AGGLUTININS FOR THE TYPHOID-PARATYPHOID GROUP IN A RANDOM SAMPLE OF THE POPULATION OF BRITISH GUIANA.

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Enteric fever in British Guiana, according to official reports, would not appear to be excessively common: the number of notifications for the whole Colony during the years 1930 and 1931 was 244 and 250 respectively on a population of 310,000.

The few observers who have studied this disease in the Colony have all insisted on its sporadic character. Rowland (1910) has clearly illustrated this peculiarity with a series of spot maps showing the distribution of enteric in Georgetown from 1909 to 1913.

In the interior of the Colony, on the Demerara River, during nearly ten years' observation, I found *Bact. typhosum* infections to be frequent; the disease was more or less always present in the region, but no definite focus of infection could be located.

This epidemiological peculiarity of enteric in British Guiana suggests the existence of a large number of undiagnosed infections which, if traced, would give the link between these apparently sporadic cases. That enteric infection should be widely distributed and prevalent in the Colony is highly probable, when one considers the very low standard of general sanitation, particularly as regards drinking water supply and sewage disposal; on the low-lying coastal areas of Demerara the distinction between the so-called "sweet water trenches," which form the main source of the drinking water supply in many of the villages and plantations, and the sewer trenches is often more marked in theory than in practice.

I have reported elsewhere on the occurrence and epidemiology of paratyphoid C in British Guiana (1927–9), pointing out the relations between this disease and the epidemiology of malaria. Recently (1933) I have dealt in greater detail with this important character. Enteric fever due to Hirschfeld's bacillus appears to follow in the train of malaria and occasionally of other endemic or epidemic diseases. From a clinical and epidemiological study of this disease *Bact. paratyphosum* C appears, under ordinary conditions, to be only mildly pathogenic, but if an epidemic of malaria, relapsing fever or typhus, brings about a phase of diminished herd-resistance, it may give rise to fulminating and very fatal disease.

https://doi.org/10.1017/S0022172400018684 Published online by Cambridge University Press

One of the most striking characteristics of the outbreak which I studied in British Guiana was the sudden appearance of a large number of serious cases of the disease over an extensive tract of country soon after the onset of a very severe epidemic of malaria in 1926. With few exceptions these cases were sporadic in their distribution. These peculiarities in the mode of onset of the outbreak and the distribution of cases suggest that the paratyphoid infection was pre-existent to the malarial outbreak and was already widely distributed in the district; the malarial epidemic supplied the opportunity which caused the infection to become clinically apparent.

The existence in British Guiana of widespread infection with *Bact. typhosum* and *Bact. paratyphosum* C offered an obvious opportunity of studying the frequency of antibodies acting on these organisms in the blood of the general population, and comparing the results with those obtained by other workers among the population in England, where the frequency of these infections is very different. Such a comparative study should throw light on two important and interdependent immunological problems. Do the natural antibacterial antibodies—in this case natural agglutinins—arise as the result of latent infection, or in some other way? Does a survey of the frequency of natural agglutinins among a given population afford a reliable index of the frequency of the corresponding infections?

Agglutinins for the typhoid-paratyphoid group in normal subjects in England.

Rosher and Fielden in 1922, working in England, in a series of 181 normal, non-inoculated subjects, obtained positive agglutination in dilutions of 1 : 20 or over, for *Bact. typhosum* in 3 per cent.; for *Bact. paratyphosum* A in 0 per cent.; for *Bact. paratyphosum* B in 4 per cent.

A similar investigation was carried out by Smith, MacVie and Newbold (1930) in Manchester; only the figures obtained for females can be of interest for our purpose, as the results obtained for males were still markedly influenced by preventive inoculation administered during the war. Table I summarises the results obtained from the examination of 146 females.

Table I.

	Titre*					
	1:20	1:40	1:80	1:160	1:320	1:640 +
Bact. typhosum	2·0	2·0	—	0·7	—	—
Bact. paratyphosum A	—	—	—	—	—	—
Bact. paratyphosum B type	0·7	1·3	—	—	—	—
Bact. paratyphosum C type	—	—	0·7	—	—	—
Group phase	2·7	0·7	—	0·7	0·7	—

* The figures under each titre are the percentage of cases reacting at that dilution but not above.

It may be noted that none of the 154 sera obtained from males agglutinated *Bact. paratyphosum* C in the type phase, so that it is clear that this particular

agglutinin is very rarely present among the normal English population. Table II summarises the findings of Gardner and Stubington (1932) in a series of 50 normal subjects in England, tested with "O" suspensions.

Table II.

Suspensions	Number of sera showing trace titres			
	0–25	25–50	50–100	100–200+
Bact. typhosum "O"	31	16	2	1
Bact. aertrycke "O" (*para.* B "O")	44	5	1	0
Bact. paratyphosum A "O"	50	0	0	0

AGGLUTININS FOR THE TYPHOID-PARATYPHOID GROUP IN NORMAL SUBJECTS IN THE SOUTHERN UNITED STATES.

In Alabama, in the Southern U.S.A., where enteric is very prevalent, Havens and Mayfield (1931) have recorded a very high incidence of agglutinins for the typhoid-paratyphoid group in normal, non-selected individuals: agglutinins for *Bact. typhosum* were found in 23 per cent. of the sera examined in titres of 1 : 40 or over. These authors believe that the agglutinins found in the blood of normal individuals are the result of previous infection.

AGGLUTININS FOR THE TYPHOID-PARATYPHOID GROUP IN NORMAL SUBJECTS IN BRITISH GUIANA.

Material.

The sera of 350 normal subjects have been studied. The term "normal" is here applied to individuals not suspected of suffering from enteric at the time of examination, who gave a negative history for enteric and for suspicious prolonged fever, and who had not received anti-typhoid vaccination. The reliability of such negative histories, obtained from natives, is obviously limited. Anti-typhoid vaccination is not common in the Colony, being more or less limited to contacts of notified cases who voluntarily submit to inoculation. On the Demerara River I carried out two extensive vaccination campaigns in 1928 and 1930; very thorough card-index records of these inoculations were kept; so that it is certain that no inoculated person from this area was included among those examined during this enquiry.

Most of the cases examined were surgical or malarial patients admitted to Mackenzie Hospital; as regards race and sex they can be classified as follows:

	Males	Females	Total
Negroes	90	68	158
Mixed	40	36	76
East Indians	38	15	53
Portuguese	16	12	28
Aboriginal Indians	14	16	30
Chinese	2	3	5

As regards residence, the cases can be classified as follows:

Demerara River	231
Coastal Villages and Plantations	65
City of Georgetown	54

The majority of cases were labourers, but a sufficient number of middle-class natives (clerks, shopkeepers, etc.) were also examined; this element tends to prevail in the Georgetown group. The whole series of cases may be taken as a fair average sample of the native population of the Colony.

Technique.

The macroscopic agglutination method was used, dilutions ranging from 1 : 10 upwards; the usual control tests were carried out and readings were taken after 4 and 24 hours in the water bath at 56° C. Each serum was tested against the following "H" antigens: *Bact. typhosum*, *Bact. paratyphosum* A, *Bact. paratyphosum* B type, *Bact. paratyphosum* C type, and the common group phase antigen. The following "O" suspensions were also used: *Bact. typhosum*, *Bact. paratyphosum* B, *Bact. paratyphosum* C.

"H" antigen suspensions for *Bact. typhosum*, *Bact. paratyphosum* A and monophasic *Bact. suipestifer* (group phase antigen), were prepared by killing 18-hour broth cultures with formalin and by heating for 2 hours in the water bath at 56° C. "H" antigen suspensions for the type phases of *Bact. paratyphosum* B and C were prepared in the same way from broth cultures grown at 25° C. from type colonies, selected by plating and by a preliminary agglutination test.

"O" antigen suspensions were prepared, after testing cultures for roughness, by washing off 24-hour cultures grown on 1 : 800 phenol agar with absolute alcohol; centrifuging and storing in the form of a thick suspension in 50 per cent. alcohol. From these stock alcoholic suspensions, saline suspensions of the required opacity were made at the time of use.

I wish here to thank Dr R. Lovell, of the Department of Bacteriology of the London School of Hygiene and Tropical Medicine, for a great deal of valuable advice on the technique which has been followed in this research, and for supplying me with a full set of cultures and of specific sera.

Table III summarises separately the results obtained from the examination of the blood of 150 females and 200 males.

The sex factor does not appear to exercise any influence on the occurrence and distribution of agglutinins, as a comparison of the two sections of the table evidently demonstrates. The race factor, too, has no importance in this respect.

It will be noted that the incidence of "H" agglutinins is high; they are particularly frequent for *Bact. typhosum*, for the type phase of *Bact. paratyphosum* C. and for the common group phase antigen. Agglutinins for *Bact. paratyphosum* A are less frequent, and those for the type phase of *Bact. paratyphosum* B are distinctly uncommon. In a great many cases and with all antigens, but more especially for typhoid, paratyphoid C type, and the common group antigen, agglutination occurs in titres which might easily be regarded as "diagnostic."

The incidence of "O" agglutinins is very high for all the three antigens

Table III.

Antigen	Negative	Serum dilutions 1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	% of sera positive for agglutinins	% of sera agglutinating in a titre of 1:40 or over
						Females (150)							
Bact. typhosum "H"	93	21	9	14	8	4	1	—	—	—	—	31·3	18·0
Bact. paratyphosum A "H"	134	7	3	2	3	1	—	—	—	—	—	10·6	4·0
Bact. paratyphosum B type "H"	143	1	4	—	1	1	—	—	—	—	—	4·6	1·3
Bact. paratyphosum C type "H"	115	6	6	11	2	6	2	—	—	1	—	22·6	14·6
Common group phase "H"	90	7	19	11	10	11	1	—	1	—	—	40·0	22·6
Bact. typhosum "O"	99	24	24	2	1	—	—	—	—	—	—	34·0	2·0
Bact. paratyphosum B "O"	85	27	26	7	5	—	—	—	—	—	—	43·3	8·0
Bact. paratyphosum C "O"	102	15	15	13	2	1	2	—	—	—	—	32·0	12·0
						Males (200)							
Bact. typhosum "H"	137	13	21	13	7	4	5	—	—	—	—	31·5	14·5
Bact. paratyphosum A "H"	165	11	6	9	4	5	—	—	—	—	—	17·5	9·0
Bact. paratyphosum B *type* "H"	179	6	6	3	3	3	—	—	—	—	—	10·5	4·5
Bact. paratyphosum C type "H"	167	11	4	4	2	6	5	—	—	1	—	16·5	9·0
Common group phase "H"	135	15	14	14	4	11	5	1	1	—	—	32·5	18·0
Bact. typhosum "O"	141	29	20	8	1	—	1	—	—	—	—	29·5	5·0
Bact. paratyphosum B "O"	135	32	25	6	1	—	1	—	—	—	—	32·5	4·0
Bact. paratyphosum C "O"	147	18	20	9	2	3	—	—	—	—	1	26·5	7·5

Table IV.

Antigen	All colony 350 sera: % positive for agglutinins	All colony 350 sera: % agglutinating 1:40 or over	Demerara River 231 sera: % positive for agglutinins	Demerara River 231 sera: % agglutinating 1:40 or over	City of Georgetown 54 sera: % positive for agglutinins	City of Georgetown 54 sera: % agglutinating 1:40 or over	Coast villages and plantations 65 sera: % positive for agglutinins	Coast villages and plantations 65 sera: % agglutinating 1:40 or over
Bact. typhosum "H"	34·2	16·0	36·3	16·4	33·3	24·7	27·6	7·6
Bact. paratyphosum A "H"	14·5	6·8	13·4	5·6	14·8	9·2	18·4	9·2
Bact. paratyphosum B type "H"	8·0	3·1	5·6	2·5	9·2	3·7	15·3	4·6
Bact. paratyphosum C type "H"	19·4	11·7	24·6	15·1	7·4	7·4	10·7	3·0
Common group ant. "H"	35·7	20·0	39·3	22·5	25·9	14·8	30·7	15·3
Bact. typhosum "O"	31·4	3·7	32·0	3·0	24·0	3·7	35·3	6·1
Bact. paratyphosum B "O"	37·1	5·7	34·6	5·1	44·4	7·4	40·0	6·1
Bact. paratyphosum C "O"	28·8	9·4	32·0	13·0	25·9	3·7	20·0	1·5

tested, but in the great majority of cases these are only found in the lower dilutions (1 : 10, 1 : 20); agglutination in high titres, on the contrary, is exceptional; even in medium titres (1 : 40 to 1 : 320) "O" agglutinins are much less frequent than the corresponding H agglutinins. Titres above 1 : 80 were very uncommon for *Bact. typhosum* and *Bact. paratyphosum* B but much less so for *Bact. paratyphosum* C. The presence in normal sera of "O" agglutinins acting to low titre on *Bact. typhosum* and *Bact. paratyphosum* B has been recorded by many observers. So far as I am aware no extensive data have been recorded in regard to the frequency of "O" agglutinins acting on *Bact. paratyphosum* C.

In Table IV results have been classified according to the habitual residence of the individuals studied: the incidence of the various agglutinins is shown for the whole Colony (350 sera), for the Demerara River (231), for the City of Georgetown (54), for the coastal villages and plantations (65). The two latter groups are small and figures must be accepted with reserve. Agglutinins appear to be frequent in all groups, but H agglutinins for *Bact. typhosum* appear to be considerably more frequent in the Georgetown group, while "H" agglutinins for the type phase of Hirschfeld's bacillus are much more frequent in the Demerara River group. The latter fact corresponds to clinical experience and might possibly find its explanation in the distribution of malaria, which is much more prevalent and severe in the river districts of the interior than in Georgetown and the well populated coastal belt.

A very considerable number of sera were found which contained agglutinins for the antigens of more than one species. In Table V the details of such cases are given. Many of these are, in all probability, the result of double infection, but in most instances it is difficult to offer a satisfactory explanation.

Discussion and conclusions.

Agglutinins for the typhoid-paratyphoid group are evidently very frequently found in the blood of normal subjects in British Guiana. Such a fact appears more forcibly if a comparison is made with the results of similar researches in England. Hirschfeld's bacillus has not been isolated in England and agglutinins for this species may be regarded as altogether exceptional in this country. In British Guiana, on the contrary, paratyphoid C is endemic and on 350 normal subjects agglutinins for the "H" type antigen of Hirschfeld's bacillus existed in the blood of 19·4 per cent.; for the common "H" group antigen in 35·7 per cent., and for the "O" antigen in 28·8 per cent. If we only take into consideration the cases in which the agglutination titre was such as might easily be found in actual infection (1 : 40 or over), the above figures would still read 11·7, 20 and 9·4 per cent. respectively.

Such results strongly suggest that the agglutinins found in the blood of normal subjects are, to a great extent at least, the consequence of *sub-clinical infection*. In British Guiana enteric infection must be very much more widely spread and common than is indicated by clinical experience and by morbidity returns; this is particularly the case for paratyphoid C.

We now have a satisfactory explanation of the sporadic distribution of enteric reported from the city of Georgetown and from the Colony in general: it is only the clinical manifestation of infection which is sporadic, but infection is widespread. This explains also the sudden appearance of paratyphoid C in a virulent form, over a wide area but still with an apparently sporadic distribution during the severe malarial epidemic of 1926–7. Infection with Hirschfeld's bacillus may evolve with only the mildest clinical signs; I have recorded

Table V*.

	"H" antigens					"O" antigens		
Case No.	*Bact. typhosum*	*Bact. paratypho-sum* A	*Bact. paratypho-sum* B type	*Bact. paratypho-sum* C type	Common group antigen	*Bact. typhosum*	*Bact. paratypho-sum* B	*Bact. paratypho-sum* C
1	320	—	—	1280	320	—	—	160
2	40	—	—	160	160	—	10	40
3	—	40	10	—	40	—	—	20
4	320	—	—	—	—	40	320	—
5	—	160	20	—	40	40	40	—
6	320	40	160	—	160	40	40	—
7	40	160	10	—	10	80	10	—
8	80	80	—	—	—	—	—	—
9	80	—	20	20	40	20	—	—
10	160	20	80	160	160	—	—	20
11	160	—	40	—	160	10	10	10
12	160	10	—	10	40	—	20	—
13	80	10	160	20	160	—	—	—
14	160	—	—	20	—	20	20	—
15	40	—	—	—	—	40	10	40
16	40	—	—	40	—	—	—	40
17	40	160	—	—	—	—	—	—
18	20	40	—	—	—	20	—	40
19	40	—	—	40	40	—	40	10
20	—	80	—	—	80	10	10	—
21	160	40	—	—	10	—	—	10
22	80	—	20	—	160	—	—	—
23	40	—	—	20	10	10	10	80
24	—	80	160	—	40	—	—	—
25	40	—	—	—	—	10	80	10
26	20	40	—	—	20	10	20	20
27	10	20	—	40	10	10	40	—
28	10	10	20	—	20	20	20	20
29	40	20	—	—	—	10	10	—
30	320	20	—	—	—	—	10	40
31	—	80	20	—	—	20	40	—
32	—	—	20	40	80	—	—	—
33	—	—	—	—	40	20	40	40

* The numbers under each antigen correspond to the end-titre of agglutination.

ambulatory cases giving a positive haemoculture with only slight fever, of the duration of 3–5 days. Such forms are probably more the rule than the exception, and in a country like British Guiana, where malaria fever prevails, it is scarcely surprising if they escape recognition.

The high incidence of agglutinins for the common group phase "H" antigen should be noted; these agglutinins are often found alone, *i.e.* without type agglutinins for either *Bact. paratyphosum* B or C; group agglutinins in fact are more frequently found than both these type agglutinins taken jointly.

The possibility of infection with other members of the Salmonella group should therefore be entertained.

From what has been said it is evident that the interpretation of positive findings in the Widal reaction for the routine laboratory diagnosis of enteric in British Guiana is beset with difficulties. Tests carried out according to the older technique, in which no differentiation was made between the type and group phases of the flagellar antigens, would frequently yield misleading results; the use of separate "H" antigens for the type phases of *Bact. paratyphosum* B and C is essential for the proper differentiation of the specific agglutinins of these two organisms, both of which exist in the Colony. The incidence of "O" agglutinins in significant titre in the blood of normal subjects being considerably less frequent than is the case for "H" agglutinins, greater value can be placed on the findings of high "O" titres.

Qualitative receptor analysis in the conditions which prevail in British Guiana presents, without doubt, many advantages on the old technique of the Widal reactions, but even so not much reliance can be placed on positive results obtained in a single test. Repeated examinations of the blood should be carried out; cultural methods are here more imperatively required than elsewhere and should always be attempted, whenever possible. In the case of paratyphoid C infection it is well to remember that the disease is typically a blood infection; the specific organism can be easily grown from the blood throughout the course of the disease, and from the urine, often, after the clinical signs have abated and the patient has entered convalescence.

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(*MS. received for publication* 27. IV. 1933.—Ed.)