SEROLOGICAL RELATIONSHIP BETWEEN PNEUMOCOCCUS TYPE I AND AN ENCAPSULATED STRAIN OF ESCHERICHIA COLI*

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During the course of experimental work dealing with freshly isolated pneumococci, a culture was obtained which had been diagnosed serologically as Type I pneumococcus. Upon further examination the culture proved to be composed of Gram-negative bacilli, subsequently identified as *Escherichia coli*, which, however, were agglutinated by Type I antipneumococcic horse serum. The exact source of the organism has not been traced but presumably it grew out in a heart's blood culture from a mouse injected with sputum from a suspected case of lobar pneumonia.

The apparent serological relationship between this colon bacillus and Type I pneumococcus suggested the possibility of a situation somewhat analogous to that demonstrated by Avery, Heidelberger, and Goebel (1) between Type II pneumococcus and a member of the Friedländer group, later classified as Type B by Julianelle (2). This paper describes various characteristics of the colon bacillus, hereafter designated as Strain 8-a, and its connection with Type I pneumococcus.

Morphological and Cultural Characteristics.—The cultures used were transfers from a single, well isolated colony on an agar plate. Examination of Gram-stained smears suggested that the organism might be one of the colon-typhoid group. Eosin-methylene blue agar colonies were medium sized, flattened, and possessed the metallic sheen typical of colon bacilli. Acid and gas were formed in dextrose, maltose, mannite, and lactose broths, but not in sucrose. Brom-cresol purple milk was acidified and coagulated with gas bubbles in the curd.

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Indol was formed, and nitrates were reduced to nitrites. Gelatin was not liquefied. Inulin was not attacked. There was no discoloration in lead acetate agar. In Russell's double sugar agar acid was formed throughout with gas bubbles in the butt of the tube. The organism was Voges-Proskauer negative and methyl red positive. Citrate was not utilized.

No motility was observed in preparations made on several different occasions.

Under the conditions usually employed it was not possible to demonstrate the presence of capsules. By the Neufeld method, however, using anti-*coli* 8-a rabbit serum, a definite *Quellung* reaction was obtained. For further evidence, 0.5 cc. of the culture was injected intraperitoneally into a mouse. 4 to 6 hours later the peritoneal exudate, when mixed with anti-*coli* 8-a rabbit serum and stained by Welch's method, showed the presence of distinct and well stained capsules surrounding the bacilli.

Well defined areas of hemolysis were formed around colonies in blood agar pour plates. Subsurface colonies had a gross appearance somewhat similar to those of pneumococci.

The characteristics described clearly permit the identification of this culture as an encapsulated strain of *Esch. coli* (*B. coli communis*).

Virulence of Esch. coli 8-a.—The intraperitoneal injection of 0.5 cc. of undiluted, and 10^{-1} dilution of a 4½ hour blood broth culture killed duplicate white mice, weighing 16 to 20 gm., in less than 48 hours. Mice injected with higher dilutions survived for at least 72 hours. Plate counts indicated approximately 1 billion organisms per cc. in the original culture.

Agglutination Reactions

Agglutination tests were conducted by mixing 0.5 cc. of serum dilutions with an equal amount of 18 hour broth cultures of the various organisms. The results recorded are readings made after 2 hours incubation in a 40°C. water bath. Storing the mixtures in the refrigerator overnight resulted in no essential changes in the relationships. Table I illustrates the cross-agglutination between Type I antipneumococcic horse serum and *Esch. coli* 8-a.

The results shown in Table I indicate that the non-specific agglutination in this instance is limited to Type I antipneumococcic horse serum since with Types II and III serums no reaction occurred with the colon bacillus and, curiously enough, Type I antipneumococcic rabbit serum also failed to agglutinate the organism. In order to determine whether the ability of Type I antipneumococcic horse serum to agglutinate *Esch. coli* 8-a is a general phenomenon tests were conducted with seven samples from different horse bleedings. Tests were also

made with two antimeningococcic serums, two samples of scarlet fever antitoxin, one of diphtheria antitoxin, and one of normal horse serum. All of the antipneumococcic horse serums agglutinated the colon bacillus to varying degrees, but none of the other serums used brought about the reaction. These results furnish added evidence that this

TABLE I

Agglutination of Esch. coli 8-a in Type I Antipneumococcic Horse Serum

Antipneumococcic serums	Cultures	Final dilutions of serums				
		1:5	1:10	1:20	1:40	1:80
Pn I horse serum D-30	*Coli* 8-a	4+	4+	3+	2+	1+
	Pn I	3+	3+	3+	3+	2+
Pn I horse serum 576-4	*Coli* 8-a	4+	3+	3+	1+	–
	Pn I	2+	3+	3+	3+	2+
Pn I rabbit serum R 241	*Coli* 8-a	–	–	–	–	–
	Pn I	4+	4+	4+	4+	3+
Pn II horse serum D-21	*Coli* 8-a	–	–	–	–	–
	Pn II	4+	4+	4+	1+	–
Pn II horse serum 546-2	*Coli* 8-a	–	–	–	–	–
	Pn II	4+	2+	1+	–	–
Pn III horse serum D-22	*Coli* 8-a	–	–	–	–	–
	Pn III	4+	3+	3+	2+	1+
Pn III horse serum D-39	*Coli* 8-a	–	–	–	–	–
	Pn III	4+	2+	1+	1+	–

4+ indicates compact, disc-like agglutination, not easily broken; 3+ a compact, disc-like agglutination which breaks up into large clumps; 2+, smaller clumps; 1+, a definite, but finely granular agglutination; – no agglutination visible to the naked eye.

case of non-specific agglutination is a characteristic only of Type I antipneumococcic horse serum.

Agglutinin Adsorption.—Using a monovalent Type I antipneumococcic horse serum, tests were made for adsorption of agglutinins for Type I pneumococcus and *Esch. coli* 8-a. Equal parts of the cultures and serum dilutions were mixed and incubated 2 hours in a 40°C. water bath and overnight in the refrigerator.

Readings were made and the mixtures centrifuged. The clear supernatants were transferred in the same order to duplicate agglutination tubes. The serum treated with each culture was then retested for the presence of agglutinins for each organism as before. The results are shown in Tables II and III.

It can be readily observed that, by this procedure, most of the agglutinins for *Esch. coli* 8-a were removed by adsorption with *Esch. coli* 8-a, but those for Type I pneumococcus were relatively unaffected. Similar treatment of the serum with Type I pneumococci effectively removed agglutinins for both organisms.

TABLE II

Agglutinins for Type I Pneumococcus and Esch. coli 8-a in Type I Antipneumococcic Horse Serum before Adsorption

Type I antipneumococcic horse serum	Cultures	Final dilutions of serum					
		1:5	1:10	1:20	1:40	1:80	1:160
576-4	Pn I	3+	4+	4+	4+	3+	1+
	Coli 8-a	4+	4+	4+	3+	1+	±

TABLE III

Agglutinins for Type I Pneumococcus and Esch. coli 8-a in Type I Antipneumococcic Horse Serum after Adsorption

Serum adsorbed by	Cultures added to supernatants	Final dilutions of serum					
		1:10	1:20	1:40	1:80	1:160	1:320
Pn I	Pn I	3+	2+	−	−	−	−
	Coli 8-a	3+	−	−	−	−	−
Coli 8-a	Pn I	2+	3+	3+	2+	−	−
	Coli 8-a	2+	−	−	−	−	−

Serological Tests with Anti-Coli 8-a Serum.—Rabbits were immunized with heat-killed vaccines made from *Esch. coli* 8-a. Two courses of fifteen doses each were given. The schedules and amounts were similar to those used in preparing anti-pneumococcic rabbit serums (3). The rabbits were bled by cardiac puncture 7 days after the last dose in each course of injections. The serums were pooled and preserved by the addition of 0.3 per cent tricresol.

This pooled serum was tested against the homologous organisms, Types I, II, and III pneumococci, Type I SSS, and against a filtrate of the supernatant from a lot of *Esch. coli* 8-a vaccine. In addition, a Type I antipneumococcic horse serum was tested against the *coli* 8-a vaccine filtrate and against Type I SSS.

The agglutination titer of the serum for the homologous organism was 1:5120, and its precipitin titer for the vaccine filtrate was 1:80. No agglutination occurred with Types I, II, and III pneumococci, nor was there any precipitation of Type I SSS. A Type I antipneumococcic horse serum which, in a dilution of 1:160, precipitated Type I SSS (1:10,000 dilution) also precipitated, in a serum dilution of 1:80, a vaccine filtrate of *coli* 8-a.

These results indicate that the cross-reactions between Type I antipneumococcic horse serum and *Esch. coli* 8-a are not reciprocal, and further suggest that a soluble capsular material is elaborated by the colon bacillus.

TABLE IV

Agglutination of Virulent and Avirulent Strains of Coli 8-a and Stock Strain of Esch. coli by Various Serums

Serums	Cultures	Final dilutions of serums								
		1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Anti-*coli* 8-a	Virulent *coli* 8-a	3+	3+	3+	3+	3+	2+	1+	1+	−
	Avirulent *coli* 8-a	1+	1+	1+	1+	1+	1+	1+	1+	1+
	Stock *coli* K-12	±	±	−	−	−	−	−	−	−
Pn I horse serum 576-4	Virulent *coli* 8-a	3+	3+	2+	1+	±	−	−	−	−
	Avirulent *coli* 8-a	1+	1+	1+	−	−	−	−	−	−
Pn I horse serum 576-5	Virulent *coli* 8-a	4+	3+	3+	1+	−	−	−	−	−
	Stock *coli* K-12	1+	1+	1+	1+	−	−	−	−	−
Pn I and II horse serum 610-17	Virulent *coli* 8-a	4+	3+	2+	−	−	−	−	−	−
	Stock *coli* K-12	1+	1+	1+	1+	1+	−	−	−	−
Pn I and II horse serum 615-23	Virulent *coli* 8-a	3+	2+	−	−	−	−	−	−	−
	Stock *coli* K-12	1+	1+	1+	1+	1+	−	−	−	−

Agglutination Tests with Other Coli Strains.—For further testing, *Esch. coli* 8-a was transferred through twenty serial passages in 10 per cent Type I antipneumococcic horse serum broth. Although microscopic examination of agar plate cultures of this serum broth culture failed to reveal evidence of rough colony formation, 0.5 cc. of the culture failed to kill mice, and so is designated as an avirulent strain of *coli* 8-a. Agglutination tests tended to indicate that the loss of virulence was accompanied by alterations in reactivity usually associated with strains intermediate between the "S" and "R" forms. An old stock culture of *Esch. coli* (K-12) was also tested for its agglutinability by various serums. The results of these tests are presented in Table IV.

The agglutination reactions of the virulent *coli* 8-a were of the flocculent nature, while those of the avirulent *coli* 8-a and of the K-12 strain were of the finely granular type, and give added evidence that passage of the virulent *coli* 8-a through serum broth had at least partially degraded the organism.

As a matter of interest, the anti-*coli* 8-a rabbit serum was tested against Types A, B, and C Friedländer's bacilli. All tests were completely negative.

DISCUSSION

The foregoing data are presented to illustrate another example of the heterogenetic specificity described by Avery, Heidelberger, and Goebel. Their results demonstrated a relationship between Type II pneumococci and Type B Friedländer's bacilli; the present communication establishes a serological connection between Type I pneumococci and an encapsulated strain of *Esch. coli*. The two reports suggest a possible explanation for some of the discrepancies in typing suspected pneumonia sputums by various methods, particularly when anti-pneumococcic horse serums are used in diagnosis.

The occurrence of encapsulated strains of *Esch. coli* is not uncommon. Their existence is mentioned by Ford (4*a*), and extensive studies of their characteristics were made by Smith and his associates (4*b*). Precipitin tests with a filtrate of vaccine made from the 8-a strain indicate that the capsular material may be elaborated in soluble form. Such an observation is in conformity with the findings of Tomcsik (5) who prepared purified polysaccharides from encapsulated colon and *aerogenes* bacilli. Work is in progress in attempts to isolate the carbohydrate fraction of *Esch. coli* 8-a.

The source of the colon bacillus herein described is unknown. It is probably of fecal origin as indicated by its inability to utilize citrate (6), and the negative Voges-Proskauer and positive methyl red reactions. The possibility that the organism entered as a contaminant from the mouse cannot be overlooked. Assuming, however, that both pneumococci and colon bacilli were present in the upper respiratory tract of the human subject, it is not unlikely that the colon organisms would obliterate the pneumococci during mouse passage. The substitution of the Neufeld *Quellung* reaction in diagnosing suspected

pneumonia sputums for the mouse passage method would, however, overcome such a difficulty.

It is of interest to note that the lack of serological specificity observed in this study is a characteristic limited to Type I antipneumococcic horse serum. The facts cited suggest that Type I pneumococci contain a reactive substance not possessed by the colon bacillus, and which may be antigenic in horses but not in rabbits. This offers one possible explanation for the greater specificity commonly assumed for rabbit serums used in the Neufeld *Quellung* test.

SUMMARY

An encapsulated strain of *Escherichia coli* has been isolated which is hemolytic, pathogenic for mice, and which has served to illustrate further evidence of heterogenetic specificity. The relationship appears to be limited to the serological reactions between the colon organism and Type I antipneumococcic horse serum. Type I antipneumococcic rabbit serum failed to agglutinate the organism and no reactions occurred with Types II and III antipneumococcic horse serums, normal horse serum, and a variety of other immune horse serums. Serum from rabbits immunized with the colon bacillus agglutinated the homologous organism and precipitated its soluble substance, but failed to cause agglutination of Type I pneumococci or to precipitate Type I pneumococcic polysaccharide. The evidence indicates a connection somewhat analogous to that between Type II pneumococcus and Type B Friedländer's bacillus.

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