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The Role of Complement in the Immune Reactions of *Paramecium aurelia* and *Tetrahymena pyriformis*

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Summary. The immobilizing effect of normal guinea pig serum and some serum fractions on *P. aurelia* is correlated with their haemolytic effect on sensitized erythrocytes, indicating that the activity is due to complement. Complement fixation tests were used to compare *P. aurelia* and *T. pyriformis* antigens. Complement was found to be fixed by the external antigens of *T. pyriformis* but not by those of *P. aurelia*. *Breis* formed by macerating both species of ciliate fixed complement and cross-reactions between the two *breis* and the non-homologous antisera were shown to occur. No difference, as shown by complement fixation tests, was found between the internal antigens of two varieties of *P. aurelia*. The effect of complement on the immune reactions involving *P. aurelia* and *T. pyriformis* and their homologous antisera was examined and no lytic reaction at high dilutions of the antisera was found.

INTRODUCTION

MUCH of the work done on the genetics of *Paramecium aurelia* by Sonneborn (1947) and Beale (1954) has been carried out on antigenic characters. The immunological side of this work has, however, lagged behind and for this reason the following study was undertaken.

The most obvious effect of the immune reaction in *P. aurelia* is immobilization of the animals and for this reason little is known about the other effects. In the experiments here described, complement fixation tests were used to detect the immune reaction. The action of normal serum on *P. aurelia* was also investigated in some detail.

A comparison was made of the antigens found on *P. aurelia* and *Tetrahymena pyriformis* with respect to their ability to fix complement in the presence of various antisera.

METHODS AND MATERIALS

The material used consisted of:

Paramecium aurelia, Variety 1—serotypes 103D and 103G.

P. aurelia, Variety 5—Stock 63 (serotype unknown). The stock was grown at 32° C. and designated 63X. For references to the nomenclature see Beale (1954).

Tetrahymena pyriformis, Variety 1 (Gruchy 1955) serotypes WH 14 and WH 52. Antisera to 103D, 63G and WH 14 were available.

A dried lettuce medium, extracted with water and inoculated with *Aerobacter aerogenes* was used to culture *P. aurelia* and clones of *T. pyriformis*. Other samples of *T. pyriformis* were grown on a sterile 2 per cent peptone medium at 32° C. Antiserum to the WH 14 strain was produced by injecting a rabbit with a total of 4 million animals. Nine injections were given intravenously. Two types of antisera were produced, one from animals grown

on peptone (anti-14P) and the other from animals grown on the bacteria-lettuce medium (anti-14B). Margolin, Loefer and Owen (1957) have found differences in the immobilizing antigens of *T. pyriformis* grown on the bactericidal and axenic media.

None of the reactants used in the complement fixation tests was found to be haemolytic; that is, they were not found to cause lysis of the sensitized erythrocytes. However, most of the strong *breis* used were to some extent anticomplementary, fixing complement in the absence of antiserum. In these tests *P. aurelia* cultures were concentrated, unless otherwise stated, to about a million organisms/ml. and *T. pyriformis* to between 4 and 10 million organisms/ml. Peptone cultures of *T. pyriformis* were used in the experiments owing to their high concentration of animals and freedom from bacteria.

Before each experiment the activity of the fresh guinea-pig serum was tested by incubation with sheep's erythrocytes, sensitized with rabbit antisheep serum. Serial dilutions of the guinea pig serum were added to test suspensions of this haemolytic system. The percentage of cells lysed in each tube, after incubation at 37° C. for 30 minutes, was obtained by measuring the optical density of the free haemoglobin with an EEL colorimeter and green filter. A graph was then drawn of the dilution of guinea pig serum used against the percentage lysis obtained. From this graph was found the amount of guinea pig serum which would produce 50 per cent lysis of the erythrocytes when added to the standard haemolytic system. This 50 per cent unit is used as the arbitrary standard throughout these experiments.

RESULTS

1. THE EFFECT OF COMPLEMENT ON THE IMMUNE REACTION OF *P. aurelia* 103D AND *T. pyriformis* WH 14 (GROWN ON PEPTONE)

The test animals were placed in replicates of serial dilutions of their homologous antiserum; 1/100–1/102,400 for the antiserum against *P. aurelia* 103D; and 1/10–1/10,240 for the antiserum against *T. pyriformis*. Eight complement dilutions, ½–1/512 were used in eight successive experiments.

The results showed that the addition of a dilution of complement, which by itself had no effect on the animals, did not increase the titre of the antiserum. There was also no sign of a new lethal zone in the higher dilutions of the antiserum. Where the complement dilution used did have an effect of its own, there was no evidence that the titre of the antiserum was enhanced.

One cumulative effect was observed, however, in the case of *T. pyriformis*, when the conditions were close to lethal. The animals became completely immobile for several hours, after which they were found to have formed rigid sheaths which retained their shape even after the animals had made their escape.

2. THE EFFECT OF NORMAL SERUM ON *P. aurelia* AND *T. pyriformis*

Both species of protozoa were tested with serial dilutions of fresh guinea pig serum. The maximum effect of the serum was reached, in the case of *P. aurelia*, after two hours, while the effect on *T. pyriformis* was at its greatest within half an hour. Table 1 gives these results, read at the maximum for each animal.

This lysis can be inhibited by heating the serum. However the swelling of *P. aurelia* in the ½ dilution of serum appears to be independent of heating and thus must be due to some factor other than the heat-labile complement.

TABLE 1

THE EFFECT OF NORMAL GUINEA PIG SERUM ON *P. aurelia* AND *T. pyriformis*

Animals used	*Complement dilution*						
	1/4	*1/8*	*1/12*	*1/32*	*1/64*	*1/128*	*No C'*
P. aurelia 103D	+ +++ swelling	+ +++	+ +++	+++(+)	++	—	—
P. aurelia 63X	+ +++ swelling	+ +++	+++(+)	+++(+)	++	—	—
T. pyriformis WH 14P ..	+ +++ lysis	+ +++ lysis	+ +++ swelling	+++(+)	++	(+)	—

Key to Table 1 and subsequent tables:

++++ Immobilized
+++ slight movement
+ Impeded
+++(+) Almost immobilized
++ Slow movement
(+) Retarded

It can be seen from Table 1 that *T. pyriformis* is more sensitive to normal serum than *P. aurelia*. The titre is slightly greater and lysis of the *T. pyriformis* takes place.

The following experiments were designed to show that there is a correlation between the presence of intact complement in the normal guinea pig serum and the immobilization of the ciliates by the serum. Intact complement was demonstrated by its lytic effect on suspension of sensitized erythrocytes.

Different components of complement were destroyed and the resulting serum fractions were tested against *P. aurelia*. The two heat-labile components, C'1 and C'2, of complement were destroyed by heating at 56° C. Samples of the serum were immersed in a water-bath for 5, 10 and 20 minutes at this temperature. *P. aurelia* were then tested for immobilization in the presence of these serum fractions (Table 2).

TABLE 2

THE EFFECT OF HEAT ON THE ACTION OF COMPLEMENT ON *P. aurelia*

Time heated at 56° C.	*Complement dilution*					
	1/1	*1/2*	*1/4*	*1/8*	*1/16*	*1/32*
0 min.	+ +++	+ +++	+ +++	+++(+)	+++(+)	++
5 min.	+ +++	+++(+)	++	(+)	—	—
10 min.	+ +++	+++(+)	(+)	—	—	—
20 min.	+ +++	+	(+)	—	—	—

The serum fractions containing C'3+C'4; C'1+C'2+C'3 and C'1+C'2+C'4 were prepared, following Kabat and Mayer (1948), the heat-stable components C'3 and C'4 being removed separately from the serum using Zymosan and Ammonia respectively.

TABLE 3

IMMOBILIZING EFFECT OF VARIOUS SERUM FRACTIONS ON *P. aurelia*

Serum fraction	*Serum dilution*					
	1/2	*1/4*	*1/8*	*1/16*	*1/32*	*1/64*
Complete serum (C') ..	+ +++ +	+ +++	+++(+)	++	+	(+)
C'1, C'2, C'3+C'1, C'2, C'4	+++ +	+++(+)	++	++	+	(+)
C'3, C'4+C'1, C'2, C'3 ..	+++ +	+++(+)	++	(+)	(+)	—
C'3, C'4+C'1, C'2, C'4 ..	+++	+++(+)	++	(+)	—	—
C'1, C'2, C'4	+++(+)	++	(+)	—	—	—
C'1, C'2, C'3	+	(+)	(+)	—	—	—
C'3, C'4 (heated 10 min.)	+++(+)	+	—	—		—

The volumes of the various sera were the same (0·1 ml.) in each test and ten paramecia of the 103D serotype were added to each, using a micropipette. The haemolysis curves for the serum fractions and mixtures are shown in Figs. 1–3.

The results (Table 3) suggest that the immobilization of *P. aurelia* is caused by complement and is not merely a non-specific effect of any serum protein. The immobilization

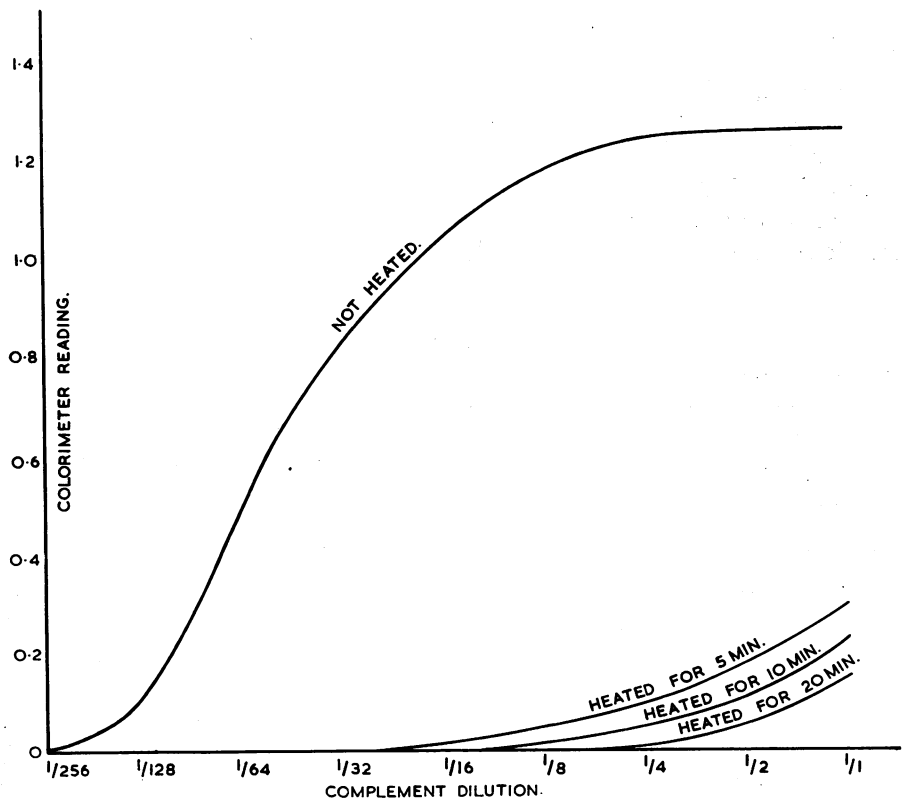

FIG. 1. The effect of heat on the complement of normal guinea pig serum.

titres of the various treated and untreated sera can be correlated with the amount of haemolysis which each produces in the presence of sensitized erythrocytes.

3. COMPLEMENT FIXATION TEXTS

A. With P. aurelia

Using a concentration of 800,000/ml. of the 103D serotype, a *brei* was produced. This concentration was used as a control in an experiment to determine whether the specific immobilizing antigens on *P. aurelia*, found on the surface of the animals, fixed complement in the presence of the homologous antiserum. The result is given in 50 per cent units of complement. The amount of complement fixed by the antigens was found by subtracting the complement fixed by the animal alone from the total. This gives the amount fixed specifically by the antigen-antibody complex (Table 4).

TABLE 4

COMPARISON OF AMOUNT OF COMPLEMENT FIXED BY THE INTERNAL AND EXTERNAL ANTIGENS

No. of animals used:	*800,000/ml.*		*400,000/ml.*	
State of animals	*Entire*	Brei	*Entire*	Brei
Units of complement fixed by animals alone ..	0·36	1·77	0·31	1·04
Units of complement fixed by animals with anti-103D (*P. aurelia* antiserum)	0	0·53	0	0·15
Units of complement fixed by animals with anti-14P (*T. pyriformis* antiserum)	0	0·19	0	0

Thus none of the external antigens of the 103D serotype fix complement in the presence of the homologous antiserum. However, when the same number of animals are macerated and the internal antigens exposed, a fair amount of complement is fixed even with the non-homologous antiserum, anti-14P, which is active against *T. pyriformis* WH 14 grown on peptone.

A further experiment showed that there was no detectable difference, as revealed by complement fixation, between the internal antigens of Variety 1 and 5. *Breis* made from animals from both varieties were tested with both types of antisera. The results are expressed here (Table 5) as the amount of complement fixed specifically with anti-63G (a Variety 5 antiserum) taken as a percentage of the amount fixed with anti-103D, the Variety 1 antiserum.

TABLE 5

COMPLEMENT FIXED WITH ANTI-63G (*P. aurelia*) AS PERCENTAGE OF COMPLEMENT FIXED WITH ANTI-103D (*P. aurelia*) BY VARIOUS *Brei*

Brei *used*	*Mean per cent anti-63G × 100/anti-103D*	Brei *dilutions*				
		1/1	*1/2*	*1/4*	*1/8*	*1/16*
	%	%	%	%	%	%
103D	80	84	68	76	82	92
103G	66	49	66	69	70	75
63X (1st sample) ..	66	55	66	69	84	58
63X (2nd sample) ..	76	93	88	88	92	50

From these results it can be seen that, although the scatter is fairly wide (49–93 per cent), there is no significant difference between the ratios of the amount fixed by the *breis* using two different antisera. Thus there can be no significant fixation of complement which involves the immobilizing antigens of 103D, otherwise the amount of complement fixed using anti-103D would be greater and the percentage (anti-63G × 100/anti-103D)

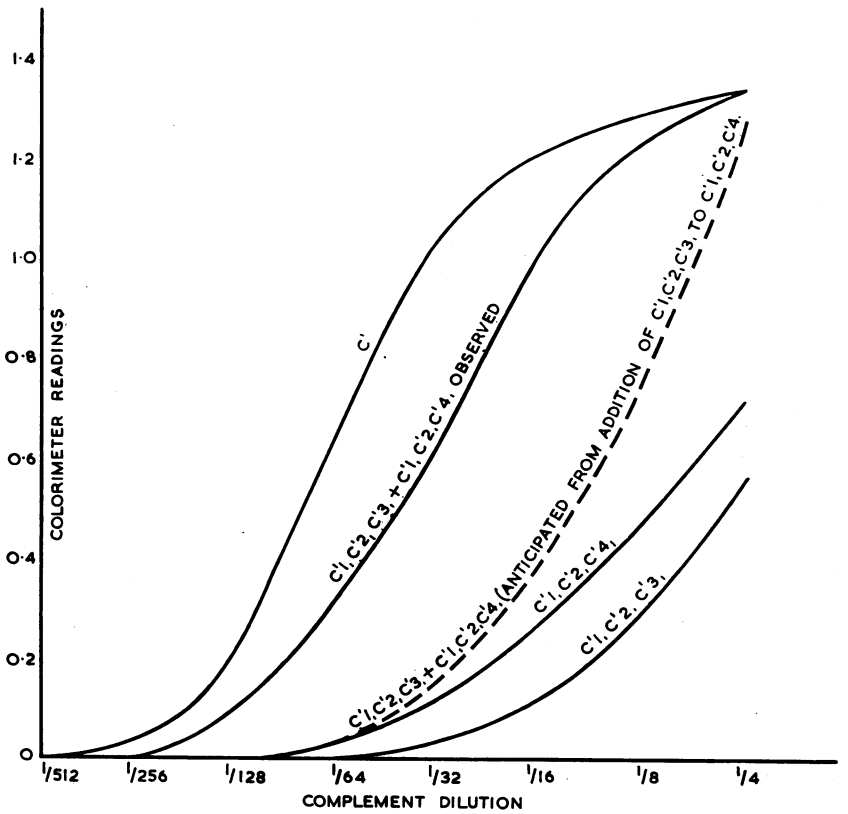

FIG. 2. The effects of removing different components of complement as shown by curves of haemolysis.

for 103D would be smaller. Similarly if there had been a significant difference in the internal antigens of 63X, 103D and 103G, the percentages of the complement fixed by the 63 stock would have been higher than those of the 103 stock. In fact the overall percentages, 73 per cent for stock 103 and 71 per cent for stock 63, show no such trend.

The actual difference in the amount of complement fixed with the two antisera using the same *brei* seems to indicate that the anti-63G has about 70 per cent of the activity of the anti-103D. This was borne out to a certain extent by using a Tetrahymena *brei* (52P) with the two antisera. The percentage anti-63G × 100/anti-103D was found to be 61 per cent.

B. With Tetrahymena pyriformis

Similar experiments were carried out using *T. pyriformis* as the antigen. Here wide cross-reactions are encountered between serotypes, and the immobilizing antigens are much less specific than those found on *P. aurelia* (Margolin).

From Table 6 it can be seen that the amount of complement fixed by *P. aurelia* antiserum (anti-103D) is of the same order as that fixed by the *T. pyriformis* anti-14B. The other interesting point is the appearance of a prozone at the highest concentrations of the *brei*. In this system the maximum amount of complement is fixed when a concentration of about a million macerated animals/ml. is used with a 1/10 dilution of antiserum.

It appears that anti-14P is of greater activity than anti-14B, anti-14B fixing only

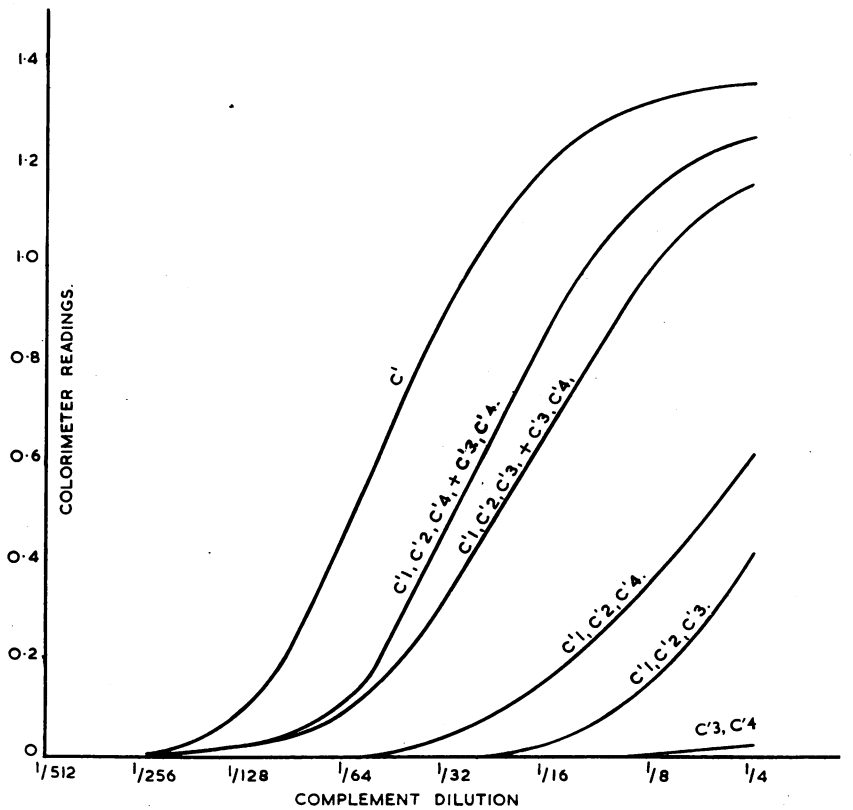

FIG. 3. The effects of removing different components of complement as shown by curves of haemolysis.

TABLE 6

COMPLEMENT FIXED BY 14P *Brei* AND VARIOUS ANTISERA

Antiserum used	*Number of macerated animals/ml.*				
	5×10^6/ml.	$2{\cdot}5 \times 10^6$/ml.	$1{\cdot}25 \times 10^6$/ml.	625,000/ml.	312,500/ml.
Units of C′ fixed by 14P animals alone	1·58	0·92	0·44	0·0	0·0
Units of C′ fixed by 14P with anti-103D (*P. aurelia*)	0·17	0·80	1·00	0·83	0·53
Units of C′ fixed by 14P with anti-14P (*T. pyriformis*)	0·25	0·80	1·16	1·07	0·17
Units of C′ fixed by 14P with anti-14B (*T. pyriformis*)	0·10	0·67	0·90	0·88	0·0

78 per cent of that fixed by anti-14P. The results obtained from the best antiserum (anti-14P) indicate that the *P. aurelia* antiserum fixes 84 per cent of that fixed by the homologous antiserum.

From the figures in Table 7 there can be no doubt that complement is fixed by the external antigens of *T. pyriformis*. The fixation of a small amount of complement with the *P. aurelia* antiserum may be the result of a common antigen which is not picked up easily in the *P. aurelia* material or is unable to fix complement when in association with other *P. aurelia* materials. Another more probable solution is that a small number of the animals lyse, freeing their internal antigens. However, this cannot explain all of the fixation, since the anti-103D × 100/anti-14P ratio has dropped from 84 to 16 per cent, indicating that the homologous antiserum is much more active. This can only be explained on the basis of most of the common antigens being inaccessible to both antisera.

TABLE 7

COMPLEMENT FIXED BY WHOLE ANIMALS (*T. pyriformis*) AND VARIOUS ANTISERA

Antiserum used	*Number of 14P animals/ml.*				
	5×10^6/ml.	$2{\cdot}5 \times 10^6$/ml.	$1{\cdot}25 \times 10^6$/ml.	625,000/ml.	312,500/ml.
Units of C′ fixed by 14P without antiserum	0·44	0·63	0·44	0·21	0·21
Units of C′ fixed by 14P with anti-103D (*P. aurelia*)	0·21	0·23	0·21	0·10	0·10
Units of C′ fixed by 14P with anti-14P (*T. pyriformis*)	1·47	1·08	1·23	1·10	0·59
Units of C′ fixed by 14P with anti-14B (*T. pyriformis*)	1·52	1·14	1·23	0·95	0·54

Another point of interest arises when the action of the *T. pyriformis* antisera on the whole animals is examined. The activity of anti-14B is comparable with that of the anti-14P antiserum. This indicates that anti-14B contains poorer complement fixing antibodies to the internal antigens but carries just as many to the external antigens.

A complement fixation test was run using WH 52 animals of *T. pyriformis* (grown on peptone), which show a heavy cross-reaction with WH 14P animals as judged by their agglutination with anti-14P serum. It was found that small but significant amounts of complement were fixed using whole animals (WH 52P) with anti-14P serum.

DISCUSSION

Unfortunately it proved to be impossible to obtain confirmation of Robertson's (1939a) results on the effect of complement on the immune reaction of certain serotypes of *T. pyriformis* with their homologous antiserum. In these experiments complement was added in dilutions which had no visible effect on the animals when used alone. However, when added to serial dilutions of the antiserum, a new lethal peak was found at a high dilution of the antiserum. The action of the antiserum alone at this dilution had no perceptible effect, but when the complement was added, agglutination followed by lysis of all the animals took place.

The failure in the present experiments to find lysis at high dilutions of the antiserum in the presence of complement may be due to several factors. The antisera were not prepared

in the same way and the antisera produced were of a much lower titre than those which Robertson used, although comparable with the titres obtained by other workers (Margolin, Loefer and Owen). Further the *T. pyriformis* stocks were different. However, the formation of tough, rigid sheaths round the animals was confirmed when both complement and antiserum were added to the test animals. Similar experiments with *P. aurelia* stocks were completely negative; mucus was extruded but no sheaths were formed.

It can be seen from the results that removal of part of the serum containing one component of complement is sufficient to destroy both the immobilizing reaction and the haemolytic effect. Thus it would appear that the immobilizing reaction of normal serum is dependent on the presence of at least three fractions which correspond to those of complement itself. It must be remembered, however, that this type of immobilization can be differentiated from that caused by immune serum. Normal serum loses its immobilizing capacity when heated to 56° C. for half an hour, while immune serum is unaffected.

Turning to the complement fixation experiments, a clear-cut difference has been shown to exist between the external antigens of *P. aurelia* and *T. pyriformis*, only the latter fixing complement in the presence of homologous antiserum. This accentuates the differences between the antigens on the two ciliates which have already been demonstrated by the lack of a definite agglutination and sheath formation in *P. aurelia* when acted upon by homologous antiserum.

It seems characteristic of *breis* that a definite prozone is formed when more than 1·25 million *T. pyriformis*/ml. are used with a 1/10 dilution of their antiserum or 250,000 *P. aurelia*/ml. are used with a 1/50 dilution of their antiserum. These concentrations seem fairly close to equal volumes of the actual material in the different *breis*. However, it must be remembered when comparing the amount of complement fixed by the *P. aurelia* and *T. pyriformis breis* that the antisera are not of the same strength, and while 1/50 *P. aurelia* antiserum and 1/10 *T. pyriformis* antiserum have relatively the same effect on their respective animals, i.e. each can be diluted, serially, seven times before they lose their effect on the whole animals, this is not a good criterion on which to judge the total concentration of the antibody involved in the reaction with the *brei*.

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REFERENCES

BEALE, G. H. (1954). *Genetics of* P. aurelia. Cambridge University Press, Cambridge.

GRUCHY, D. F. (1955). 'The breeding system and distribution of *T. pyriformis*.' *J. Protozool.*, **2**, 178.

MARGOLIN, LOEFER and OWEN. Unpublished.

ROBERTSON, M. (1939). 'A study of the reactions *in vitro* of certain ciliates belonging to the *Glaucoma-Colpidium* group to antibodies in the sera of rabbits immunized therewith.' *J. Path. Bact.*, **48**, 305–22.

ROBERTSON, M. (1939). 'An analysis of some of the antigenic properties of certain ciliates belonging to the *Glaucoma-Colpidium* group as shown in their response to immune serum.' *J. Path. Bact.*, **48**, 323–38.

SONNEBORN, T. M. (1947). 'Recent advances in the genetics of *Paramecium* and *Euplotes*.' *Adv. in Gen.*, **1**, 244–358.

KABAT, E. A. and MAYER, M. M. (1948). *Experimental Immunochemistry*. C. C. Thomas, Springfield, U.S.A.