ADSORPTION TITRATION AS A SPECIFIC SEMI-QUANTITATIVE ASSAY FOR SOLUBLE AND BOUND *PARAMECIUM* SEROTYPIC ANTIGEN

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Summary

The immobilization of paramecia when mixed with appropriate antisera has been utilized as a specific and semi-quantitative assay for the serotypic antigens (adsorption titration). While serotypic antigen in both soluble and particle-bound forms is detected by adsorption titration, only soluble antigen is detected in gel-diffusion analysis. $500\,g$, $3000\,g$, $12{,}000\,g$, and $105{,}000\,g$ particulate fractions as well as $105{,}000\,g$ and $150{,}000\,g$ cell supernatant fractions were prepared by differential centrifugation. Analyses of these fractions indicate:

(1) Relatively little of the antigen in the cell is present in soluble form.

(2) A considerable proportion of the antigen is present in a form tightly associated with the $105{,}000\,g$ precipitate.

(3) This antigen is not solubilized using the method of serotypic antigen purification described, but is liberated as soluble material by sodium deoxycholate.

(4) Over two-thirds of the antigen is present in a form loosely bound to cell particulates.

(5) About 40% of the antigenic activity is obtained by the purification procedure.

The possibilities that the serotypic antigen associated with the $105{,}000\,g$ precipitate is in the process of synthesis, is a structural–ribosomal protein, or is associated with a particle distinct from the ribosome are discussed.

I. INTRODUCTION

Bound to the surface of the ciliated protozoan *Paramecium* are a group of soluble proteins called the serotypic antigens [Sonneborn 1948; Beale 1952; Preer 1959*a*, 1959*b*; see also Beale (1954, 1957) and Beale and Wilkinson (1961) for reviews of the subject]. Normally a given cell exhibits only one of the group at a time although it has the genetic potential for synthesizing them all. Antisera prepared either against the purified protein or against homogenates of cells affect the motility of homologous cells; a typical antiserum will immobilize cells at a dilution of 1 in 400. At progressively higher dilutions, the reduction of motility will be less, until finally no effect is detectable. Antisera prepared against cell homogenates from clones exhibiting different serotypic antigens seldom show cross-reactions, suggesting that normally only one antigenic substance takes part in the immobilization reaction.

Therefore, it appeared feasible to develop an assay which would specifically and differentially detect serotypic antigens in heterogeneous cell fractions. Such fractions might be expected to adsorb immobilizing antibody only if they contain homologous serotypic antigen and such adsorption would be reflected in a lowering of the

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immobilization potency of the antiserum. In addition, the system would permit the analysis of particulate as well as soluble cell fractions and, if coupled with gel-diffusion techniques, would allow an estimate of the ratio of soluble to bound serotypic antigen.

II. METHODS AND MATERIALS

(a) Cultures

Paramecium aurelia (stock 51, syngen 4) expressing serotypic antigens 51A, 51B, or 51D were mass-cultured in a medium which had been autoclaved at 17 lb/in^2 for 40 min and consisted of 0·3% (w/v) Vegemite (Kraft Foods Ltd., Australia) plus a laboratory-isolated strain of flavobacterium. The cultures, grown at 25°C, were handled aseptically and were checked for contaminating microorganisms by plating. When the cultures had reached peak population (7–8 days, 4×10^3 cells/ml) they were harvested by centrifugation at 250 g for 5 min. The packed cells were washed once with 0·0083M Tris-HCl buffer, pH 7·5 (Tris 83), and then resuspended in Tris 83 to give a concentration of 2×10^6 cells/ml and either stored at $-60°C$ until needed or used immediately.

(b) Preparation of Purified Serotypic Antigen

Serotypic antigens 51A, 51B, and 51D were extracted and purified by modifying the original method of Preer (1959b). Preer's initial salt–ethanol extraction was replaced by homogenization of the cells in Tris 83, and the final ammonium sulphate precipitate, which was dissolved and then dialysed against 0·1M potassium phosphate buffer, pH 7·0, was passed through a DEAE-cellulose column equilibrated with the buffer. The antigen came through immediately behind the hold-up volume. Analysis of the final product by ultracentrifugation and moving boundary electrophoresis indicated that its purity was better than 97%. It gave a single band in paper electrophoresis (pH 5·0 and 8·6) and a single precipitate band in agar immuno-electrophoresis (pH 8·9). Yields averaged 1 mg per 10^7 cells.

(c) Gel-diffusion Tests

Two dimensional double gel-diffusion analyses (Ouchterloney 1948) were set up in 5-cm petri plates as previously described (Reisner and Sobey 1962). The plates were incubated in a water-saturated atmosphere at 37°C and examined over a 72-hr period. For quantitative antigenic analyses log$_2$ serial dilutions were used. The greatest dilution of a sample giving a detectable band was taken as the end-point. Two or three replicates were run for all tests.

(d) Adsorption Titration Tests

The buffer used for all dilutions (E.M.F. buffer) consisted of 0·04% NaCl, 0·01% KCl, 0·02% CaCl$_2$.H$_2$O, 0·01% MgSO$_4$.7H$_2$O, 0·005% KH$_2$PO$_4$, and 0·005% K$_2$HPO$_4$, pH 6·8 (all percentages w/v).

Antisera against 51A, 51B, and 51D animals were log$_2$ serially diluted and the highest dilution of antiserum causing complete immobilization of homologous cells

was taken as the immobilization end-point. Bulk dilutions were made up at twice the end-point concentrations, frozen in 5 ml lots, and stored at $-20°C$. These "standard sera" were thawed only once. They gave no evidence of cross-reactivity.

For adsorption titration analyses log_2 serial dilutions of the test materials were prepared. $0 \cdot 2$ ml of standard serum was mixed with $0 \cdot 2$ ml of each dilution and the mixtures were incubated in depression plates for 1 hr at 25°C. Then $0 \cdot 04$-ml portions

TABLE 1

COMPARISON OF ADSORPTION TITRATION WITH OUCHTERLONEY
GEL-DIFFUSION ANALYSES

Antigenic Type	Antigen Concentration (μg/ml)	
	Adsorption Titration*	Gel-diffusion Analysis†
51A	$1 \cdot 6$	$5 \cdot 0$
51B	$2 \cdot 0$	$4 \cdot 0$
51D	$0 \cdot 8$	$7 \cdot 0$

* Antigen concentration raising motility 1 unit.

† Antigen concentration at end-point.

of a homologous cell suspension (50–100 cells) were added and the incubation was continued an additional 2 hr. The motility of the cells in each mixture was scored from 0 to 6 using the criteria given by Sonneborn (1950):
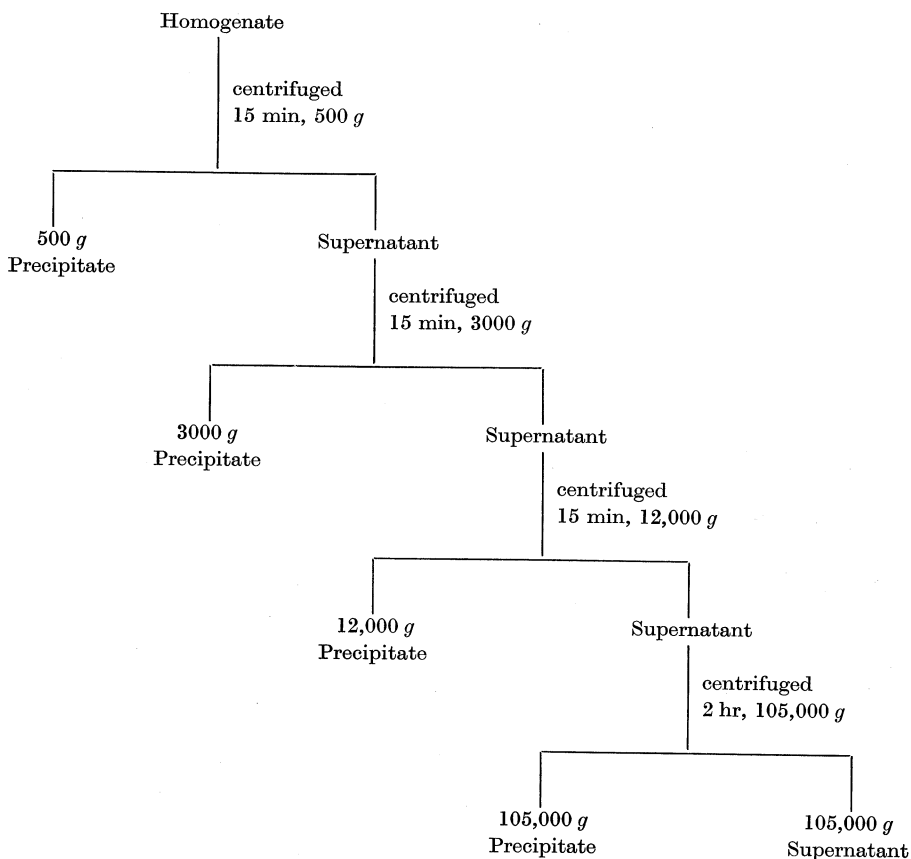
0 Complete immobilization;

1 Cells unable to leave bottom of the depression;

2 Cells swim very slowly in circles with diameters less than twice body length;

3 Cells swim very slowly throughout the depression;

4 Cells swim throughout the depression but are obviously slow;

5 Cells are barely affected;

6 Cells swim at a normal rate.

In adsorption titration a score of 6 indicates that all available immobilizing antibody is removed from solution by the fraction under test.

Preliminary tests of the standard sera indicated that day to day variation could occur in the sensitivity of homologous cells, i.e. unadsorbed standard sera gave motility scores between 0 and 2. However, adsorption titrations with purified antigen showed that reproducible results were obtained if the adsorption titration end-point was taken as 1 unit above the standard serum motility score, e.g. if the standard serum gave a motility score of 1 the adsorption titration end-point equalled 2. For every assay, standard serum was log_2 serially diluted to check simultaneously its effect on tester cells. To examine cell fractions for toxicity, separate dilution series were made in which the standard sera were replaced by E.M.F. buffer.

(e) Cell Fractionation

All steps in the cell fractionation procedure were carried out at 0–4°C. Between 50 and 100 million cells, after being concentrated as described, were immediately transferred to conical centrifuge tubes and centrifuged at 3000 g for 15 min. The tightly packed cells were resuspended in medium A (0·25M sucrose, 0·025M KCl, 0·005M MgCl$_2$, and 0·05M Tris, adjusted to pH 7·6 with HCl)—1 ml of medium A per 5×10^6 cells. After homogenizing the suspension in a glass–Teflon tissue grinder (0·15 mm clearance) for 90 sec at 500 r.p.m. (>99% cell breakage) the procedure outlined in the flow diagram below was followed:

```
                        Homogenate
                            |
                            |  centrifuged
                            |  15 min, 500 g
              _____|_____
             |                             |
          500 g                        Supernatant
        Precipitate                        |
                                           |  centrifuged
                                           |  15 min, 3000 g
                            _____|_____
                           |                               |
                        3000 g                         Supernatant
                      Precipitate                          |
                                                           |  centrifuged
                                                           |  15 min, 12,000 g
                                            _____|_____
                                           |                               |
                                        12,000 g                       Supernatant
                                      Precipitate                          |
                                                                           |  centrifuged
                                                                           |  2 hr, 105,000 g
                                                            _____|_____
                                                           |                               |
                                                       105,000 g                       105,000 g
                                                      Precipitate                      Supernatant
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The 500 g precipitate, which consisted of whole cells, large cell fragments, and macro- and micronuclei, was retained only for the work on unwashed fractions (Table 2). The other precipitates were washed once or twice in E.M.F. buffer and then resuspended in 4 ml of E.M.F. buffer. For the examination of some soluble cell fractions the 12,000 g supernate of cell homogenates was centrifuged at 150,000 g for 40 min. The top two-thirds of supernatant was removed and dialysed overnight against Tris 83.

(f) Protein Estimations

The concentration of purified antigen was calculated using the spectrophotometric method of Warburg and Christian (1942). The correction factor of 0·85 determined by Preer (1959a) for 51A was used for 51B as well when it was found that equal weights of the lyophylized antigens dissolved in equal volumes had almost identical E_{260} and E_{280} values. 51D on the other hand required no correction factor.

The protein concentration of cellular fractions was estimated using the method of Lowry *et al.* (1951).

(g) Preparation of Antisera

Rabbit antisera against homogenates of cells expressing 51A, 51B, or 51D serotypes were prepared according to the method of Sonneborn (1950).

Antisera against the 105,000 *g* precipitate were prepared as follows: Twice-washed fresh 105,000 *g* material obtained from 51A cells was emulsified with an equal volume of Freund's complete adjuvant (Freund 1956). The emulsion was injected into rabbits subcutaneously and intramuscularly. After 3 weeks a booster injection was given and the animals were bled 7 days later.

All antisera were heated at 56°C for 45 min before use.

TABLE 2

PROTEIN AND ANTIGEN CONTENT OF UNWASHED 51A CELL FRACTIONS
OBTAINED BY DIFFERENTIAL CENTRIFUGATION*

Fraction	Protein (mg)	Antigen (mg)	Antigen (as % protein)	$\dfrac{\text{Antigen}}{\text{Total Antigen}} \times 100$
500 *g*	512	9·8	1·9	35·5
3,000 *g*	38·4	9·8	25	35·5
12,000 *g*	41·2	2·5	5·9	9
105,000 *g*	53·7	3·4	6·4	12
Supernatant	115	2·2	1·9	8
Totals (means)	760	27·7	(3·6)	100

* The mass culture consisted of 105×10^6 cells of which 99·9% were serotype 51A.

(h) Sodium Deoxycholate Treatment of the 105,000 g Precipitate obtained from 51A Cells

A freshly prepared 105,000 *g* precipitate was resuspended and washed twice in medium A and then resuspended in medium A to give 0·86 mg/ml protein. 1 ml of the preparation was mixed with an equal volume of 1% (w/v) aqueous sodium deoxycholate solution while another 1 ml was mixed with an equal volume of water. The preparations were held at 1°C for 1 hr, then made up to 12 ml with medium A and centrifuged for 2 hr at 105,000 *g*. The top two-thirds of the two supernatants were carefully pipetted into dialysis tubing and dialysed exhaustively against Tris 83. The remaining supernatant was discarded and the precipitates washed twice with

medium A by resuspension in 12 ml of buffer followed by centrifugations for 1 hr at 105,000 g. The final precipitates were resuspended in 3 ml of E.M.F. buffer. Adsorption titration curves of the four final fractions are shown in Figure 2.

III. Results

(a) Sensitivity and Specificity of Adsorption Titration

Table 1 shows the sensitivity of adsorption titration when used to assay purified antigen preparations; it demonstrates that the technique is somewhat more sensitive than our double gel-diffusion assay. As to the specificity of the assay, neither particulate fractions (3000 g, 12,000 g, and 105,000 g) nor the 105,000 g supernatant showed demonstrable cross reactions. In addition, when cell

TABLE 3

ANTIGENIC CHARACTERIZATION OF TWICE-WASHED PARTICULATE FRACTIONS FROM 51D PARAMECIA

| Fraction | Protein (mg/ml) | Antigen (mg/ml)* | | Antigen (% protein) | Loosely Bound Antigen / Total Antigen |
		Adsorption Titration	Gel Diffusion		
3,000 g	3·2	0·050	0	1·6	0
	6·5	0·10	0	1·5	0
	5·1	0·10	0	2·0	0
12,000 g	0·2	0·013	0	6·5	0
	0·3	0·025	0	8·3	0
	6·8	0·41	0·014	6·0	0·03
	6·9	0·21	0·007	3·0	0·03
105,000 g	0·5	0·026	0	5·2	0
	0·5	0·10	0	20·4	0
	2·8	0·21	0	8·9	0
	4·2	0·41	0	9·8	0

* Loosely bound antigen was determined by the gel-diffusion assay, total antigen by adsorption titration assay.

fractionations were performed in medium A to which purified heterologous antigen had been added in concentrations 100–300 times above the detectable minimum, no heterologous antigen was detected on the particulate fractions (once-washed). Within the limits of the assay, all of the heterologous antigen could be demonstrated in the 105,000 g supernatant. Thus, non-specific adsorption of soluble protein to the cell particulates during fractionation appears unlikely.

(b) Association of Serotypic Antigen with Cell Fractions

The distribution of antigen in unwashed cell fractions obtained from 51A cells is shown in Table 2. The data demonstrate that about 3·5% of the cell's protein is antigen and that the bulk of it is associated with the 500 g and 3000 g fractions (71%).

The total antigen in the preparation amounted to about 28 mg or $2 \cdot 6$ mg/10^7 cells. The antigen purification procedure yields about 1 mg/10^7 cells, i.e. about 40% of the antigenic activity is obtained by the procedure.

Table 3 summarizes the data obtained from twice-washed particulate fractions obtained from 51D cells. Virtually all antigen detectable by gel diffusion is eliminated but adsorption titration demonstrated that significant amounts of the antigen are bound to each of the particulate fractions. Results obtained using fractions from 51A and 51B animals were essentially similar to those reported in Table 3 except that the 105,000 g precipitate from 51A cells appears richer in serotypic antigen. 51A antigen expressed as percentage protein of the 105,000 g fraction is 28 $\pm$ 16%, based on six determinations.

TABLE 4

SEROTYPIC ANTIGENIC PROPERTIES OF 150,000 g SUPERNATANT COMPARED WITH PURIFIED SOLUBLE ANTIGEN*

| Fraction | Protein Concentration at End-point (μg/ml) | | B/A | Antigen as % Protein (adsorption titration) | Antigen as % Protein (gel diffusion) |
	Adsorption Titration (A)	Gel Diffusion (B)			
Supernatant (51A)	80	640	8	$1 \cdot 8$	$0 \cdot 8$
Purified 51A	$1 \cdot 4$	$5 \cdot 4$	4	100	100
Supernatant (51B)	46	370	8	$0 \cdot 2$	$0 \cdot 4$
Purified 51B	$0 \cdot 1$	$1 \cdot 6$	16	100	100

* The anti-51A and anti-51B sera used for analyses are different from those used in the other studies reported, thus requiring separate end-point determinations for the purified antigen.

Analyses of two *Paramecium* soluble fractions (150,000 g supernatants) are given in Table 4. The data indicate that there is no detectable difference between purified antigen and the antigen present in the soluble fraction. Furthermore, both of the soluble fractions as well as the purified antigens were able to remove all immobilizing antibody when they were used in sufficiently high concentrations. The total calculated amount of antigen present in the 150,000 g supernatants was $3 \cdot 1$ mg for 51A and $0 \cdot 7$ mg for 51B, which is about 5–20% of the amount of purified antigen obtained when the same number of cells is subjected to the complete purification procedure.

(c) Detailed Examination of the 105,000 g Particulate Fraction

Electron microscopy of the 105,000 g fraction (Fig. 1) revealed that it consists predominately of particles about 175 by 225 Å and aggregates of these particles. The particles have the characteristic appearance of ribosomes.

To test the possibility that the more rigorous conditions used in gel-diffusion assay compared to adsorption titration destroyed antigenic activity in the preparation, a twice-washed preparation was halved and one-half was subjected immediately to adsorption titration while the other was incubated for 72 hr at 37°C in the presence of 0·01% (w/v) Merthiolate prior to assay. The results indicated that not more than 50% of the activity was lost.
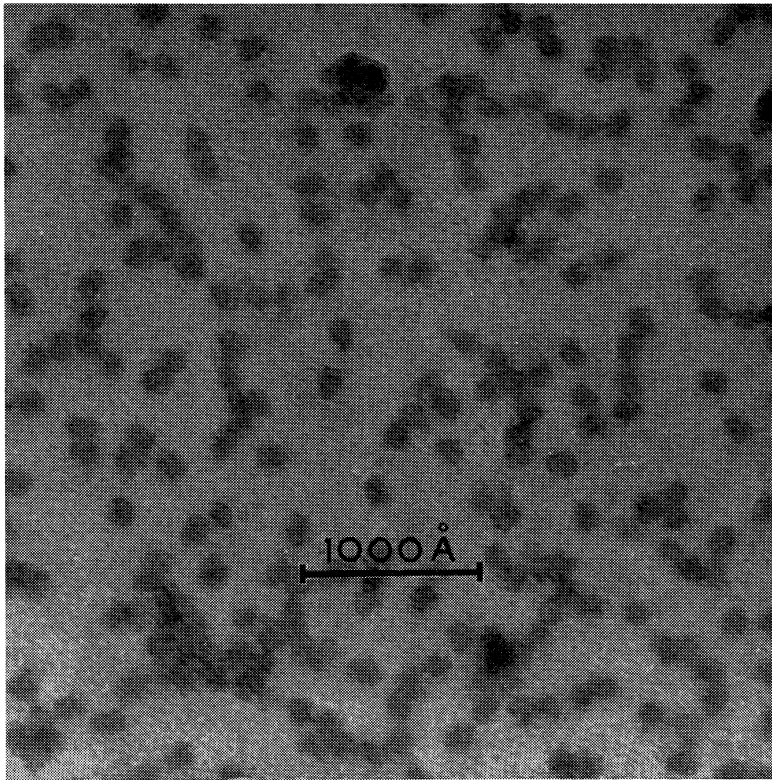

Fig. 1.—Electron photomicrograph of twice-washed 105,000 *g* pellet stained with phosphotungstic acid.

(d) *Exposure of the 105,000* g *Precipitate to Sonic Disintegration, to the Antigen Purification Procedure, and to Sodium Deoxycholate*

Several investigators have reported the liberation of nascent protein from microsomal material after sonic treatment (Campbell, Greengard, and Kernot 1960; Campbell 1961; von der Decken and Campbell 1961; von der Decken 1963). However, subjecting a 105,000 *g* fraction from 51A cells to sonic disintegration for 2 min failed to liberate any antigenic activity from it.

Similarly when washed 105,000 *g* fractions were subjected to the antigen purification procedure no antigenic activity was recovered in the final supernatant. Two such fractions were obtained and subjected to the entire purification procedure

except that column chromatography was omitted. Less than 2% of the fractions' protein was obtained and there was no serotypic antigenicity even though adsorption titration of the starting materials indicated 20 mg of antigen in one fraction and 5 mg in the second.

The effect of 0·5% sodium deoxycholate on a washed 105,000 g precipitate is shown in Figure 2. It demonstrates that a twice-washed 105,000 g pellet resuspended in the absence of sodium deoxycholate and centrifuged at 105,000 g retained over 98% of its antigenicity. However, when the pellet is resuspended in 0·5% deoxycholate all of the antigenicity appears in the 105,000 g supernatant. Within the limits of the assay no antigenic activity was either lost or gained through solubilization. Electron photomicrographs of treated preparations failed to reveal any structural changes in the ribosomes.
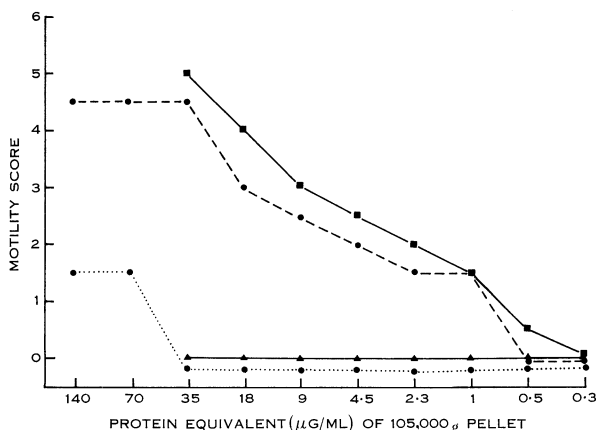

Fig. 2.—Adsorption titration curves obtained from twice-washed resuspended 105,000 g pellets subjected to a third centrifugation at 105,000 g for 2 hr. For details of the preparation see Section II.

● · · · · · · ●   Supernatant from untreated material.
● – – – – ●   Pellet from untreated material.
■ ———— ■   Supernatant from material resuspended in 0·5% deoxycholate.
▲————▲   Pellet from material resuspended in 0·5% deoxycholate.

(e) *Ability of the 105,000 g Fraction to Elicit Immobilizing Antibody*

Antisera against a twice-washed 105,000 g precipitate from 51A cells was prepared as described in Section II. Although the material used for injection had no demonstrable antigenic activity in gel-diffusion analysis, it produced antisera of considerable potency and specificity. The data are summarized in Table 5. In addition to the immobilization tests the three antisera were run in Ouchterloney plates against purified antigens 51A, 51B, and 51D. Precipitation bands were observed only with 51A antigen.

IV. DISCUSSION

The basic assumption for the investigation described is that the serotypic antigens are uniquely detected by the immobilization reaction. Therefore, once it

had been demonstrated with purified serotypic antigens that adsorption titration analysis was sufficiently sensitive (Table 1), it remained to determine the specificity of the test. The heterologous antigenic analyses indicated no detectable degree of cross-reaction between cell fractions obtained from cells expressing different serotypes. Furthermore, if adsorption of soluble serotypic antigen to cell particulates occurs during preparation, it must be of a highly specific character, for heterologous soluble antigen introduced into the system is undetectable except in the supernatant fraction (a fraction which contains a minor fraction of the homologous antigen). The additional demonstration that the 105,000 g fraction binds antigen in such a way that neither sonic disintegration nor the complete antigen purification procedure is able to liberate the bound antigen indicates that the fraction's antigenicity is not an artefact of adsorption.

TABLE 5

ANTIBODY PRODUCTION IN RABBITS USING 105,000 g PRECIPITATE FROM 51A CELLS

Injection	Serum 1	Serum 2	Serum 3
Initial			
Protein content (mg)	3·0	1·5	0·75
Estimated antigen equivalent (μg)	800	400	200
Booster			
Protein content (mg)	5·7	2·8	1·4
Estimated antigen equivalent (μg)	3,200	1,600	800
Highest serum dilution causing complete immobilization of:			
51A cells	1 : 640	1 : 640	1 : 640
51B and 51D cells	< 1 : 10	< 1 : 10	< 1 : 10
Highest serum dilution causing detectable reduction of motility of:			
51A cells	1 : 5000	1 : 2500	1 : 1300
51B cells	1 : 20	1 : 40	1 : 40
51D cells	< 1 : 10	< 1 : 10	< 1 : 10

The utilization of adsorption titration in conjunction with gel diffusion in the analysis of *Paramecium* cell fractions demonstrates the serotypic antigen to be present in three forms: (1) in solution in the cytoplasm; (2) loosely bound to cell particulates; and (3) tightly bound to cell particulates. The data presented in Table 2 demonstrate that less than one-tenth of the detectable antigen is found in the cell's soluble fraction and the antigenicity is indistinguishable from that exhibited by the purified product (Table 4). Assuming that homogenization has some tendency to liberate loosely bound antigen into the soluble fraction, the actual percentage of antigen present in solution in the cell may be very small indeed.

With regard to antigen loosely bound to cell particulates the 3000 g fraction is of particular interest. Over one-third of the total antigenicity is obtained in this fraction (Table 2) and the antigen accounts for 25% of the total protein in the fraction.

Two washes remove about 95% of the antigenic activity (Table 3) relative to the fraction's protein content, i.e. there is a highly disproportionate loss of antigenic activity indicating its loose binding to the fraction. Since it is not unlikely that the antigenic activity in the 500 g fraction is also predominantly loosely bound, it would appear that over two-thirds of the cell's antigen exists in this form. Finally, it should be noted that microscopic examination of the 3000 g fraction revealed it to be composed predominantly of what appear to be pellicular fragments. Here it is of interest to note that Preer and Preer (1959) and Finger *et al.* (1960) surveyed serotypic antigenicity of *Paramecium* cell fractions using, however, unwashed cell fractions and utilizing the techniques of gel diffusion and antibody production. In the former work only the loosely bound antigen would have been detected while the latter study would not differentiate between loosely and tightly bound antigen. Nevertheless, both studies revealed that cell wall and cilia preparations were highly antigenic.

While washing removed essentially all of the antigenic activity demonstrable by gel diffusion, adsorption titration revealed that significant antigenic activity remained associated with all the particulate fractions examined (Table 3) and the washed 105,000 g fraction was demonstrated to elicit specific immobilizing antibody (Table 5). For several reasons our attention became centred on the 105,000 g fraction. First, the amount of tightly bound antigenicity, particularly in material obtained from 51A cells, was large. Second, electron photomicrographs indicated that the fraction appears homogeneous, consisting of particles and aggregates of particles indistinguishable from the ribosomes seen in other organisms. Third, we have found (Reisner and Macindoe 1967) that this fraction, together with the 105,000 g supernatant, GTP, and an ATP generating system, is able to incorporate amino acid into protein. Finally, the possibility that the tightly bound antigenicity exhibited by the 3000 g and 12,000 g fractions was due to trapped 105,000 g particles, could not be excluded.

The fact that the assay conditions used in gel diffusion could not account for the total lack of detection of diffusible antigen in the washed 105,000 g fraction coupled with the observations that neither sonic disintegration nor the antigen purification procedure were able to solubilize the antigenic activity, indicated that the activity was indeed tightly bound to the particulate material and did not contribute to the soluble product obtained by purification. Two obvious interpretations can be placed on the data. First, that the serotypic antigenicity is due to nascent protein in the process of synthesis. Second, that the serotypic antigen is a ribosomal structural protein. The knowledge that the antigens have an isoelectric point of about 4 (Steers 1961) and a molecular weight of 250,000 (Preer 1959a) together with the observation that deoxycholate treatment, although virtually stripping all the antigenicity from the 105,000 g fraction (Fig. 2), caused no obvious change in the appearance of the particles in the electron microscope renders the second possibility highly unlikely. On the other hand, the conjecture that the antigenicity is due to nascent protein is difficult to reconcile with the very large amounts present—28% of the total protein in the case of preparations from 51A cells. On the assumptions that all antigen molecules in the process of synthesis exhibit antigenic potency equal to the purified finished protein (see Fig. 2), and that the

ribosome is about 50% protein and 50% ribonucleic acid and has a particle weight of about 3×10^6 daltons while the serotypic antigen has a molecular weight of 250,000 (Preer 1959a), we see that there are somewhat less than two ribosomes per antigen molecule. Furthermore, we have recently found (Macindoe and Reisner, unpublished data) from studies of the reduced and carboxymethylated form of the antigen that it is probably composed of at least three polypeptide chains. Assuming that a ribosome synthesizes one polypeptide at a time it is clear that there are insufficient ribosomes for the amount of antigen detected to be in the process of synthesis. Thus it would seem that we are dealing with a particle distinct from the ribosome but not distinguishable from it in the electron microscope, either because it is identical in appearance with the ribosome or because it has not been rendered observable by the techniques employed.

V. Acknowledgments

VI. References

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