A NEW ANTIGENIC RELATIONSHIP AMONG FAECAL BACILLI DUE TO A COMMON β ANTIGEN

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(With Plate 9)

In the course of a study of faecal bacilli a close antigenic relationship was noticed between a number of paracolon, *Escherichia coli* and *Shigella flexneri* Y strains. This affinity was found to be due to the presence of a common antigenic factor, named for convenience β antigen. Although this antigen showed some similarities to the L antigen of Kauffmann (1943) and the α antigen of Stamp & Stone (1944), the differences were conspicuous enough to warrant a closer investigation. This has revealed most interesting results in demonstrating the similarity of the somatic β antigen to both H and O antigens, and its ability to stimulate antibodies of a high titre in normal human and rabbit sera.

EXPERIMENTAL

Agglutination reactions with β antisera

The following strains were selected for the preparation of antisera: three paracolon, two *Esch. coli* and *Sh. flexneri* Y. Table 1 presents the biochemical and other characters of the paracolon and *Esch. coli* strains used in this study.

Antisera were prepared by immunizing rabbits with bacterial suspensions in saline, heated at 56° C. for 30 min. and preserved with 0·2 % formalin. All suspensions cross-agglutinated with the heterologous antisera to a high titre (1/12,000 to 1/100,000), the tests being carried out in a water-bath at 52° C. for 30 min. The noteworthy feature of these reactions was the rapid agglutination, which was practically complete in 5–30 min., the titre being only slightly raised on additional incubation at 52° C. overnight. The agglutination was abundant, resembled flagellar reactions and varied with strains from a light floccular type to a coarser semi-floccular form. This was interesting in view of the non-motile character of a number of strains used for the production of antisera, viz. *Sh. flexneri* Y and two of the paracolon bacilli, the latter remaining non-motile after numerous attempts to enhance motility.

In order to investigate the 'flagellar' character of the described reactions additional agglutination tests were performed with β strains and homologous β antisera. Table 2 shows the results with three representative organisms, namely, paracolon 40, *Esch. coli* 101 and *Sh. flexneri* Y.

Three kinds of suspension in saline were prepared from the same 24 hr. culture on nutrient agar and standardized to 1000 million organisms per ml. The β suspensions were made by heating at 56° C. for 30 min., the O (boiled) by boiling for 1 hr. and the O (alcohol) by heating in alcohol at 65° C. for 1 hr. To each suspension formalin was added to give final concentration of 0·2 %.

As mentioned previously, β suspensions (heated at 56° C. for 30 min.) gave a rapid abundant floccular or semi-floccular agglutination (Plate 9, *a*). Boiling for 1 hr. either completely inactivated the antigen or resulted in an atypical reaction requiring 1–2 hr. for development, the aggregate resembling a small clump of cotton-wool (Plate 9, *b*) and the titre being significantly lower than with β suspensions. It may be added that β suspensions boiled for 15 min only, as well as those boiled for 3 hr., produced a 'cotton-wool clump' type of reaction even after prolonged incubation at 52° C. Alcoholized suspensions gave negative results, or a granular somatic type of agglutinate was formed in 1–2 hr. (Plate 9, *c*), the titre being lower than with β suspensions.

Additional tests were performed with β, O (boiled) and O (alcohol) suspensions prepared from different subcultures of the selected strains, but using the same culture on nutrient agar for each set of suspensions. It was noticed that some β suspensions, especially those derived from less recently isolated strains, gave a granular, finer type of agglutinate and that the titre of reactions with O suspensions varied significantly within the same strain.

Further tests were made with β, O (boiled) and O (alcohol) suspensions of the selected strains against heterologous antisera. Table 2 shows a typical pro-

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Table 1. *Biochemical and other characters of paracolon and coliform β strains used for preparation of antisera.*

Strain	Fermentation of							Indol	Methyl red	Vosges-Proskauer	Citrate (Koser's medium)	Gelatin liquefaction	H_2S (Kligler's medium)
	Motility	Lactose	Mannite	Glucose	Sucrose	Maltose	Salicin						
Paracolon 30	−	−(30)*	AG	AG	−	AG	AG	+	+	−	−	−	−
Paracolon 40	−	−(30)	A	A	−	A	A	+	+	−	−	−	−
Paracolon 41	+	AG (4)	AG	AG	−	AG	AG	+	+	−	−	−	−
Esch. coli 101	+	AG	AG	AG	AG	AG	AG	+	+	−	−	−	−
Esch. coli 103	+	AG	AG	AG	AG	AG	AG	+	+	−	−	−	−

* Numbers in brackets indicate days of incubation.

Table 2. *Agglutination reactions of β and O suspensions with homologous and heterologous β antisera*

β antiserum from	Agglutination titre against suspension of								
	Paracolon 40			*Esch. coli* 101			*Sh. flexneri* Y		
	β	O (boiled)	O (alcohol)	β	O (boiled)	O (alcohol)	β	O (boiled)	O (alcohol)
Paracolon 40	100,000	Negative to 800	Negative to 3200	12,000	Negative to 400	Negative to 640	50,000	Negative to 100	Negative to 400
Esch. coli 101	12,000	Negative to 200	Negative to 800	12,000	200 to 1600	Negative to 3200	12,000	Negative to 160	200 to 800
Sh. flexneri Y	50,000	Negative	Negative to 400	10,000	200	Negative to 800	50,000	200 to 1280	200 to 1280

tocol of the results of cross-agglutination reactions with the three representative organisms, paracolon 40, *Esch. coli* 101 and *Sh. flexneri* Y. On the basis of numerous tests the following conclusions were reached: (1) β antigen is thermolabile and is largely destroyed after boiling for 1 hr., but a more resistant component is occasionally present. (2) β antigen is predominantly a surface antigen as indicated by the rapidity of β agglutination and rapid antibody response to immunization in which it resembles H antigen; β antigen may, however, resemble the O antigen, since in some strains stablity in warm alcohol was demonstrated. (3) Cross-agglutination reactions between paracolon, *Esch. coli* and *Sh. flexneri* Y strains, as shown in Table 2, are apparently due to β antigen. (4) β antigen masks the formation of O antibodies as demonstrated by negative or low-titre reactions of O suspensions with the homologous β antisera after overnight incubation at 52° C.

Some other characters of β antigen were investigated. β suspensions heated at 60° C. for 1 hr. were less sensitive to agglutination with β antisera than those heated at 56° C. as judged by the slower rate of reaction, while β suspensions heated at 75° and 85° C. for 1 hr. gave not only slower reactions but also a lower agglutination titre. These findings indicated that β agglutination is progressively diminished when β suspensions are heated above 60° C.

Formalin preserves β antigen. Formolized and subsequently boiled β cultures retained their β antigen, but its sensitivity was slightly diminished.

Agglutination reactions with somatic antisera of β strains

In order to produce somatic antisera from β forms rabbits were immunized with boiled suspensions of paracolon 30, paracolon 40, *Esch. coli* 101 and *Sh. flexneri* Y. Agglutination tests with the above sera were performed at 52° C., allowing overnight incubation and using β and O (boiled) suspensions of the selected organisms.

Results presented in Table 3 are of interest in showing that: (1) β suspensions give no or slight agglutination with the heterologous O antisera, thus confirming the previous finding, that cross-agglutinations shown in Table 2 were due to reactions with β antibodies. (2) β suspensions may be agglutinated by the homologous O antiserum, giving usually an incomplete O type of agglutination due to the presence of O agglutinogen in the suspensions. However, in one case (paracolon 30) the presence of β antigen was apparently responsible for O inagglutinability.

Agglutinin-absorption tests

A number of agglutinin-absorption tests were performed with β antisera of paracolon 30, paracolon 40, *Esch. coli* 101 and *Sh. flexneri* Y, using for absorption β and O (boiled) thick suspensions of organisms and the 'double absorption' method.

Table 4 shows the results of one representative set of experiments with the β antiserum from *Esch. coli* 101 strain, similar results being obtained with other sera. It will be seen that, in general, absorption of β antiserum with β suspensions of heterologous organisms removed common agglutinins for all β strains, whereas absorption with O suspensions had no effect. This establishes the distinctiveness of the β and O antigens. Furthermore, O suspensions gave negative or low-titre agglutinations with homologous β antiserum absorbed with heterologous β suspensions, confiming the previous finding (Table 2) that β antigen masks the development of O antibodies. It is fair to conclude that β antigen is probably more superficially placed in the bacterial cell than O antigen.

Thus β antibody is best prepared with a non-motile β strain and absorbed with the homologous O (boiled) suspension. In practice, however, unabsorbed β antisera of the non-motile bacilli proved to be reliable for detection of β strains, since β agglutination is rapid and of a high titre. H antigen of motile strains did not interfere with β reactions.

Occurrence of β antigen in freshly isolated coliform and paracolon strains

Faecal specimens from cases of gastro-enteritis and from normal individuals were plated out on MacConkey, plain desoxycholate agar and 'SS' medium. Colonies were tested for β antigen by slide agglutination and tube agglutination with broth cultures of organisms isolated from the plates. An examination was made of 704 colonies comprising 596 coliform and 108 paracolon bacilli from 119 faecal specimens. β antigen was detected in 103 out of 596 coliforms (17 %) and in 12 out of 108 paracolon strains (11 %). β forms were isolated from faecal specimens of normal individuals and from cases of gastro-enteritis as well as from faeces of rabbits and cats. There did not appear to be any increase of β forms in pathological specimens when compared with normal samples.

Occurrence of β antigen in stock cultures of faecal bacilli

A number of stock cultures of faecal bacilli previously maintained on agar slopes under paraffin oil for at least 2 years were tested for the presence of β antigen.

Table 5 shows that 10 out of 110 paracolon bacilli and 3 out of 50 *Proteus* strains contained β antigen. This antigen was absent in 65 *Salmonella* cultures of different strains and present in 3 out of 22

Table 3. *Agglutination reactions of β and O suspensions with O antisera*

	Agglutination titre against suspension of							
	Paracolon 30		Paracolon 40		*Esch. coli* 101		*Sh. flexneri* Y	
O antiserum from	β	O	β	O	β	O	β	O
Paracolon 30	—	2,500	—	—	—	—	—	—
Paracolon 40	—	40	2,500	2,500	—	—	—	—
Esch. coli 101	—	—	—	40	2,500	10,000	—	—
Sh. flexneri Y	—	—	—	—	—	—	1,280	2,500

Table 4. *Agglutination reactions of β and O suspensions with unabsorbed and absorbed antiserum of* Esch. coli 101

β antiserum from *Esch. coli* 101	Agglutination titre against β suspension of				O (boiled) suspension of *Esch. coli* 101
	Esch. coli 101	Paracolon 30	Paracolon 40	*Sh. flexneri* Y	
Unabsorbed	12,000	6,000	12,000	12,000	400
Absorbed with β suspension of:					
Esch. coli	—	—	—	—	—
Paracolon 30	200	—	100	—	200
Paracolon 40	—	—	—	—	200
Sh. flexneri Y	400	—	—	—	400
Absorbed with O (boiled) suspension of *Esch. coli* 101	12,000	6,000	6,000	12,000	—

Table 5. *Occurrence of β antigen in stock cultures of faecal bacilli*

	Paracolon bacilli		*Proteus*		*Shigella*		*Salmonella*	
β antigen	No. of cultures	%	No. of cultures	%	No. of cultures	%	No. of cultures	%
Present	10	9	3	6	3	14	0	0
Absent	100	91	47	94	19	86	65	100

Table 6. *Incidence of β antibodies in* 100 *normal sera*

No. of sera non-agglutinating	No. of sera agglutinating	No. of sera agglutinating at titre of							
		1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
29	71	11	13	13	12	7	11	3	1

Table 7. *Agglutination of β suspensions from four paracolon strains and* Sh. flexneri Y *by normal human and rabbit sera*

			Sera											
					Percentage agglutinating at titre									
β suspension from	Source	No.	Percentage non-agglutinating	Percentage agglutinating	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
Paracolon sp. (4 strains)	Human	25	11	89	17	21	19	14	5	5	6	1	1	0
Sh. flexneri Y			0	100	0	20	20	24	12	8	4	4	4	4
Paracolon sp. (4 strains)	Rabbit	28	64	36	15	13	4	0	2	0	2	—	—	—
Sh. flexneri Y			0	100	4	25	21	32	14	0	4	—	—	—

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Shigella strains, viz. in *Sh. flexneri* Y, *Sh. flexneri* V 1 and D 19. The agglutination with suspensions from stock cultures was occasionally floccular, but more often a granular type of reaction was encountered, especially with *Proteus* strains.

Occurrence of β antibodies in normal human and rabbit sera

Considering the relatively frequent occurrence of β forms of coliform and paracolon bacilli isolated from stools of normal individuals, it was thought probable that circulating β antibodies may be found in blood. To this end 100 sera from normal human adults were investigated, using a sensitive β suspension of paracolon 40 strain, which gave a floccular type of agglutination.

As shown in Table 6 a survey of 100 blood specimens justified this supposition, for 71 sera showed agglutination. Of these sera 49 had a titre 1/80 or lower, while 22 had a higher titre, between 1/160 and 1/1280 after 1 hr. at 52° C. It was proved that the agglutination was due to β antibodies, because the sera were negative when tested with the same organism devoid of β antigen.

When β antibodies had been demonstrated in normal human sera, another survey was conducted with additional samples of human and rabbit sera in order to compare β antibody level using β suspensions of 4 paracolon strains and *Sh. flexneri* Y.

Table 7 shows that β antibodies were encountered in rabbit as well as in human sera, but more frequently in the latter. The titres with the 4 β paracolon strains were similar, reaching 1/2560 while the β suspensions of *Sh. flexneri* Y proved to be a more sensitive antigen giving some agglutination with all sera and a titre of 1/5120 in 4 out of the 25 human sera. A number of control tests were made with *Sh. flexneri* Y, Oxford suspension devoid of β antigen, with results similar to those obtained with our *Sh. flexneri* Y strain which did not contain β antigen. The titres in each case were 1/10 to 1/80.

These observations indicate that care must be taken in evaluation of antibody response in human and rabbit sera, as some reactions may be due to the presence of natural antibodies, such as β antibody, resulting from antigenic organisms comprising the normal flora of the alimentary tract.

Morphology of β strains

A number of β cultures showed on plating out a distinct dissociation into opaque and semi-transparent colonies. Suspensions prepared from these colonies exhibited a variability in agglutination reactions towards β antisera, the suspensions from opaque colonies giving the typical floccular type of agglutination, while agglutinations with the semi-transparent colonies were atypical, scanty and of a granular character or negative. The colonies giving negative or atypical agglutination were regarded as β minus. Broth cultures of β forms showed a greater opacity than β minus forms of the same strains as demonstrated by the photoelectric colorimeter, for example, paracolon 40, β culture gave 65·5 units of galvanometer deflexion, while β minus gave 69·9 units. *Esch. coli* 101 gave for β and β minus forms readings of 57 and 66 units respectively. A control with sterile broth gave 100 units.

It was thought that the presence of β antigen in the bacterial cell might be connected with the formation of a capsule. In order to investigate this point β and β minus bacilli were stained by Lawson's, Anthony's and indian ink methods; no distinct and constant difference could be observed with regard to capsulation between the two forms of any one strain. This indicated that the presence of β antigen is not connected with the formation of a capsule.

Dissociation of β strains

Recently isolated strains exhibited a great and unpredictable variability in β antigenic structure. Some colonies on MacConkey medium lost their β antigen after 2–3 days at room temperature without subculture, while other colonies preserved it for 2–3 weeks under the same conditions. Numerous contradictory observations were made with regard to the presence of β antigen in subcultures. Some colonies which were recorded as devoid of β antigen on primary isolation, produced β variants when transferred to broth and vice versa. Some strains retained β antigen through successive subcultures in broth, while others showed a prompt loss of it or contained only a fraction of β antigen as demonstrated by a poor, granular character of agglutination with a 'trailing off' indistinct end-point. Examination of β paracolon strains stored for 1 year on Dorset's egg medium and on agar slopes under paraffin oil showed that β antigen was better preserved on the first medium.

This tendency toward irregular dissociation without reference to environmental changes raised peculiar technical difficulties in studying the β antigen. In an attempt to induce dissociation under controlled conditions β strains were grown on phenol agar, but β antigen was not inhibited on this medium. Success was achieved, however, by cultivation of β strains in broth media, containing a heterologous β antiserum in 1/200 dilution. Two strains (paracolon 30 and *Esch. coli* 101) were used in these experiments; the fifth successive subculture yielded β minus forms.

The next step was an attempt to restore β antigen to β minus forms. Because β antigen showed most characteristics of a surface constituent it was decided

to apply the method of Crossley, Ferguson & Brydson (1946), who suggested cultivation of rough forms of bacteria on soluble starch media to restore those organisms to smooth types. However, successive subcultures on starch media did not achieve restoration of β antigen.

Virulence of β strains

As the observations made by several workers indicated that some somatic thermolabile antigens might be associated with virulence of the organisms, for example Vi antigen (in *Salmonella typhi*) of Felix & Pitt (1934*a*, *b*), L antigen of Kauffmann (1943), toneally into mice which were then observed for a period of 14 days.

As demonstrated in Table 8, β forms showed a significantly higher virulence to mice than β minus forms as judged by the mortality rate of inoculated animals. Septicaemia was usually observed within 1–3 days, and the causal organism was recovered post-mortem from heart, spleen and liver.

To test the endotoxic nature of the β antigen broth cultures of β forms were prepared as described above. Each culture was divided into two; one part was used for inoculation of mice, while the other one was heated at 56° C. for 1 hr. and tested for sterility prior

Table 8. *Virulence of β and β minus forms of paracolon and coliform bacilli*

	Mice*				Mice*		
β strains	No. inoculated	No. of deaths	Mortality (%)	β minus strains	No. inoculated	No. of deaths	Mortality (%)
Paracolon 30	20	11	55	Paracolon 30	20	1	5
Paracolon 40	40	15	38	Paracolon 40	40	2	5
Esch. coli 214	25	20	80	*Esch. coli* 214	25	7	28
Esch. coli 214*a*	40	29	73	*Esch. coli* 214*a*	40	11	28
Esch. coli 215	16	12	75	*Esch. coli* 215	16	5	31
Total	141	87	62	Total	141	26	18

* Observed for a period of 14 days.

Table 9. *Mortailty per batch of ten mice inoculated with corresponding living and killed β strains*

Living cultures	Mice* No. of deaths	Killed cultures	Mice* No. of deaths
Paracolon 30	5	Paracolon 30	0
Paracolon 40	2	Paracolon 40	0
Esch. coli 215	9	*Esch. coli* 215	0

* Observed for a period of 14 days.

it was decided to test the virulence of some β strains used in this study.

In this investigation paracolon 30 and 40, and more recently isolated strains of *Esch. coli* type I (strains 214, 214*a* and 215), were tested with regard to their virulence for mice, using β and β minus forms of each particular strain. The β and β minus cultures used for inoculation of mice were obtained under controlled conditions by seeding a 10 ml. broth medium with a drop of an 18 hr. broth culture of the corresponding organism, using a calibrated Pasteur pipette. The broth cultures were incubated at 37° C. for 18 hr., standardized to 1000 million organisms per ml. opacity and tested for β agglutination. Doses of 0·2 ml. of culture were administered intraperito inoculation. The living and killed cultures were administered intraperitoneally into mice in 0·2 ml. doses and the animals were under observation for 14 days. The results presented in Table 9 indicate that pathogenicity was associated with the living cultures.

Comparison of the virulence of paracolon and coliform β strains used in this study with unrelated strains devoid of β antigen, which were tested in the course of another investigation, showed that the latter were occasionally more virulent. Hence the assumption that the mere presence of β antigen does not indicate that the organism is more virulent than another strain lacking this antigen. But from the results in Table 8 it can be predicted that the loss of

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β antigen will lower the virulence of that particular strain.

A few experiments were carried out to test the necrotizing ability of β forms by administering intradermally into rabbits doses of 0·05 ml. of 18 hr. broth cultures. The results were not consistent. Occasionally pustules were formed and the organism was recovered from pus but true necrosis was not observed.

Relationship of β antigen to Vi and α antigen

The possibility of serological relationship to Vi (Felix & Pitt, 1934*a*, *b*) and α antigen (Stamp & Stone, 1944) was considered. Several α cultures (*Bact. wakefield*, 1, 2 and 3, *Proteus morgani*, strains Fergusson, Fairbridge and 3915) and α antiserum were obtained by courtesy of Dr H. J. Bensted, Director of Central Public Health Laboratory, London. In addition α antisera were prepared with strains 3915 and *Bact. wakefield* 1. Reciprocal cross-agglutination and agglutinin-absorption tests proved dissimilarity of β, Vi and α antigens. Furthermore, Dr Bensted kindly examined our representative β strains, paracolon 30 and 40, and also β antiserum, and confirmed our results by personal communications, that our cultures possess 'labile superficial antigens different from Stamp & Stone's'.

DISCUSSION

Numerous records are found in literature regarding the great complexity of antigenic structure in coliform and paracolon bacilli. However, various workers demonstrated several hitherto unrecognized antigens, thus contributing to the understanding of this problem. Cruickshank (1939) described a thermostable X antigen, reported first by Topley & Ayrton (1924). In 1943, Kauffmann demonstrated a new thermolabile antigen, named by him L, while Stamp & Stone (1944) gave an account of α antigen found in motile and non-motile lactose and non-lactose-fermenting coliform bacilli but distinct from H and O antigens. Knipschildt (1946) described a new thermolabile antigen B, appearing in three forms. A number of workers studied capsular substances in coliform bacilli, for example, Julianelle (1937) examined the antigenic structure of *Bact. aerogenes*, while Knipschildt (1945) demonstrated a thermostable capsular antigen A, probably similar to the one referred to earlier by Smith & Bryant (1927) and Smith (1928).

The β antigen described in this work is distinct from other known antigens and a brief comparison of a few outstanding features will show the dissimilarity. The characteristic points of the β antigen are as follows: (1) It is present in motile and non-motile strains of some coliform, paracolon, *Proteus* and also in *Shigella* bacilli, but was not detected in the examined *Salmonella* types. (2) With freshly isolated β strains a rapid floccular or semi-floccular type of agglutination is produced in 5–20 min. at 52° C. (3) It is not destroyed at 60, 75 or 85° C. for 1 hr. but with rising temperatures progressive damage follows, so that after exposure to 100° C. it is inactivated or it cannot be demonstrated in its typical form. Occasionally after this treatment at 100° C. an atypical 'cotton-wool clump' type of agglutination is observed. (4) It is inactivated by warm alcohol or it gives a granular somatic kind of agglutination. (5) The presence of β antigen interferes with the production of O antibodies. (6) A living or formolized β culture usually reacts with its homologous O antiserum on slide or in tube, but occasionally agglutination may be impaired. (7) Boiling destroys the antibody-binding property of β antigen. (8) Presence of β antigen in the bacterial cell is not connected with the formation of a capsule. Two other noticeable features are the frequent occurrence of β antibodies in normal human and rabbit sera and a higher virulence to mice of β than β minus forms of the same strain.

In comparing β with other known antigens only a few characteristics are quoted, sufficient to indicate dissimilarity. Presence of β factor in non-motile strains excludes it from the H group of antigens. Thermolability at 100° C. distinguishes it from O and X antigens; this property makes it also different from Knipschildt's A capsular antigen. Lack of cross-agglutination with Vi antiserum and the rapidity of β agglutination shows that β and Vi antigens are not related.

A few more somatic antigens of the thermolabile group remain to be discussed. The L antigen of Kauffmann differs from β antigen in a number of points, for example, in production of low-titre antisera with L antigen in contrast to high-titre antisera with β antigen; in granular agglutination reactions in contrast to floccular β agglutination: in more frequent O inagglutinability of the living L cultures; in the optimum temperature of 37° C. for L agglutination as against 52° C. for β agglutination. The α antigen of Stamp & Stone proved to be serologically distinct from β antigen; the lack of α antibodies in normal human sera contrasts with the frequent occurrence of β antibodies. The β antigen of Knipschildt is distinct in its antibody-binding property after boiling. On these grounds there seems little doubt that the β antigen is a hitherto undescribed factor found in a number of faecal bacilli.

The interesting point about β antigen is its similarity to both H and O antigens. A certain irregularity in agglutination reactions may be due either to the position of β antigen in the bacterial

cell (viz. on the surface or deeper) or to the complexity of structure of β antigen which may contain several factors. It should be noted that in the L group of antigens Kauffmann (1944) has recognized seventeen serologically different types and in a later survey (1947) he mentioned seven additional L antigens, found by Knipschildt.

The occurrence of β forms in coliform, paracolon, *Proteus* and *Shigella* strains leads to the possibility of committing errors in bacteriological diagnoses, for example, a β anaerogenic paracolon bacillus may cross-agglutinate with some *Shigella* antisera if these contain β antibodies. A precedent was created with regard to *Bact. wakefield* (Berger, 1945), which was included in the dysentery group of organisms on the basis of its antigenic relationship to *Sh. flexneri* X and Y and to Boyd's type P 119, the common factor proving to be α antigen (Bridges & Taylor, 1946). Similarly, some forms of *Sh. flexneri* Y, *Sh. flexneri* V 1 and D 19 were shown to be serologically allied to certain paracolon and coliform bacilli by sharing the common β antigen. Further, in the interpretation of serological reactions with patients' sera one cannot neglect the frequent occurrence of β antibodies, capable of agglutinating formalized *Shigella* suspensions which may contain β antigen. For these reasons the problem of β antigen should not be overlooked in the field of diagnostic bacteriology and serology.

SUMMARY

1. The presence of a new thermolabile an named β antigen has been demonstrated in motile and non-motile coliform, paracolon *Proteus* strains, and in *Shigella* bacilli (*Sh. flexne Sh. flexneri* V 1 and D 19). This antigen ca frequently found in the above types.

2. The characteristics of the β antigen have described, and its dissimilarity to H, O, Vi, A, B and α antigens has been established.

3. A high incidence of β antibodies was de strated in normal human and rabbit sera.

4. β strains of paracolon and coliform b injected intraperitoneally into mice were show be more virulent to mice than β minus forms o same strains.

5. It is suggested that unrecognized presen β antigen in cultures and β antibody in human rabbit sera may cause errors in bacteriological serological diagnoses of enteric infections.

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a *b* *c* *d*

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EXPLANATION OF PLATE 9

Plate 9. Types of agglutination reactions of β and O suspensions with β antisera. Organism: paracolon 40. Showing agglutination with (*a*) β suspension, heated at 56° C. for 30 min.; (*b*) O (boiled) suspension; (*c*) O (alcoholized) suspension; (*d*) β suspension with no antiserum.

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