BACTERIUM PARATYPHOSUM C AS A CAUSE OF ENTERIC FEVER IN EGYPT

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Historical

The term *Bacterium paratyphosum* C has been variously applied by different investigators. In this paper it is understood to refer to the organism so designated under the Kauffmann-White Scheme of classification as (*c*) Specific "H" antigen and (1, 4, 5) non-specific "H" antigen and thus to be identical with the organism isolated by Hirschfeld in 1916 from the blood of a Serbian soldier of the Royal Serbian army, who had been taken ill with a paratyphoid-like fever.

Since then it has been isolated by various other workers from similar cases in Macedonia (Dudgeon & Urquhart, 1920), in Albania (Weil, 1917), in Baghdad (MacAdam, 1919), in Palestine (Schutze, 1920), in East Africa (Garrow, 1920), in British Guiana (Giglioli, 1930), etc.

The geographical distribution of these cases has led to the organism becoming known as the "Eastern European type of *Bact. paratyphosum* C" in contradistinction to *Bact. cholera-Suis* var. *Kunsendorff* of the Kauffmann-White Scheme or the "Western European type of *Bact. paratyphosum* C".

Hitherto, its existence has not been recognized in Egypt, and it is the purpose of this paper to draw attention to the fact that it exists here.

Origin of the organisms isolated

During the summer of 1937, while doing routine work on samples of urine and faeces sent to the Public Health Central Laboratories, Cairo, for bacteriological examination, an organism was isolated from the urine of a patient suffering from an enteric form of fever, which corresponded morphologically, biochemically and serologically with Hirschfeld's *Bact. paratyphosum* C. In the same summer as well as in the early part of the present year six more strains of this organism were isolated either from the urine or faeces of patients suffering from the same form of enteric fever. The distribution, the source, and the period of isolation are illustrated in Table I.

Table I. *Distribution and source of organisms isolated*

No.	Isolated from	Locality	Date	Clinical picture
1	Urine	Tanta	May, 1937	Enteric fever
2	,,	,,	,, ,,	,,
3	,,	Zagazig	June, 1937	,,
4	,,	Benha	July, 1937	,,
5	,,	Giza	January, 1938	,,
6	Faeces	Teh El Barud	February, 1938	,,
7	Urine	Kom Hamada	,, ,,	,,

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Bacteriological investigations

The following are the characteristics of the organisms isolated from the urine in six and from the faeces in one of the above cases.

Morphological characteristics

They are short, motile, non-capsulated, Gram-negative coliforms.

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.

Old cultures give rise to similar colonies, slightly opaque, with a serrated edge or of a vine-leaf shape, flat or with a slight convexity and a beaten copper surface.

Broth cultures are homogeneously turbid with no characteristic odour.

Biochemical reactions

In Table II we give the biochemical reactions of four of the strains isolated, together with those of a strain of *Bact. paratyphosum* C (Hirschfeld) received from the N.C.T.C. of the Lister Institute.

Table II. *Biochemical reactions*

	Organism Tanta I	Organism Tanta II	Organism Zagazig	Organism Benha	Hirschfeld's *Bact. paratyphosum* C, N.C.T.C. of Lister Institute
Lactose	–	–	–	–	–
Sucrose	–	–	–	–	–
Inositol	–	–	–	–	–
Xylose	+	+	+	+	+
Arabinose	+	+	+	+	+
Dulcitol	+	+	+	+	+
Glucose	+	+	+	+	+
Mannitol	+	+	+	+	+
Laevulose	+	+	+	+	+
Maltose	+	+	+	+	+
Galactose	+	+	+	+	+
Raffinose	–	–	–	–	–
Dextrin	–	–	–	–	–
Inulin	–	–	–	–	–
Adonitol	–	–	–	–	–
Salicin	–	–	–	–	–
Rhaminose	+	+	+	+	+
Lead acetate agar	Blackened	Blackened	Blackened	Blackened	Blackened
Indole	–	–	–	–	–

N.B. + = acid and gas; – = no acid, no gas.

The action of the organisms on maltose and rhamnose was somewhat delayed. All turned milk acid and failed to liquify gelatine. The "sugars" were discarded after 7 days' incubation.

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 III.

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

Organism	*Bact. typhosum* O serum	*Bact. typhosum* H Sp. serum	*Bact. para-typhosum* A serum	*Bact. para-typhosum* B H Sp. serum	Hirsch-feld's *Bact. para-typhosum* C H Sp. serum	*Bact. aertrycke* O serum	*Bact. aertrycke* H Sp. serum	*Bact. enteri-tidis* serum	Saline (control)
Tanta I	0	0	0	0	1/250	0	0	0	0
Tanta II	0	0	0	0	1/250	0	0	0	0
Zagazig	0	0	0	0	1/250	0	0	0	0
Benha	0	0	0	0	1/250	0	0	0	0
Hirschfeld's *Bact. paratyphosum* C	0	0	0	0	1/250	0	0	0	0

Agglutination tests with sera locally prepared against the "H" emulsions of the organisms isolated

Four different sera were prepared by inoculating rabbits intravenously with "H" emulsions of the organisms isolated from the first four cases, and the agglutination tests were carried out as is shown in Table IV.

The non-specific "H" agglutinins for *Bact. paratyphosum* B and *Bact. aertrycke* 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 tested and proved to be free from all agglutinins except only the "H" specific agglutinins of the organism as is shown in Table V.

Table IV. *Agglutination tests with sera locally prepared*

Sera prepared locally	Hirsch-feld's *para-typhosum* C H diphasic emulsion	*Bact. typhosum* O emulsion	*Bact. typhosum* H emulsion	*Bact. para-typhosum* A emulsion	*Bact. para-typhosum* B H diphasic emulsion	*Bact. aertrycke* O emulsion	*Bact. aertrycke* H diphasic emulsion	*Bact. enteri-tidis* emulsion	Saline (control)
Tanta I	1/250	0	0	0	1/125	0	1/125	0	0
Tanta II	1/250	0	0	0	1/250	0	1/250	0	0
Zagazig	1/250	0	0	0	1/250	0	1/250	0	0
Benha	1/250	0	0	0	1/125	0	1/125	0	0
Hirschfeld's	1/250	0	0	0	1/250	0	1/250	0	0

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

Antisera against organisms	Hirsch-feld's *para-typhosum* C emulsion	Typhoid O emulsion	Typhoid H emulsion	*Para-typhosum* A emulsion	*Para-typhosum* B emulsion	*Aertrycke* O emulsion	*Aertrycke* H emulsion	*Enteri-tidis* emulsion	Control (saline)
Tanta I	1/250	0	0	0	0	0	0	0	0
Tanta II	1/250	0	0	0	0	0	0	0	0
Zagazig	1/250	0	0	0	0	0	0	0	0
Benha	1/250	0	0	0	0	0	0	0	0
Hirschfeld's *paratyphosum* C	1/250	0	0	0	0	0	0	0	0

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With such treated sera the final tests for identification were carried out; and thus the cross-agglutination test (as is shown in Table VI) and the cross-absorption test (as is shown in Table VII) defined more precisely the co-relation of the organisms examined with one another and with Hirschfeld's *Bact. paratyphosum* C; and finally rendered obvious that the organisms we are dealing with are quite identical with Hirschfeld's *Bact. paratyphosum* C, diphasic containing (C) specific "H" antigen and (1, 4, 5) non-specific "H" antigen.

Table VI. *Cross-agglutination test*

Antisera	Tanta I H emulsion	Tanta II H emulsion	Zagazig H emulsion	Benha H emulsion	Hirschfeld's *para-typhosum* C H emulsion	Control (saline)
Tanta I	1/250	1/250	1/250	1/250	1/250	0
Tanta II	1/250	1/250	1/250	1/250	1/250	0
Zagazig	1/250	1/250	1/250	1/250	1/250	0
Benha	1/250	1/250	1/250	1/250	1/250	0
Hirschfeld's *para-typhosum* C	1/250	1/250	1/250	1/250	1/250	0

Table VII. *Cross-absorption test*

Antisera from which agglutinins are absorbed	Hirschfeld's para-typhosum C emulsion	Tanta I emulsion	Tanta II emulsion	Zagazig emulsion	Benha emulsion
Hirschfeld's *Bact. paratyphosum* C antiserum absorbed by Hirschfeld's *paratyphosum* C emulsion	0	0	0	0	0
Hirschfeld's *Bact. paratyphosum* C antiserum absorbed by Tanta I emulsion	0	0	0	0	0
Hirschfeld's *Bact. paratyphosum* C antiserum absorbed by Tanta II emulsion	0	0	0	0	0
Hirschfeld's *Bact. paratyphosum* C antiserum absorbed by Zagazig emulsion	0	0	0	0	0
Hirschfeld's *Bact. paratyphosum* C antiserum absorbed by Benha emulsion	0	0	0	0	0

Similar results were obtained by treating the other antisera (Tanta I, Tanta II, Zagazig and Benha) in the same way as tabulated above.

Vital agglutination test

Over and above the examinations done for every one of the first four cases as detailed above, the serum of every patient of all the seven cases was tested for agglutination simultaneously against Hirschfeld's *Bact. paratyphosum* C and the organisms isolated from each case in question. Positive results, as is shown in Table VIII, were obtained in all cases, except one, to the titre of 1 in 250 and it may have gone higher but this was considered enough for a positive result.

Discussion

As is indicated by its very name, the organism is mainly found in eastern Europe, yet it has been met with in such Asian localities as came to be connected with the east of Europe during the Great War, for example, Palestine,

Table VIII. *Vital agglutination test*

Blood serum of patients	Emulsion of organisms: Hirschfeld's paratyphosum C	Tanta I	Tanta II	Zagazig	Benha
Tanta I	1/250	1/250	1/250	1/250	1/250
Tanta II	1/250	1/250	1/250	1/250	1/250
Zagazig	1/250	1/250	1/250	1/250	1/250
Benha	1/250	1/250	1/250	1/250	1/250

Similar results were obtained by treating the sera of the other cases in the same way as tabulated above.

Baghdad and elsewhere; and still more some workers came across it in British Guiana, Algeria and East Africa; and it was only natural to expect its appearance in Egypt owing to the free intercourse that existed between it and the other fields of action during the war.

Thorough investigations, carried out in reference to these cases, proved that the patients came from totally different localities and that no relation whatsoever obtained amongst them that would point to the existence of a single common source of infection.

Stimulated by these results, it was decided to examine the sera, which are sent to the Laboratories for the Widal's test, and which are usually put up against *Bact. typhosum*, *Bact. paratyphosum* A and *Bact. paratyphosum* B, for the presence of agglutinins against *Bact. paratyphosum* C.

Out of fifty cases, nine sera agglutinated Hirschfeld's *Bact. paratyphosum* C: three to a titre of 1 in 50; two to a titre of 1 in 125; and four to a titre of 1 in 250.

Samples of urine and faeces from these positive cases were then asked for. Specimens from seven cases only were received and the findings are given in Table IX.

Table IX. *Findings of specimens of urine and faeces received from seven cases which gave positive Widal reaction*

Titre of agglutination	No. of cases	*Bact. paratyphosum* C isolated from
1 in 250	2	Urine
1 in 125	1	,,
1 in 125	1	Faeces

From such data one may infer that paratyphoid C is spread all over the country. As regards its frequency the figures in Table X show that it is comparable with that of paratyphoid A and paratyphoid B.

Table X. *The frequency of paratyphoid* C *in Egypt*

Number of specimens of faeces and urine received from 1. v. 37 to 31. vii. 37	Number of cases positive for paratyphoid organisms: *Bact. paratyphosum* A	*Bact. paratyphosum* B	*Bact. paratyphosum* C
1420	10	2	4

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The number of positive cases in the above table may seem to be few compared with the number of the specimens examined, but this may be attributed, first, to the fact that in the Central Laboratories we derive our material from certain restricted areas and, secondly, because it is invariably the routine of the medical officers to request an examination of the stools and urine, losing sight of the fact that blood culture, in all cases, afford the best means for the detection of the organism in the earlier period of the disease.

Now that the existence of *Bact. paratyphosum* C infection in Egypt has been proved, the question of its inclusion in prophylactic vaccines, along with the usual "T.A.B.", is worthy of serious consideration.

Summary

1. The paper records, for the first time, the isolation of *Bact. paratyphosum* C in Egypt.

2. It has been isolated from the urine of nine patients and the faeces of two patients suffering from enteric fever during the summer of 1937 and the early part of the present year.

3. Its frequency is comparable with that of *Bact. paratyphosum* A, and may exceed that of *Bact. paratyphosum* B.

4. Its inclusion in prophylactic vaccines, along with the usual "T.A.B." vaccine is worthy of serious consideration.

REFERENCES

Andrewes, F. W. & Neave, S. (1921). *Brit. J. exp. Path.* **2**, 157.
Dudgeon, L. S. & Urquart, A. L. (1920). *Lancet*, **2**, 15.
Garrow, R. P. (1920). *Lancet*, **1**, 1221.
Giglioli, G. (1930). *J. Hyg., Camb.*, **29**, 273.
Hirschfeld, L. (1919). *Lancet*, **1**, 296.
Iwascheuzoff, G. (1926). *Arch. Schiffs- u. Tropenhyg.* **30**, 1.
Kauffmann, F. (1931). *Zbl. ges. Hyg.* **25**, 273.
—— (1934). *Ergebn. Hyg.* **15**, 219.
MacAdam, W. (1919). *Lancet*, **2**, 189.
Mackie, F. P. & Bown, G. J. (1919). *J. R. Army med. Cps*, **33**, 145.
Neukirch, P. (1918). *Z. Hyg. InfektKr.* **85**, 103.
Schutze, H. (1920). *Lancet*, **1**, 93.
—— (1930). *Brit. J. exp. Path.* **11**, 34.
Topley, W. W. C. & Wilson, G. S. (1936). *The Principles of Bacteriology and Immunity.*
Weigmann, F. (1925). *Zbl. Bakt.* **95**, 396.
—— (1925). *Zbl. Bakt.* **97**, Beiheft 299.
Weil, E. (1917). *Klin. Wschr.* **30**, 1061.
White, P. B. (1926). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 103.
System of Bacteriology (1930).

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