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The Surface Antigens of *Paramecium aurelia*

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SUMMARY: A surface antigen preparation obtained from *Paramecium aurelia* absorbs from a concentrated antiserum approximately 74 % of the total antibody absorbed by the intact animal. An antiserum prepared against this preparation immobilized *P. aurelia* of the same serotype.

Antisera against the various serotypes of *Paramecium aurelia* are prepared by injecting homogenates of mechanically disrupted organisms into a rabbit (Sonneborn, 1950). These antisera contain antibodies against antigens other than the immobilization antigen (van Wagtendonk & van Tijn, 1953). In an effort to obtain partially purified preparations of the immobilization antigen, preparatory to the determination of its chemical constitution, a method was devised to obtain the antigen without disrupting the cell. This antigen preparation which includes the trichocysts, but not the cilia, will be referred to as the 'surface antigen preparation'. The properties of this preparation are described in this communication.

RESULTS

Paramecium aurelia, var. 4, stock 51.7, serotype A was grown in a bacterized lettuce extract medium. Several litres of a 24 hr. old culture were first concentrated by filtration through a Berkefeld filter. The organisms were then concentrated and partially freed from bacteria by electromigration through a sterile salt solution (van Wagtendonk, Simonsen & Zill, 1952). The volume of the concentrated organisms was carefully measured and the number of *P. aurelia* present determined by a count of samples of a suitable dilution. Molar NaCl solution was added to the suspension to reach a final concentration of 0.06 M. The mixture was gently shaken for 1 min. and centrifuged for 2 min. at 600g. The supernatant fluid, containing the trichocysts (microscopical examination showed the absence of cilia) was carefully decanted and saved. The sediment was resuspended in 0.06 M-NaCl solution and treated in the same way. The two supernatant suspensions were combined, centrifuged for 1 hr. at 2000g in a refrigerated centrifuge, dialysed for 24 hr. against running distilled water and finally concentrated by lyophilization.

The number of organisms present after the first treatment and after the second treatment was determined by a count of the organisms present in a suitable dilution and the loss by lysis calculated. It can be seen from Table 1

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that no lysis took place during the first salt treatment and that the loss due to lysis during the second treatment varied from 2 to 9 %, with a mean of 5.5 %.

The lyophilized preparations were taken up in 10 ml. of a salt solution according to Ringer (diluted 1 : 10, v/v) and 1 ml. portions were incubated with 1 ml. of 51A antiserum (diluted 1 : 2, v/v with diluted salt solution according to Ringer) for 1 hr. at 32° and left standing at 4° for 24 hr. The precipitate was centrifuged, washed once with the diluted salt solution and nitrogen determined using the micro-Kjeldahl method and the digestion mixture of Brüel, Holter, Linderstrom-Lang & Rozits (1947). The nitrogen content of the surface-antigen preparation was also determined.

Table 1. *Effect of salt treatment on the lysis of Paramecium aurelia*

No. of organisms before salt treatment	No. of organisms after 1st treatment	No. of organisms after 2nd treatment	Percentage lysis
12,800	12,800	12,300	3.9
13,500	13,500	13,150	3.0
39,700	39,700	36,100	9.1
37,000	37,000	34,400	7.0
174,000	174,000	159,000	8.6
106,000	106,000	97,000	6.6
93,000	93,000	91,000	2.2
120,000	120,000	116,000	3.3
Mean			5.5

Incubation of the antigen preparation with antisera prepared against homogenates of serotypes 51B and 51D did not result in the formation of a precipitate. Furthermore, the antigen preparation formed only one ring when incubated with 51A antiserum according to Oudin (1948).

Untreated *Paramecium aurelia*, serotype A, were incubated with the same serum and the amount of antibody absorbed determined. It can be seen from Table 2 that the absorption of antibody by whole organisms from a concentrated antiserum is only slightly greater than the absorption by the surface antigen preparation.

Table 2. *Absorption of antibody from an antiserum prepared against a homogenate of Paramecium aurelia, var. 4, serotype 51A by various preparations obtained from P. aurelia*

Preparation	No. of <i>P. aurelia</i> $\times 10^6$	Total N in preparation ($\mu\text{g.}$)	N per organism ($\mu\text{g.} \times 10^6$)	Total N absorbed ($\mu\text{g.}$)	N absorbed per organism ($\mu\text{g.} \times 10^6$)
Live	17.4	350.4	201	34.5	20
	12.0	266.3	222	55.0	46
	13.5	315.2	233	34.9	26
	12.0	298.3	249	32.8	27
	Mean				29.7
Surface antigen	15.9	9.2	5.8	24.0	15
	9.1	8.6	9.5	44.1	48
	11.6	8.6	7.4	34.0	29
	12.0	5.2	4.3	28.2	24
	Mean				29.0
Supernatant of homogenate	14.0	226.8	162	72.1	52

Finally, surface-antigen preparations were injected into a rabbit and an antiserum prepared. This antiserum immobilized *Paramecium aurelia*, serotype A, in a dilution of 1 : 200 after 2 hr.

DISCUSSION

The experiments demonstrate that the surface antigen preparation from *Paramecium aurelia*, serotype 51A, does absorb antibodies from a serum prepared against homogenates of the same type, and that an antiserum prepared against the surface antigen preparation does react specifically with organisms of the same serotype in causing immobilization of the organisms. The fact that the Oudin test shows only a single band indicates that the preparation may contain a single antigenic substance. It must be pointed out that the preparation contains trichocysts and any soluble surface substances associated with the organism. Whether the antigenic activity of the preparation should be ascribed to the trichocysts, to soluble substances, or to both, is not known.

A correction can be made in the value for the absorption of antibodies by the antigen preparation. If it be assumed that 10 % of the originally present organisms lysed, these animals could have contributed to the absorption value of the preparation. In an earlier publication (van Wagtendonk & van Tijn, 1953) the absorption by a supernatant fluid from a homogenate of *Paramecium aurelia* was determined. This value is given in Table 2. The supernatant fluid of 14,000 lysed *P. aurelia* would contribute 7×10^{-5} $\mu\text{g.}$ of absorbed nitrogen to the determined value for the absorption by the surface antigen preparation. Thus the surface antigen prepared from one *Paramecium* would absorb 22.0×10^{-5} $\mu\text{g.}$ or approximately 74 % of the total antibody absorbed by the intact organism.

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