THE OCCURRENCE OF NATURAL ANTIBODIES IN RABBIT SERA IN RELATION TO THE PARACOLON GROUP OF ORGANISMS

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THE OCCURRENCE OF NATURAL 'H' ANTIBODY AND NATURAL 'O' ANTIBODY TO PARACOLON BACILLI IN RABBIT SERA

In two mirror tests made in 1945 to determine the antigenic relation between certain strains of paracolon bacilli (8144, 1748 and 4308; Schwabacher, 1949), the anomalous results shown in Table 1 were obtained. In both tests, the serum to strain 8144, after full absorption with homologous suspension, agglutinated the heterologous suspensions substantially.

To ensure complete absorption of antibody, the serum was absorbed repeatedly with massive doses. Table 2 records that the homologous strain failed to remove the residual antibody, though one of the heterologous strains (4308) absorbed the antibody shared by both heterologous strains. Paracolon 4308 was not agglutinated by α antibody (serum Fairbrother), proving that the antibody was not identical with the α antibody described by Stamp & Stone (1944).

Table 1. *Agglutination titres obtained in mirror tests between paracolon bacilli strains* 8144, 1748 *and* 4308

1 in 40 dilution antiserum against	Formolized absorbing suspensions	Titre against test formolized suspension of	
		Strain 8144	Strain 1748
8144	Nil	5120	2560
8144	8144	< 160	1280*
8144	1748	5120	< 160
1748	Nil	2560	5120
1748	8144	< 160	5120
1748	1748	< 160	< 160
		Strain 8144	Strain 4308
8144	Nil	2560	2560
8144	8144	< 160	1280*
8144	4308	320	< 160
4308	Nil	640	640
4308	8144	< 160	640
4308	4308	< 160	< 160

* Anomalous agglutination by an antiserum fully absorbed by the homologous strain.

The agglutination may have been due to natural rabbit antibody. To test the possibility, the sera of ten stock rabbits were tested against 'O' suspensions of nine strains of paracolon bacilli (8144, 1748, 8179, 1136, 4308, 1111, 7893, 7924, 7973; Schwabacher, 1949). Each animal weighed more than 2500 g. and had been in stock for at least 18 months. Three sera failed to agglutinate any of the strains, while six sera each agglutinated two strains and one serum agglutinated one strain. Four strains of paracolon bacilli were not agglutinated, whereas titres of 1 in 10 were obtained with five sera against 4308, three sera against 7893, one serum against 8144, and one serum against 1748. Suspensions 8144 and 1111 were agglutinated by two sera at a dilution of 1 in 20. These natural antibodies were present in very low titres (Table 3).

The presence of natural antibodies to paracolon bacilli was tested in the sera of eight rabbits immunized with eight different strains of *Proteus vulgaris*. Of these eight flagella sera, two contained antibodies for 4308 and 1748, and two others contained antibodies for 1136. When the anti-*Proteus* sera were absorbed with their homologous strains, they retained the antibodies to the paracolon strains which they had agglutinated before absorption (Table 4).

Presumptive evidence was also found of natural antibodies in a *Salmonella* serum sent from the Emergency Public Health Laboratory in Oxford.

Dr Joan Taylor very kindly examined the nine paracolon strains to ascertain whether they con-

Table 2. *Heterologous agglutinin titres against paracolon strains* 4308 *and* 1748 *obtained with a serum against strain* 8144 *after homologous and heterologous absorption with formolized suspensions*

Dilution of serum 8144 absorbed	Absorbing suspension and dose per ml.		Titre against suspension of strain		
	First absorption	Second absorption	8144	4308	1748
1 : 20	Nil	Nil	2560	2560	2560
1 : 10	8144 : 40×10^9	Nil	< 40	1280	640
1 : 10	8144 : 82×10^9	8144 : 82×10^9	< 80	320	320
1 : 10	8144 : 82×10^9	4308 : 82×10^9	< 80	< 80	< 80

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Table 3. *Titres of normal stock rabbit sera against 'O' suspensions of paracolon bacilli*

Suspension	Rabbit no. 1	2	3	4	5	6	7	8	9	10
4308	10	10	< 10	< 10	< 10	10	< 10	10	10	< 10
7893	< 10	< 10	< 10	< 10	< 10	10	< 10	10	< 10	10
8144	< 10	< 10	20	< 10	< 10	< 10	< 10	< 10	10	< 10
1111	< 10	< 10	20	< 10	< 10	< 10	< 10	< 10	< 10	20
1748	< 10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	40
8179, 7924, 7973, 1136	All less than 10									

Table 4. *Titres of anti-*Proteus *flagella rabbit sera against suspensions of paracolon bacilli, with and without homologous absorption*

1 in 5 dilution serum	Absorbing suspension and dose per ml.	Agglutinating suspension PR_3	PR_{19}	PR_9	PR_{26}	4308	1748	1136
PR_3	Nil	> 5000	—	—	—	80	40	< 10
PR_{19}	Nil	—	> 5000	—	—	80	40	< 10
PR_9	Nil	—	—	> 5000	—	< 10	< 10	20
PR_{26}	Nil	—	—	—	> 5000	< 10	< 10	40
PR_3	$PR_3 : 128 \times 10^9$	< 20	—	—	—	20	20	< 20
PR_{19}	$PR_{19} : 128 \times 10^9$	—	< 20	—	—	20	20	< 20
PR_9	$PR_9 : 128 \times 10^9$	—	—	< 20	—	< 20	< 20	20
PR_{25}	$PR_{26} : 128 \times 10^9$	—	—	—	< 20	< 20	< 20	20

— = no observation.

Table 5. *Somatic antigens shared by strains of paracolon bacilli and salmonellas*

Salmonella somatic serum	Agglutinating *Salmonella* suspension	Titre	Agglutinating paracolon suspension	Titre
enteritidis	*enteritidis*	1600	1748	< 50
aberdeen	*aberdeen*	1600	1748	< 50
tel aviv	*tel aviv*	1600	1748	100 (trace 200)
urbana	*urbana*	1600	1748	< 50
hvittingfoss	*hvittingfoss*	1600	1136	1600
urbana	*urbana*	1600	1136	< 50
poona	*poona*	400	7924	< 50
poona	*poona*	400	7973	< 50
urbana	*urbana*	1600	7973	< 50
newport	*newport*	1600	4308	< 50
poona	*poona*	400	4308	< 50

tained any *Salmonella* 'O' antigens. Table 5 shows that tube agglutination yielded titres significant of antigenic relationship in only two instances, namely 1748 and *Salm. tel aviv*, and 1136 and *Salm. hvittingfoss*. Positive slide agglutinations with the other *Salmonella* sera listed were not confirmed by tube agglutination.

In view of the high titre given by 1136 with *Salm. hvittingfoss* serum, a mirror test was made with the two organisms. The titres for *Salm. hvittingfoss* obtained in Watford were not as high as those reported from Oxford, but the results were clear-cut and otherwise in agreement. Table 6 shows that the two strains share a common antigen and each has a specific antigen of its own. However, there was an anomaly similar to that in Table 1; the Oxford *Salm. hvittingfoss* serum absorbed with its homologous strain still retained antibodies for paracolon 1136, indicating that the rabbit's serum contained antibodies which were presumably not evoked by *Salm. hvittingfoss*.

Absorption of the *Salm. hvittingfoss* serum with 1136 removed all antibodies for 1136, leaving only the specific antibody for *Salm. hvittingfoss*. It is therefore apparent that there is a second antigen in 1136 which absorbs the corresponding antibody from

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› Oxford prepared *Salm. hvittingfoss* serum. To ıfirm this, a fresh rabbit was chosen for immuniza- n at Watford. Before immunization with *Salm. ttingfoss* 'O' suspensions killed at 100° C., the ›bit's serum was found to be free of antibodies for › nine paracolon type suspensions in dilutions of ı 5 to 1 in 40. This Watford *Salm. hvittingfoss* serum ›lded an 'O' titre of 3200 both to *Salm. hvittingfoss* d paracolon suspension. After absorption with × 10^9 somatic *Salm. hvittingfoss* per ml. (steamed 100° C. for 2 hr.), no antibodies remained for the mologous strain or for 1136 (Table 7).

shared by lactose fermenting and non-lactose fermenting coliform bacilli. Stamp & Stone stressed the need to remove the α antibody from diagnostic sera by appropriate absorption. Four strains of coliform bacteria containing the α antigen were kindly sent by Dr Joan Taylor and nine paracolon type sera (prepared to determine an association between antigenic and biochemical type; Schwabacher, 1949), were tested for α antibody. Agglutinating suspensions were grown at 37° C. on nutrient agar overnight, suspended in saline and heated for 1 hr. at 55° C. Three of the strains were agglutinated to a titre of

'able 6. *Titres obtained in mirror test between* Salm. hvittingfoss (*Oxford antiserum*) *and paracolon* 1136

Serum	Dilution of serum absorbed	'O' absorbing suspension	Test 'O' suspension: *Salm. hvittingfoss*	Test 'O' suspension: 1136
Salm. hvittingfoss (Oxford)	1/10	Nil	640	640
	1/5	1136	320	< 20
	1/5	*hvittingfoss*	< 20	320
1136	1/10	Nil	320	2560
	1/5	1136	< 20	< 20
	1/5	*hvittingfoss*	< 20	640

Table 7. *Titres of agglutination obtained with anti-*Salm. hvittingfoss *serum* (*prepared at Watford*)

Serum	Dilution of serum absorbed	'O' absorbing suspension	Test 'O' suspension: *Salm. hvittingfoss*	Test 'O' suspension: 1136
Salm. hvittingfoss (Watford)	1/10	Nil	3200	3200
	1/10	1136	640	< 40
	1/10	*hvittingfoss*	< 40	< 40

Table 8. *Titres obtained in mirror test between a coliform bacillus possessing an α antigen and a paracolon bacillus*

Serum	Absorbed with	Agglutination test suspension: Fairbrother 55° C.	Fairbrother 100° C.	Paracolon 8144 55° C.	Paracolon 8144 100° C.
8144	Nil	80	< 10	320	640
	8144 (55° C.)	< 10	< 10	< 10	< 10
	Fairbrother (55° C.)	< 10	< 10	160	640
Fairbrother	Nil	2560	320	80	< 10
	8144 (55° C.)	640	320	< 10	< 10
	Fairbrother (55° C.)	< 10	< 10	< 10	< 10

Temperatures are those at which suspensions were heated for times stated in text.

It is clear that the antiserum to *Salm. hvittingfoss* epared in Oxford contained a natural antibody hich could be removed by the corresponding anti- n in paracolon 1136.

THE PRESENCE OF THE α ANTIGEN OF STAMP AND STONE IN *PARACOLON BACILLI*

amp & Stone (1944) demonstrated natural anti- dies to α antigen in rabbits' sera. This antigen was

40 and the fourth strain (Fairbrother) to a titre of 80 by serum 8144. The agglutination was finely granular in type. The serum failed to agglutinate suspensions of the four strains when they had been steamed at 100° C. for 2 hr.

With a view to determining whether the antigen shared by 8144 and the strain Fairbrother was α in type, an immune serum was prepared against the strain Fairbrother. Cells held at 54° C. for 1 hr. were used for immunization. An initial dose of 100 × 10^6 was given intravenously. Injections were given

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twice a week until the animal had received 1000×10^6 organisms. Before the immunizing course was started the rabbit's serum was tested and found to be free from α antibody for strain Fairbrother, and from 'α', 'H' and 'O' antibody for paracolon 8144. The Fairbrother serum yielded a typically higher titre to the α antigen than to the 'O' antigen. When absorbed with a heterologous suspension the 'O' titre remained unaltered.

The 8144 serum prepared with organisms killed at 60° C. contained α antibody. Stamp & Stone state that the α antigen is destroyed in 1 hr. at 95° C. and in 15 min. at 100° C.

The mirror test (Table 8) shows that the paracolon 8144 shares the α antigen of strain Fairbrother.

DISCUSSION

The finding of natural antibodies in the rabbit to paracolon bacilli has a practical application. Kauffmann (1937, 1941), Schiff, Bornstein & Saphra (1941), Peluffo, Edward & Brunner (1942), Wheeler, Stuart, Rustigan & Bormann (1943), and Kauffmann (1944*a*, *b*, *c*) describe strains of *Bact. coli* and late-lactose fermenters which share somatic antigens with the *Salmonella* group. Sevitt (1945) and Wheeler, Stuart & Ewing (1946) record paracolon bacilli which share antigens with *Shigella* strains. It follows that, as a precautionary measure, tests should be made for natural antibodies before preparing immune serum in a rabbit. Lovell (1934) in testing forty normal rabbits' sera failed to find agglutinins to a series of *Salmonella* suspensions but obtained evidence of naturally occurring *Salmonella* agglutinins in the sera of healthy swine, cattle, sheep and horses which had been taken to the slaughter house. Emslie-Smith (1948) demonstrated coliform and paracolon antibodies in the sera of two uninoculated rabbits. In fact, by absorption experiments, using lactose fermenting and late-lactose fermenting strains from human faeces and contaminated war wounds, he was able to recognize at least seven distinct agglutinins in each of the two sera investigated. It has been shown (Schwabacher, 1949) that paracolon strains have multiple antigens, hence a non-immunized rabbit's serum reacting with a strain to be used for antibody production, may contain multiple antibodies. Such rabbits should not be used for immunization.

Messer (1943), Francis (1944) and Fairbrother (1945), have stressed the dangers inherent in rapid slide agglutination methods because of the α antibody present in rabbits' sera.

CONCLUSION

In selecting a rabbit for the production of immune serum, the rabbit's serum should be subjected to two tests. First, the serum should be examined for the presence of natural agglutinins to the strain about to be used as antigen. If they are found, the rabbit should be discarded on account of the possibility of other non-specific antibodies.

Secondly, the serum should be tested for agglutinins against an organism bearing the α antigen of Stamp & Stone. If a rabbit, the serum of which contains α antibody, has to be used, the α antibody will have to be absorbed out in order to avoid 'false positive' agglutinations in slide agglutination tests.

SUMMARY

1. Naturally occurring agglutinins to paracolon bacilli have been found in seven out of ten sera from non-immunized rabbits and in one anti-*Salmonella* and two anti-paracolon immune rabbits' sera after complete absorption with the immunizing strain.

2. The α antigen of Stamp & Stone, which is known to occur as a natural agglutinin, has been demonstrated in one out of nine paracolon bacilli.

3. Rabbits which before immunization show agglutinins to the immunizing strain or to an α antigen should not be used for the preparation of diagnostic antisera for intestinal Gram-negative bacilli.

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