STUDIES ON THE "H" ANTIGENS OF ESCHERICHIA COLI

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INTRODUCTION

The species, Escherichia coli, includes strains with definite characteristics which form a part of a large group of gram negative bacilli often discussed in the literature under such names as "the coli group", "the coliform organisms", etc. This large group is limited for the most part by fermentation of lactose. Efforts have been made for many years to classify this group of organisms into species that would have some useful and constant relationship to such practical problems as specific disease production and fecal pollution of water and food. The earlier attempts to make use of the antigen-antibody reactions all led to the same conclusion, namely, that classification based on that property would lead to the establishment of a very large number of groups with no relation to the above mentioned practical problems.

In recent years the group has been divided into Aerobacter, intermediates, and Escherichia sections. For this classification a group of tests is used to which Parr (1936) has given the mnemonic "Imvic". The tests are (I) indole production; (M) the methyl-red reaction; (V) the Voges-Proskauer test; and (C) the utilization of sodium citrate as the sole carbon source. Thus the Escherichia section has Imvic reactions +·+· which means that the strains of that section produce indole from the decomposition of tryptophane, produce a final pH in glucose broth of 4.5 or less, fail to produce acetyl-methyl carbinol from dextrose and are unable to utilize sodium citrate as the sole carbon source. The Aerobacter section gives the Imvic reactions −·−· and the intermediates section includes strains which have one or more Escherichia characteristics and one or more of those attributed to Aerobacter. Slow lactose fermenting strains of all three sections are recognized. Among the latter are the paracolon strains, frequently isolated from man, especially in gastro-enteritis outbreaks.

These paracolons occasionally do not ferment lactose or ferment it slowly with the formation of acid, or acid and gas. The motabile strains are also sometimes included among the paracolon bacilli. These ferment lactose late and on a lactose medium such as eosin methylene blue agar give off from the non-lactose fermenting mother colony a small lactose fermenting colony or papilla.

Although Smith and Keogh (1903) and Boyer and Keogh (1904) demonstrated the difference between flagellar and somatic antigen it was not until the work of Weil and Felix (1917) with a particular strain of proteus in relation to typhus that serologists became interested and fully aware of flagellar and somatic antigens. However, those studying the serological relationships of the coliform group of organisms do not appear to have made any particular effort to limit their reactions to either flagellar or somatic antigens or, when they have, the results have not been reported separately as such.

Herrold and Culver (1919) in a study of motile, indole negative paracolons and motile and non-motile "coli", practically all indole positive, found both groups heterogeneous, but the organisms in the paracolon group more closely related than those in the colon group. The authors do not state definitely how the antigens for antiserums were prepared or identify the type of agglutination. The agglutination may have been due to O antigens or both H and O antigens as the agglutination tests were incubated for 3 hours at 37° C. and allowed to stand 24 hours at room temperature before being read.

Dudgeon, Wordley and Bourtree (1922) found that most of their hemolytic strains of coliform bacteria isolated from urinary infections were agglutinated by a serum prepared from one of them but the non-hemolytic strains did not show such a relationship. The motilities of their strains were not noted. Their agglutination tests were incubated at 50 - 55° C.

for 5 hours and left 1 hour at room temperature and the resulting aggregates were "small". From this one concludes that they were dealing chiefly with somatic antigens and O antiserums.

Stuart, Baker, Zimmerman, Brown and Stone (1940) added cellobiose fermentation to the Ewie reactions and found a correlation between the serological and the biochemical groupings. Although both motile and non-motile cultures were used by these authors and antiserums were prepared with living cultures they made no distinction between flagella and somatic agglutination in their paper.

Stuart, Wheeler, Kustigian and Zimmerman (1943) tested Escherichia coli, and strains belonging to the paracolon group with the Ewie reactions ++—. Each group was tested for agglutination with its own antiserums. The paracolon organisms showed more relationship within the group than did Escherichia coli. The paracolon group, as defined by the authors, consists of strains which ferment lactose slowly or with the formation of acid only and of non-lactose fermenting coliforms isolated from man, especially in gastro-enteritis outbreaks. This study included motile and non-motile strains, all immunizations were done with living cultures and the authors in their summary state, "Because of their complex serological structure classification of paracolon bacteria on the basis of H and O antigens is impracticable." However, the definiteness of this conclusion does not seem to be fully justified by their published data.

Wallick and Stuart (1943) isolated 650 Escherichia coli strains with Ewie reactions ++— from one individual and tested these with ten antiserums produced against 10 of these strains. Subsequent absorptions indicated that 85.3% were antigenitically identical. From each of 100 persons unrelated to the above individual a strain was isolated and the 100 strains tested with the same ten antiserums. Eight strains agglutinated to a titer suggesting close relationship but absorption studies proved

only one to be identical with any of the ten strains. The authors incubated their motile strains for 2 hours at 37° C. followed by 12 to 18 hours at 55° C. while non-motile cultures were incubated at 55° C. only. From this one may assume that flagellar agglutination was noted when present. However, it is not reported separately which makes it difficult to tell how much relationship is due to flagellar antigens and how much to somatic antigens.

Taylor (1941) who took especial care to limit her reactions to flagellar agglutinations tested 50 coliform strains with antisorums prepared from three of these. Her strains all produced acid and gas in glucose, failed to ferment sucrose and fermented lactose, though some gave fermentation of the latter sugar only after several days incubation. These late lactose fermenters were motabile strains. She found that 22 strains or 44%, including those strains used to make the antisorums, gave flagellar agglutination with one or more of the three antisorums.

As so little has been recorded of flagellar antigen relationship in the coliform group it seemed to us worthwhile to continue the work of Taylor by studying the serological relationship due to flagellar antigens of one definite section of the coliform group. The Escherichia coli section giving the Ivic reactions ++-- was chosen. The strains used include E. coli motabile as well as E. coli.

MATERIALS AND METHODS

The work reported in this paper consists of a study of the flagellar antigens of 80 strains of Escherichia coli (Imvic ++--) by means of straight agglutination tests and absorption tests using serums prepared against 10 of the strains. Of the 80 strains, 12 were of the motabile type.

Table I lists all of the 80 strains with data relative to source and biochemical reactions at 37°C. Strains marked "stock" in the table had been in the culture collection for years and were all originally isolated from man. The other strains were isolated from pathological specimens and stool specimens of man at various times during the year and a half during which time this study was conducted.

All of the 80 strains exhibited active motility, but there was considerable variation in the proportion of organisms in the hanging drop field which showed motility at the time of examination. Examinations were made of broth cultures that had been incubated at 37° C. for 15 to 17 hours and proportions of motile organisms varied. Some strains gave hanging drops in which practically all organisms seemed to be motile, others showed only a few motile individuals. Nearly all proportions between these extremes were observed in the entire group.

Antigens: To prepare an antigen, either for immunizing animals or for agglutination tests the stock culture was streaked on a moist extract agar plate and incubated overnight at 37° C. From this plate a smooth moist colony was selected and inoculated into a tube of extract broth. Six to eight hours later the culture was observed for motility. If motile, a loopful of the culture was transferred to a flask containing 150 c.c. of infusion broth. This was incubated at 37°C. for 15 to 17 hours If at the end of that time the culture still showed motile organisms, a

TABLE I

Source and Characteristics of the 30 Strains of Escherichia coli Investigated

AC = acid and gas

Strain	Lactose	Motility	Indole	Methyl-Red	Voges-Proskauer	Growth in Koser's Citrate	Source
146	AG late	+	+	+	-	-	Stock
70	AG late	+	+	+	-	-	Stock
100	AG late	+	+	+	-	-	Stock
262B	AG late	+	+	+	-	-	Stock
269B	AG late	+	+	+	-	-	Stock
269H	AG late	+	+	+	-	-	Stock
269S	AG late	+	+	+	-	-	Stock
412	AG late	+	+	+	-	-	Stock
912	AG late	+	+	+	-	-	Stock
Hogan	AG late	+	+	+	-	-	Stool (stock)
Lockwood	AG late	+	+	+	-	-	Stool (stock)
5	AG	+	+	+	-	-	Stock
271	AG	+	+	+	-	-	Stock
272	AG	+	+	+	-	-	Stock
269P	AG	+	+	+	-	-	Stock
Allen	AG	+	+	+	-	-	Stool
Baldman	AG	+	+	+	-	-	Stool (stock)
Bowie	AG	+	+	+	-	-	Stool
Bradshaw	AG	+	+	+	-	-	Stool
Brigg	AG	+	+	+	-	-	Urine
Brownlee	AG	+	+	+	-	-	Stool
Chamberlain	AG	+	+	+	-	-	Stool
Chatbough	AG	+	+	+	-	-	Urine
Cole	AG	+	+	+	-	-	Stool
Cooper	AG	+	+	+	-	-	Urine
Crits	AG	+	+	+	-	-	Urine
Cruse	AG	+	+	+	-	-	Urine (stock)

TABLE I (Continued)

Source and Characteristics of the 30 Strains of Escherichia coli Investigated

AG = acid and gas

Strain	Lactose	Motility	Indole	Methyl-Red	Voges-Proskauer	Growth in Koser's Citrate	Source
Edwards	AG	+	+	+	-	-	Urine
Ellis	AG	+	+	+	-	-	Stool
Fall	AG	+	+	+	-	-	Stool
Foster	AG	+	+	+	-	-	Stool
Gamble I	AG	+	+	+	-	-	Stool
Gamble II	AG	+	+	+	-	-	Stool
Goodman	AG	+	+	+	-	-	Urine
Gordan	AG	+	+	+	-	-	Stool
Goss	AG	+	+	+	-	-	Stool
Grady	AG	+	+	+	-	-	Stool (stock)
Halen	AG	+	+	+	-	-	Stool (stock)
Hawks	AG	+	+	+	-	-	Urine
Huky	AG	+	+	+	-	-	Exudate
Hill	AG	+	+	+	-	-	Urine
Hiller	AG	+	+	+	-	-	Urine (stock)
Hershey	AG	+	+	+	-	-	Abscess
Hoopman	AG	+	+	+	-	-	Stool
Johnson, J.	AG	+	+	+	-	-	Appendix
Kellner	AG	+	+	+	-	-	Stool
Merrill	AG	+	+	+	-	-	Stool
Kirk	AG	+	+	+	-	-	? (recent)
Koechert	AG	+	+	+	-	-	Stool
Kridler	AG	+	+	+	-	-	Urine
Lash	AG	+	+	+	-	-	Urine
Martin II	AG	+	+	+	-	-	Stool (stock)
Maltese	AG	+	+	+	-	-	Urine
McCallum	AG	+	+	+	-	-	Stool
Mercer	AG	+	+	+	-	-	Urine

TABLE I (Continued)

Source and Characteristics of the 60 Strains of Escherichia coli Investigated

AG = acid and gas

Strain	Lactose	Motility	Indole	Methyl-Red	Voges-Proskauer	Growth in Koser's Citrate	Source
Merrick	AG	+	+	+	-	-	Urine
Moreland	AG	+	+	+	-	-	Urine (stock)
Murray	AG	+	+	+	-	-	Stool (stock)
Newman	AG	+	+	+	-	-	Knee wound
Oliver	AG	+	+	+	-	-	Stool (stock)
Osgood	AG	+	+	+	-	-	Stool
Osterhaudt	AG	+	+	+	-	-	Stool
Pfau	AG	+	+	+	-	-	Stool
Platt	AG	+	+	+	-	-	Stool
Pullman	AG	+	+	+	-	-	Urine
Ray	AG	+	+	+	-	-	Urine
Riley	AG	+	+	+	-	-	Wound
Robbins	AG	+	+	+	-	-	Blood culture
Robertson	AG	+	+	+	-	-	Urine
Rowe	AG	+	+	+	-	-	Stool
Sears	AG	+	+	+	-	-	Stool
Smith, F.	AG	+	+	+	-	-	Urine
Smith, A.	AG	+	+	+	-	-	Urine
Swanson	AG	+	+	+	-	-	Stool (stock)
Shupe	AG	+	+	+	-	-	Stool
Veazie	AG	+	+	+	-	-	Stool
Vudem	AG	+	+	+	-	-	Stool
White	AG	+	+	+	-	-	Stool
Williams	AG	+	+	+	-	-	Urine
Wright	AG	+	+	+	-	-	Urine

gram stained smear was examined to check for contamination and an extract agar slant culture was made for stock. Formalin was added to the flask of antigen to a concentration of 0.2% and it was stored in the refrigerator.

Antiserums: The 10 strains used for the preparation of antiserums are listed below.

Strain	Variety	Source
100	E. coli mutabile	stock
2629	E. coli mutabile	stock
Edwards	E. coli	urine
412	E. coli mutabile	stock
912	E. coli mutabile	stock
269B	E. coli mutabile	stock
5	E. coli	stock
Goodman	E. coli	urine
Johnson	E. coli	appendix
Pullman	E. coli	urine

All antiserums with the exception of one were made from antigens which showed practically every organism motile at the end of 15 to 17 hours incubation at 37° C. The exception, Edwards, was made from an antigen which came from two colonies, one of which yielded a culture containing few motile organisms and the other yielded a culture in which practically all organisms were motile. These cultures were used in equal parts.

Rabbits used for preparation of antiserums weighed from 3 3/4 to 6 pounds. Increasing doses of antigen were given into the ear vein daily for three days. The doses were 0.5 c.c., 1 c.c., and 2 c.c., respectively. Occasionally an animal became ill after the first

injection. In such cases the injections were discontinued and later started again beginning with the 0.5 c.c. amount. Five or six days after the last injection the rabbit was bled from the ear and the antibody titer of the serum determined. If the titer was satisfactory the rabbit was bled from the carotid. The serum was placed in a sterile vaccine vial and merthiolate added to 1-10,000 dilution. The serums were stored in the refrigerator. If after one series of injections the titer was lower than 1-3200, a second series was given and the titer again determined five days after the last injection. This did not in every case raise the antibody titer. One serum was therefore used at a titer below this figure.

Antigens for Absorptions. These were made by inoculating Blake bottles of 2% agar with 1 to 2 c.c. of an extract broth culture which had been incubated overnight and diluted to 75 to 100 c.c. with extract broth. The inoculated Blakes were incubated about 22 hours; the bacteria washed off with 1% formalinized saline and left standing overnight in centrifuge tubes at room temperature. These were then centrifugalized at high speed for at least 1½ hours, the supernatant fluid poured off and the organisms resuspended in a small amount of 0.2% formalinized saline. These antigens were stored in the refrigerator. They were always used within 3 or 4 days following centrifugalization.

Absorption Tests. For the first absorption of each serum the bacteria were not repacked by centrifugalization because it was thought possible that some flagella might be lost in manipulation. To 4 c.c. of the desired bacterial suspension 1 c.c. of the undiluted serum to be absorbed was added and mixed well. This was incubated at 37° C. for 2 to 3 hours and then stored in the refrigerator overnight. The next morning it was centrifugalized at high speed for 1½ hours. The amount of packed cells was noted and if this was 1 c.c. the dilution was 1 to 4. If there was

more than 1 c.c. of packed cells 0.2% formalinized saline was added to bring the serum dilution to 1 to 4. If it was necessary to absorb the serum a second or third time the diluted serum was added to a known volume of packed bacteria. The final dilution of the absorbed serum in such instances was still regarded as 1 to 4.

Agglutination tests. H or flagellar agglutination takes place very rapidly and the agglutinated bacteria appear as large, buoyant clumps which settle to the lower portion of the tube. The aggregates are supposedly composed of bacilli which are held together by their tangled flagella and which re-disperse readily on shaking.

In this study agglutination tests previous to absorptions were set up in tubes which were then incubated at 50° C. for one hour and read without the aid of a lens or mirror. Agglutination tests using absorbed serums were set up in the same manner but read with the aid of a mirror and oftentimes artificial light, depending on what light was best. Agglutination which appeared in one hour but which did not re-disperse on shaking was not considered flagellar agglutination. Where titers were to be determined, either before or after absorption, serial dilutions of the serums were made in the usual manner, each succeeding dilution being double that of the preceding one. The highest of these dilutions giving unmistakable agglutination of the H type was taken as the titer of the serum. The tubes used were ¼ inch by ⅜ inch serum tubes.

EXPERIMENTAL WORK

To classify the 80 motile strains on a basis of flagellar agglutination, the first procedure was to test each of the 80 strains with each of the 10 antiserums. Since it was anticipated that many strains would not agglutinate in any dilution with any of the 10 antiserums, a preliminary test was first set up consisting of one tube for each antigen. The dilution of the antiserum in this preliminary test was 1:50. Antigens failing to agglutinate in this test were discarded without further study. Antigens agglutinating in this preliminary test were subsequently set up with dilutions of antiserum in the manner described above to determine the titer. It was thought possible that such tests might be negative in 1:50 dilutions but positive in a higher dilution, that is, that they might exhibit the so called "zone" phenomenon. However, agglutination tests on 40 of the strains, using a range of dilutions, gave no evidence of such a phenomenon. Table II gives the results of titrations on all antiserums giving a positive result in the preliminary one tube test.

To study the relationship of the flagellar antigens giving positive agglutination tests in the same antiserums, absorption tests were carried out, some of which were reciprocal. To determine completeness of absorption, the absorbed antiserum was tested in a 1:50 dilution with the absorbing antigen, the result being read with the aid of a mirror. If this proved negative, the absorbed antiserum was tested with the various antigens which it had agglutinated before being absorbed. The results of all absorption tests are given in Tables III, IV, V, VI, VII, VIII and IX.

TABLE II

Agglutination Titers of Ten Antiserums with 21 Antigens

ANTIGENS	ANTISERUMS									
	100	262B	Edwards	412	912	Johnson	Pullman	269B	Goodman	5
100*	3200	3200	1600	6400	800	0	0	0	0	0
262B*	6400	3200	1600	6400	3200	0	0	0	0	0
Edwards	6400	3200	3200	3200	800	0	0	0	0	0
412*	3200	800	800	6400	1600	0	0	0	0	0
912*	3200	1600	1600	6400	1600	0	0	0	0	0
140*	3200	3200	1600	12800	1600	0	0	0	0	0
Kirk	3200	1600	1600	6400	1600	0	0	0	0	0
Fall	800	1600	1600	6400	1600	0	0	0	0	0
Cruse	800	800	800	3200	800	0	0	0	0	0
269H*	3200	3200	Not tested	3200	Not tested	0	0	0	0	0
Pullman	0	0	0	0	0	800	3200	0	0	0
Johnson	0	0	0	0	0	12800	800	0	0	0
Osgood	0	0	0	0	0	200	1600	200	0	0
Hicky	0	0	0	0	0	3200	1600	0	0	0
Gamble	0	0	0	0	0	0	800	0	0	0
Pfen	0	0	0	0	0	3200	3200	0	0	0
269B*	0	0	0	0	0	0	0	6400	200	0
Goodman	0	0	0	0	0	0	0	3200	6400	0
Kelmer	0	0	0	0	0	0	0	0	50	0
Lockwood*	0	0	0	0	0	0	0	400	0	0
5	0	0	0	0	0	0	0	0	0	6400

*Indicates mutabile strains

RESULTS

An examination of Table II shows that the 21 strains fall into four groups which are either not related at all or very slightly so.

One of these groups contains only one E. coli strain, strain 5. There are two other small groups, one containing five strains, two of which are motabile, and the other containing six strains. The titers of the antiserums for the various antigens vary considerably, indicating that no two strains in either of these groups have identical flagellar antigen. While it appears that the strains of each of these two groups share flagellar antigens, different strains contain them in varying amounts and in combination with other antigens for which there are no antibodies in the ten antiserums. Tables VIIIand IX give the results of absorption tests. Although only one reciprocal absorption was carried out, the results agree with the above statement. The strains vary as to source and include those from an appendix, an empiete, stools and a urine.

The group which appears most striking includes 10 strains, all of which are agglutinated by the same five antiserums, 100, 262B, h12, 912 and Edwards, in dilutions comparable with the titers of the serums for their homologous antigens. It was to be expected that strains containing the same flagellar antigens as the homologous strain, when agglutinated by the antiserum would give a titer that would be one dilution above or below the homologous titer. However, some of these antigens were agglutinated by an antiserum in a dilution two dilutions lower than the homologous titer. Thus it appears possible that they may have very closely related but not identical flagellar antigens. In looking over Table II one finds both antigens h12 and Cruse agglutinated with antiserums 262B and Edwards to titers which are two dilutions below the homologous titer. Both the Fall and Cruse antigens agglutinate with antiserum 100 to titers two

TABLE III

Absorption Tests with Antiserum 912

Tested with antigen	Antiserum 912								
	Unabsorbed	Absorbed with							
		Edwards	262B	100	412	14G	Fall	Kirk	Cruse
Edwards	800	0	0	0	0	0	0	0	0
262B	3200	0	0	0	0	0	0	0	0
100	800	0	0	0	0	0	0	0	0
912	1600	200	100	100	0	0	0	0	0
412	1600	200	100	100	0	0	0	0	0
14G	1600	200	100	100	0	0	0	0	0
Fall	1600	200	100	100	0	0	0	0	0
Kirk	1600	200	100	100	0	0	0	0	0
Cruse	800	200	100	100	0	0	0	0	0

TABLE IV

Absorption Tests with Antiserum 412

Tested with antigen	Antiserum 412								
	Unabsorbed	Absorbed with							
		Edwards	262B	100	912	14G	Fall	Kirk	Cruse
Edwards	3200	0	0	0	0	0	0	0	0
262B	6400	0	0	0	0	0	0	0	0
100	6400	0	0	0	0	0	0	0	0
912	6400	800	800	400	0	0	0	0	0
412	6400	800	800	800	0	0	0	0	0
14G	12800	800	800	800	0	0	0	0	0
Fall	6400	400	400	400	0	0	0	0	0
Kirk	6400	400	800	400	0	0	0	0	0
Cruse	3200	800	800	400	0	0	0	0	0

TABLE V

Absorption Tests with Antiserum 100

Tested with culture	Antiserum 100								
	Unabsorbed	Absorbed with							
		262B	Edwards	412	912	140	Fall	Kirk	Cruse
100	3200	0	0	800	400	800	800	800	800
262B	6400	0	0	800	800	800	800	800	800
Edwards	6400	0	0	800	800	1600	800	1600	800
412	3200	0	0	0	0	0	0	0	0
912	3200	0	0	0	0	0	0	0	0
140	3200	0	0	0	0	0	0	0	0
Fall	800	0	0	0	0	0	0	0	0
Kirk	3200	0	0	0	0	0	0	0	0
Cruse	800	0	0	0	0	0	0	0	0

TABLE VI

Absorption Tests with Antiserum 262B

Tested with culture	Antiserum 262B								
	Unabsorbed	Absorbed with							
		100	Edwards	412	912	140	Fall	Kirk	Cruse
262B	3200	0	0	400	400	400	400	200	200
100	3200	0	0	400	400	400	400	400	200
Edwards	3200	0	0	200	400	400	400	400	200
412	800	0	0	0	0	0	0	0	0
912	1600	0	0	0	0	0	0	0	0
140	3200	0	0	0	0	0	0	0	0
Fall	1600	0	0	0	0	0	0	0	0
Kirk	1600	0	0	0	0	0	0	0	0
Cruse	800	0	0	0	0	0	0	0	0

TABLE VII

Absorption Tests with Antiserum Edwards

Tested with culture	Antiserum Edwards								
	Unabsorbed	Absorbed with							
		100	2628	412	912	14G	Fall	Kirk	Cruse
Edwards	3200	0	0	200	200	200	200	200	400
2628	1600	0	0	200	200	200	200	200	200
100	1600	0	0	200	400	400	400	400	200
412	800	0	0	0	0	0	0	0	0
912	1600	0	0	0	0	0	0	0	0
14G	1600	0	0	0	0	0	0	0	0
Fall	1600	0	0	0	0	0	0	0	0
Kirk	1600	0	0	0	0	0	0	0	0
Cruse	800	0	0	0	0	0	0	0	0

TABLE VIII

Absorption Tests with Antiserum Pullman

Tested with culture	Antiserum Pullman					
	Unabsorbed	Absorbed with				
		Osgood	Johnson	Hicky	Gamble	Pfen
Pullman	3200	100	1600	0	800	1600
Osgood	1600	0	800	0	0	1600
Johnson	800	0	0	0	400	0
Hicky	1600	50	0	0	800	200
Gamble	800	0	800	0	0	800
Pfen	3200	100	0	0	800	0

TABLE IX

Absorption Tests with Antiserum Johnson

Tested with culture	Antiserum Johnson				
	Unabsorbed	Absorbed with			
		Osgood	Pullman	Hicky	Pfen
Johnson	12800	6400	6400	3200	6400
Pullman	800	200	0	0	0
Osgood	200	0	0	0	0
Hicky	3200	1600	0	0	0
Pfen	3200	3200	0	50	0

dilutions below the homologous titer. Tables IV, VI and VII give the results of reciprocal absorption tests of strains 412, 262B and Edwards. These show that 412 is closely related to, but does not have the same flagellar antigens as 262B and Edwards, which have identical flagellar antigens. Although there are no reciprocal absorption tests using strains Cruse and Fall results of the tests given in Tables III, IV, V, VI and VII show that while related to, they do not have the same flagellar antigens as strain 100. They appear more like strains 912 and 412. Some antigens though they do not have identical flagellar antigens have been agglutinated by antiserum to homologous or to higher titers. Thus it appears that although a titer two dilution below the homologous titer may indicate that the strain does not have identical flagellar antigens with the strain producing the antiserum, nevertheless identical titers of an antiserum for two or more different antigens still does not prove that the strains possess identical flagellar antigens. As reciprocal absorption tests were not done with the Cruse strain, it is not known that it does not possess an antigen or antigens which the other strains do not have. However, in the absorptions done it appears like strains 912 and 412 and if it contains identical flagellar antigens, the consistently low titer with all antiserums would seem to be due to the fact that it is a less sensitive antigen than the others or contains the antigens in different proportions. In the absorption tests, none of the absorption doses were adjusted so that it was possible to demonstrate a quantitative difference in the strains containing closely related or identical flagellar antigens.

As the results in the tables show, the two stock mutabile strains, 262B and 100, have the same flagellar antigens as the recently isolated urine E. coli strain Edwards, and that the two related stock mutabile strains, 912 and 412, have identical flagellar antigens. Thus this

grouping of antigens is dependent on tests with only two serums, each containing a large amount of common antibody and what appears to be a smaller amount of antibody specific for each serum. Other strains in the group, Kirk, Fall, Cruse and 1140, are from varying sources. Absorptions indicate that they most nearly resemble the mutabile strains 112 and 912. However, reciprocal absorptions were not carried out on these strains so it is possible that they also contain other antigens. No absorption tests were carried out with strain 2698.

One of the very striking results of this study is the high percentage of mutabile strains which gave positive agglutination tests. Of the 11 mutabile strains 8 or 72.7% gave a positive agglutination test while of the other 69 strains, only 13 or 18.8% gave a positive agglutination test with one or more antiserums. The antiserums were made from 5 mutabile strains and 5 strains which produced acid and gas in 24 hours. Two of the antiserums were made from mutabile strains having identical flagellar antigens. Three antiserums were made from 2 mutabile strains and 1 strain fermenting lactose in 24 hours, all containing identical flagellar antigen. Therefore, antiserums containing different antibodies numbered only seven, which may be represented by antiserums of four rapid lactose fermenting strains, two mutabile strains, and one, either by a mutabile strain or a rapid lactose fermenting strain. Thus it is very evident that the large percentage of positive agglutination tests given by mutabile strains is not due to the use of a larger number of antiserums made from mutabile strains than from other strains containing different flagellar antigen, but to the fact that in this small group a high percentage have related or identical antigens which may also be contained in some other E. coli strains.

SUMMARY AND CONCLUSIONS

To determine the serological relationship of the flagellar antigens of Escherichia coli, a study was made of 80 motile strains isolated from man and having the Imvic reactions ++--. Of the 80 strains 11 were of the mutabile type. Antisorums were prepared against formalinized broth antigens of ten of the strains which were very motile. Five of these strains were of the mutabile type. Each of the antisorums was used to test a formalinized broth antigen of each strain for flagellar agglutination. Absorption tests, some of which were reciprocal, were carried out with most of the strains which gave a positive agglutination test in one or more antisorums.

An analysis of the relationship brought out in the results of this study show that in so far as this investigation has gone, out of the 80 E. coli strains, only 21 possess flagellar antigens represented by antibody in the 10 antisorums. A study of these 21 strains showed that some sorums behaved as if they contained the same flagellar antibodies. Thus the 21 strains were grouped by sorums containing antibodies for seven flagellar antigen complexes. The 21 strains fell into four groups which were either not related at all or very slightly so. The largest, and antigenically the most complete of these groups was formed by 6 mutabile strains and 4 other strains. Absorption tests carried out with the strains of this group proved that mutabile strains and rapid lactose fermenting strains may contain identical flagellar antigens or may have very closely related flagellar antigens; that each of the two antisorums of this group contained antibodies for at least two antigenic components, one of which was common to all strains and another more specific; that antigens may have closely related but not identical flagellar antigens and be agglutinated by the antisorum to homologous titer; that strains from different

sources and isolated years apart may have identical or closely related flagellar antigens.

One group consisted of only one strain. Two other groups contained organisms from various sources which were agglutinated by the antiserums to varying titers, indicating that the different strains contained the flagellar antigens in different amounts together with other antigens for which the antiserums contained no antibodies.

A marked difference was found in the number of mutabile strains and rapid lactose fermenting strains agglutinating in the antiserums. Of the 11 mutabile strains 8 or 72.7% gave positive agglutination tests, while of the other 69 strains only 13 or 18.8% gave positive agglutination tests. Although this is a very small number of strains, it appears that there may be a tendency for the mutabile strains to contain flagellar antigens which are fewer in number and less diverse in composition than are those of the other E. coli.

Two E. coli mutabile strains were found which were very unstable with respect to motility. An all motile and agglutinable broth culture of 8 to 16 hours when used for an inoculum to a broth medium gave a culture containing approximately one-third very motile organisms that would not agglutinate with the homologous antiserum which had been made against an all motile culture. The instability of the motility of these strains in such a short time and the failure of some cultures to agglutinate makes necessary a study of flagella and motility in relation to agglutination of E. coli before any further work on the flagellar antigenic relationship is undertaken. The details of the observations made on this phenomenon are given in the appendix.

APPENDIX

Does Phase Variation with Respect to Motility Exist in the E. coli?

Flagellar agglutination has been used mainly in the study of E. typhosus and Salmonella group of organisms which are usually motile. There are both motile and non-motile E. coli strains and practically no work has been done on the motility and H antigens of these organisms. It has been assumed that a formalinized broth culture of a motile E. coli strain would be agglutinated by its homologous antibody in a manner corresponding to that of E. typhosus or any of the Salmonella strains. Topley and Wilson (1937) say of Salmonella, "A formolized broth culture of a flagellated species readily agglutinates in the presence of the homologous H agglutinins." Craigie (1931) in his study of the serological reactions of E. typhosus shook motile typhosus bacilli and found as the bacilli became denuded of flagella and the flagella underwent fragmentation, the amount of flagellar clumping in antiserum became less. After his strain had been in use for some time, it became less motile and a definite granular element appeared in the agglutination. Taylor (1941) in her study of flagellar antigens of E. coli found agglutination of strains showing varying amounts of motility. Gard (1937) and Gard and Brilliusen (1939) found in normal coliform non-specific flagellar antigen with no phase variation of the Salmonella type. Peluffo, Edwards, and Bruner (1942) have reported the presence of monophasic type of Salmonella flagellar antigen in slow lactose fermenters with Kwic reactions -+-+.

In this study it was assumed that formalinized broth cultures of E. coli with varying degrees of motility would show some agglutination with homologous antiserums. It was not until the straight agglutination tests had been completed that the question of motility in relation to agglutination arose.

The original antigen of the motobile strain 100 gave a typical flagellar agglutination with its homologous antiserum. Nine months later when the stock culture which had been inoculated from the culture used to make the original antigen was inoculated to infusion broth and incubated 15 to 17 hours at 37° C. no agglutination occurred with this same homologous antiserum. When the strain was passed from one broth to another, two each day for two days, using a series of infusion broth and another series of extract broth, neither of the final cultures agglutinated in the homologous antiserum. When a streak plate was made using an inoculum from one of the broths or from the stock culture and one colony picked to infusion broth which was incubated from 15 to 17 hours, there was no agglutination with the homologous antiserum, even though approximately one-third of the organisms appeared very motile.

Although no phase variation has been found, specific and non-specific H antigens have been reported in the coliform group. These findings suggested the possibility that the failure to agglutinate with the homologous antiserum might be due to phase variation. The following experiment was done with that in mind. An extract agar plate was streaked with an inoculum from the stock culture, incubated for 16 hours and 15 colonies were each transferred to a 2 c.c. volume of infusion broth. The broths were incubated for 6 hours and from these macroscopic agglutination tests were set up using the homologous antiserum at a dilution of 1:100. Three cultures gave a positive agglutination test and when hanging drop preparations were made for observing motility, every organism in the field of these cultures appeared motile. Hanging drop preparations of those cultures which gave no agglutination contained approximately one-third or less motile organisms. There did not appear to be any difference in the degree of motility of the individual motile organisms in the agglutinable and non-agglutinable cultures. The above results with slight variations

in the number of positive agglutinations were observed at least three times. In one experiment, when an agglutinable and all motile broth culture was used the same day it was tested to inoculate another broth which was incubated overnight, an agglutinable antigen resulted. This was tried several other times and could not be repeated. To obtain a satisfactory antigen from an inoculum of an all motile and agglutinable culture, it was necessary to make a streak plate and incubate overnight. Then 6 to 10 colonies were each transferred to a 20 to 30 c.c. volume of infusion broth which were incubated for 8 hours. Each culture was tested separately for motility and agglutination, and those found satisfactory were pooled for an antigen. To make certain the strain was not rough, a non-agglutinable culture showing approximately one-third motile organisms was inoculated to an agar slant. The inoculated agar was incubated overnight, the organisms were washed off with saline and heated. This showed no clumping of bacteria.

A portion of the stock culture became moldy and a new stock was made directly from a non-moldy portion of the stock culture. Four experiments were done in which the new stock was used for the inoculum. In each experiment 15 to 20 colonies were transferred from a 16 hour streak agar plate, each to a 2 c.c. volume of infusion broth; incubated for 6 to 8 hours and then tested for agglutination with the homologous antiserum. All were negative and no culture contained more than approximately one-third motile organisms.

Strain 100 absorption antigen was made from an inoculum containing approximately one-third motile organisms at the end of 6 hours incubation. This antigen absorbed antiserum but when diluted failed to agglutinate in any dilution of the homologous antiserum.

Strain 262B is a mutabile strain which contains the same flagellar antigens as strain 100. Until recently 262B gave an agglutinable culture

when inoculated directly from the stock culture to infusion broth. To secure an agglutinable antigen it became necessary to plate out the culture, pick colonies to broth and incubate. From these cultures the most motile culture was selected and used to make another streak plate and after the plate was incubated overnight 10 to 15 colonies were again transferred to broth and incubated for 6 to 8 hours after which each culture was tested for motility. This was continued until an all motile culture was obtained which agglutinated with the homologous antiserum. Direct inoculation of this culture to a broth media did not give an agglutinable antigen. The agglutinable culture was streaked on an extract agar plate and incubated overnight. Six to 10 of the colonies were each transferred to a 20 to 30 c.c. volume of extract broth and incubated overnight or to infusion broth and incubated for eight hours. Each culture was tested separately and most of them would agglutinate with the homologous antiserum and would contain all motile organisms.

When two mutabile cultures, 2623 and 100, were inoculated into broth and yielded a culture containing approximately one-third motile organisms, it would not agglutinate in the presence of the homologous antiserum which had been made against an all motile culture. When the proportion of motile organisms in these cultures is compared with the proportion found in many Salmonella cultures which give flagellar agglutination with homologous antiserum, there is no obvious difference. The fact that these two E. coli mutabile strains do not agglutinate unless every organism in the field appears motile very definitely raises the question as to whether this failure to agglutinate with an homologous antiserum is due to phase variation, to the amount and distribution of flagellar substances or to a combination of these factors. Also, is this a characteristic which can be found only in the mutabile type of E. coli.

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