CRUMPTON, M. J., DAVIES, D. A. L. & HUTCHISON A. M. (1958). *J. gen. Microbiol.* **18**, 129-139

The Serological Specificities of *Pasteurella pseudotuberculosis* Somatic Antigens

BY M. J. CRUMPTON, D. A. L. DAVIES AND ANN M. HUTCHISON

Microbiological Research Establishment, Porton, Wiltshire

SUMMARY: The serological specificities of lipopolysaccharides obtained from representatives of each of the five serological groups of *Pasteurella pseudotuberculosis* have been examined by haemagglutination methods. At concentrations of 500 $\mu\text{g./ml.}$ solutions of the lipopolysaccharides failed to give full sensitization of erythrocytes, but after treatment with alkali full sensitization was obtained at concentrations in the range of 50 to 3 $\mu\text{g./ml.}$ By using antisera prepared in rats by immunization with living *P. pseudotuberculosis*, the capacity of each 'Smooth' lipopolysaccharide to inhibit the agglutination of sensitized erythrocytes by each group antiserum was determined. Absolute group specificity was found by this method. It is considered that the lipopolysaccharides bear the specificity of the somatic antigens on which the serological grouping of the species is based. 'Smooth' *P. pseudotuberculosis* lipopolysaccharides failed to inhibit the agglutination by *P. pestis* antisera of erythrocytes sensitized with *P. pestis* lipopolysaccharide, but inhibition of this system was obtained with lipopolysaccharides of 'Rough' *P. pseudotuberculosis* strains derived from various serological groups. These 'Rough' lipopolysaccharides were closely related serologically to one another and to the *P. pestis* product but were not identical in specificity. It is considered that in Smooth *P. pseudotuberculosis* strains the 'Smooth' somatic antigen does not overlie a 'Rough' somatic antigen.

In the Smooth state of *Pasteurella pseudotuberculosis* heat-stable somatic antigens have been detected, characteristic of one of the five serological groups into which this species has been subdivided (Arkwright, 1927; Schütze, 1928; Kauffmann, 1932; Thal, 1954). It has been considered that in 'Smooth' cultures a 'Smooth' somatic antigen overlies a 'Rough' somatic antigen which is exposed on the surface of 'Rough' organisms and has a specificity common to all 'Rough' cultures derived from any of the serological groups. The specificity of the 'Rough' component is also common to all strains of *P. pestis* (Schütze, 1932; Bhatnagar, 1940; Thal, 1954, 1956). These conclusions were based on precipitation, agglutination, serum absorption and immunological studies with whole organisms or heated autolysed culture filtrates.

It has been suggested that the 'Rough' somatic antigen common to *Pasteurella pseudotuberculosis* and *P. pestis* may be composed of two serologically distinct components (Chen & Meyer, 1955; Bhagavan, Chen & Meyer, 1956); this idea was based on the results of diffusion-precipitin analysis. By this method Crumpton & Davies (1956) showed that the two species possessed at least 7 antigens in common, but heat stability with respect to antigenicity, as opposed to haptenic reactivity, could be clearly demonstrated for only one of these components when antisera against cultures of Rough *P. pseudotuberculosis* and *P. pestis* were compared (Crumpton & Davies, to be published). This heat-stable component was isolated from *P. pestis* and purified by Davies

(1956); it is considered to be the 'Rough' somatic antigen postulated by Schütze (1932).

Somatic antigens derived from strains representing each of the serological groups of *Pasteurella pseudotuberculosis* have been extracted and purified (as lipopolysaccharides), and described by Davies (1958). These materials did appear to carry group specificity, but in precipitin tests they cross-reacted in some instances with one another and also with analogous products of 'Rough' strains. Nevertheless, the indications from chemical and physical data were that the materials were single chemical entities.

Of the methods available for studying the serological properties of polysaccharides the 'indirect bacterial hemagglutination' method (Neter, 1956) has certain distinct advantages. In this test material is adsorbed on to erythrocytes which thus acquire the specificity of the adsorbed substance. This overcomes difficulties associated with the rather poor solubility of lipopolysaccharides extracted by the phenol method (Westphal, Lüderitz & Bister, 1952); alternative methods of extraction are not very satisfactory where surface protein antigens are present. The haemagglutination method has also proved in many instances to be the most sensitive one for the detection and titration of antibodies (Neter *et al.* 1955) and in modified form can be used for the detection of very small amounts of material (Neter & Gorzynski, 1954). In the present work the haemagglutination and haemagglutination inhibition methods have been used to examine the serological specificities of the *Pasteurella pseudotuberculosis* 'Smooth' and 'Rough' lipopolysaccharides described in the preceding paper by Davies (1958).

METHODS

Organisms. Strains of *Pasteurella pseudotuberculosis* used for lipopolysaccharide extraction and for immunization of animals were kindly supplied by Dr E. Thal (Veterinärmedizinischen Staatsinstitut, Stockholm). These represented each of the 5 serological groups (Thal, 1954) and in accordance with the International Bacteriological Code of Nomenclature, serological groups have been lettered. Cultures have thus been designated, e.g. 14/A/S, indicating strain number/serological group/colony form. Rough strains from some serological groups were isolated by using tryptic meat agar + peptic sheep's blood. *P. pestis* strain Tjiwidej Smooth (TS) was used for production of *P. pestis* polysaccharide which does not differ in different strains of this species (Davies, 1956).

Lipopolysaccharides. The lipopolysaccharides of *Pasteurella pseudotuberculosis* used were those described by Davies (1958). These materials have somewhat similar properties to the 'endotoxins' which have been extracted from various species of the Enterobacteriaceae (e.g. Davies, Morgan & Mosimann, 1954; Davies, 1955). The 'Rough' somatic lipopolysaccharide of *P. pestis* is that described by Davies (1956). These preparations are rather poorly soluble in water and were heated at 100° for 5 min. for use in haemagglutination inhibition tests. This treatment increases the degree of dispersion to give a

stable but heavily opalescent solution. For sensitization of erythrocytes lipopolysaccharides were first treated with alkali (Macpherson, Wilkinson & Swain, 1953; Neter *et al.* 1954; Landy, Trapani & Clark, 1955). Solutions at 1 mg./ml. were heated at 37° for 18 hr. in 0.02N-NaOH and neutralized to pH 7.0 with 0.2N-HCl.

Antisera. For the production of antisera against whole living organisms, white rats were used because they are not susceptible to *Pasteurella pseudotuberculosis* infection. Groups of 7 rats were injected weekly by the intra-peritoneal route with organisms of each of the 5 serological groups and several Rough strains, suspended in 0.5 ml. saline (0.9%, w/v). After 2 injections of organisms equivalent to 5 µg. dry wt., the dose was raised to 100 µg.; sera from rats of each group were pooled after 9 injections had been given. Antisera against the isolated lipopolysaccharides were prepared in rabbits after combining the materials with the lipoprotein component of the *Shigella dysenteriae* 'O' somatic antigen (Partridge & Morgan, 1940; Davies, 1956). For use in the haemagglutination test sera were inactivated by heating at 56° for 30 min. and absorbed twice for 1 hr. at 37° with 10% (v/v) of washed packed group 'O' Rh negative human erythrocytes.

Haemagglutination test. Human group 'O' Rh negative blood (citrated) was stored at 2° and replaced by a fresh stock after 30 days. Before use the erythrocytes were washed 3 times with saline and resuspended in saline at 2.5% (v/v). In order to sensitize erythrocytes, equal volumes of this suspension and a range of serial 1/2 dilutions of alkali-treated lipopolysaccharide dissolved in isotonic phosphate buffered saline (pH 7.2) were mixed and incubated at 37° for 1 hr. These sensitized erythrocytes were washed 3 times with 10 vol. of a 1/250 dilution of normal rabbit serum in saline to remove excess lipopolysaccharide and resuspended at 0.5% (v/v). Serial 1/2 dilutions of antiserum were prepared in 0.2 ml. volumes using a 1/100 dilution of normal rabbit serum in saline as diluent; an equal vol. of the 0.5% sensitized erythrocyte suspension was added to each dilution of antiserum. The tubes were shaken, incubated at 37° for 1 hr. and allowed to stand at 2° overnight. Unsensitized erythrocytes for use in control tests were prepared in a similar way, but substituting buffered saline for the dilution of alkali-treated lipopolysaccharide. Controls were set up in each test, namely serum dilutions with unsensitized erythrocytes and sensitized erythrocytes with the serum diluent; when any agglutination was detected in control tubes the test was discarded. Agglutination was read microscopically, the greatest dilution at which agglutination could be detected was taken as the end-point. The sensitizing activity is recorded as the least concentration (in µg./ml.) of lipopolysaccharide which when added to the 2.5% erythrocyte suspension gave agglutination to the maximum serum dilution. The reciprocal of this serum dilution will be referred to as the haemagglutination titre.

Haemagglutination inhibition test. This test was carried out in a similar way to that described by Morgan & van Heyningen (1944) for measurement of the serological activity of blood group substances. To serial 1/2 dilutions of heated homologous and heterologous lipopolysaccharides in saline (0.2 ml. volumes) an equal volume of serum was added suitably diluted in a 1/100 dilution of

normal rabbit serum in saline to contain 3–4 haemagglutinating doses, as determined from the haemagglutination titre of the serum with the homologous lipopolysaccharide. The tubes were shaken and incubated for 1 hr. at 37°; 0.2 ml. of a 0.5% (v/v) suspension of erythrocytes was then added to each tube, these cells having been sensitized with the homologous lipopolysaccharide at twice the concentration corresponding to its sensitizing activity. After shaking, the tubes were reincubated for 1 hr. at 37° and left at 2° overnight. Controls were: (a) serum with sensitized erythrocytes, which consistently gave complete agglutination; (b) initial dilutions of each lipopolysaccharide and serum with unsensitized erythrocytes. When agglutination occurred in control (b) the test was discarded. Agglutination was read microscopically and the first tube showing detectable agglutination was taken as the end-point; the dilution (inhibition titre) corresponding to this tube in the homologous system was taken to represent an activity of 100%. The inhibiting activities of heterologous lipopolysaccharides tested have been expressed as a percentage of this activity (i.e. inhibition titre heterologous polysaccharide/inhibition titre homologous polysaccharide $\times 100$). Differences between end-points greater than 7 tubes have been taken to indicate lack of cross-reactivity, since this would correspond to less than 1% of the activity of the homologous standard where twofold geometrical dilutions were employed. One tube differences between end-points have been taken as significant only when the same result has been obtained at least twice.

RESULTS

Sensitizing activities of lipopolysaccharides

When representative 'Smooth' and 'Rough' *Pasteurella pseudotuberculosis* lipopolysaccharides (25/E/S and 32/D/R) were tested without alkali treatment for their ability to sensitize erythrocytes for haemagglutination, only at the highest concentration tested (500 $\mu\text{g./ml.}$) was significant (but not maximal) sensitization obtained. The *P. pestis* lipopolysaccharide, unlike any others which have been investigated, was, however, fully active (sensitizing activity 3 $\mu\text{g./ml.}$) without any alkali treatment.

Table 1. *The titration of the sensitizing activity of Pasteurella pseudotuberculosis 32/D/R lipopolysaccharide*

Lipopoly- saccharide ($\mu\text{g./ml.}$)*	Serum dilution (reciprocal)							
	5	10	20	40	80	160	320	640
	Degree of agglutination†							
25	4	4	4	4	4	3	1	0
12.5	4	4	4	4	4	2	1	0
6	4	4	4	4	2	2	1	0
3	4	4	4	3	2	0	0	0
1.5	2	0	0	0	0	0	0	0

* Concentration of solution added to an equal volume of a 2.5% erythrocyte suspension.

† Degree of agglutination: 4, complete; 3, few free cells; 2, half of cells clumped; 1, most cells free; 0, no reaction.

The sensitizing activity of each of the lipopolysaccharides after alkali treatment has been determined. These results are not given in full, but a typical test on one of the preparations (32/D/R) is shown in Table 1. From this test the sensitizing activity is taken to be 6 $\mu\text{g.}/\text{ml.}$, below this concentration the haemagglutination titre of the serum falls off sharply, above this concentration there is no significant increase in serum titre.

The sensitizing activities determined in this way for the series of lipopolysaccharides studied are shown in Table 2. They vary between 50 and 3 $\mu\text{g.}/\text{ml.}$ These figures are independent of the particular serum being used; the haemagglutination titres obtained in this series using the homologous rat antisera are also shown in this table.

Table 2. *Sensitizing activities of alkali-treated lipopolysaccharides*

Lipopolysaccharide	Sensitizing activity ($\mu\text{g.}/\text{ml.}$)*	Serum haemagglutination titre†
<i>Pasteurella</i> <i>pseudotuberculosis</i>		
14/A/S	50	160
16/B/S	25	320
16/B/R	25	160
27/C/S	25	320
43/C/R	50	80
32/D/S	12.5	320
32/D/R	6	320
25/E/S	6	160
<i>Pasteurella pestis</i>	3	1280

* Least concentration of solution added to an equal volume of a 2.5 % erythrocyte suspension necessary to give agglutination up to maximum serum titre.

† Reciprocal of maximum serum titre.

Determination of serological specificities

The serological specificities of the *Pasteurella pseudotuberculosis* and *P. pestis* lipopolysaccharides were examined by using the haemagglutination inhibition test. For each lipopolysaccharide determinations were made of the inhibition titres as previously defined, using sera produced against each group of *P. pseudotuberculosis* and against *P. pestis*. An example of one of the 9 tests is shown in Table 3, where all the *P. pseudotuberculosis* 'Smooth' and 'Rough' lipopolysaccharides are tested for their capacity to inhibit the *P. pestis* system. In this and the following table 0 % serological activity is recorded when the inhibition titres of the homologous and heterologous lipopolysaccharides differ by 7 or more tubes. The data in Table 3 is summarized in the last line of Table 4. The data shown in Table 4, lines 1 to 5, indicate that the materials which were isolated from representatives of each 'Smooth' *P. pseudotuberculosis* group are, indeed, group specific antigens showing no overlap of specificity. In lines 6-9 (Table 4) it can be seen further that only one of the 'Smooth' group specific lipopolysaccharides show any significant inhibition of the 'Rough' *P. pseudotuberculosis* or *P. pestis* systems. In the latter part of Table 4 figures show that the lipopolysaccharides isolated from 'Rough' strains originally derived from groups B, C and D cultures and from *P. pestis* show different

Table 3. *Inhibition by Pasteurella lipopolysaccharides of the agglutination by Pasteurella pestis antiserum of erythrocytes sensitized with P. pestis lipopolysaccharide*

Antiserum against *P. pestis* was prepared in rabbits by immunization with living organisms of an avirulent strain (TS), and was used at a dilution of 1/75 (3–4 haemagglutinating doses). Degrees of agglutination as in Table 1.

Inhibiting lipopoly-saccharide	Lipopolysaccharide dilution (reciprocal $\times 10^3$)										Serological activity (%)	
	2	4	8	16	32	64	128	256	512	1024		2048
	Degree of agglutination											
<i>P. pestis</i>	0	0	0	0	0	0	0	0	0	0	3	100
<i>P. pseudotuberculosis</i>												
14/A/S	1	2	2	3	4	4	4	4	4	4	4	0
16/B/S	0	0	1	1	2	3	3	4	4	4	4	0
16/B/R	0	1	2	3	4	4	4	4	4	4	4	0
27/C/S	0	0	2	3	3	3	3	4	4	4	4	0
43/C/R	0	0	0	0	0	0	0	0	0	3	4	50
32/D/S	0	0	1	2	2	3	3	4	4	4	4	0
32/D/R	0	0	0	0	0	0	0	0	2	3	4	25
25/E/S	2	2	2	2	2	4	4	4	4	4	4	0

Table 4. *The specificities of lipopolysaccharides isolated from Pasteurella pestis and 'Smooth' and 'Rough' strains of P. pseudotuberculosis*

Sensitizing lipopoly-saccharide	Dilution of* homologous antiserum	Inhibiting lipopolysaccharide (% serological activity)								
		14/A/S	16/B/S	16/B/R	27/C/S	43/C/R	32/D/S	32/D/R	25/E/S	<i>P. pestis</i>
14/A/S	1/20	100	0	0	0	0	0	0	0	0
16/B/S	1/20	0	100	0	0	0	0	0	0	0
27/C/S	1/20	0	0	0	100	0	0	0	0	0
32/D/S	1/15	0	0	0	0	0	100	0	0	0
25/E/S	1/15	0	0	0	0	0	0	0	100	0
16/B/R	1/15	0	0	100	0	12	0	100	0	2
43/C/R	1/5	0	0	0	0	100	6	100	0	100
32/D/R	1/30	0	0	3	0	50	0	100	0	3
<i>P. pestis</i>	1/75	0	0	0	0	50	0	25	0	100

* Dilution to give 3–4 haemagglutinating doses.

degrees of cross-reactivity but nevertheless possess closely related specificities. Thus, for example, the 16/B/R and *P. pestis* systems only interact very weakly, but the intermediate 32/D/R lipopolysaccharide inhibits both the 16/B/R and *P. pestis* systems.

Examination of Pasteurella pseudotuberculosis antisera for antibodies related to the 'Rough' Pasteurella somatic antigens

Antisera prepared in rats by immunization with living virulent *Pasteurella pseudotuberculosis* and in rabbits by immunization with living avirulent *P. pestis* were examined by the haemagglutination test for their ability to react with erythrocytes sensitized with *P. pseudotuberculosis* 'Rough' and *P. pestis* lipopolysaccharides. The sensitized erythrocytes were added to 1/2 serial dilutions of each serum, and the haemagglutination titres of the 8 'S' and 'R'

antisera are shown in Table 5. These results show that antisera against the 'Smooth' *P. pseudotuberculosis* strains (except 16/B/S) contain no detectable antibody for the 'Rough' lipopolysaccharides 16/B/R and *P. pestis* and only at relatively high serum concentrations ($\approx 1/5$) could antibody be detected against 32/D/R sensitized erythrocytes. In the case of 16/B/S some agglutination ($\approx 1/10$) was observed with each of the 'Rough' lipopolysaccharide sensitized erythrocytes. Results obtained using 43/C/R antiserum did not differ significantly from those recorded in Table 5 for *P. pestis* antiserum. Sera against the 'Rough' *P. pseudotuberculosis* organisms (16/B/R and 32/D/R) and *P. pestis* contain antibodies which possess closely related but not identical specificities, a result which agrees with that obtained in the inhibition tests.

Table 5. Examination of antisera to *Pasteurella pseudotuberculosis* for the presence of antibodies to 'Rough' *Pasteurella* lipopolysaccharides

Sensitizing lipopolysaccharide	Antiserum						
	14/A/S	16/B/S	16/B/R	27/C/S	32/D/S	32/D/R	25/E/S
	Haemagglutination titre						
16/B/R	0*	5	80	0	0	320	0
32/D/R	2.5	10	80	2.5	5	320	0
<i>P. pestis</i>	0	2.5	0	0	0	160	0

* Zero titre signifies no detectable agglutination at the greatest serum concentration used (1/2.5).

When antisera produced in rabbits by immunization with isolated 'Smooth' and 'Rough' *Pasteurella pseudotuberculosis* lipopolysaccharides (after combination with 'Shiga' lipoprotein) were examined in the same way, agglutination of *P. pestis* sensitized erythrocytes was obtained with both 'anti-Rough' and 'anti-Smooth' sera at dilutions down to 1/40, a result which agrees with the cross reactivity found with the same sera when precipitin tests were used (Davies, 1958).

DISCUSSION

To obtain full sensitization of erythrocytes with *Pasteurella pseudotuberculosis* lipopolysaccharides it has been necessary first to treat the materials with dilute alkali. It has been suggested (Landy *et al.* 1955) that the presence of a substantial lipid component in some of these lipopolysaccharides prevents adsorption to the erythrocyte surface and that alkali treatment affects the lipid in some way to overcome this inhibition. This is difficult to reconcile with the presence in the *P. pestis* product of c. 50% lipid (Davies, 1956), since this material requires no alkali treatment to elicit its full sensitizing activity, although alkaline conditions were carefully avoided during extraction and purification. The extracellular slime polysaccharide of *Klebsiella aerogenes* requires alkali treatment before it will adsorb to erythrocytes (Macpherson *et al.* 1958), but this substance contains no detectable lipid (D. A. L. Davies, unpublished observations). This is equally difficult to reconcile with the

conclusions of Vogel (1957), who considered that lipid is essential for erythrocyte sensitization.

The results of haemagglutination inhibition tests show that the purified lipopolysaccharides isolated from representatives of the 5 *Pasteurella pseudotuberculosis* serological groups (Davies, 1958) are the group-specific 'Smooth' somatic antigens detected by previous workers using various serological methods. No cross-reaction was detected among the products of 'Smooth' strains using sera prepared by immunization with living *P. pseudotuberculosis* organisms.

By the phenol extraction method (Westphal *et al.* 1952) it is possible to obtain lipopolysaccharides from both 'Smooth' and 'Rough' cultures (Westphal & Lüderitz, 1954; Davies, 1957 *a*). If a 'Rough' somatic antigen were present beneath the 'Smooth' somatic antigen in 'Smooth' forms, both 'Smooth' and 'Rough' components would be expected in the extracts of 'Smooth' strains. No evidence has been obtained to substantiate this classical view of S $\rightarrow$ R loss variation. The 'Smooth' *Pasteurella pseudotuberculosis* products have shown no significant neutralization of antibody against 'Rough' *P. pseudotuberculosis* strains or *P. pestis*, and antisera against *P. pseudotuberculosis* 'Smooth' strains failed to give significant agglutination of erythrocytes sensitized with 'Rough' *Pasteurella* lipopolysaccharides. Sera prepared by immunization of rabbits with the isolated 'Smooth' lipopolysaccharides, however, agglutinated red cells sensitized with *P. pestis* lipopolysaccharide. This is not easily interpreted but it seems possible, in view of the fact that in composition the 'Smooth' materials contain the components of the 'Rough' materials, with certain additional sugars, that 'Rough' specificity might be associated with the basic chain structure (glucosamine and aldohexose) and 'Smooth' specificity associated with additional side chains (deoxy sugars in particular). If this is true it would be necessary to postulate further that in the intact organism, but not in the isolated product, the basic chain structure is not exposed for antibody induction. That the S $\rightarrow$ R variation is not the loss of an antigen in the sense of a discrete molecular entity, but reflects a change of polysaccharide structure, including the loss of deoxysugars, has been more clearly demonstrated in *Shigella dysenteriae* (Davies, 1957 *a, b*).

In *Pasteurella pseudotuberculosis* the change from the virulent smooth colony form to an avirulent rough state involves more than a change in the heat-stable somatic antigen. A protein antigen is present which also appears to contribute to smoothness of colonial form; only when this antigen and the polysaccharide 'Smooth' somatic antigen are both absent is colonial form frankly rough (Crumpton & Davies, 1957). Loss of either of these two components alone does not affect the colony appearance greatly. The 'Smooth'/'Rough' protein (antigen 4) is present also in smooth colony strains of *P. pestis* which does not possess a 'Smooth' somatic polysaccharide component (Crumpton & Davies, 1956; Davies, 1956).

Examination of lipopolysaccharides extracted from 'Rough' strains of *Pasteurella pseudotuberculosis* derived from three different serological groups (B, C and D) showed that they had lost group specificity. The specificities of

the 'Rough' products and the *P. pestis* lipopolysaccharide are closely related but not identical. The 43/C/R material could not be distinguished directly from that of *P. pestis*, but 32/D/R material has a specificity related to both although it is more like that of 43/C/R than the *P. pestis* product. Thus the 32/D/R lipopolysaccharide inhibited 43/C/R antiserum as effectively as 43/C/R itself, and inhibited *P. pestis* antiserum almost as effectively as 43/C/R lipopolysaccharide. But whereas the 43/C/R material inhibited 32/D/R serum as effectively as the homologous substance, *P. pestis* lipopolysaccharide reacted poorly in this test. The specificity of material 16/B/R seemed further removed from 43/C/R and *P. pestis* than 32/D/R. Thus whereas 43/C/R and *P. pestis* materials inhibit 16/B/R antisera rather weakly, 32/D/R material is as effective as the homologous substance. This overlap of specificity was also demonstrated by the cross-reactivity of antisera to 'Rough' *Pasteurella* strains in the agglutination of cells sensitized with the 'Rough' *Pasteurella* lipopolysaccharides. In the extreme case anti 16/B/R serum showed no capacity to agglutinate red cells sensitized with *P. pestis* lipopolysaccharide.

Although the 'Rough' *Pasteurella* lipopolysaccharides appear very similar in chemical composition (Davies, 1956, 1958) the haemagglutination technique shows that they form a spectrum of overlapping specificity in the order *Pasteurella pseudotuberculosis* 16/B/R-32/D/R-43/C/R-*P. pestis*. These are differences which could not readily be resolved by other methods. A somewhat similar situation has been described by McCarty & Lancefield (1955) for the group A specific *Streptococcus* polysaccharide and products of very similar chemical composition from group A variants.

The examination of antigenic patterns in bacteria by agar diffusion precipitin methods has shown in many instances a complexity not previously recognized. This is true of *Pasteurellas* (Crompton & Davies, 1956, and to be published) and the presence of antigens not known to early workers in the field may account for some of the difficulties in interpreting their results, from experiments necessarily carried out with crude extracts or autolysates. Apart from the conclusion that a 'Rough' somatic antigen does not underlie the 'Smooth' somatic antigen in 'Smooth' *Pasteurella pseudotuberculosis*, and the observation that the 'Rough' products are not identical in specificity, the general picture from earlier findings agrees for the most part with the results now obtained by using purified bacterial products.

REFERENCES

- ARKWRIGHT, J. A. (1927). The importance of motility of bacteria in classification and diagnosis, with special reference to *Bacillus pseudotuberculosis rodentium*. *Lancet*, i, 13.
- BHAGAVAN, N. V., CHEN, T. H. & MEYER, K. F. (1956). Further studies of antigenic structure of *Pasteurella pestis* in gels. *Proc. Soc. exp. Biol.*, N.Y. **91**, 353.
- BHATNAGAR, S. S. (1940). Bacteriological studies on *Pasteurella pestis* and *Pasteurella pseudotuberculosis*. Part II. The serology of *Pasteurella pestis* and *Pasteurella pseudotuberculosis*. *Indian J. med. Res.* **28**, 17.

- CHEN, T. H. & MEYER, K. F. (1955). Studies on immunization against plague. X. Specific precipitation of *Pasteurella pestis* antigens and antibodies in gels. *J. Immunol.* **74**, 501.
- CRUMPTON, M. J. & DAVIES, D. A. L. (1956). An antigenic analysis of *Pasteurella pestis* by diffusion of antigens and antibodies in agar. *Proc. Roy. Soc. B*, **145**, 109.
- CRUMPTON, M. J. & DAVIES, D. A. L. (1957). A protein antigen associated with smooth colony forms of some Pasteurellas. *Nature, Lond.* **180**, 863.
- DAVIES, D. A. L. (1955). The specific polysaccharides of some Gram-negative bacteria. *Biochem. J.* **59**, 696.
- DAVIES, D. A. L. (1956). A specific polysaccharide of *Pasteurella pestis*. *Biochem. J.* **63**, 105.
- DAVIES, D. A. L. (1957*a*). Isolation of a Rough somatic antigen from *Shigella dysenteriae*. *Biochem. biophys. Acta*, **26**, 151.
- DAVIES, D. A. L. (1957*b*). The composition and distribution of Rough somatic antigens in some Gram-negative bacteria. *J. gen. Microbiol.* **17**, vi.
- DAVIES, D. A. L. (1958). The 'Smooth' and 'Rough' somatic antigens of *Pasteurella pseudotuberculosis*. *J. gen. Microbiol.* **18**, 118.
- DAVIES, D. A. L., MORGAN, W. T. J. & MOSIMANN, W. (1954). Studies in immunochemistry. 13. Preparation and properties of the 'O' somatic antigen of *Shigella dysenteriae* (Shiga). *Biochem. J.* **56**, 572.
- KAUFFMANN, F. (1932). Vergleichende Untersuchungen an Pseudotuberkulose-, Paratyphus-, Pasteurella- und Pestbacillen. *Z. Hyg. InfektKr.* **114**, 97.
- LANDY, M., TRAPANI, R. J. & CLARK, W. R. (1955). Studies on the O antigen of *Salmonella typhosa*. III. Activity of the isolated antigen in the hemagglutination procedure. *Amer. J. Hyg.* **62**, 54.
- MCCARTY, M. & LANCEFIELD, R. C. (1955). Variation in the group specific carbohydrate of group A streptococci. I. Immunochemical studies on the carbohydrates of variant strains. *J. exp. Med.* **102**, 11.
- MACPHERSON, I. A., WILKINSON, J. F. & SWAIN, R. H. A. (1953). The effect of *Klebsiella aerogenes* and *Klebsiella cloacae* polysaccharides on hemagglutination by and multiplication of the influenza group of viruses. *Brit. J. exp. Path.* **34**, 603.
- MORGAN, W. T. J. & VAN HEYNINGEN, R. (1944). The occurrence of A, B and O blood group substances in pseudo-mucinous ovarian cyst fluids. *Brit. J. exp. Path.* **25**, 5.
- NETER, E. (1956). Bacterial hemagglutination and hemolysis. *Bact. Rev.* **20**, 166.
- NETER, E. & GORZYNSKI, E. A. (1954). Erythrocyte-modifying capacity of *Shigella dysenteriae* (Shiga) antigen and its polysaccharide component. *Proc. Soc. exp. Biol., N.Y.* **85**, 503.
- NETER, E., GORZYNSKI, E. A., ZALEWSKI, N. J., RACHMAN, R. & GINO, R. M. (1954). Studies on bacterial hemagglutination. *Amer. J. publ. Hlth*, **44**, 49.
- NETER, E., WESTPHAL, O., LÜDERITZ, O., GINO, R. M. & GORZYNSKI, E. A. (1955). Demonstration of antibodies against enteropathogenic *Escherichia coli* in sera of children of various ages. *Pediatrics*, **16**, 801.
- PARTRIDGE, S. M. & MORGAN, W. T. J. (1940). Immunization experiments with artificial complexes formed from substances isolated from the antigen of *Bact. shigae*. *Brit. J. exp. Path.* **21**, 180.
- SCHÜTZE, H. (1928). *Bacterium pseudotuberculosis rodentium*. Rezeptoranalyse von 18 Stämmen. *Arch. Hyg.* **100**, 181.
- SCHÜTZE, H. (1932). Studies in *B. pestis* antigens. II. The antigenic relationship of *B. pestis* and *B. pseudotuberculosis rodentium*. *Brit. J. exp. Path.* **13**, 289.
- THAL, E. (1954). *Untersuchungen über Pasteurella pseudotuberculosis*. Lund, Sweden: Berlingska Boktryckeriet.

- THAL, E. (1956). Relations immunologiques entre *Pasteurella pestis* et *Pasteurella pseudotuberculosis*. *Ann. Inst. Pasteur*, **91**, 68.
- VOGEL, R. A. (1957). The role of lipid in hemagglutination by *Candida albicans* and *Saccharomyces cerevisiae*. *Proc. Soc. exp. Biol., N.Y.* **94**, 279.
- WESTPHAL, O. & LÜDERITZ, O. (1954). Chemische Erforschung von Lipopolysacchariden gramnegativer Bakterien. *Angew. Chem.* **66**, 407.
- WESTPHAL, O., LÜDERITZ, O. & BISTER, F. (1952). Über die Extraktion von Bakterien mit Phenol-Wasser. *Z. Naturf.* **7b**, 148.

(Received 26 July 1957)