STUDIES ON THE O ANTIGENS OF TYPHOID, PARATYPHOID, AND COLON BACILLI

I. INCIDENCE OF O AND H AGGLUTININS FOR BACTERIUM PARATYPHOSUM B IN NORMAL HUMAN SERUMS

II. OBSERVATIONS ON THE ORIGIN OF THE BACTERIUM PARATYPHOSUM B AGGLUTININS IN NORMAL HUMAN SERUMS

by

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II. OBSERVATIONS ON THE ORIGIN OF THE BACTERIUM PARATYPHOSUM B AGGLUTININS IN NORMAL HUMAN SERUMS[1]

Introduction

The ability of normal serum to agglutinate bacteria in suspension was observed by many of those early workers who studied the properties of the immune agglutinins (Bordet,1899, Gengou, 1899, Jatta, 1900). No systematic investigation of this natural phenomenon was apparently made at that time. Points of difference between natural and immune agglutinins were described by Landsteiner and Calvo (1902), and Landsteiner and Reich (1905). In the serological study of particular organisms their agglutination by normal serum was mentioned and its extent noted, as for example, in the case of Pf. malloi, by M'Fadyean (1896), and Eastman (1908). In the case of the streptococci, normal agglutinins were described by Moser and V. Pirquit (1902). Durham (1902) demonstrated the presence of normal coli agglutins in human serum.

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1. Throughout this paper the nomenclature of the Kauffmann-White scheme of classification in the Salmonella group has been used. For organisms not included in this group the nomenclature of the fifth edition of Bergey's Manual is employed.

Investigations of Parischa (1936), Felix (1928), Giglioli (1933), Pijper (1930) and Lewin (1936) have demonstrated the occurrence of normal agglutinins to the Salmonella group. From this we can conclude that the phenomenon of normal agglutinins is world wide and not restricted to any one locality.

The occurrence of agglutinins in the serum of normal humans has been observed by many workers. Rosher and Fielden (1922) found that of 276 male serums, 42 per cent showed the presence of O agglutinins for Bact. typhosum and 26 per cent for Bact. paratyphosum B. Smith, MacVie and Newbold (1930), give the following results for the flagellar or H agglutinins of 302 serums. Of this total 14.9 per cent agglutinated Bact. typhosum, 5.9 per cent agglutinated Bact. paratyphosum B. Havens and Mayfield (1931) tested 1136 serums for O agglutinins and found 23 per cent of the total to agglutinate Bact. typhosum, 14 per cent in a 1:40 dilution, six per cent in 1:80, and three per cent in a 1:160 dilution or higher. Fishberg (1922) studied 60 human serums and reports that 20 per cent of the total agglutinated Bact. typhosum in a 1:20 dilution. In three cases the agglutination occurred in a 1:50 dilution. Giglioli (1933) states that of 150 serums from a random sample of the population of British Guiana:

31.3% agglutinated Bact. typhosum H

4.6% agglutinated Bact. paratyphosum B H

43.3% agglutinated Bact. paratyphosum B O

34.0% agglutinated Bact. typhosum O.

Alves (1936) in a series of 530 non-inoculated Southern Rodesian natives, found that Bact. typhosum H agglutination was present in 5.1 per cent in a minimum dilution of 1:50. Lewin (1938) records the following results of 442 human serums:

Serum Dilution	H Agglutination		O Agglutination	
	Number Showing Agglutination	Per Cent Showing Agglutination	Number Showing Agglutination	Per Cent Showing Agglutination
1:25	47	10.6	199	45.0
1:100	13	2.9	20	4.5
1:200	5	1.1	9	2.0
1:400	2	0.4	8	1.8

Gardner and Stubbington (1938) report that in 50 human serums tested, granular O agglutination occurred in a 1:25-50 dilution as follows: 30 per cent showed Bact. typhosum agglutinins, and 10 per cent showed Bact. paratyphosum B agglutinins. Dreyfanlesu, Lebesu and McCrady (1938) state that five of 100 male, but none of 100 female serums gave agglutination in a dilution of 1:50. Cruickshank (1939) upon the examination of 200 Wassermann serums reports that 11.5 per cent agglutinated Bact. paratyphosum B, and 15.5 per cent agglutinated Bact. typhosum in the granular somatic O phase. Gregory and Atkenson (1936) disclose that of 500 serums sent into the laboratory for the Wassermann test and titered for the presence of H and O agglutinins for Bact. typhosum, Bact. typhosum H and Bact. paratyphosum A as follows: 17.3 per cent of the total agglutinated Bact. paratyphosum B, 0.6 per cent Bact. paratyphosum A, and 30.6 per cent agglutinated Bact. typhosum in the O phase. H agglutination occurred as follows: for Bact. paratyphosum B

3.5 per cent, Bact. paratyphosum A 0.4 per cent and Bact. typhosum 3.6 per cent. Sears and Phillips (1936) of this laboratory have reported an unusually high incidence of O agglutinins taken from a random sample in this area. The study reported in the present paper was undertaken to confirm and extend the studies of these investigators in respect to this organism.

Objectives of This Thesis

This investigation deals with: (1) The occurrence of normal human agglutinins for Bact. paratyphosum B; (2) The behavior of such serum with other related antigens; (3) The specific nature of normal agglutinins.

Incidence of Agglutinins for Bact. Paratyphosum B in Normal Human Serums.

Method and Technique

Serums taken for the routine Kahn test were obtained from the Multnomah County Hospital. Serums that showed any trace of hemolysis were not used, nor were the serums of patients having a past history of enteric fever. Positive Kahn serums were likewise eliminated.

Antigens used for the agglutination tests were as follows:

Bact. paratyphosum B 8006 - Obtained from Kauffmann (Copenhagen)

Bact. paratyphosum B 869B - Obtained from California State Laboratory (Berkley)

Bact. typhosum 0901 - Obtained from Felix (London)

Bact. typhosum H901 - Obtained from Felix (London)

The H and O antigens were prepared essentially according to Coleman (1936).

Preparation of the H Antigen

Care was taken to employ actively motile smooth strains. One or two of the smooth colonies were suspended in saline and then transfered to Blake bottles. After incubation for 18 to 20 hours at 37° C., the growth was washed off with 0.85 per cent saline containing 2 per cent formaline. Sterility tests were made and the suspension was then standardized to give a turbidity equal to ten times the Barrium Sulfate standard No. 3. Upon using, the suspension was diluted 1:10 with saline thus reducing the formalin content to 0.2 per cent.

However, it was found that this method was not suitable for the preparation of pure specific and non-specific H antigens. When these antigens were prepared in the above manner it was noted that the specific phase always contained a small amount of non-specific and vice versa. To eliminate this error it was found advisable to use a six to eight hour broth culture from single colonies to which was added formalin to a final concentration of 0.2 per cent.

Preparation of O Antigens

The same procedure was used as in the preparation of the H antigens. The O antigen differs in that 0.5 per cent phenol in 0.85 per cent saline was used to wash off the growth on the Blake bottles. Alcohol was added to give a final concentration of 30.0 per cent. The standardization was

carried out as in the preparation of the H antigen. The agglutinability of the suspension was tested in comparison with a previous lot which had proven to be satisfactory.

Preliminary Testing of Human Serums

The purpose of this experiment was to discover those serums which might contain agglutinins for Bact. paratyphosum B in any of its smooth antigenic forms. Each serum was subjected to a preliminary test using an O antigen of Bact. paratyphosum B 8006, and also an H antigen of the same strain in which specific and group phases were prepared separately and mixed. Each serum was tested in a single dilution of 1:20 against each antigen. Serums which gave no agglutination with these antigens were discarded and are not considered further in this paper. Those which have a positive reaction with either the H or O antigen were investigated further as described later.

In these preliminary tests, as in all other tests described, three by three-eighths inch agglutination tubes were used in which the total volume was maintained at 0.5 cc. The incubation in the water bath was at 50° C. The tubes were immersed so that only one-fourth to one-half of the column was below the water level, thus facilitating the mixing by convection current.

H agglutination was read at the end of two hours, and the O agglutination after twenty hours in the water bath. All of the tubes were read

with the naked eye and also with the bottom lens of a 10X microscopeocular. The degree or amount of agglutination present was recorded as follows:

+4 -- Clear supernatant fluid; complete agglutination

+3 -- Clear supernatant fluid; definite agglutination

+2 -- Slightly cloudy fluid; definite agglutination

+1 -- Cloudy supernatant fluid; agglutination barely discernible to the eye

1L -- Agglutination visible only by the use of a lens.

A total of 233 serums was examined in the preliminary test and of these 156 gave negative results. Table I gives the details of the 77 serums that agglutinated one or both of the antigens used in the preliminary test.

Table I

DETAILED RESULTS ON 77 SERUMS SHOWING H OR O AGGLUTINATION IN A PRELIMINARY TEST IN A 1:20 DILUTION

Serum Number	Bact. paratyphosum B 8006	
	O Antigen	H Antigen
89315	+1	0
93263	+2	0
93624	+1	0
81036	+4	0
93649	+3	0
93292	+2	0
93664	+3	0
93671	+2	0
93662	+4	+2
93672	+2	0
93675	+3	0
93674	+4	0
1735	+4	0
93667	+2	0
89071	+3	0
16027	+2	0
91333	+2	0
78 MCH	+4	0
93951	+2	0
74970	+4	0
93950	+4	0
75336	+1	0
93939	+4	0
16934	+3	0
5480	+2	0
93948	+4	+2
93976	+2	0
80974	+4	0
54956	+4	0
94009	+4	0
37 MCH	+3	0
94022	+4	0
93926	0	+2
94019	+4	0
19 MCH	+2	0
96343	+2	0
96341	+3	0
93967	0	+2

Table I (Continued)

Serum Number	Bact. paratyphosum B 8006	
	O Antigen	H Antigen
80087	+3	0
94003	+4	0
92659	+3	+1
94406	+2	0
94413	+2	0
94331	+2	0
94430	+1	0
85754	+4	0
12 MCH	+2	0
22 MCH	+3	0
95037	+4	0
94869	+2	0
95165	+1L	0
95412	+4	0
82459	+4	0
39593	+2	0
96346	+3	0
95382	+4	0
13043	+3	0
95470	+2	0
95388	+2	0
95423	+2	0
88841	+1L	0
95485	+1	0
95481	+4	0
28 MCH	+1	0
54558	+1	0
85642	+2	0
95800	+3	0
95663	+3	0
31 MCH	+2	+2
61687	+1L	0
95557	+2	0
85937	+4	0
38 MCH	+3	0
M.R. MCH	+4	0
R.V.MCH	+3	0
18169	+4	0
96330	+3	0
96376	+2	0
96457	+2	0

Complete Titration of Serums Positive in the Preliminary Test

Serums which were positive in this preliminary test were examined further as follows:

O agglutinins were titrated in dilutions 1:20 to 1:320 inclusive, against the separate O antigens of Bact. paratyphosum B 8006, Bact. paratyphosum B 289B, and Bact. typhosum 0901.

Serums that showed the presence of H agglutinins in the preliminary test were titrated against H antigens of Bact. typhosum H901, and specific and group phase antigens separately of the two strains of Bact. paratyphosum B. The dilutions were also 1:20 to 1:320 inclusive.

Inspection of Table II reveals the detailed titration of the O positive serums. In this table the 1:320 dilution is omitted since none of the serums tested gave agglutination in this dilution. Examination of Table II shows that the highest titer in the majority of the serums was low. However, upon the inspection of Table IV, we see that an appreciable portion of the serums agglutinated one or more of the antigens in dilutions as high as 1:160, the figure being 20.7 per cent in the case of O antigens of Bact. paratyphosum B 8006, and 17 per cent in the case of Bact. typhosum 0901. It appears that the O antigen of Bact. paratyphosum B 289B is less agglutinable than is Bact. paratyphosum B 8006 since none of the serums agglutinated the former antigen to a dilution of 1:160, and since seven serums agglutinating Bact. paratyphosum B 8006 failed to agglutinate Bact. paratyphosum B 289B even in a 1:20 dilution.

In a 1:80 dilution only 17 per cent of the total serums agglutinate Bact. paratyphosum B 299B, whereas the corresponding figure for Bact. paratyphosum B 6006 is 30 per cent. In the two lower dilutions the figures for Bact. paratyphosum B 299B are greater than for Bact. paratyphosum B 6006.

Referring to Table II, it is seen that the greater sensitiveness of the Bact. paratyphosum B 6006 O antigen is manifested also in the greater completeness of agglutination even in the lower dilutions, since a high per cent of the serums are recorded one or more plusses higher for the Bact. paratyphosum B 6006 than for the Bact. paratyphosum B 299B O antigen.

Table II

O AGGLUTINATION REACTION OF ALL THE POSITIVE SERUMS FOUND IN THE PRELIMINARY 1:20 DILUTION

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	8006	289B	
29315	1:20 - 1L	0	1:20 - 1L
93263	1:20 - +2 1:40 - +1	1:20 - +1 1:40 - 1L	1:20 - +2 1:40 - +2
93264	1:20 - +2 1:40 - +1	1:20 - +2 1:40 - +1	1:20 - 1L
21036	1:20 - +3 1:40 - +1	1:20 - +2 1:40 - +1	0
93649	1:20 - +3 1:40 - +3 1:80 - +1	1:20 - +2 1:40 - +1	1:20 - +4 1:40 - +2 1:80 - +1
93664	1:20 - +3 1:40 - +2 1:80 - +1	1:20 - +3 1:40 - +1	1:20 - +1
93672	1:20 - +3 1:40 - +3 1:80 - +1	1:20 - +2 1:40 - +1	1:20 - +2 1:40 - +2 1:80 - +1 1:160-1L
93662	1:20 - +4 1:40 - +2 1:80 - 1L	1:20 - +3 1:40 - +1	0
93675	1:20 - +4 1:40 - +3 1:80 - +1 1:160- 1L	1:20 - +3 1:40 - +2 1:80 - 1L	1:20 - +3 1:40 - +3 1:80 - +2

Table II (Continued)

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	8006	259B	
95135	1:20 - +2	1:20 - +1	1:20 - +2 1:40 - +2 1:80 - +1 1:160 - IL
22 MCH	1:20 - +4 1:40 - +2 1:80 - IL	1:20 - +3 1:40 - IL	0
94869	1:20 - +2	1:20 - +1	0
9516	1:20 - +1 1:40 - IL	0	0
95282	1:20 - +4 1:40 - +2 1:80 - +1 1:160 - +1	1:20 - +3 1:40 - +3	0
95412	1:20 - +4 1:40 - +4 1:80 - +2 1:160 - IL	1:20 - +2 1:40 - +3	1:20 - IL
95346	1:20 - +4 1:40 - +2	1:20 - +3 1:40 - +2	0
39693	1:20 - +3 1:40 - +2 1:80 - +2	1:20 - +4 1:40 - +2	0
82459	1:20 - +4 1:40 - +4 1:80 - +3 1:160 - +3	1:20 - +4 1:40 - +3 1:80 - +2	0
13043	1:20 - +2 1:40 - +2 1:80 - +1	1:20 - +3 1:40 - +2 1:80 - +1	0

Table II (Continued)

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	0006	239B	
93951	1:20 - +2 1:40 - +1	1:20 - +1	1:20 - +1
16934	1:20 - +4 1:40 - +3 1:80 - +2 1:160 - +1	1:20 - +3 1:40 - +2	1:20 - +3
76336	1:20- +2	1:20 - +1	0
19 MCH	1:20 - +2 1:40 - +1	1:20 - +1	0
93950	1:20 - +4 1:40 - +4 1:80 - +2	1:20 - +4 1:40 - +3 1:80 - +1	1:20 - +2 1:40 - +2 1:80 - +1
80974	1:20 - +4 1:40 - +3 1:80 - +2 1:160 - +1	1:20 - +4 1:40 - +2 1:80 - +1	1:20 - +4 1:40 - +3 1:80 - +2
78 MCH	1:20 - +4 1:40 - +4 1:80 - +2 1:160 - +2	1:20 - +2 1:40 - +2 1:80 - +1	1:20 - +2 1:40 - +2 1:80 - +1
93978	1:20 - +2	1:20 - 1L	1:20 - +2 1:40 - +2 1:80 - +1
74970	1:20 - +4 1:40 - +3 1:80 - +1 1:160 - +1	1:20 - +3 1:40 - +2 1:80 - +1	1:20 - +3 1:40 - +2 1:80 - +1
94033	1:20 - +4 1:40 - +2 1:80 - +1	1:20 - +3 1:40 - +1	1:20 - +3 1:40 - +1
94001	1:20 - +4 1:40 - +4 1:80 - +2 1:160 - +1	1:20 - +4 1:40 - +3 1:80 - +1	1:20 - +4 1:40 - +2 1:80 - +1 1:160 - +1

Table II (Continued)

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	8006	289B	
80087	1:20 - +3 1:40 - +1	1:20 - +1	0
5480	1:20 - +4 1:40 - +2 1:80 - +1	1:20 - +3 1:40 - 1L	1:50 - +1
94022	1:20 - +3	1:20 - +2	0
37 MCH	1:20 - +4 1:40 - +2 1:80 - +1	1:20 - +4 1:40 - +1	1:20 - +3 1:40 - +2
94406	1:20 - +2 1:40 - +2 1:80 - +1	1:20 - +2 1:40 - +1	1:20 - +3 1:40 - +2 1:80 - +2 1:160 - +1
94413	1:20 - +3 1:40 - +2	1:20 - +1	0
94444	1:20 - +4 1:40 - +2 1:80 - +1	1:20 - +2 1:40 - +1	0
94430	1:20 - +1	0	0
86754	1:20 - +4 1:40 - +4 1:80 - +3 1:160 - +1	1:20 - +4 1:40 - +3	0
94351	1:20 - +3 1:40 - +1	1:20 - +1 1:40 - +1	1:20 - +4 1:40 - +3 1:80 - +3
12 MCH	1:20 - +2 1:40 - +1	1:20 - +1	0
95037	1:20 - +3 1:40 - +2 1:80 - 1L	1:20 - +3 1:40 - 1L	1:20 - +3 1:40 - +2 1:80 - +1 1:160 - 1L

Table II (Continued)

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	8006	289B	
93674	1:20 - +3 1:40 - +1 1:80 - +1	1:20 - +1	1:20 - +1 1:40 - +1
93684	1:20 - +2 1:40 - +1	1:20 - 1L	0
93671 6	1:20 - +3 1:40 - +2 1:80 - +2	1:20 - +2 1:40 - +1	1:20 - +1 1:40 - +1
1745	1:20 - +4 1:40 - +3 1:80 - +2 1:160- +2	1:20 - +3 1:40 - +1	1:20 - +2
93667	1:20 - +3 1:40 - +2 1:80 - +1 1:160 - +1	1:20 - +3 1:40 - +2 1:80 - +1	0
29071	1:20 - +3 1:40 - +1	1:20 - +2	1:20 - +2 1:40 - 1L
16027	1:20 - +4 1:40 - +4 1:80 - +1 1:160 - 1L	1:20 - +2 1:40 - +2 1:80 - +1	0
91335	1:20 - +1	0	1:20 - +4 1:40 - +1 1:80 - +1
93946	1:20 - +4 1:40 - +2	0	1:20 - +4
92659	1:20 - +3 1:40 - +3 1:80 - +1	1:20 - +3 1:40 - +2	1:20 - +3 1:40 - +2 1:80 - +2
93939	1:80 - +2 1:40 - +1	1:20 - +3 1:40 - +2	1:20 - +4 1:40 - +3 1:80 - +2

Table II (Continued)

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	8006	2893	
95388	1:20 - +4 1:40 - +4 1:80 - +2 1:160 - 1L	1:20 - +3 1:40 - +3	1:20 - +4 1:40 - +2 1:80 - +1
95423	1:20 - +2	1:20 - +1	1:20 - +4 1:40 - +3
95481	1:20 - +4 1:40 - +4 1:80 - +2	1:20 - +4 1:40 - +3	1:20 - +4 1:40 - +3 1:80 - +1
95470	1:20 - +3 1:40 - +1	1:20 - +3 1:40 - 1L	1:20 - +3 1:40 - +3
88641	1:20 - 1L	1:20 - +1	1:20 - +1
95485	1:20 - +1	1:20 - +1	1:20 - +1
95800	1:20 - +3 1:40 - +2 1:80 - 1L	1:20 - +2 1:40 - +1	1:20 - +2 1:40 - +2 1:80 - +2
28 MCH	1:20 - +1 1:40 - +1	1:20 - +2 1:40 - +1	0
85642	1:20 - +2 1:40 - +2	1:20 - +1	0
54568	1:20 - +1 1:40 - +1	1:20 - +1	1:20 - 1L 1:40 - 1L
95683	1:20 - +2 1:40 - +2	1:20 - +1 1:40 - 1L	1:20 - +3 1:40 - +3 1:80 - +2 1:160 - +1
31 MCH	1:20 - +2 1:40 - 1L	0	1:20 - +2 1:40 - 1L
95587	1:20 - +1	1:20 - 1L	1:20 - +2 1:40 - +1

Table II (Continued)

Serum Number	Bact. paratyphosum B		Bact. typhosum 0901
	2006	2278	
61657	1:20 - 1L	0	1:20 - +2 1:40 - +2 1:80 - +1
33 MCH	1:20 - +3 1:40 - +1	1:20 - +1	1:20 - +2 1:40 - +1
85937	1:20 - +4 1:40 - +3 1:80 - +2 1:160 - 1L	1:20 - +4 1:40 - +2 1:80 - 1L	1:20 - +3 1:40 - +2 1:80 - +1
M. R. MCH	1:20 - +4 1:40 - +2 1:80 - +1	1:20 - +4 1:40 - +1 1:80 - 1L	1:20 - +4 1:40 - +4 1:80 - +3 1:160 - +1
96341	1:20 - +3 1:40 - +2 1:80 - 1L	1:20 - +3 1:40 - +1	1:20 - +4 1:40 - +2 1:80 - 1L
96343	1:20 - +2	1:20 - +2 1:40 - 1L	1:20 - +3 1:40 - +1
96467	1:20 - +3 1:40 - +1	1:20 - +3 1:40 - 1L	1:20 - +4 1:40 - +1 1:80 - +1
96376	1:20 - +2 1:40 - 1L	1:20 - +1 1:40 - 1L	1:20 - +3 1:40 - +1
96330	1:20 - +3 1:40 - +3	1:20 - +2 1:40 - 1L	1:20 - +4 1:40 - +2 1:80 - +1 1:160 - 1L
18169	1:20 - +3 1:40 - +3 1:80 - +1	1:20 - +2 1:40 - 1L	1:20 - +4 1:40 - +4 1:80 - +1 1:160 - 1L
R. S. MCH	1:20 - +3 1:40 - +1	1:20 - +3 1:40 - +1	1:20 - +3 1:40 - +2

Table III

H AGGLUTINATION REACTION OF ALL THE POSITIVE SERUMS FOUND IN THE PRELIMINARY DILUTION OF 1:20

Serum Number	Bact.paratyphosum 8006		Bact.paratyphosum 289D		Bact.typhosum H901
	Specific Phase	Non-specific Phase	Specific Phase	Non-specific Phase	Specific Phase
93662	1:20 - +3 1:40 - +2 1:80 - +2 1:160 - +2	1:20 - +4 1:40 - +4 1:80 - +3 1:160 - 1L	1:20 - +4 1:40 - +4 1:80 - +3	1:20 - +4 1:40 - +4 1:80 - +3 1:160 - 1L	0
93967	1:20 - +3 1:40 - +2 1:80 - +2 1:160 - +2	0	1:20 - +4 1:40 - +3 1:80 - +2 1:160 - +2	0	0
93926	0	1:20 - 1L	0	1:20 - 1L	0
93948	1:20 - +3 1:40 - +1	1:20 - +1	1:20 - +4 1:40 - +2	1:20 - +1	0
92659	0	1:20 - 1L	0	0	0
31 MCH	1:20 - +2 1:40 - +1	1:20 - +2 1:40 - +2	1:20 - +3 1:40 - +2	1:20 - +1	0

Table III shows the agglutination reaction of the six positive H serums. The table shows that one of the six serums agglutinated both strains of Bact. paratyphosum B in the specific phase only. Two of the six serums agglutinated Bact. paratyphosum B 6006 in the non-specific phase, whereas for Bact. paratyphosum B 289B only one of the serums was found in the non-specific phase. Three of the total serums occurred in both phases. In none of the positive H serums was the H antigen of Bact. typhosum agglutinated. However, as only a very small number of H agglutining serums were found, the above ratio is not significant.

Table IV

HIGHEST TITERS FOR DIFFERENT O ANTIGENS SHOWN BY 77 NORMAL HUMAN SERUMS, WHICH AGGLUTINATED AN O ANTIGEN OF BACT. PARATYPHOSUM B 8006 IN THE PRELIMINARY TEST.

Highest Titer of Serum	Antigens					
	*Bact. typhosum 901 O		Bact. paratyphosum B 8006 O		*Bact. paratyphosum B 295 B O	
	No. of Serums	Per cent of total Positives	No. of Serums	Per cent of Total Positives	No. of Serums	Per cent of Total Positives
1:20	11	21	14	18.2	20	28.5
1:40	16	30	27	35.1	38	54.2
1:80	17	32	20	25.9	12	17.1
1:160	9	17	16	20.7	0	0
Total Positives	53	100	77	99.9	70	99.8

*These results are not for the total number of serums tested, but only for those serums that showed Bact. paratyphosum B 8006 agglutinins.

Table V

INCIDENCE OF AGGLUTININS FOR O AND H ANTIGENS OF

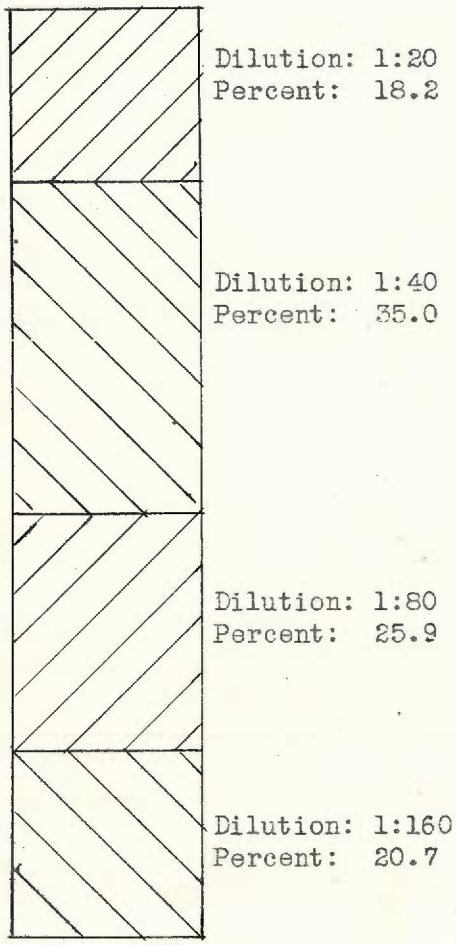
HACT. PARATYPHOSUM B 8006 IN 233 NORMAL HUMAN SERUMS.

	Number		Per Cent	
	O	H	O	H
Negative in 1:20	156	227	67.0	97.4
Positive in 1:20	77	6	33.0	2.6
Total	233	233	100	100

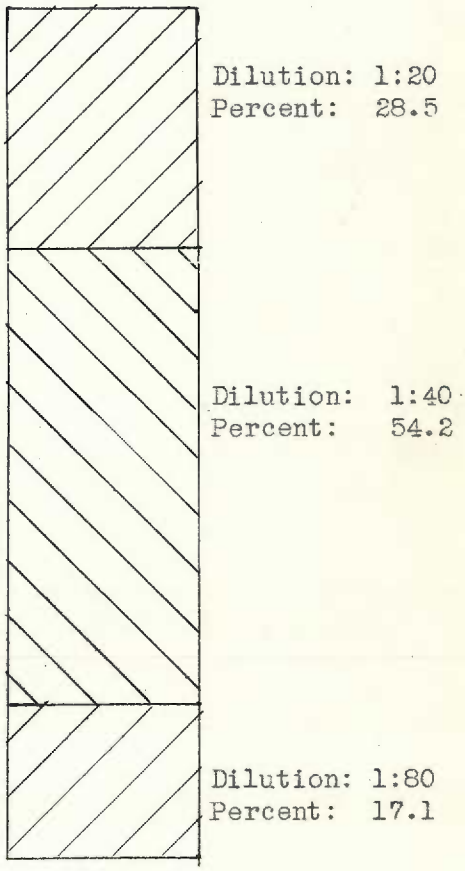
Graph I

GRAPHIC REPRESENTATION OF THE PERCENT INCIDENCE OF HIGHEST TITER IN THE 77 O AGGLUTININ POSITIVE SERUMS.

Bact. paratyphosum B
8006



Bact. paratyphosum B -
289B



Significance of Results

Relationship to Sero-Diagnosis

With the information presented in the tables, it is evident that no single arbitrary titer can be selected at, or above, which an agglutination can be considered as positive, and below which it can be regarded as negative.

Previous investigators have attempted to set an arbitrary titer which would rule out all non-specific factors. Coleman (1936) suggests the following technique for the reading of the agglutination reaction:

A. Report that definite agglutination occurred --
   1. When both granular and flocular agglutination (+3 or +4) is obtained in a 1:80, or higher, dilution.
   2. When either granular or flocular agglutination (+3 or +4) is obtained in a 1:160, or higher, dilution.

B. Report that partial agglutination occurred --
   1. When reactions less than those reported as definite agglutination are obtained, provided (+3 or +4) one or both types occur in a 1:40 dilution.

C. Report that agglutination of diagnostic significance did not occur --
   1. When reactions less than those regarded as partial are obtained.
   2. When no agglutination is obtained.

Topley and Wilson (1936) regard a titer of 1:250 for both H and O antigens as well above the normal level, and is usually quite safe to use. Zinsser and Bayne Jones (1934) record that a positive O agglutination titer above 1:50 is significant, while for H agglutination, titers of 1:80 or above appear to have some diagnostic significance. Sherwood (1935) publishes that the titer should be 1:80 to suggest active infection. Stitt, Clough and Clough (1938) point out that agglutination in a dilution of 1:100 is diagnostic of active infection.

From only a few citations one realizes that the titer which represents an active infection appears to vary with each individual author. It is quite necessary that if an arbitrary titer is to be set for a positive serum, the titer must be high enough to exclude the range of normal agglutinins. The normal agglutinin level will vary with different localities. The serum titer of normal humans will be much higher where there is a continuous spread of the disease agent than in a locality showing a low case incidence. The technique employed in different laboratories is not constant, and as a result, the reports will show considerable variations. The stage of the disease will effect the serum titer as well as a previous vaccination to typhoid or paratyphoid. If such an arbitrary titer were to be selected for this area, then from the results of this research we would be inclined to set an O titer of 1:320 or above as being diagnostic, for below this we enter the range of normal agglutinins.

It is also significant that a serum showing no H agglutinins, but an O titer of 1:160 is not an indication of infection. However, from the scarcity of normal H agglutinins (Table V), a serum showing an H titer of 1:160 is more apt to be indicative of infection than a positive O at a 1:160 dilution. This in turn brings out the value of employing both H and O titration of all suspected serums.

The variation of results from different parts of the world are in all probability due to differences in technique and materials used. From the information in Table IV one can see that different strains of the same antigen show marked variations in sensitivity when applied to the agglutination reaction. From this discussion, it is evident that experimental results from different parts of the world can not be accurately compared unless standard technique was employed by all.

Possible Origin of Normal Agglutinins

History

There has been much discussion in the literature as to the origin of agglutinins found in a random sample of population for a specific antigen. One of the favorite theories is that the agglutinins in the serum of normal persons arise due to a prior exposure to the specific infectious agent either through vaccination or as a result of natural infection, clinical or subclinical. Many workers seem to favor this viewpoint. Rosher and Fielden (1922) whose results have previously been

discussed, attribute this high percentage of normal agglutinins to the wholesale vaccination during the World War. Giglioli (1933) concludes that a sub-clinical infection was probably responsible for the agglutinins present. Neill, Fleming, and Gasperi (1931) observed that about 25 per cent of adults have agglutinins against the diphtheria bacillus in a dilution of 1:10 or higher. These authors conclude that it is hard to believe people would develop normal agglutinins to diphtheria except as a result of a past infection. Havens and Mayfield (1931) have disclosed that in agglutination tests for Bact. typhosum with the serum of 1,136 supposedly normal persons, 23 per cent yielded positive results. From these experimental results the above authors do not feel that the normal agglutinins are caused by non-specific factors but rather by an association with the specific organism. Paricha, Panja and Lal (1935) show that the occurrence of agglutinins in the serum of apparently healthy individuals can be considered as evidence of exposure to the specific infectious agent either through artificial means or as a result of natural infection, clinical or sub-clinical. Pijper (1929) is also of the opinion that normal agglutinins are the result of a previous infection. Savage (1919) reports that since the serum of a new-born child is largely devoid of agglutinins that are found in later life, agglutinins may, after all, be acquired properties. Lewin (1938) bases his conclusion on the fact that in his area Bact. paratyphosum B is quite common and that the normal agglutinins are probably due to a past infection.

There are, however, observations which have been interpreted as evidence of non-specific stimulation. Gilbert and Coleman (1930) have noted increased typhoid agglutinins during the course of infection such as undulant fever, in persons, some of whom gave no history of typhoid vaccination or an attack of typhoid fever. Kilduff and Kerscler (1919) found a high incidence of typhoid agglutinins in tuberculosis patients, 40 per cent of which could not be explained by vaccination or an attack of typhoid fever. Damon (1937) and Madgwich and Portner (1932) have also published similar results. Gregory and Atkinson (1936) conclude that the presence of normal O agglutinins for Bact. paratyphosum B, in human serums, was due to some other organism than Bact. paratyphosum B or a foreign protein. Sears and Phillips (1939) in their work on normal agglutinins suggest their experimental results as being due to non-specific factors rather than to the specific disease agent. Jordan (1933) concludes normal agglutinins may be present in the blood without infection and without the entry parenterally or enterally of an antigenic substance. Ingalls (1937) attributes the development of normal agglutinins to the existance of a haptenic fraction.

Theories on the Origin of Normal Agglutinins

These two modes of origin, namely: (1) the specific stimulation of agglutinins by actual infection with the corresponding organism; and (2) infection with some other organism that shares a common antigenic component, do not exhaust all other possibilities. Topley and Wilson (1936)

list two other theories besides the above two: (3) the entrance to the tissues via the intestinal tract, or possibly other routes, of non-living antigenic material capable of stimulating the production of an antibody with the active group in question; and (4) the formation of such antibodies as a by-product in the normal functioning of the antibody-forming apparatus altogether apart from any specific external stimulus.

Interpretation of Theories

In view of the findings in the first part of this thesis, it would be difficult to accept the theory that agglutinins from a random sample of population arise in response to the infectious disease agent. From this theory one would interpret the high percentage of normal Bact. paratyphosum B O agglutinins as an index of the incidence of Bact. paratyphosum B infection. This theory can not be the explanation of the high incidence of normal agglutinins as every evidence tends to point to the fact that Bact. paratyphosum B as well as other enteric infections in this area are rare. The records of the public health and hospital laboratories bring out very clearly the low incidence of Bact. paratyphosum B. The state laboratories report that in the last five years, Bact. paratyphosum B was successfully isolated from patients either through blood, stool or urine in only one instance. The city laboratory reports for the last five years are negative for isolation of Bact. paratyphosum B.

The state health department states that infection with Bact. paratyphosum B in this region is very rare. The city laboratory has made approximately 10,000 routine stool examinations on food handlers for carriers of infectious Gram negative bacilli, and in no instance was a positive stool found.

From the extreme rarity of this infection, vaccination for Bact. paratyphosum B has been seldom performed.

Existance of Antigenic Components Common to Bact. Paratyphosum And the Colon Bacilli

In the first part of this thesis data was presented on the incidence of agglutinins in a sample of normal serums for Bact. paratyphosum B. These results indicated the probability that the origin of these agglutinins was to be sought in some antigenic component common to Bact. paratyphosum B, and other widely distributed intestinal organisms.

It is the purpose of this part of the thesis to investigate the possibility of the existence of such common components with the various strains of the most widely occurring intestinal organism, namely Esch. coli.

Methods and Technique

A preliminary test was carried out on several normal human serums. This test consisted of using stock mixtures of Esch. coli labeled X and Y. Stock mixture X contained a mixture of O antigens from one-half of the total number of coli strains, and mixture Y contained O antigens from the

remaining half of the coli strains. This procedure was introduced to reduce the amount of work necessary to test each serum with separate O antigens of the eight coli strains employed. When a serum failed to agglutinate either of the two stock mixtures, the serum was then discarded and considerable time was saved. Serums showing agglutinins for either of the stock mixtures were then titrated with separate O antigens of the eight coli antigens. All antigens were prepared as listed in part one. The technique for the agglutination reaction was carried out according to the method employed in the fore part of this thesis. The Esch. coli strains were isolated chiefly from the feces and urine, and upon examination gave cultural and biochemical reactions, Table VI, that were typical of Esch. coli. The strains differ from each other only in minor characteristics.

Table VII gives the complete results of the preliminary test. It becomes quite evident that the normal human serum contains a rather high percentage of Esch. coli agglutinins, and some show agglutination in titers as high as 1:320. The limitation of using stock mixtures X and Y was noted, and since the greater majority of serums appear to contain coli agglutinins, this test was eliminated.

The question then arose as to whether these coli agglutinins were in some way similar to the Bact. paratyphosum B agglutinins. To ascertain if such were the case, serums were obtained from the hospital and agglutination

Table VI

CULTURAL REACTIONS OF THE ESCH. COLI STRAINS*

Coli Strains	Dextrin	Lactose	Saccharose	Adonitol	Dulcitol	Sorbitol	Arabinose	Xylose	Rhamnose	Salicin	Gelatin	Indol	H_2S	M. R.	V. P.	Motility	Inosite	Glycerine	Raffinose
No. 5	AG	AG	-	AG	-	-	AG	AG	AG	+2 -	-	+	Sl	+	-	+	-	Sl	-
Hillman	AG	AG	-	-	Sl AG	AG	AG	AG	-	-	-	+	Sl	+	-	+ -	-	+2 Sl	AG
Swanson	AG	AG	-	-	+2 -	AG	AG	+3 -	AG	-	-	+	Sl	+	-	+	-	+2 Sl	-
Grady	AG	AG	-	-	AG	AG	AG	AG	AG	+2 -	-	+	Sl	+	-	+	-	Sl	+3 -
Ballman	AG	AG	-	-	-	AG	AG	AG	AG	+2 -	-	+	Sl	+	-	+	-	Sl	-
Miller	AG	AG	AG	-	AG	AG	AG	Sl AG	AG	-	-	+	+	+	-	+	-	+3 Sl	AG
Flynn	AG	AG	AG	-	+3 -	AG	AG	AG	AG	-	-	+	Sl	+	-	+	-	+2 Sl	AG -
No.271	AG	AG	-	-	-	AG	AG	AG	AG	-	-	+	Sl	+	-	+	-	Sl	-

*AG = Acid and Gas
- = Negative
Sl = Slight Acid and Gas
+3 = Three days before reaction occurred
+2 = Two days before reaction occurred.

Table VII

THE PRELIMINARY TEST OF NORMAL HUMAN SERUMS WITH STOCK MIXTURES OF COLI ANTIGENS

Serum Number	Antigens	
	X	Y
38	1:20 - +2 1:40 - +2 1:80 - +2 1:160 - +2 1:320 - 1L	1:20 - +3 1:40 - +2 1:80 - +2 1:160 - +2 1:320 - 1L
52	1:20 - +2 1:40 - +2 1:80 - +2 1:160 - +1 1:320 - +1L	1:20 - +3 1:40 - +2 1:80 - +1 1:160 - +1 1:320 - 1L
41	1:20 - +2 1:40 - +2 1:80 - +1 1:160 - +1 1:320 - 1L	1:20 - +4 1:40 - +2 1:80 - +1L
48	1:20 - +2 1:40 - +2 1:80 - +3 1:160 - +3 1:320 - +1	1:20 - +2 1:40 - +3 1:80 - +3 1:160 - 1L
47	1:20 - +2 1:40 - 1L	1:20 - +1L
45	1:20 - +3 1:40 - +2 1:80 - +2 1:160- +2	1:20 - +4 1:40 - +2 1:80 - +2 1:160 - +1L
37	1:20 - +3 1:40 - +3 1:80 - +3 1:160 - +3 1:320 - 1L	1:20 - +4 1:40 - +2 1:80 - +3 1:160 - 1L 1:320 - 1L
34	1:20 - +2 1:40 - +2 1:80 - +2 1:160 - +2 1:320 - +2	1:20 - +2 1:40 - +2 1:80 - +2 1:160 - +2 1:320 - +2

X solution contained Miller, Ballman, Grady, Hillman B. coli strains. Y. solution contained Flynn, Swanson, 271, 5 B. coli strains.

Table VIII

PRELIMINARY TEST ON 56 HUMAN SERUMS FOR

BACT. PARATYPHOSUM B AND ESCH. COLI O AGGLUTININS

Serum Number	Esch. coli	Bact. paratyphosum B
	Hillman	8006
10246	+3	0
10251	+4	0
29355	+4	0
102453	+4	0
102526	+4	0
102518	+4	0
61075	+4	0
3252	+3	0
47247	+4	M.
63247	0	0
36251	+4	0
18139	0	0
102543	+4	0
102383	+3	0
104 MCH	0	0
49490	+2	0
52963	+4	0
102713	+4	+1
54106	+3	0
102537	+1	0
102530	+4	0
102544	+4	+1
54805	0	0
102545	0	0
102436	0	0
105 MCH	+4	0
101306	+1	0
102548	+4	+2
15 MCH	+4	+3
102538	+4	0

Table VIII (Continued)

Serum Number	Esch. coli	Bact. paratyphosum B
	Hillman	8006
78801	+4	0
19 MCH	+4	+1
23668	+4	+2
22 MCH	+2	1L
24 MCH	+1	1L
31150	+1	0
2 MCH	+4	+4
9851	+3	0
3404	+4	0
3405	+1	0
93777	+2	0
90778	0	0
1181	+4	0
102609	+4	+4
102627	+2	0
102652	+4	0
101865	+4	+4
67436	+4	+2
162166	+4	0
92743	+4	+2
81149	+3	0
102613	+4	+2
47722	+3	+1
102614	+4	+2
79521	+4	1L
102616	+4	+1
37218	+4	+3
80318	+4	0
102608	+4	0

tests, using O antigens of Bact. paratyphosum B and Esch. coli, were conducted on each serum. After 58 serums had been tested (Table VIII) and 19 serums found that showed Bact. paratyphosum B agglutinins, absorption tests were conducted on ten of the positive serums.

Technique for the Absorption Test

Three Blake bottles were inoculated with each of the eight Esch. coli strains, that had been tested previously for smoothness. After 20 hours incubation at 37° C., the growth was removed with 10 c.c. of 0.85 per cent saline. The growth from all the bottles was pooled and thoroughly mixed. From this, 10 c.c. portions were pipetted into centrifuge tubes and centrifuged. To the packed cells, serum was added and also 0.5 c.c. of five per cent phenol, and diluted with saline so that five c. c. of serum represented a 1:10 dilution. The thoroughly mixed cells and serum after incubation for two hours at 37° C., followed by refrigeration over night, was sedimented by centrifuging and the clear supernatant serum was tested for completeness of absorption. Reabsorption was preformed when necessary. A serum control was run with each absorbed serum.

Table IX contains the detailed results for the positive Bact. paratyphosum B normal serums that were absorbed with mixtures of eight coli strains and re-titered for O agglutinins of Bact. paratyphosum B. Five of the serums subjected to this test showed no Bact. paratyphosum B O agglutinin removal after absorption by Esch. coli. Two serums showed a partial reduction in titer, and three of the ten serums tested were almost completely freed of O agglutinins for Bact. paratyphosum B.

Table IX

COMPLETE TITRATION OF THE COLI ABSORBED BACT. PARATYPHOSUM B

NORMAL HUMAN SERUM

Antigen	Dilution of Serum 1:20	1:40	1:80	1:160
	Serum No. 92743			
389B	1L	0	0	0
Control	+2	1L	0	0
8006	+1	0	0	0
Control	+3	+2	1L	0
B. typhimurium	0	0	0	0
Control	0	0	0	0
	Serum No. 97218			
389B	+2	+1	0	0
Control	+3	+3	+1	1L
8006	+4	+3	1L	0
Control	+4	+4	+1	1L
B. typhimurium	+2	0	0	0
Control	+2	1L	0	0
	Serum No. 102814			
389B	0	0	0	0
Control	+1	1L	0	0
8006	0	0	0	0
Control	+2	+1	0	0
B. typhimurium	0	0	0	0
Control	0	0	0	0

Table IX (Continued)

Antigen	Dilution of Serum 1:20	1:40	1:80	1:160
		Serum No. 102613		
289B	0	0	0	0
Control	+1	1L	0	0
8006	+1	0	0	0
Control	+3	+2	1L	0
B. typhimurium	0	0	0	0
Control	0	0	0	0
		Serum No. 102609		
289B	+3	+2	+1	0
Control	+4	+3	+1	1L
8006	+4	+4	+1	0
Control	+4	+4	+3	+1
B. typhimurium	+2	0	0	0
Control	+2	0	0	0
		Serum No. 101865		
289B	+1	+1	1L	1L
Control	+4	+3	+1	1L
8006	+2	+3	+2	+1
Control	+4	+4	+3	+2
B. typhimurium	0	0	0	0
Control	+2	1L	0	0
		Serum No. 102536		
289B	+3	+1	0	0
Control	+4	+2	+1	0
8006	+4	+1	0	0
Control	+4	+4	+2	+1
B. typhimurium	+2	0	0	0
Control	+2	0	0	0

Table IX (Continued)

Antigen	Dilution of Serum 1:20	1:40	1:80	1:160
	Serum No. 15 MCH			
289B	+3	+1	0	0
Control	+3	+1	0	0
8006	+3	+1	0	0
Control	+3	+2	0	0
S. typhimurium	0	0	0	0
Control	0	0	0	0
	Serum No. 23568			
289B	+2	+1	0	0
Control	+2	+1	0	0
8006	+1	+1	0	0
Control	+2	+1	0	0
S. typhimurium	0	0	0	0
Control	0	0	0	0
	Serum No. 102548			
289B	1L	1L	0	0
Control	+2	+1	0	0
8006	+2	1L	0	0
Control	+1	1L	0	0
S. typhimurium	1L	1L	1L	0
Control	1L	1L	0	0

The interpretation of this experiment presents evidence that Esch. coli is not the major factor involved in the production of normal O agglutinins for Bact. paratyphosum B. If Esch. coli were the dominant factor, then the above absorption tests should have presented more serums showing a complete removal of agglutinins. However, considering the fact that no two coli strains possess the same serological reactions, we may also assume that if more than eight Esch. coli strains had been used there may have been more serums with titer reductions. Inasmuch as three of the ten serums from this test did show titer reductions, the possibility of an antigenic relationship between Esch. coli and Bact. paratyphosum B seems to exist. The extent of this relationship is to be determined by further investigation.

Serological Relationship Between Esch. coli and Bact. paratyphosum B.

History

The ability of coli strains to be agglutinated by a variety of serums has been shown by various investigators. Wilson (1909) points out that when the serum of typhoid fever patients was examined with regard to its agglutinative action on other bacilli (e.g., Esch. coli, Bact. enteritides) it was found that these microbes at times were agglutinated. Denes and Denes (1932) reported that a variant of Esch. coli was agglutinated in high titer by an antiserum prepared from Bact. typhosum. Engel and Olin (1929) published that patients suffering from pernicious

anemia will often show the presence of coli agglutinins. Kristensen, Bojlen and Kjaer (1936) show that from a systematic study of 1,104 coli strains, 164 of these strains agglutinate with one or more of the Salmonella O antiserums. Some agglutinated in titers as high as the homologous Salmonella. Mackie (1937) and Hayashi (1936) have both shown a relationship to exist between certain coli strains and Shigella dysenteriae. Habs and Arjona (1935) reported that a strain of Esch. coli was agglutinated by the serum of the Salmonella group D. By cross agglutination and absorption it was determined that Esch. coli contained antigens belonging to the Salmonella group D and B.

Esch. coli Absorption of Salmonella Immune Serums.

To determine the antigenic relationship between strains of Esch. coli and Bact. paratyphosum B, antiserums were prepared for Bact. paratyphosum B and various other members of the Salmonella group. When the prepared serums were titered by each of the eight coli strains, a wide range of agglutination occurred. Table X presents the results of this test. It is interesting to note that no two coli strains exhibit the same agglutinative power. The Hillman strain of coli appears to agglutinate in titers well beyond that shown by the remaining strains. Grady and Flynn coli strains show relatively no agglutination, whereas the remaining strains of coli were intermediate in their ability to agglutinate in the presence of various Salmonella immune serum. It was also noted

that coli agglutination occurred mainly with serums prepared from Bact. paratyphosum B and Bact. typhosum. Some of the coli strains agglutinated in titers as high as 1:2560. The results from Table X would indicate that Esch. coli shares antigenic components with Bact. paratyphosum B and Bact. typhosum, and to a lesser degree with other Salmonella groups.

In order to ascertain the extent of the antigenic relationship between Esch. coli and Bact. paratyphosum B, it was felt advisable to absorb Bact. paratyphosum B and allied immune serum with two strains of Esch. coli, namely Hillman and Swanson. Table XI shows that when the Salmonella immune serums were absorbed with Esch. coli and then re-titered with their own homologous antigens, no drop in titer could be noted. This is quite striking due to the fact that coli antigens showed agglutinins to a relatively high titer with the above serums.

The results from Table XI lends some evidence which would indicate that the agglutinins removed by coli absorption were not of sufficient quantity to reduce the serum titer to its own homologous antigen. It may also be possible that Esch. coli shares only one of the several O antigenic factors of the Salmonella serum. Thus by removing but one of Bact. paratyphosum B several antigenic factors, the remaining factors being intact are still of sufficient quantity to enable the serum to show no titer reduction.

Table X

AGGLUTINATION OF EIGHT ESCH. COLI STRAINS BY SALMONELLA SERUMS

Antingens of Esch. coli	Bact. paratyphosum B 8006	Bact. paratyphosum B 289B	Bact. typhosum 0901	Bact. aberdeen 1:10,240	Bact. poona 1:5,120	Bact. london 1:5,120=+4	Bact. oranienberg 1:2,560=1L
Hillman	1:1,280	1:1,280=1L	1:2,560=1L	1:2,560	1:320=1L	1:80=1L	1:160=1L
Swanson	1:20=0	1:160=1L	1:160=1L	1:20=0	1:20=1L	1:20=0	1:20=0
271	1:160=1L	1:20=0	1:20=1L	1:20=+1	1:20=0	1:20=1L	1:20=1L
Miller	1:160=1L	1:640=1L	1:80=1L	1:20=0	1:640=1L	1:80=1L	1:20=+1
Grady	1:20=0	1:20=0	1:20=1L	1:20=0	1:20=1L	1:20=0	1:20=0
Baldman	1:160=1L	1:20=0	1:20=1L	1:20=0	1:20=0	1:20=0	1:20=0
5	1:160=1L	1:2,560=+1	1:20=0	1:20=0	1:20=0	1:40=+4	1:20=0
Flynn	1:20=0	1:20=0	1:20=1L	1:20=0	1:20=0	1:20=0	1:20=1L

Table XI

TITRATION OF SALMONELLA SERUMS WITH ITS OWN HOMOLOGOUS ANTIGEN AFTER ABSORPTION BY TWO ESCH. COLI STRAINS

	Salmonella Serums			
	Bact. typhosum 338	Bact. typhimurium	Bact. paratyphosum B 3006	Bact. Virchow
Absorbed by Hillmann				
Before	1:20,480	1:10,240	1:20,480	1:10,240
After	1:20,480	1:10,240	1:20,480	1:10,240
Absorbed by Swanson				
Before	1:20,480	1:10,240	1:20,480	1:10,240
After	1:20,480	1:10,240	1:20,480	1:10,240

It has previously been pointed out that certain Esch. coli strains show a striking power of agglutination with Salmonella serums to a relatively high titer. This wide range of agglutination was first believed to occur as a result of Hillman coli containing a heterophile antigen, but experimental results showed this was not the case (see Appendix). However, to obtain more complete information on their antigenic relationship, mirror agglutination and absorption tests were performed. Coli immune serums were prepared for Hillman and Swanson strains of Esch. coli. When these coli immune serums were titered with the various Salmonella antigens, it was observed that agglutination failed to occur much above the normal agglutinin level as seen from Table XII. All of the agglutination in this experiment could be accounted for on the basis of the normal agglutinins present before immunization began and therefore must be excluded. This inability of coli immune serum to agglutinate antigens of the Salmonella group, tends to cast doubt upon the assumption that coli and Bact. paratyphosum B share a common antigenic factor. There exists, however, a possibility that Bact. paratyphosum B shares a deep somatic antigen with a surface antigen of the coli group. If such a phenomenon were to exist then the inability of Bact. paratyphosum B to agglutinate in the presence of coli immune serum could be accounted for.

The wide range of immune serums specific for members of the intestinal group of pathogens which agglutinate Esch. coli, indicate the extreme complexity of the latter organism. It would appear quite improbable that Esch. coli would share antigens common to all such groups. Topley and Wilson (1936) list the Kauffmann-White classification of the Salmonella

Table XII

COMPLETE TITRATION OF TWO B.COLI SERUMS BY SALMONELLA ANTIGENS

B.coli Serums	Antigens							
	Bact. paratyphosumB 6006	Bact. paratyphosum B 2899	Bact. typhosum 0901	Bact. london	Bact. oranienberg	Bact. virchow	Bact. poona	Bact. aberdeen
Hillman	1:80	0	1:160	0	1:80	1:80	-	-
Normal agglutinins	1:60	-	1:80	0	1:40	1:40	-	-
Swanson	0	0	0	0	0	0	0	1:80
Normal agglutinins	0	0	0	0	0	0	0	0

group. Each of the seven different groups listed contain two or more antigenic factors which are specific for that group. Inasmuch as Esch. coli agglutinates in the presence of serum from several of these groups it was necessary to specifically absorb serum from the groups Esch. coli reacts upon. The specifically absorbed serum was then titered with Esch. coli to ascertain the specific components with which coli reacts. When such serums were prepared and tested the results from Table XIII show such a complexity as to shed but little light upon this subject. With the information derived from this table one can see that certain coli strains apparently share antigenic factors common to all the Salmonella groups tested. Upon a close examination of Table XIII it was noted that when all of the antigens were removed from Bact. paratyphosum B except factor V, no extensive agglutination occurred. Thus one can assume that Esch. coli contains no factor V. When the same serum was absorbed and factors IV and V were left intact, relatively no agglutination occurred. In other words, if Esch. coli does share antigenic factors common to the Salmonella group, one would from this experiment be inclined to limit such relationship to factor XII. However, Bact. virchow which contains no factor XII does show a relationship to Esch. coli which can not be attributed to factor XII but more to factor VI. With the Salmonella strains available in this laboratory, it was impossible to prepare a specific absorbed serum containing only factor XII. If such a serum were available, more complete information may have been derived. It is quite possible that if such a serum were available, no coli agglutination would have occurred, and thus one could either eliminate factor XII or show that it

Table XIII

SPECIFIC ABSORBED SALMONELLA SERUMS TITERED WITH FOUR ESCH. COLI STRAINS *

Esch. coli Antigens	Bact. virchow serum absorbed by Bact. newport	Bact. typhosum 0901 serum absorbed by Bact. paratyphosum B 6006	Bact. paratyphosum B 6006 serum absorbed by Bact. abortus equi	Bact. paratyphosum B Serum absorbed by Bact. typhosum 0901
No. 271 Control	1:40=0 1:40 =0	1:40=0 1:40=0	1:40=0 1:40=0	1:40=0 1:40=0
Hillman Control	1:80 1:1,280	1:160 1:1,280	1:320 1:1,280	1:80 1:1,280
Swanson Control	1:320 1:1,280	1:160 1:320	1:80 1:80	1:40 1:80
Hiller Control	1:40=0 1:40=0	1:80 1:80	1:40=0 1:40=0	1:40=0 1:40=0
Remaining Antigens	(VII)	(IX)	(V)	(IV,V)

*Bact. paratyphosum B (IV,V, (XII)) absorbed by Bact. typhosum 0901 (IX, (XII)) ——— IV, V
Bact. paratyphosum B (IV,V, (XII)) absorbed by Bact. abortus equi (IV, (XII)) ——— V
Bact. typhosum (IX, (XII)) absorbed by Bact. paratyphosum B(IV,V, (XII))——— I X
Bact. virchow (VI, VII) absorbed by Bact. newport (VI, VIII)——— VII

was the factor which is commonly shared. If factor XII were the responsible agent then more than 33 per cent of all the serums tested in the first part of this thesis should show Bact. paratyphosum B normal agglutinins. This can be emphasized from the fact that almost all of the serums tested for normal coli agglutinins were positive. If coli and Bact. paratyphosum B were to share factor XII, the same proportion of these serums should show normal Bact. paratyphosum B agglutinins. This, however, was not the case and therefore places a limitation on the value of the above phenomenon.

It has already been shown that Esch. coli strains are agglutinated in the presence of Salmonella immune serums, but Salmonella antigens do not agglutinate when titered against coli immune serums. Since Salmonella immune serums possess coli agglutinins, the question arose as to whether these coli agglutinins were of a specific nature. To demonstrate the specificity of the coli agglutinins present in Salmonella immune serum, two such serums were absorbed with their own homologous antigens. When all the Salmonella agglutinins were removed, the serum was re-titered for the presence of any existing coli agglutinins. Table XIV includes the results from the above test and shows that the coli agglutinins were not completely removed from the Bact. paratyphosum B or Bact. typhosum serum upon absorption with their own homologous antigens. This did not hold true where such coli agglutinins exist in a 1:80 or lower titer. Before immunization to Bact. paratyphosum B 269B, the Hillman coli agglutinin titer was 1:160. This represents the normal Hillman titer. After immunization with 269B,

Table XIV

ESCH. COLI TITER OF BACT. PARATYPHOSUM B 2892 AND BACT. TYPHOSUM 0901 SERUMS AFTER ABSORPTION WITH THEIR HOMOLOGOUS ANTIGENS

Esch. coli Antigens	Bact. paratyphosum B 2892 Absorbed by Bact. paratyphosum B 2892	Bact. typhosum 0901 Absorbed by Bact. typhosum 0901
Flynn	0	0
Control	0	0
Baldman	0	0
Control	0	0
No. 8	1:320	0
Control	1:2,560	0
Grady	0	0
Control	0	1:80
Swanson	1:80	1:80
Contol	1:160	1:320
No. 271	0	0
Control	0	0
Hiller	1:80	1:80
Control	1:640	1:160
Hillman	1:1,280	1:160
Control	1:2,560	1:2,560

the Hillman titer had increased to 1:1,280. This marked increase of coli agglutinins can be attributed to the stimulating effect of the Bact. paratyphosum B 289B immunization. The injection of the rabbit with 289B has stimulated the production of Hillman coli agglutinins. These coli antibodies in Salmonella immune serum cannot be considered as Bact. paratyphosum B mino agglutinins but are specific coli agglutinins. As a result of this coli agglutinin stimulation in the 289B immune serum, it is only natural for such serum to show a high titer for the Hillman coli antigen. If this phenomenon exists, then all the antigenic relationships existing in the immune serum for coli and Bact. paratyphosum B must be questioned. In all likelihood, such relationship is due to the anamnestic reaction and not to an antigenic component common to both organisms. This phenomenon explains why Bact. typhosum and Bact. paratyphosum B immune serum show such a high titer to coli antigens, and why Bact. typhosum and Bact. paratyphosum B will not agglutinate in the presence of coli immune serum. Normal rabbits in their relatively short span of life do not come in contact with Bact. paratyphosum B consequently showing no agglutinins to this organism. Coli immunization in rabbits showing normal coli agglutinins and no Bact. paratyphosum B agglutinins, will obviously increase the coli titer but will have no stimulating effect on the Bact. paratyphosum B agglutinins, as none were present at the start of the immunization. Experimental results do not indicate that we are dealing with antigenic relationships but are normal agglutinins that have been stimulated by the anamnestic

reaction.

Therefore, it is extremely necessary for one doing research on antigenic relationships between Salmonella and the coli group, to test the experimental animals before immunization for Esch. coli agglutinins. To merely test the normal serum with one or two coli antigens is not sufficient as the coli group is so heterogenitic.

During the course of this investigation the discovery of a typhoid case whose serum had a high coli agglutinin titer presented an opportunity to test this phenomena with a human anti-typhoid serum. Serum was obtained from the patient and titered with various antigens of the Salmonella and Esch. coli groups. Table XV contains the detailed results of this experiment. It is seen that certain coli strains are agglutinated in titers equally as high as that shown by Bact. typhosum 0901. The titer in each case was 1:640. All of the Esch. coli strains employed were agglutinated in titers of 1:40 or higher. These results are from the typhoid serum early in the course of the disease. A lapse of twelve days ensued before a second sample was taken. This was performed to ascertain if a lapse of time would effect the Esch. coli titer of the human anti-typhoid serum. It has been shown previously that rabbits immunized to Bact. typhosum will stimulate the production of coli agglutinins, and this human serum presents an opportunity to determine if the same is true with a typical human anti-typhoid serum. Table XVI contains the results obtained from the human anti-typhoid serum after a lapse of twelve days from that of the serum

Table XV

HUMAN TYPHOID ANTI-SERUM TITERED WITH

NUMEROUS SALMONELLA AND ESCH. COLI O ANTIGENS

Antigens	Serum Dilution						
	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280
Bact. typhosum 0901	+4	+4	+4	+3	+2	+1	0
Bact. paratyphosum B 8006	+4	+4	1L	0	0	0	0
Bact. virchow	0	0	0	0	0	0	0
Bact. orienberg	0	0	0	0	0	0	0
Bact. poona	+2	0	0	0	0	0	0
Bact. london	+4	+4	0	0	0	0	0
Bact. typhimurium	+4	+4	0	0	0	0	0
Bact. aberdeen	+3	+3	+1	+1	0	0	0
Bact. paratyphosum A	0	0	0	0	0	0	0
Esch. coli Miller	+4	+3	+2	1L	0	0	0
Esch. coli Flynn	+2	1L	0	0	0	0	0
Esch. coli No. 5	1L	1L	0	0	0	0	0
Esch. coli Hillmon	+3	+4	+3	+3	1L	0	0
Esch. coli No. 271	+4	+4	+3	+3	+3	1L	
Esch. coli Grady	+4	+4	+4	+3	+2	1L	

Table XVI

HUMAN TYPHOID ANTI-SERUM TITERED WITH O ANTIGENS FROM THE SALMONELLA AND ESCH. COLI ANTIGENS

(Serum taken 12 days after that of Serum from Table XV)

Antigens	Serum Dilutions					
	1:40	1:80	1:160	1:320	1:640	1:1,280
Bact. typhosum 0901	+4	+4	+4	+4	1L	0
Bact. paratyphosum B 8006	+3	1L	0	0	0	0
Bact. aberdeen	0	0	0	0	0	0
Esch. coli Miller	+3	+3	+3	+3	0	0
Esch. coli No. 271	+4	+4	+4	+4	+4	+3
Esch. coli Swanson	+3	+3	+3	1L	0	0
Esch. coli Grady	+3	+4	+4	+4	+1	1L
Esch. coli Hillman	+4	+3	+3	+2	1L	

tested in Table XV. Upon comparison of the two tables, one can see that the lapse of time has facilitated the increased production of coli agglutinins. Certain coli strains have increased in titer from 1:640 to 1:1,280. Other strains of coli show no apparent change in titer. These results lend confirmation to those obtained from rabbit immune serum. Interpretation of these results presents evidence that the typhoid condition has stimulated the increased production of coli agglutinins.

Absorption Tests on a Human Anti-Typhoid Serum

Several absorption tests were conducted upon the human anti-typhoid serum to determine if the increased production of coli agglutinins was the result of the anamnestic reaction and not to an antigenic relationship between Esch. coli and Bact. typhosum. In an earlier part of this thesis, it was shown that coli agglutinins present in Bact. typhosum and Bact. paratyphosum B immune serum were not removed with the serums, but were absorbed with their own homologous antigens. This human anti-typhoid serum presents an opportunity to investigate, and determine if such reaction were also to be observed in human anti-typhoid serum. When the above serum was absorbed with Bact. typhosum 0901, Table XVII shows that the removal of all typhoid agglutinins has but little effect upon the coli titers. All of the coli strains employed in this experiment agglutinated in titers just as high, after Bact. typhosum 0901 absorption, as they did before the test was conducted. This absorption does, however, remove all of Bact. paratyphosum B

Table XVII

HUMAN TYPHOID ANTI-SERUM ABSORBED BY BACT. TYPHOSUM 0901 AND TITERED WITH O ANTIGENS FROM MEMBERS OF THE SALMONELLA AND ESCH. COLI GROUP.

Antigens	Serum Dilutions					
	1:40	1:80	1:160	1:320	1:640	1:1,280
Bact. typhosum 0901 Contol	0 +4	0 +4	0 +4	0 +4	0 1L	0 0
Esch. coli Miller Control	+3 +3	+3 +3	+3 +3	+1 +3	0 0	0 0
Esch. coli Hillman Control	+4 +4	+3 +4	+3 +4	+2 +4	1L 1L	0 0
Esch. coli Grady Control	+4 +3	+4 +3	+4 +3	+4 +3	+1 +2	1L +2
Esch. coli No. 271 Control	+4 +4	+4 +4	+4 +4	+4 +4	+4 +4	+3 +4
Esch. coli Swanson Control	+3 +4	+3 +4	+3 +4	1L +3	0 0	0 0
Bact. aberdeen Control	0 0	0 0	0 0	0 0	0 0	0 0
Bact. paratyphosum B 8006 Control	0 +3	0 1L	0 0	0 0	0 0	0 0
Bact. paratyphosum B 289B Control	0 +3	0 1L	0 0	0 0	0 0	0 0

agglutinins. This is to be expected since Bact. typhosum and Bact. paratyphosum B share a common antigen, and the removal of this antigenic component led to the removal of Bact. paratyphosum B agglutinins. The information derived from this experiment presents evidence that Esch. coli and Bact. typhosum are in no manner antigenically related. Also, the increased production of coli agglutinins in the human anti-typhoid serum was in response to the anamnestic reaction.

To definitely exclude any possible antigenic relationship between Esch. coli and Bact. typhosum, the human anti-typhoid serum was absorbed by two different strains of Esch. coli. The results from Table XVIII show that when this anti-serum was absorbed by Hillman strain of Esch. coli, there was no titer reduction for either Bact. typhosum or Bact. paratyphosum B. This again presents evidence that Esch. coli and Bact. typhosum or Bact. paratyphosum B are not antigenically related to each other. Some very interesting facts are brought out by Table XVIII regarding certain Esch. coli strain relationships. Absorption by Hillman has removed almost entirely all Swanson and Hiller agglutinins, thus showing that the above two coli strains contain antigenic factors which are common to Hillman strain of coli.

The contents of Table XIX show the titer of various antigens after the anti-serum was absorbed by Swanson strain of coli. Here again one can observe that the above absorption did not change the titer of either Bact. typhosum or Bact. paratyphosum B. This will confirm the discussion

Table XVIII

HUMAN TYPHOID ANTI-SERUM ABSORBED BY HILLMAN STRAIN OF ESCH. COLI

Antigens	Serum Dilutions						
	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
Esch. coli Hillman	1L	0	0	0	0	0	0
Control	+4	+4	+4	+3	1L	1L	
Esch. coli Grady	+2	+3	1L	0	0	0	0
Control	+4	+4	+4	+4	+4	+3	1L
Esch. coli No. 271	+4	+4	+4	+4	+4	+4	1L
Control	+4	+4	+4	+4	+4	+4	+1
Esch. coli Miller	1L	0	0	0	0	0	0
Control	+4	+4	+4	+4	+1	0	0
Esch. coli Swanson	0	0	0	0	0	0	0
Control	+4	+4	+4	1L			
Bact. paratyphosum B 8006	+1	1L	0	0	0	0	0
Control	+1	1L	0	0	0	0	0
Bact. paratyphosum B 289B	1L	0	0	0	0	0	0
Control	+1	0	0	0	0	0	0
Bact. typhosum 0901	+3	+3	+2	1L			
Control	+4	+4	+4	1L			

Table XIX

HUMAN TYPHOID ANTI-SERUM ABSORBED BY SWANSON STRAIN OF ESCH. COLI

Antigens	Serum Dilutions						
	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
Bact. typhosum 090l	+4	+4	+3	0	0	0	0
Control	+4	+4	+4	1L	0	0	0
Bact. paratyphosum B 9006	+2	0	0	0	0	0	0
Control	+1	1L	0	0	0	0	0
Bact. paratyphosum B 289B	1L	0	0	0	0	0	0
Control	+1	0	0	0	0	0	0
Esch. coli No. 271	+4	+4	+4	+4	+4	+4	+1
Control	+4	+4	+4	+4	+4	+4	1L
Esch. coli Hiller	+4	+4	+4	+4	1L	0	0
Control	+4	+4	+4	+4	1L	0	0
Esch. coli Grady	+2	1L	1L	0	0	0	0
Control	+4	+4	+4	+4	+4	+4	1L
Esch. coli Hillman	+4	+3	+3	+2	1L	0	0
Control	+4	+4	+4	+3	1L	1L	0
Esch. coli Swanson	0	0	0	0	0	0	0
Control	+4	+4	+4	1L	0	0	0

Table XX

HUMAN TYPHOID ANTI-SERUM ABSORBED BY BACT. PARATYPHOSUM B 2838

Antigens	Serum Dilution					
	1:40	1:80	1:160	1:320	1:640	1:1,280
Bact. paratyphosum B 2838 Control	0 +3	0 1L	0 0	0 0	0 0	0 0
Bact. paratyphosum B 8006 Control	0 +3	0 1L	0 0	0 0	0 0	0 0
Bact. typhosum 0901 Control	+4 +4	+4 +4	+4 +4	1L +4	0 1L	0 0
Bact. aberdeen Control	0 0	0 0	0 0	0 0	0 0	0 0
Esch. coli No. 871 Control	+4 +4	+4 +4	+4 +4	+4 +4	+4 +4	+4 +4
Esch. coli Miller Control	+3 +3	+3 +3	+3 +3	+1 +3	0 0	0 0
Esch. coli Hillman Control	+4 +4	+4 +4	+4 +4	+4 +4	+2 +4	0 1L
Esch. coli Swanson Control	+4 +4	+3 +4	+3 +4	1L +3	0 0	0 0
Esch. coli Grady Control	+3 +3	+3 +3	+3 +3	+2 +3	1L +2	0 +2

given for Table XVIII. Swanson absorption had no appreciable effect on any of the coli strains except Grady which showed a marked reduction in titer.

The final absorption on this anti-serum was performed by absorbing with Bact. paratyphosum B 2898. There existed a possibility that Bact. paratyphosum B absorption would reduce the coli titer; however, Table XX shows that there was no coli titer reduction. One can notice the slight reduction in titer for Bact. typhosum which can be accounted for since both organisms share a common antigenic factor.

CONCLUSION

Of 233 specimens of human serums submitted to a clinic for the serological test for syphilis, 77 or 33 per cent were found to agglutinate an O antigen and six or 2.6 per cent an H antigen of Bact. paratyphosum B.

From the six serums which showed H antibodies, one showed only the non-specific type, two showed only the specific type, while three showed both specific and non-specific types. Two of these serums gave titers as high as 1:160, and of the others, 1:40 was the highest titer given.

Of the 77 specimens showing O agglutination, 20.7 per cent gave titers to 1:160, 25.9 per cent gave titers to 1:80 only, 35.0 per cent gave titers to 1:40, and 18.2 per cent agglutinated only in the lowest dilution employed, namely, 1:20.

A difference in the agglutinability of the two strains of Bact. paratyphosum B used was evident, strain 8006 showing a higher sensitivity than strain 229B.

Only the serums tested with O antigens of Bact. paratyphosum B were titrated against an O antigen of Bact. typhosum. From these serums, 64 per cent of the total showed the presence of Bact. typhosum agglutinins.

Eight serums not included in the above were tested for their capacity to agglutinate two polyvalent antigens of Esch. coli, each consisting of a mixture of four strains. Both antigens were agglutinated by all the serums tested. An additional 58 serums were tested for the presence of Bact. paratyphosum B O agglutinins and for O agglutinins of a single strain of Esch. coli. Nineteen of this total were positive in a 1:20 dilution for Bact. paratyphosum B and 53 for the Esch. coli antigen.

Ten of the serums containing Bact. paratyphosum B agglutinins were absorbed by a mixture of eight Esch. coli strains. Three of these lost all agglutinins for Bact. paratyphosum B antigen, two showed only a reduction, while five remained unchanged.

When rabbits were immunized to certain typhoid and Salmonella strains their serums frequently agglutinated Esch. coli antigens in dilutions much higher than did the serums of the same animals previous to immunization. When serums showing this rise in titer for Esch. coli were absorbed with their own homologous antigen, the coli titer was generally unchanged.

Absorption of these Salmonella serums with Esch. coli strains removed the agglutinins for the absorbing strain, but general failed to affect the titer of the serum for its homologous antigen or for other Salmonella antigens.

The effect of Esch. coli absorption upon the titer of these serums for coli strains other than the absorbing one was variable. Some had their agglutinins completely removed, others showed a slight reduction in titer, while for still others the titer remained unchanged.

An attempt was made to discover the specific O component of the Salmonella serums which was responsible for agglutination of the coli strains by the use of serums rendered specific for individual antigenic components by selective absorption. The results indicated that none of these components with the possible exception of XII could be credited with causing this phenomenon. With the strains available in this laboratory, it was difficult to exclude the possibility that factor XII was the responsible agent though certain observations threw considerable doubt upon this assumption. The fact that only 84 per cent of the serums positive for Bact. paratyphosum B agglutinated Bact. typhosum suggests that factor XII is not the only agent involved.

Animals immunized to certain coli strains showed no increase over their normal O agglutinin content for antigens of the typhoid and Salmonella strains.

The above observations fail to confirm the theory that the O agglutinins in normal serums for Bact. paratyphosum B are frequently the result of immunization with normal intestinal organisms sharing common antigenic components with Bact. paratyphosum B. In fact the results leave this problem almost entirely untouched. The observed tendency, widely noted by others also, for Esch. coli agglutinins to increase greatly in the serums of animals immunized to typhoid and Salmonella strains likewise appears not to

be due to common antigenic components in these organisms, but rather suggests that this phenomenon is a type of anamnestic reaction. It is suggested that the widespread presence of coli agglutinins in the serums of lower animals is due to specific stimuli arising from their own intestinal tracts and that subsequent intensive immunization of such animals against any antigen may have the result of reactivating the mechanism for Esch. coli antibody formation.

Studies made on the serum of one case of typhoid fever revealed during the course of the disease, a marked rise of Esch. coli agglutinins which were not removed when the serum was absorbed by a stock typhoid antigen. This observation suggests that the phenomenon in animals discussed above, occurs also in human beings.

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APPENDIX

Several experiments were conducted during the course of this investigation which were not of sufficient value to be included in the main body of this thesis. The results of these experiments are recorded here for future reference.

It was noted during this investigation that the Hillman coli strain exhibited a high degree of sensitivity when titered with various Salmonella immune serums. This wide range of agglutination was first believed to occur as the result of Hillman coli containing a heterophile antigen. Therefore, a test was made for the presence of a heterophile antibody in the Hillman coli immune serum.

To 0.5 c.c. of a 1:10 dilution of Hillman immune serum, 0.5 cc. of a 0.5 per cent suspension of sheep cells was added and after thorough mixing, 0.5 c.c. of a 1:10 dilution of guinea pig compliment was added. The tubes were read after incubation for one hour at 37°C. in the water bath. The usual controls (sheep cells, compliment and serum) were included. No hemolysis occurred after the required length of incubation. The controls were all satisfactory. This experiment shows that the Hillman strain of coli does not contain a heterophile antigen.

An attempt was made to estimate the sensitivity of antigens prepared by various methods. The Hillman strain of coli was used for this test, and the antigens were prepared as follows:

1. Live antigens.

2. Live antigens heated to 60° C. for 30 minutes.

3. Live antigens plus phenol (final concentration of 0.5 per cent).

4. Hillman antigen prepared according to the method given in the main part of this these (standardized).

These four antigens were titrated by Hillman immune serum.

Table Ib

TITRATION OF HILLMAN IMMUNE SERUM BY LIVE, HEATED, PHENOLIZED AND STANDARDIZED HILLMAN ANTIGENS

Hillman coli Antigens	Hillman Serum Dilution					
	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240
Live	+4	+4	+4	+3	+2	+1
Heated	+4	+4	+4	+3	+3	1L
Phenolized	+4	+4	+4	+3	+2	+2
Standardized	+4	+4	+4	+4	+3	+1

From the results of this experiment, one can see that all four antigens exhibit about the same degree of sensitivity.

An experiment was devised to determine if the time factor was involved in the production of minoragglutinogens by members of the Salmonella group. Antigens were prepared for various strains of the Salmonella group at time intervals from 12 to 96 hours inclusive. When these antigens of different

growth periods were titered with coli immune serum, no difference in titer was observed. Evidently the time factor is not involved in the production of minor agglutinogen by the Salmonella group. During this investigation a total of 26 rabbit serums was tested for the presence of coli and Salmonella normal agglutinins. Of this total 22 or 84.6 per cent showed normal agglutinins for the Hillman strain of coli, 12 or 46.1 per cent showed normal agglutinins for the Swanson strain of coli, 10 or 38.4 per cent contained normal agglutinins for Bact. paratyphosum B 8006, 9 or 34.6 per cent showed normal agglutinins for Bact. typhosum 0901, and 9 or 34.6 per cent of this total contained normal agglutinins for Bact. virchow.

Typed by: Zona Cobb