenic strain emulsion	0	0	0

Corresponding results were obtained by treating the antisera of aerogenic and anaerogenic strains in the same way.

Agglutination tests with the patient's serum. The patient's serum agglutinated Hirschfeld's *Bact. paratyphosum* C and the aerogenic and anaerogenic strains up to a titre of 1/250, the highest tested.

Permanence of anaerogenesis

The anaerogenic strain was tested by culturing on liquid media made up with different specimens of peptone (Park Davis peptone, bacto-peptone (Difco), Lemco-peptone (Witte)) and containing different "sugars". After 24 hr. incubation at 37° C. acid but no gas was produced. After 14 days further incubation at 37° C. no gas was produced.

These tests were repeated from time to time for about 3 years, during which sixty-two successive subcultures were made and kept at room temperature, which varied markedly in the different seasons, but no gas was ever produced.

Discussion

The existence of anaerogenic strains of paratyphoid types which are normally aerogenic has long been recognized; Ledingham, Penfold & Woodcock (1915) stated that "many paratyphoid strains give little or no gas in the sugars they normally ferment", and Bruce White (1929) reported that "anaerogenic strains of all the commonly occurring aerogenic types have been reported with varying frequency".

My serological tests have shown no antigenic difference between the anaerogenic and the aerogenic strains; and the serum to the aerogenic strain absorbed with the anaerogenic strain was deprived of its agglutinins in the same way as antiserum to the anaerogenic strain absorbed with the aerogenic strain. At the same time biological tests have shown complete identity of the two strains in all respects except the inability of the anaerogenic strain to produce gas from any "sugar".

Summary

1. The existence of an anaerogenic strain of *Bact. paratyphosum* C is recorded in Egypt.

2. It was isolated in August 1937, together with an aerogenic strain, from the urine of a patient suffering from enteric fever.

3. Biologically the anaerogenic strain was identical with the aerogenic except that it did not produce gas from any "sugar".

4. The anaerogenic strain retained its inability to produce gas from "sugars" for the three years during which tests were carried out, subculturing on various fluid media containing different sugars failing to cause any gas production.

5. Serologically there was no indication of any antigenic difference between the anaerogenic and the aerogenic strains.

6. The anaerogenic strain contained the Vi antigen and retained it for three years when kept at room temperature.

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