AN AGGLUTINOGEN COMMON TO CERTAIN STRAINS OF LACTOSE AND NON-LACTOSE-FERMENTING COLIFORM BACILLI

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The experiments recorded in the present paper are based on certain observations made during the course of an investigation into an outbreak of paratyphoid fever.

It was found that the diagnostic serum employed for the identification of colonies of *Bact. paratyphosum* B produced a marked degree of agglutination on the slide with certain non-lactose-fermenting colonies isolated from faeces, which on further investigation proved not to belong to the Salmonella group. These were only very occasionally encountered and were found to be either *Proteus morgani* or paracolon bacilli. On examining lactose-fermenting colonies, a somewhat greater proportion reacted in the same way. Agglutination tests carried out in the water-bath with heat-killed broth cultures of these strains gave titres of from 1 in 200 to 1 in 400, whereas the serum used had a specific para B, 'H', titre of 1 in 6400 and a group Salmonella titre of less than 1 in 50. No agglutination occurred with alcoholized 'O' suspensions.

Tests with other diagnostic sera showed that the agglutinin responsible was not confined to *paratyphosum* B antisera. Certain agglutinating sera against *Bact. typhi-murium*, *Bact. typhosum*, *Bact. paratyphosum* C and even *Bact. flexneri* and *Br. melitensis* were also found to agglutinate the same organisms. The titres obtained in the water-bath against heat-killed broth cultures ranged from 1 in 50 to 1 in 800, and, as with the *paratyphosum* B antiserum, no agglutination occurred against alcoholized 'O' suspensions.

The reaction, therefore, appeared to be non-specific and to be due possibly to the presence of naturally occurring agglutinins in the rabbit sera, rather than to any antigenic relationship between the group of organisms agglutinated and those from which the antisera had been prepared. That this was the case was also suggested by the result of absorption tests. It was found that the non-specific agglutinin was completely absorbed out by any of the group strains but not by the organism from which the serum had been prepared. Conversely, the specific agglutinin was removed only by absorption with the homologous organism.

These results would have suggested that the non-specific agglutination was due to the presence of a common flagellar antigen in the reacting strains but for the fact that, though it developed fairly rapidly, it was fine and granular in type. Moreover, a characteristic of all the strains was their deficient motility, many being completely non-motile under the most varied conditions. It was decided to investigate further the nature of the antigen concerned and the results obtained are set out in the present paper.

EXPERIMENTS

A few strains were selected for detailed study and their biochemical and other characteristics are set out in Table 1. For convenience they are hereafter referred to as α strains.

Preparation of agglutinating antisera

Antisera were prepared against each of these strains by inoculation of rabbits with suspensions washed off 24 hr. agar slopes and heated to 56° C. for 1 hr. Doses of 250 million, 500 million and 1000 million were given intravenously at weekly intervals and the animals bled out 1 week after the last dose. Before immunization, the sera of the rabbits were examined for the presence of agglutinins against each of the strains under investigation.

Table 1. *Biochemical and other characteristics of strains selected for investigation*

	Fermentation of						Indol production	Vosges-Proskauer reaction	Methyl-red reaction	Growth in Koser's citrate	Motility	Haemolysis on rabbit blood plates
	Glucose	Maltose	Mannitol	Lactose	Sucrose	Salicin						
Bact. coli, 1044	AG	AG	AG	AG	AG	0	−	−	+	−	−	−
Bact. coli, 1055	AG	AG	AG	AG	0	AG	+	−	+	−	±	−
Intermediate, type 1, 1818	AG	AG	AG	AG	AG	AG	−	−	+	+	±	−
Bact. aerogenes, 1084	AG	AG	AG	AG	AG	AG	−	+	−	+	±	−
Bact. aerogenes, 1087	AG	AG	AG	AG	AG	AG	−	+	−	+	−	−
Proteus morgani, 1721	AG	0	0	0	0	0	−	−	+	−	−	−

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Table 2. *Agglutination of α strains by corresponding antisera, employing saline suspensions heat-killed at* 56° C.

Anti-sera	Agglutination titre against suspensions of 1044	1055	1818	1084	1087	1721
1044	12800	6400	6400	6400	12800	12800
1055	6400	3200	12800	3200	12800	12800
1818	6400	3200	3200	3200	3200	6400
1084	25600	12800	25600	25600	12800	25600
1087	3200	1600	3200	3200	3200	3200
1721	6400	3200	3200	3200	3200	6400

Table 2 shows the agglutination produced in the water-bath by these sera with saline suspensions, washed off agar plates grown at 37° C. for 24 hr., and killed by heating at 56° C. for 1 hr. It will be seen that cross-agglutination occurred with all strains, in nearly all cases to full titre. Agglutination was finely granular in most cases, the particles tending to stick to the sides of the lower half-inch of the Dreyer tubes. With one or two suspensions it was semi-floccular, particularly with the homologous antiserum. It was complete in 2–4 hr.

The tests were repeated with broth cultures, grown at 22° C., formolized and heated to 55° C. for 20 min. Similar results were obtained with these 'H' suspensions, except that agglutination, with the homologous antisera, of one or two strains which had shown evidence of motility was more definitely floccular. 'O' suspensions, prepared by washing off dry agar-plate cultures with alcohol, heating at 65° C. for 1½ hr., washing and resuspending in saline, were also tested against the same sera. The results are shown in Table 3.

Table 3. *Agglutination of α strains by corresponding antisera employing alcoholized 'O' suspensions*

Anti-sera	Agglutination titre against 'O' suspensions of 1044	1055	1818	1084	1087	1721
1044	800	400	—	—	—	—
1055	800	800	—	—	—	—
1818	—	—	1600	—	—	—
1084	—	—	—	—	800	—
1087	—	—	—	200	200	—
1721	—	—	—	—	—	800

It will be seen that the common antigen was destroyed by treatment with alcohol. Agglutination of an 'O' type occurred with the homologous antiserum and also to a limited degree with heterologous sera, indicating that certain strains possessed a common 'O' antigen. With one strain, 1084, no agglutination developed with the homologous antiserum. Possible reasons for this will be discussed later.

Heat-lability of the common α antigen

Suspensions of α strains in broth and saline were prepared and heated to different temperatures for varying periods of time. It was found that the antigen was not inactivated by a temperature of 75° C. for 1 hr., but that inactivation was complete at a temperature of 95° C. for 1 hr. or 100° C. for 15 min. Alcoholized suspensions heated to 60° C. for 1 hr. still gave a trace of agglutination, but at 65° C. for 1 hr. were completely inactivated. When 'O' suspensions were prepared by the acid inactivation method described by Duncan (1935), the α agglutination was unimpaired.

These findings afforded additional support for the view already formed that α antigen was not identical with either 'H' or 'O' antigen.

Reciprocal absorption experiments

Four of these sera having no 'O' agglutinins in common were selected for absorption tests. Thick suspensions of the corresponding strains were killed by heat at 56° C. and used to absorb each of the sera in turn. The absorbed sera were tested against formolized broth cultures of each of the strains and also against 'O' suspensions of the homologous strain. The results are shown in Table 4.

It will be seen that the absorbed sera failed to agglutinate any of the 'H' suspensions of heterologous strains to an appreciable extent, indicating that reciprocal absorption of the non-specific agglutinin was complete. It will be noted also that sera absorbed with heterologous strains also failed to agglutinate even the homologous 'H' suspension in most cases. This result could hardly be attributed to complete lack of specific 'H' antigen in the suspension or of 'H' agglutinin in the serum, since some of the strains showed definite evidence of motility, and in one serum, at least, 'H' agglutination had been demonstrated after absorption with a homologous 'O' suspension. Moreover, 'O' agglutination might have been expected to occur in the absence of 'H' agglutination, since, as shown in the table, these absorbed sera agglutinated the homologous 'O' suspension to practically full titre. These results were confirmed when the tests were repeated with non-formolized, heat-killed broth cultures. It seemed, therefore, that some factor was present which was inhibiting 'O' agglutination and probably, in some cases, 'H' agglutination also. Further instances of this phenomenon will be described later.

The effect of repeated subculture on the development of α antigen. Production of specific variants

Attempts were made to improve the motility of some of the strains under investigation, in order, if possible, to distinguish better between α and specific 'H' agglutination. The strains were subcultured daily on semi-solid nutrient agar (0·5 % agar), subcultures being made from the edge of the growth. After several subcultures one or two of the strains showed evidence of swarming and their motility appeared to be definitely increased. Others, after as many as twenty subcultures, showed no change in these respects. At intervals during the subculturing, the strains were grown in heart broth and their agglutination reactions tested against homologous and heterologous antisera. It was found, as will be seen in Table 5, that, after a variable number of subcultures, the majority of strains failed to agglutinate with any but the homologous antisera. Strain 1055 was rendered specific after two subcultures only, 1818 after three, 1084 after eleven, while 1087 and 1721 remained non-specific after twenty subcultures. This development of specific variants was found to be independent of increase in motility and improvement in 'H' agglutination, since several of the strains rendered specific showed no definite change in these respects and agglutination remained fine and granular in type, even with the homologous antiserum. Moreover, it was found that some of

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Table 4. *Cross-absorption tests between α antisera and corresponding α strains*

Antisera	Agglutination titre against: Formolized heat-killed broth cultures of 1055	1087	1721	1818	'O' suspension of homologous organism
1055 unabsorbed	6400	3200	3200	6400	800
1055 absorbed strain 1055	—	—	—	—	—
1055 absorbed strain 1087	800	—	—	—	1600
1055 absorbed strain 1721	—	—	—	—	800
1055 absorbed strain 1818	200	—	100	—	800
1087 unabsorbed	3200	1600	1600	3200	200
1087 absorbed strain 1055	—	—	—	—	200
1087 absorbed strain 1087	—	—	—	—	—
1087 absorbed strain 1721	—	—	—	—	100
1087 absorbed strain 1818	—	100	—	—	200
1721 unabsorbed	6400	6400	6400	6400	1600
1721 absorbed strain 1055	—	—	—	—	800
1721 absorbed strain 1087	200	—	—	100	800
1721 absorbed strain 1721	—	—	—	—	—
1721 absorbed strain 1818	100	—	—	—	800
1818 unabsorbed	6400	3200	6400	6400	1600
1818 absorbed strain 1055	—	—	—	100	800
1818 absorbed strain 1087	—	—	—	—	800
1818 absorbed strain 1721	200	—	100	—	800
818 absorbed strain 1818	100	—	—	100	—

Table 5. *Agglutination of specific variants by non-specific α sera*

Anti-sera	Agglutination titre against: (*a*) broth cultures heat-killed at 56° C., (*b*) alcoholized 'O' suspensions of 1055 *a*	1055 *b*	1087 *a*	1087 *b*	1721 *a*	1721 *b*	1818 *a*	1818 *b*
1055	6400	1600	—	—	—	—	—	—
1087	—	—	200	200	—	—	—	—
1721	—	—	—	—	800	1600	—	—
1818	—	—	—	—	—	—	800	800

the strains became specific when subcultured a number of times on nutrient-agar medium, a procedure which would not be expected to lead to an increase in motility.

On studying further the factors tending to produce specific variants, it was found that successive subcultures on McConkey's medium or in heart broth usually had no such effect; in many cases the non-specificity appeared to be increased by these means. When, however, agglutinating serum prepared from a heterologous strain was added to heart broth in a dilution of 1 in 250, and α strains were repeatedly subcultured in it, they usually readily became specific. Strain 1721 was made specific by this means after three subcultures only, though the other methods described had failed.

It will be seen that individual strains varied greatly in the ease with which specific variants were obtained. In general it was found that lactose-fermenting α strains tended to retain their non-specificity during prolonged subculture. Non-lactose-fermenting α organisms, on the other hand, apart from 1721, nearly always changed to the specific form after one or two subcultures and therefore could not be included in the investigation.

It was noted that agglutination of strains in the specific and non-specific phases was clean-cut, but that there existed an intervening stage in which a muddy type of agglutination occurred. When cultures in this stage were plated out, they were found to consist of a

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mixture of colonies, either entirely in the specific or in the non-specific phase, which bred true to type on further subculturing. There was, therefore, no evidence of any diphasic variation similar to that found in 'H' antigens of certain Salmonella strains.

Preparation of specific sera

It was of interest to compare the agglutinins produced by immunization with these specific variants with those in antisera to the corresponding α strains. Rabbits were therefore immunized with four selected specific strains, using the same technique as before. These specific sera were then tested against heat-killed broth cultures and also against alcoholized 'O' suspensions of the same strains in their specific and non-specific phases. The results are shown in Table 6.

Table 6. *Agglutination of α strains and their specific variants by the corresponding specific antisera*

	Agglutination titre against α strains							
	Broth cultures heat-killed at 56° C.				Alcoholized 'O' suspensions			
Anti-sera	1055	1084	1721	1818	1055	1084	1721	1818
1055	—	—	—	—	—	—	—	—
1084	—	50	—	—	—	800	—	—
1721	—	—	—	—	—	—	800	—
1818	—	—	—	800	—	—	—	1600

	Agglutination titre against specific variants							
	Broth cultures heat-killed at 56° C.				Alcoholized 'O' suspensions			
	1055	1084	1721	1818	1055	1084	1721	1818
1055	25,600	—	—	—	6400	—	—	—
1084	—	6400	—	—	—	800	—	—
1721	—	—	3200	—	—	—	1600	—
1818	—	—	—	25,600	—	—	—	3200

It was found that in the dilutions employed—from 1 in 50 upwards—cross-agglutination never occurred with broth cultures of heterologous α strains, indicating the complete absence of α antibodies in the specific sera. With broth cultures of homologous α strains, agglutination also failed to develop in most cases. This inhibition of 'O' and in one instance of 'H' agglutination in the presence of α antigen had been previously noted (Tables 3 and 4).

'O' suspensions of both specific and non-specific strains, prepared by heating in alcohol to 65° C. for 1½ hr., gave agglutination with the homologous antisera, in most cases to full titre. In one instance, however, strain 1055, inhibition of 'O' agglutination persisted even after the suspension was heated to 100° C. for an hour. Broth cultures of specific homologous strains were agglutinated to somewhat higher titres than alcoholized 'O' suspensions, probably due to their greater sensitivity. In type, however, agglutination still remained granular.

These results suggested that the presence of α antigen in a suspension exerted an inhibitory effect on the sensitivity of the 'O' agglutinogen to the corresponding antibody. Thus specific sera failed to give 'O' agglutination with heat-killed broth cultures of α strains, whereas with 'O' suspensions, in which the α antigen had been inactivated by treatment with hot alcohol or by a temperature of 100° C. for 30 min., agglutination generally occurred to full titre.

These findings were confirmed by a further experiment. When non-specific sera were tested against heat-killed broth cultures of the homologous α strain, α agglutination masked any 'O' agglutination which might have occurred. If, however, the α agglutinin was removed by absorption with a heterologous α strain, the absorbed serum should give results similar to those obtained with the corresponding specific serum. This was found to be the case with all four non-specific sera. After absorption they entirely failed to agglutinate heat-killed broth cultures of the homologous α strains, while agglutinating to full titre similar cultures of the specific variant and 'O' suspensions of both the homologous α strain and the specific variant.

It was noted that this inhibition of 'O' agglutination produced by α antigen bore a striking resemblance to the antagonism between the Vi antigen and 'O' agglutinability, described by Felix & Pitt (1934*a*) in *Bact. typhosum*. It was obviously important, therefore, to determine whether these antigens had other properties in common. As the Vi antibody had been demonstrated by Felix primarily in mouse-virulent strains, mouse-virulence tests were carried out on a number of α strains and their specific variants.

Mouse-virulence tests

Each strain was grown for 18 hr. in heart broth, diluted with Ringer's solution to an opacity of 1000 million organisms per ml. and inoculated into batches of forty mice, half receiving 0·5 ml. and half 0·05 ml. The results are shown in Table 7, and it will be seen that there is no evidence that the α strains are any more virulent than the corresponding variants free from α antigen.

Felix & Pitt further demonstrated that when Vi strains were grown on agar containing 1 in 900 phenol the Vi antigen and the 'O' inagglutinability were lost. In contrast to their findings, α strains could be repeatedly subcultured on phenol agar without either removing the α antigen or restoring 'O' agglutinability.

Table 7. *Virulence to mice of α strains and their specific variants*

	No. of deaths per batch of 20 mice occurring within 7 days after inoculation of infecting dose							
	α strains				Specific strains			
Infecting dose	1055	1087	1721	1818	1055	1087	1721	1818
0·5 c.c. of 18 hr. broth culture intraperitoneally	3	20	16	17	16	20	19	19
0·05 c.c. of 18 hr. broth culture intraperitoneally	1	4	4	4	1	0	6	9

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Unsuccessful attempts were made to restore the α agglutinability of specific variants by repeated subculturing in various media, including broth, blood agar, Dorset's egg medium and McConkey agar, and also by mouse passage. Growth in heart broth containing specific antiserum and in broth, to which thick heat-killed suspensions of the homologous non-specific strains had been added, also gave negative results.

Incidence of α strains

The original α strains were discovered in the faeces of patients suffering from paratyphoid fever and of contacts with such cases, suggesting that these strains might be in some way related to the disease. Further investigations have, however, disproved this supposition. The majority of α strains were recovered from contacts who remained perfectly healthy and were never found to excrete *Bact. paratyphosum* B in the stools. Examination of the faeces from between 50 and 100 patients suffering from other intestinal infections and from an equal number of normal persons has yielded about the same proportion of α strains as was met with in the epidemic cases. They have also been isolated from normal rabbit and guinea-pig faeces and from milk. The discovery of α strains in the first place during a paratyphoid epidemic appears to be due to the fact that this was the first occasion on which this particular serum containing α agglutinins had been used on a sufficiently wide scale to detect α strains.

Of the original cases examined, nine faeces out of 155 were found to yield lactose-fermenting α colonies. Even in these instances only occasional colonies, in many cases about 1 in 10, were found to react positively. It is, therefore, quite possible that a certain number may have been missed and that these nine positive results represent a minimal figure. It is more difficult to assess the incidence of non-lactose-fermenting strains, owing to the fact that they lost the antigen so rapidly on subculture that the results of slide agglutination could not be checked by water-bath tests.

Examinations were also made of human and rabbit sera for the presence of α agglutinins. Four batches of rabbits, numbering forty in all, and a similar number of human sera were tested. The human sera were consistently negative against α strains, as were three of the batches of rabbits. In one batch of rabbits, however, four sera out of nine gave agglutination with α strains with titres varying from 1 in 25 to 1 in 200. This particular batch of rabbits was the first tested and it was noted that they were under weight and not very healthy in appearance.

DISCUSSION

The serological analysis of the *Bacterium* genus has revealed a number of different antigenic components taking part in the agglutination reaction. First, the heat-labile 'H' or flagellar antigen, consisting of a number of antigenic factors, some of which are shared by closely related species while others appear to be species-specific. Secondly, the heat-stable 'O' or somatic antigen, likewise made up of several factors, shared only to a limited degree by very closely related species. Occasionally instances of 'H' and 'O' antigenic factors common to otherwise unrelated members of the *Bacterium* group have been described. For example, Lovell (1934) has noted that some lactose and non-lactose-fermenting coliform bacilli, on inoculation into rabbits, can stimulate the production of both 'H' and 'O' agglutinins against certain Salmonellas, and in our own experience, also, several instances have occurred.

A third agglutinogen, the Vi antigen, has been described by Felix & Pitt in mouse-virulent strains of *Bact. typhosum* (Felix & Pitt, 1934 *a*, *b*). This antigen is characterized by heat-lability, by an inhibitory action on the agglutinability of the organism by 'O' antisera, and by its ability to stimulate the production of protective antibodies. The same antigen has since been demonstrated by Kauffmann (1936) in *Bact. paratyphosum* C and in the recently described *S. ballerup* (Kauffmann & Moller, 1940). Other Vi antigens within the Salmonella group have been reported by Felix & Pitt (1936) but have not yet been confirmed.

In capsulated organisms of the *Bact. aerogenes* and *Bact. friedlanderi* groups, another antigen taking part in the agglutinin reaction has been noted. Up to the present, it has been very incompletely studied, but there appears to be several capsular antigenic substances, shared by different strains belonging to these groups and dividing them into distinct types.

Another agglutinogen, known as X antigen, has been described by Topley & Ayrton (1924), giving rise to cross-agglutination between certain members of the Salmonella group, in particular between type suspensions of *Bact. typhi-murium* and *Bact. newport.* Its production was found to be dependent largely on cultural conditions, being absent in young broth cultures grown at 22° C. but developing after more prolonged growth, particularly at 37° C. Under these conditions of growth, type suspensions of all freshly isolated strains of *Bact. typhi-murium* were shown to possess this antigen, and it is considered possible that it may also be found in many other species. More recently Cruickshank (1939) has suggested that conditions of growth may not be as important a factor in the development of X antigen as had previously been thought. This antigen is relatively heat-stable—according to Cruickshank it is not inactivated by steaming for 30 min.—and gives rise to a type of agglutination intermediate between 'O' and 'H'.

The agglutinogens so far described are found in the normal smooth strains and are generally assumed to be situated at the surface of the bacterial cell. In strains which have lost their somatic 'O' antigen and have become rough, the heat-stable 'rough' antigen, normally deeply situated in the bacterial cell and common to all species within the genus, may also take part in the agglutination reaction. Apart from this rough antigen, which is shared by organisms serologically unrelated in the normal smooth form, the occasional sharing to a minor degree of common 'H' and 'O' factors, and possibly the X antigen, up to the present no agglutinogen having a wide distribution throughout the *Bacterium* genus has been described. The identification, during the preliminary stages of this investigation, of a relatively heat-stable antigen, shared by both lactose-fermenting and non-lactose-fermenting organisms, therefore appeared to us of some interest and to justify its fuller investigation.

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Experiments showed that the α antigen did not possess the resistance to treatment with hot alcohol which is typical of 'O' antigen. Its occurrence characteristically in non-motile or poorly motile strains, the fact that methods which tend to encourage development of motility resulted in its disappearance, and the greater heat and acid stability which it displayed indicated that it was not an 'H' antigen. None of the strains exhibiting this antigen showed any evidence of roughness, nor did the antigen possess any of the characteristics of a rough antigen. It was present in freshly isolated strains and often failed to develop after subculture. Colonies were smooth and indistinguishable from those of non-α strains and suspensions showed no signs of auto-agglutinability. The α antigen also appeared to be considerably more heat-labile than rough antigen. Typical rough colonies could be developed from the α strains and these reacted quite differently from the parent organism.

α antigen was obviously not of the nature of a capsular antigen, as only a small proportion of these α strains showed capsular formation or a mucoid type of growth.

The possibility that the α antigen was a virulence antigen similar to that described by Felix & Pitt (1934 *a*, *b*) has also to be considered. Some support was given to this suggestion by the fact that both antigens, when present in the active state, exerted an inhibitory effect on 'O' agglutination and that this 'O' inagglutinability was in each case removed on inactivating the antigen by heat.

There are, however, several points of difference between the two. The presence of α antigen does not appear to be associated with increased mouse virulence. This was shown by a comparison of the deaths following the inoculation into mice of four α strains and their corresponding specific variants. It must be remembered, however, that Kauffmann (1936) has demonstrated that the Vi antigen of *Bact. paratyphosum* C and of *S. ballerup*, which is identical from the immunological standpoint with that of *Bact. typhosum*, has no relation to the virulence of these organisms. The α antigen is considerably more heat-stable than the Vi antigen. Vi agglutination is no longer demonstrable after heating the suspension to 60° C. for 1 hr. (Felix, Bhatnagar & Pitt, 1934), whereas a temperature of at least 75° C. for a similar period is required to inactivate α suspensions. The immunizing properties of the two antigens are also very different. Using living cultures, Felix & Pitt (1934*b*) obtained relatively low Vi titres only, whereas immunization with heat-killed suspensions of α strains consistently produces much higher antibody titres, from 1 in 3200 to 1 in 25,600.

The distribution of the two antigens appears to differ considerably. The Vi antigen has so far been demonstrated only in closely related species of the Salmonella group while the α antigen has been found in several species of the *Bacterium* group, systematically much less closely related and including both lactose and non-lactose fermenters. The α antigen resembles Vi in that, even with actively motile strains, formolization of the suspension does not affect the agglutination of the organism. This, together with the characteristic masking of the 'O' antigen which both produce, suggests that at any rate they may have a similar spatial relationship in the bacterial cell (Felix & Pitt, 1935).

A further possibility to be considered was that α antigen was identical with the X antigen of Topley & Ayrton (1924). The antigens bear some resemblance to one another in the degree of heat-stability which they display, though, according to Cruickshank (1939), X antigen would appear to be somewhat more heat-stable. The type of agglutination produced also is somewhat similar. The production of α antigen, however, is quite independent of conditions of growth and it is consistently present in young cultures of freshly isolated strains. In this respect it differs from the X antigen described by Topley and Ayrton. α antigen has not so far been demonstrated in strains of *Bact. typhi-murium* or other Salmonellas grown under the most varied conditions, nor has *Bact. typhi-murium*, 'X', antiserum been found to agglutinate α strains. So far as is known, the presence of X antigen has never been found to produce inhibition of 'O' agglutination, a characteristic phenomenon with α antigen. While both types of agglutination may be considered to be intermediate between 'H' and 'O', α agglutination is more finely granular and reaches a definite end-point sooner than X agglutination.

Taking these findings as a whole, therefore, it would seem very probable that α antigen is distinct from any of the antigenic components hitherto recognized in the *Bacterium* group. The fact that it has not up to the present been described is probably due to its relatively infrequent occurrence. It is possible that the antigen may not be an integral part of the bacterial cell but may be of the nature of a phage or virus attached to the cell and living in symbiosis. No definite evidence in favour of or against this view has yet been adduced.

The presence of α agglutinins in diagnostic rabbit sera raises several interesting points. The fact that the α antibody was found in the sera of certain supposedly normal rabbits and was entirely absent from others would seem to suggest that it is associated with the presence of mild infections. This idea receives additional support from the fact that the particular batch of animals which reacted positively were rather thin and unhealthy looking. On the other hand, Lovell (1934) has shown that the sera of many domestic animals may possess agglutinins for several different members of the Salmonella group, without there being any evidence that the animal has ever been infected by the corresponding organism. Diagnostic rabbit sera invariably had higher titres of antibody than sera from uninoculated rabbits, suggesting that non-specific stimulation of the α agglutinin had occurred as the result of immunization.

The occasional presence of these α agglutinins in diagnostic sera is of some importance, in view of the common practice of testing out non-lactose-fermenting colonies by the technique of slide agglutination. As an example of this may be cited the presence of α agglutinins in a polyvalent Flexner serum issued by the Standards Oxford Laboratory, the titre being practically identical with that of the homologous agglutinins.

In normal human sera submitted for Wassermann tests and in a few taken from patients suffering from enteric infections, no evidence of the presence of α antibodies has been obtained. Nevertheless, the fact

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that the α antigen was first demonstrated in organisms isolated from patients with paratyphoid fever raised the question as to whether such strains were limited to abnormal intestinal conditions. Examination of large numbers of coliform bacilli from healthy individuals revealed, however, no significant difference in the incidence of α strains in the two groups. They have also been isolated from normal rabbit and guinea-pig faeces and from milk.

Up to the present, prolonged investigation of coliform organisms with normal diagnostic sera has yielded no evidence of any other antigenic factor having the characteristics of α antigen but serologically distinct.

SUMMARY

1. Certain strains of lactose and non-lactose-fermenting coliform bacilli have been found to possess a common agglutinogen. This antigen is found in both motile and non-motile smooth strains and appears to be distinct from 'H', 'O' and rough antigens, and from the X antigen described by Topley & Ayrton. In certain respects it seems to resemble the Vi antigen of Felix & Pitt, notably in the inhibitory effect it exerts on 'O' agglutination. It is, however, not associated with virulence and is of relatively wide distribution.

2. The strains, when inoculated into rabbits, stimulate the production to high titre of an agglutinin which can be absorbed out completely by any one of the strains.

3. Subculture of the strains under certain conditions results in the loss of the antigen and the development of specific variants.

4. Agglutinins to these strains have been found in certain diagnostic sera and may prove a possible source of error, particularly if slide agglutination is relied on for identification.

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