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Opsonic, Cytophilic and Agglutinating Activity of Guinea-Pig γ_2 and γM Anti-*Salmonella* Antibodies

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Summary. Guinea-pig anti-*Salmonella typhimurium* antibodies of the γ_2 and γM classes were obtained free of each other, and their properties were studied quantitively. Macroglobulin antibodies were found to be about twenty times more efficient than γ_2, in agglutination and opsonic activity *in vivo*. The cytophilic activity of both classes of antibodies was the same.

INTRODUCTION

It is becoming increasingly clear that antibodies belonging to different immunoglobulin classes are endowed with different biological properties. These various functional capabilities are related to specific structures present in the Fc fragment of the molecule, but may be also dependent on the overall molecular configuration. Thus, macroglobulin antibodies, by reason of their plurivalence, are much more efficient than bivalent 7S ones in agglutinating or complement-fixing activity.

Guinea-pig serum contains three classes of immunoglobulins, namely γ_2, γ_1 and γM. The properties of the γ_2- and γ_1-immunoglobulins have been explored by a number of workers (Benacerraf, Ovary, Bloch and Franklin, 1963; Thorbecke, Benacerraf and Ovary, 1963) but comparatively little is known about the macroglobulin class.

The present work was done to study and compare some of the properties of 19S and 7S guinea-pig antibodies relevant to their function in immune defence mechanisms.

MATERIALS AND METHODS

Animals

Random bred guinea-pigs of both sexes, weighing 250–400 g, and Swiss mice weighing 20–25 g were used for immunization and for the performing of the opsonic test.

Antigen

Salmonella typhimurium T 34–59, grown on nutrient agar and killed in saline containing 0·5 per cent formol, was kindly supplied by Dr Le Minor of the Institut Pasteur, Paris.

Immunization

Each guinea-pig received 0·5 ml of a suspension of *Salmonella* ($4{\cdot}5 \times 10^9$ organisms) in complete Freund's adjuvant or in saline, as indicated, into each hind leg. One week later, the animals were reinoculated with the same dosage: 0·4 ml into each hind leg, and 0·1 ml intradermally in two sites on the back. Five weeks after the second injection, serum was collected by carotid section.

Bacterial agglutination titre

A volume of 0·9 ml of a suspension of *S. typhimurium* (5×10^8 organisms/ml) was added to 0·1 ml of two-fold serial dilutions of sera. Tubes were incubated for 2 hours at 37° and kept 24 hours at room temperature. The titres were expressed as the reciprocals of the highest serial dilution exhibiting agglutination.

Fractionation of guinea-pig antiserum

The γ-globulin fraction was precipitated twice at 18 per cent Na_2SO_4, dialysed against phosphate buffer 0·01 M, pH 8·0 and applied to a DEAE-cellulose column equilibrated with the same buffer. Three fractions were eluted, stepwise, at increasing buffer concentration, 0·01, 0·025 and 0·3 M, respectively.

Iodination of proteins

Guinea-pig γ_2 and γM-globulins were trace labelled with ^{125}I, according to the technique described by McConahey and Dixon (1966).

Assay of γ_2 and γM antibodies

The method described by Weiler, Hofstra, Szentivanyi, Blaisdell and Talmage (1960), as modified by Herzenberg, Warner and Herzenberg (1965), was employed. This method is based on the inhibition of the precipitation of a labelled reference antigen by specific antibody, produced by addition of unlabelled antigen to the system. Pure normal guinea-pig γ_2 and a preparation containing 70 per cent of γM were labelled with ^{125}I and used as reference standards. They were diluted in a 3 per cent bovine serum albumin solution in 0·05 M Tris (final pH 7·6). All antisera were diluted in 0·05 M Tris, containing 3 per cent bovine serum albumin, and 10 per cent normal rabbit serum, pH adjusted to 7·6. Fifty microlitres of a solution of labelled antigen, containing 1 μg protein, and 25 μl of a dilution of the corresponding antiserum were set in small tubes 6 × 60 mm. The dilution of antiserum was chosen in such a way that 50–60 per cent precipitation occurred. To the tubes were added 25 μl containing known amounts of the labelled reference antigen and buffer in the control. They were incubated at 37° for 2 hours, then centrifuged at 12,000 ***g*** for 20 minutes. Fifty microlitres of each supernatant were carefully removed and placed in a tube containing 2 ml of H_2O, and the radioactivity was measured. From these results, there was obtained a curve relating the inhibition of the precipitation of labelled antigen to the amounts of unlabelled antigen added to the tube. Analysis of anti-*Salmonella* antibodies in the various preparations was performed as follows: suspensions of *S. typhimurium* ($1{\cdot}6 \times 10^9$ organisms) were incubated at 37° for 2 hours with different amounts of γ_2 or γM antibodies. Under these conditions, 90 per cent or more of the antibody was absorbed by the organisms. As control, normal guinea-pig serum was used. After incubation and centrifugation at 3500 ***g*** for 20 minutes, the sediments, washed three times with 3 ml of phosphate buffer 1 M, pH 8·0, were resuspended in 0·1 ml Tris 0·05 M, pH 7·6; 25 μl of various dilutions of each suspension were added to the tubes containing γ_2 or γM labelled reference globulins. The antibody content was established by the degree of the inhibition of labelled antigen precipitation.

Preparation of normal guinea-pig γ_2

Normal γ_2-globulin was obtained from normal serum by DEAE-cellulose chromatography with phosphate buffer 0·0025 M, pH 8·0.

Preparation of normal guinea-pig γM

Normal guinea-pig γM-globulin was obtained as follows: animals were inoculated into the hind legs with complete Freund's adjuvant in saline, boosted after 1 week and bled 4 weeks later. This treatment enhances the level of 19S globulins in the serum (unpublished observation). Two hundred millilitres of the serum were precipitated twice with Na_2SO_4 18 per cent and dialysed against H_2O. The precipitate was washed three times with H_2O, dialysed against Tris 0·1 M–NaCl 1 M, pH 8·0, and applied to a column of Sephadex G-200 equilibrated with the same buffer. The ascendent part of the first peak was concentrated by precipitation at 18 per cent Na_2SO_4, and filtered again in Sephadex G-200. The final product was incubated for 1 hour at room temperature with heparin (13 U.I./ml) and $MnCl_2$ (final 0·025 M) to precipitate the lipoproteins. The mixture was centrifuged at 12,000 ***g*** for 20 minutes. The supernatant was dialysed against saline, and concentrated by precipitation at 18 per cent Na_2SO_4. The resultant preparation consisted of γM-globulins contaminated by other macromolecular proteins. In order to determine the degree of purity, an aliquot of the product was labelled with ^{125}I, and precipitated with specific rabbit anti-γM antiserum. The amount of radioactivity precipitated was 70 per cent. It was assumed that this percentage represented the proportion of γM-globulin in the preparation.

Reduction by mercaptoethanol

A volume of 0·2 ml of guinea-pig anti-*S. typhimurium* antiserum, or of its DEAE-cellulose chromatography fractions, was incubated in phosphate buffer 0·3 M, pH 8·0, containing 0·1 M mercaptoethanol for 60 minutes. After incubation, an excess of iodoacetamide was added and the samples were dialysed against buffered saline, pH 8·0.

Opsonic activity

The opsonic activity was measured as described by Biozzi, Stiffel, Halpern, Le Minor and Mouton (1961), using ^{125}I-labelled bacteria. The doses employed were the following: 5×10^8 organisms/guinea-pig (weight, 250 g) and 5×10^8 organisms/20 g for the mice. The animals were injected intravenously, and the curve of blood clearance of the radioactivity was obtained by analysing blood samples taken from the retroorbital plexus at various times. The phagocytic index, K was calculated by the equation:

$$K = \log \frac{C_1 - \log C_2}{t_2 - t_1}$$

where C_1 and C_2 represent the blood radioactivity at the times t_1 and t_2 respectively. The opsonic activity of the various antibody preparations was established by determining the phagocytic index after intravenous inoculation of different dilutions of each preparation, 5 minutes after injection of the labelled *Salmonella*. The enhancement of the phagocytosis was expressed as the difference in phagocytic index after and before antibody injection. At least five animals were used to establish each point.

Cytophilic activity

Peritoneal cells of guinea pigs were collected as described by Boyden (1964), washed twice with phosphate buffer 0·15 M, pH 8·0, and resuspended in the same buffer to a final concentration of $2{\cdot}5\times10^6$ cells/ml. Volumes of 0·8 ml of this suspension (2×10^6 cells) were added to 0·2-ml aliquots of ten-fold serial dilutions of guinea-pig γ_2 or γM anti-*S.*

typhimurium antibodies. As a control, normal guinea-pig serum was employed. The tubes were kept 2 hours at room temperature and were then washed three times in the cold by centrifugation with phosphate buffer 0·15 M, pH 8·0. The pellets were resuspended in 0·3 ml of the same buffer, and 0·2 ml of a suspension of *S. typhimurium* ^{125}I ($2{\cdot}5 \times 10^9$ organisms/ml) was added to each tube. After overnight incubation at 4°, the tubes were gently shaken and centrifuged in the cold for 7 minutes at 150 ***g***. The sediments were washed twice by centrifugation for 7 minutes at 150 ***g*** with 4 ml of cold phosphate buffer 0·15 M, pH 8·0. The pellets were suspended in 2 ml of H_2O and their radioactivity was measured. Some radioactivity was always found in the control tubes (cells treated with normal serum), amounting to less than 10 per cent of the radioactivity present in the preparations treated at the higher concentration of antibodies. The titre of cytophilic activity was expressed as the antibody concentration at which the radioactivity was twice that of the control tubes.

RESULTS

ANTI-*Salmonella* ANTIBODY PRODUCTION

The antibody produced in guinea-pigs following inoculation with killed *Salmonella* by different routes, is mostly of the macroglobulin class, very small amounts of 7S antibody being generally produced. In order to compare the relative activity of 19S and 7S anti-*Salmonella* antibodies, it was necessary to develop a method of immunization resulting in an important production of both classes of antibody. After trying various procedures and schedules of immunization, it was found that inoculation of *Salmonella* incorporated into complete Freund's adjuvant stimulated the formation of sizable amounts of both 19S and 7S antibodies. An example of the time-course of antibody production after intramuscular injection of *Salmonella*, with and without adjuvant, is presented in Table 1. When the animals were injected with bacteria alone, the antibody produced was entirely of the 19S class, as evidenced by its susceptibility to mercaptoethanol. On the contrary, use of complete Freund's adjuvant resulted in the production of considerable quantities of 7S antibody. It may be noted that the titre of 19S antibody was also much higher (eightfold in this experiment) in the animals inoculated with adjuvant.

TABLE 1

19S AND 7S ANTIBODY PRODUCTION IN GUINEA-PIGS INJECTED WITH *Salmonella typhimurium* IN COMPLETE FREUND'S ADJUVANT OR IN SALINE

Days of immunization*	Agglutinating titre			
	Without adjuvant		With adjuvant	
	Fresh serum	ME-treated serum†	Fresh serum	ME-treated serum
0	0	0	0	0
7	160	0	80	0
14	640	0	640	0
21	320	0	1280	80
28	160	0	5120	160
35	160	0	5120	320

* Animals injected on days 0 and 7 with 9×10^9 organisms.

† ME, 0·1 M-mercaptoethanol.

TABLE 2

SEPARATION OF γ_2 AND γM ANTI-*Salmonella* GUINEA-PIG ANTIBODIES BY CHROMATOGRAPHY ON DEAE-CELLULOSE

Fraction	Volume (ml)	Agglutinating titre	
		Before mercaptoethanol	After mercaptoethanol
Initial serum*	16	1280	320
First fraction, 0·01 M	56	64	64
Second fraction, 0·025 M	52	0	0
Third fraction, 0·30 M	86	125	0

* Pool A, from four animals.

SEPARATION OF γ_2 AND γ_M ANTIBODIES

Two different batches of guinea-pig anti-*Salmonella* antisera were fractionated by DEAE-cellulose to obtain 19S and 7S antibodies. Preparation A was a pool of serum from four animals, and preparation B, from ten animals. The agglutinating titre of pools A and B were 1280 and 10,000, respectively. After mercaptoethanol, the titres were 1 : 320 in both pools. After chromatography on DEAE-cellulose, three fractions were obtained from each pool, eluted at buffer concentration of 0·01, 0·025, and 0·3 M, pH 8·0. Immunodiffusion analysis with specific rabbit antisera revealed only γ_2 in the first fraction, and a mixture of γ_2 and γ_1 in the second one. The third fraction, eluted at 0·3 M, contained γ_2, γ_1 and γM, together with other serum components. When tested for anti-*Salmonella* antibody, agglutinating activity was found in the first and in the third fraction, but not at all in the second one. Moreover, it was found that the antibody in the first fraction was completely resistant to mercaptoethanol, while the antibody in the third fraction was completely destroyed by this treatment.

These results indicate that the first fraction contained only γ_2 antibody, and the third fraction only γM. No antibody of γ_1 class was found. The results of the fractionation of pool A is shown in Table 2. From these results it can be calculated that the recovery of antibody, after the fractionation, was 70 per cent. Quantitative analysis of γ_2 and γM anti-*Salmonella* antibodies were performed, as reported, in the corresponding chromatographic fractions from pools A and B.

AGGLUTINATING ACTIVITY

A comparison of the bacterial agglutinating activity of γ_2 and γM antibodies, from pools A and B, is shown in Table 3. γM antibody was more active than γ_2 in both preparations, on a weight basis, the average ratio being twenty times.

TABLE 3

AGGLUTINATING ACTIVITY OF γ_2 AND γM ANTI-*Salmonella* GUINEA-PIG ANTIBODIES

Antibody concentration (μg/ml)	Pool A		Pool B	
	γ_2	γM	γ_2	γM
0·800	+	+	+	+
0·400	+	+	−	+
0·200	−	+	−	+
0·100	−	+	−	+
0·050	−	+	−	+
0·025	−	−	−	+
0·012	−	−	−	−

OPSONIC ACTIVITY

The opsonic activity of γ_2 and γM antibodies was measured in guinea-pigs and mice. Varying amounts of antibody were injected intravenously in animals previously inoculated with ^{125}I labelled *Salmonella typhimurium*, and the enhancement produced in the rate of phagocytosis was measured. It was found that both γ_2 and γM were active in the two species, although the relative activity was not the same. In the guinea-pig, γM antibodies were twenty times more active than γ_2, this ratio being constant throughout the dose–response curve. When the amount of antibody inoculated was increased, the rate of phagocytosis reached a maximal value of 0·300 with both classes of antibody. The experimental data obtained with fraction 1 of Pools A and B (γ_2), and with fraction 3 of Pool B (γM), is reported in Table 4. The opsonic activity of mercaptoethanol-treated γ_2 and γM antibodies was not detectable.

TABLE 4

OPSONIC ACTIVITY OF γ_2 AND γM ANTI-*Salmonella* GUINEA-PIG ANTIBODIES IN THE HOMOLOGOUS SPECIES

μg of antibody injected animal of 250 g	Rate of phagocytosis*				
	Pool A			Pool B	
	γ_2	γM	γM-treated with mercaptoethanol	γ_2	γ_2-treated with mercaptoethanol
0·01		0·030	0·020		
0·02		0·072	0·020		
0·04		0·097			
0·09			0·020		
0·17		0·170			
0·25				0·037	
0·50	0·080	0·300		0·085	0·030
0·86		0·300			
1·00				0·094	0·030
4·00				0·217	0·030
20·00	0·300			0·300	0·030
40·00	0·300			0·300	

* Values are phagocytic indices (K).

When guinea-pig antibodies were tested in the mouse, γM was again more active than γ_2, but the ratio of activity was only five-fold. When the antibody concentration was increased, the rate of phagocytosis obtained with γM was maximal ($k = 0{\cdot}300$), but in the case of γ_2 antibodies, the value of 0·160 could not be surpassed. Contrary to the case in the homologous system, the opsonic activity in mice was not so strongly affected by the treatment of the guinea-pig γ_2 and γM antibodies with mercaptoethanol. A residual activity of about 10–20 per cent remained in both cases, as seen in Table 5. However, the enhancement of phagocytosis produced by mercaptoethanol-treated γ_2 antibodies had a particular time-course: the increase in the rate of phagocytosis occurred only after a 'latent' period, following the intravenous injection of antibody. This latent period lasted a few minutes and was inversely proportional to the dose of antibody used. An example of this effect is seen in Fig. 1.

CYTOPHILIC ACTIVITY

The cytophilic activity of γ_2 and γM antibodies was studied by measuring the activity of different dilutions of both classes of antibodies. The results obtained are summarized in Table 6.

TABLE 5

OPSONIC ACTIVITY OF γ_2 AND γ_M ANTI-*Salmonella* GUINEA-PIG ANTIBODIES IN THE MOUSE

μg of antibody injected animal of 20 g	Rate of phagocytosis†				
	Pool A			Pool B	
	γ_2	γM	γM-treated with mercaptoethanol	γ_2	γ_2-treated with mercaptoethanol
0·02		0·055			
0·04		0·083			
0·07	0·031	0·120	0·015		
0·11	0·049				
0·17		0·184			
0·24	0·097			0·093	
0·30	0·125	0·247	0·052		0·015
0·87	0·136	0·300			
1·90	0·160	0·300		0·160	0·048
5·00					0·130
10·00	0·160			0·160	0·160
20·00	0·160			0·160	

† Values are phagocytic indices (K).

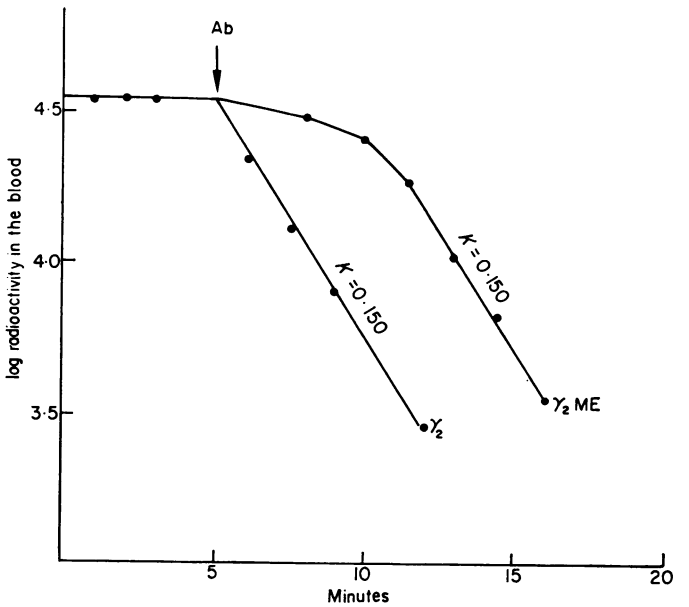

FIG. 1. Phagocytosis of *Salmonella typhimurium* (5×10^8 organisms/20 g animal) in mice inoculated with 1 μg of guinea-pig intact γ_2 antibodies or with 10 μg of guinea-pig mercaptoethanol-treated γ_2 antibodies.

It appears that γ_2 and γM antibodies possess the same degree of activity, on a weight basis. It was repeatedly found that a concentration of 0·04 μg Ab/ml was the limit of positivity of the cytophilic test employed. Treatment by mercaptoethanol followed by alkylation completely abolished the activity of γM antibodies, but did not affect the γ_2 preparation.

Since the antibody content was only a minimal fraction of the total γ-globulin present in the preparations studied, it was reasonable to assume that the observed cytophilic activity

TABLE 6

CYTOPHILIC ACTIVITY OF γ_2 AND γM ANTI-*Salmonella* GUINEA-PIG ANTIBODIES

Antibody concentration (μg/ml)	Cytophilic activity					
	Pool A		Pool B			
	γM	γ_2	γM	γ_2	γM-treated with mercaptoethanol	γ_2-treated with mercaptoethanol
4	+	+	+	+	−	+
0·4	+	+	+	+	−	+
0·04	+	+	+	+	−	+
0·004	−	−	−	−	−	−

also reflected whatever competition for the binding to the cell surface could exist between antibody and not antibody γ-globulin. To explore this possibility, cytophilic tests were performed, at all dilutions of antibody, in the presence of 400 μg/ml of normal γ_2-globulin. The cytophilic activity was the same as in the controls. This experiment suggests that, in the conditions used in the present test, the results were not affected by competition phenomena.

DISCUSSION

The biological activity of macroglobulin antibodies is not very well known, mostly on account of the difficulties to produce, quantify and isolate in pure form amounts sufficient for study. In particular, comparisons between macroglobulin and 7S antibodies are of interest to elucidate the role of the plurivalence of 19S class in the acquisition of the various biological activities. The present work was directed to investigate one of the biological properties of paramount importance in many immune defence mechanisms, namely the opsonizing capacity. The antigen selected was the O somatic antigen of *Salmonella typhimurium*, the techniques for the study of the immune phagocytosis of this bacterium having been well developed (Biozzi *et al.*, 1961). In addition, the rate of blood clearance of iodine-labelled *Salmonella* is rather low in normal guinea-pigs and, in consequence, it is easy to establish with certainty the effect of passively administered antibody.

A first requirement for this investigation was the development of an immunization method leading to the production of both 7S and 19S antibodies in sufficient amounts. It was found that immunization of animals with killed organisms, incorporated in an emulsion of complete Freund's adjuvant, resulted in a good stimulation of both 7S and 19S antibody synthesis. The antibody concentration in the serum attained comparable values of about 100–400 μg/ml. This refers to antibody directed to the whole bacteria, since the method of analysis makes use of whole bacteria as immunoadsorbent. It is to be remembered that these antibody concentrations are very small when compared to those obtained in the same animal species with protein antigen by the same immunization schedule, in which case many milligrams of antibody per millilitre are usually obtained (Binaghi, 1966).

Fractionation of the antisera by DEAE-cellulose chromatography allowed a complete separation of 7S and 19S classes. The antibody present in the 7S fraction was only of the γ_2 class, no antibody activity having been found in the fraction containing γ_1, eluted at 0·025 M phosphate, pH 8·0. This fraction also contained γ_2, a fact indicating that anti-*Salmonella* antibody belongs to the slow-moving γ_2-globulin fraction. The absence of γ_1

antibody was disappointing, since it would have been interesting to compare also the activity of this class. The same result was obtained after immunization with a *Salmonella typhi* strain T 415, possessing H and O antigen (unpublished observation). Also, Bloch, Kourilsky, Ovary and Benacerraf (1963) reported no γ_1 antibody production in guinea-pigs inoculated with *Esch. coli*. It remains to be clarified whether the failure to obtain γ_1 antibody was related to the method of immunization or to the chemical nature of these antigens.

The agglutinating activity of γM antibody was much higher than that of the γ_2. On a weight basis, the difference was about twenty times. These results agree with previous reports in the literature using rabbit antibody to red cells (Stelos and Talmage, 1957), rabbit anti-arsanilate (Groff and Shulman, 1965), rabbit anti-*Salmonella* (Robbins, Kenny and Suter, 1965) and rat anti-dinitrophenyl (Binaghi and Oriol, 1968). The reason for the high agglutinating efficiency of γM antibodies is probably a consequence of plurivalency, as shown in other systems, where the association constant for a monovalent hapten was found to be approximately the same for γ_2 and γM antibodies (Onoue, Tanigaki, Yagi and Pressman, 1965; Binaghi and Oriol, 1968).

The opsonic activity of γ_2 and γM antibodies was measured by the enhancement of phagocytosis of the corresponding bacterial antigen. The rate of phagocytosis determined by γM antibodies was about twenty times higher, at all concentrations, than that determined by γ_2 antibodies, when measured in the homologous species. Apart from this difference of activity, the maximal rate of phagocytosis observed was the same for both classes of antibodies, namely $K = 0{\cdot}300$. This value is close to the maximum physiological possibility of the animal, which is limited by the hepatic blood flow. Treatment with mercaptoethanol strongly affected the opsonic activity of both classes of antibodies.

When the guinea-pig antibodies were tested in mice, different results were obtained. The activity of γM was still higher than that of the γ_2, but the difference was only five-fold. Moreover, the maximal rate of phagocytosis was obtained with γM but not with γ_2, even at the higher concentration tested. Regarding the absolute opsonizing activity, a comparison between the two species is difficult to establish, not only because the experimental conditions were different, but also because of the unknown physiological factors involved. Another striking difference was the fact that treatment of the antibodies with mercaptoethanol only partially abolished their opsonizing activity, a residual activity of about 20 per cent remaining in both γ_2 and γM.

The reasons for these discrepancies are not clear. It may be suggested that factors promoting or affecting phagocytosis, for example, the complement system, may be operationally different in homologous and heterologous systems.

It must be mentioned that mercaptoethanol-treated guinea-pig γ_2 antibodies, although still active in the mouse, acted with a time-course different from that of intact antibodies. These ME-treated antibodies were active after a 'latent period' of a few minutes, which was inversely proportional to the amount of antibody injected. The meaning of this effect is not known. A similar 'latent period' was observed by Benacerraf and Miescher (1960) when chicken antibodies were tested in the mouse. In this case, the apparent cause was the poor capacity of chicken antibodies to fix mouse complement.

The comparison of the opsonic activity of 7S and 19S antibodies has been reported by various workers. Thus, Robbins *et al.* (1965), using a technique based on the disappearance of viable bacteria after intravenous inoculation of the antibody, has found rabbit 19S anti-*S. typhimurium* antibody 500–1000 times more efficient than 7S. Rowley and

Turner (1966), with a comparable technique, found that the minimum number of antibody molecules required to eliminate one bacterium from the peritoneal cavity was 8 for the 19S and 2200 for the γ_2. In contrast with these findings, Smith, Barnett, May and Sanford (1967), performing a test of phagocytosis *in vitro*, in the absence of heat labile serum factors, found that rabbit anti-*Proteus* γ_2 antibodies were more efficient than γM. It is likely that discrepancies of this type can be accounted for by differences in the methodology employed. The action of antibodies on bacteria and their subsequent fate is a complex phenomenon in which many host factors are involved. Components of the complement system do certainly play a role (Nelson, 1963; Spiegelberg, Miescher and Benacerraf, 1963), and it is likely that differences between the opsonic activities of γM and γ_2 antibodies are related to their abilities to fix complement. Similarly, the different behaviour of guinea-pig antibodies in the homologous animal and in mice, could be explained by assuming a varying capacity to react with homologous or heterologous complement components.

Of possible relevance to the opsonic activity of the antibodies is their cytophilic capacity. To investigate this property, a method, similar to that employed by Boyden (1964) for soluble antigens, was developed. After treatment of the macrophages with antibody, and subsequent addition of labelled bacteria, the number of bacteria attached to the cells was determined. Separation of free bacteria was easily obtained by controlled centrifugation. The results indicated that the activity of γ_2 and γM antibodies was identical, on a weight basis. It was also found that treatment of γM antibodies with mercaptoethanol destroyed their cytophilic activity, while the same treatment did not affect γ_2 antibodies. It could be established that the complement system, or other serum factors, did not mediate the cytophilic activity of γ_2 antibodies, as measured in the present test. Berken and Benacerraf, (1966), studied the cytophilic activity of guinea-pig γ_1 and γ_2 antibodies, and found that only γ_2 were cytophilic. These authors, however, observed a moderate diminution of the cytophilic activity after treatment of the γ_2 antibodies with mercaptoethanol. It appears thus, that of the three classes of antibodies recognized in the guinea-pig, namely γ_2, γ_1 and γM, cytophilic activity is present in γ_2 and γM, but not in γ_1. The possibility that attachment of antibodies to macrophages is the first step of the phagocytosis of bacteria has been suggested. In that case, γM should be more cytophilic than γ_2, since its opsonic activity is higher, but this is not the experimental finding. An alternate explanation could be that, cytophilia being the first step, the opsonic activity is directly related to the capacity to fix complement. This fact would account for the higher opsonic activity of the γM, even if its cytophilic capacity is the same as that of the γ_2, since it is well established that γM is much more efficient than γ_2 in complement fixation. Other hypotheses are worthwhile considering. For example, it is possible that phagocytosis of organisms may be greatly enhanced after formation of complexes with antibody and complement, which show sticking properties for different cell surfaces. A mechanism of this type, which has been observed in some experimental models (Nelson, 1963; Lay and Nussenzweig, 1968), could be determinant in the phagocytic activity of γM antibodies.

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