JOURNAL OF BACTERIOLOGY, Apr., 1966
Vol. 91, No. 4

Printed in U.S.A.

Nonabsorbable Rabbit Anti-*Salmonella typhimurium* Antibody as Detected by the Complement-Mediated Bactericidal Reaction

MENDEL HERZBERG, KATHRYN V. KENNY, AND JOHN B. ROBBINS
Departments of Bacteriology and Pediatrics, University of Florida, Gainesville, Florida

Received for publication 2 October 1965

ABSTRACT

HERZBERG, MENDEL (University of Florida, Gainesville), KATHRYN V. KENNY, AND JOHN B. ROBBINS. Nonabsorbable rabbit anti-*Salmonella typhimurium* antibody as detected by the complement-mediated bactericidal reaction. J. Bacteriol. **91**:1548–1555. 1966.—A portion of antibody active in the complement-mediated bactericidal reaction against *Salmonella typhimurium* from hyperimmune rabbit serum has been shown to be nonabsorbable by repeated serial absorptions with whole heat-killed or living bacteria. The first two absorptions remove 90 to 95% of the activity, but 1 to 5% cannot be removed by subsequent absorptions. The nonabsorbable antibody appears to be a macroglobulin by density-gradient centrifugation and by comparison of activity and absorbability of purified γ-M and γ-G immune globulins. Alternative hypotheses involving low avidity antibody or antibody to minor cell antigenic components are offered in explanation of the phenomenon.

The specific absorption of an activity of antiserum by homologous antigen has long been used as one of the criteria for implicating antibody as responsible for that activity. The corollary, that failure to absorb the activity means that antibody is not involved, is implied. However, a number of studies have shown that successive serial absorptions of an antiserum do not always remove all of the antibody. A nonprecipitating fraction of antibody has been demonstrated in the cases of rabbit antibody to serum albumin (10), horse antibody to serum albumin (27) and egg albumin (6, 24), and human diphtheria antitoxin (15). Failure to remove all antibody by precipitation with salmonella polysaccharide antigen was also demonstrated (25). Such nonprecipitating antibody was still capable of provoking systemic or cutaneous passive anaphylaxis with egg albumin in the guinea pig and with diphtheria toxin (9, 14, 23), but the passive arthus reaction was not induced (4), demonstrating a partitioning of activities.

Since the bactericidal reaction involves antibody against surface components of the bacteria, notably the O-antigen complex (11, 22), and since indeed the antigen need not be an integral part of the surface (1, 2), one would expect that the antibody could be easily removed by absorption with whole bacterial cells. This has been shown in the case of normal serum bactericidal activity by many studies (18) with the exception of a report by Sterzl et al. (26), who reported an inability to remove normal precolostral calf bactericidal activity by successive absorptions.

MATERIALS AND METHODS

Bacterial strain. The organism employed was a smooth strain of *Salmonella typhimurium*, designated strain 7 SucLL and previously described (7, 8). Stock cultures were maintained at 4 C on Trypticase Soy Agar (BBL) slants.

Immunization procedure and collection of serum. Rabbits were immunized with washed suspensions of live bacteria. Daily intravenous injections of 5×10^8 cells were given to each of three rabbits for a period of 4 days. After a 3-day rest period, intravenous injections containing 10^9 organisms were given on 4 successive days. After a second 3-day rest period, the four intravenous injections were repeated, and blood was collected from the ear vein 3 days after the final injection. The serum designated "Herz" was separated, filtered through a Millipore apparatus, distributed in samples, and stored at −25 C. The antiserum designated "Rob" was prepared by immunization with killed bacteria, as described by Robbins et al. (24*a*).

Absorption of serum with bacteria. The test organism was cultivated on Trypticase Soy Agar in Roux

bottles for 24 hr at 37 C. The organisms were washed from the surface of the agar with 0.85% sterile saline, heated for 1 hr in a boiling-water bath, washed three times, and resuspended in 0.85% sterile saline to the desired concentration as determined by optical density (OD) readings. This suspension of *S. typhimurium* organisms was centrifuged, and the resulting pellet was resuspended in serum to the concentration of bacteria per milliliter of serum desired. Absorption was carried out for a period of 1 hr at 4 C, with frequent mixing. The absorbed serum was centrifuged at 4 C, filter-sterilized through Millipore filters, and then stored at −25 C.

Preparation of purified γ-G and γ-M antibodies specific for S. typhimurium strain 7. Fractionation of serum and isolation and characterization of antibodies specific for the cell surface of the test organism were carried out according to the procedure described by Robbins et al. (24*a*). Essentially, this involved the absorption of antibody from serum globulin fractions onto whole heat-killed cells, with subsequent elution of the antibody at acid *p*H. Further purification of the immunoglobulins was achieved by anion-exchange chromatography with diethylaminoethyl cellulose for the γ-G and molecular sieving through G-200 Sephadex for the γ-M antibody. Both antibodies were shown to be single components by analytical and density-gradient ultracentrifugation and immunodiffusion with specific antiglobulins.

Assay for bactericidal activity. The assay method employed in this investigation was the photometric assay of Muschel and Treffers (21), which determines viability on the assumption that the resulting growth of a culture is directly correlated with the size of the original inoculum when all growth conditions are held rigorously constant. Certain modifications in source of complement, magnesium ion concentration, and amount of antibody used in the test system were described previously (24*a*). A 0.3-ml amount of a log-phase bacterial culture (8×10^7 bacteria per milliliter) was added to 1.3 ml of precolostral calf serum and 0.1 ml of a magnesium saline diluent (Mg^{++} = 44 μg/ml). After incubation in a water bath at 37 C for 60 min (kill period), 5.0 ml of Brain Heart Infusion (Difco) was added to each tube. The tubes were then reincubated for a 3-hr growth period, and the viable populations were measured by their OD at 650 mμ in a Bausch & Lomb Spectronic-20 colorimeter. The survival was determined by comparison with the OD of the control tube, which contained all components except serum, and was assumed to represent 100% survival. Bactericidal activity is reported as the volume of serum necessary to kill 50% of the original inoculum, as determined by interpolation or extrapolation from a probit versus log milliliter of serum curve. The activity of the serum was calculated by employing the ED_{50} volume as one unit of activity, dividing it into 1.0 to obtain the number of ED_{50} units per milliliter of serum.

Agglutination titer determination. Serum agglutinins were assayed in two ways. In one case, 0.5 ml of a suspension of heat-killed organisms at a concentration in saline of 10^9 cells per milliliter was added to 0.5 ml of a series of twofold dilutions of antiserum. The tubes were incubated for 2 hr at 37 C and centrifuged at 1,400 × *g* for 5 min. End-point titers were measured as the highest serum dilution to show macroscopic agglutination upon resuspension by gently shaking the tube.

The second method was described previously (24*a*). Twofold dilutions of antiserum (0.25 ml) were mixed with an equal volume of heat-killed, washed bacterial suspension containing 5×10^8 cells per milliliter in Kimax culture tubes (13 × 100 mm). These mixtures were incubated at 37 C for 1 hr, and at 4 C overnight. Titers were determined according to settling pattern, and are expressed as the reciprocal of the highest dilution showing activity.

Sucrose gradient. Ultracentrifugation in a sucrose gradient and analysis of the individual fractions for protein and agglutinating activity was done by methods described for immune proteins and whole serum (16).

RESULTS

Absorption phenomenon. The residual bactericidal activity observed after repeated serial absorptions of hyperimmune sera is presented in Table 1, experiments A and B. In these experiments, antiserum was absorbed with heat-killed bacteria at a final concentration of 10^{11} bacteria per milliliter of serum for 1 hr at 4 C. This was repeated four successive times, and the serum was tested after each absorption. Agglutinating activity was removed after the first absorption, but the antiserum retained considerable bactericidal activity (less than one log reduction) through the third and fourth absorptions.

On the assumption that a heat-labile antigen might have been destroyed in preparing the absorbing suspension, the experiment was repeated with a live absorbing antigen with essentially the same results. Since this activity appeared to be nonabsorbable by both heat-killed and live bacteria, the possibility existed that the substance responsible for activity was not "classical" antibody, which should have been absorbable. Furthermore, if the bactericidal effect was due to antibody, then it should have been complement-dependent. Thus, heat-inactivated complement was compared with unheated complement to determine if heat inactivated the bactericidal activity. That this residual activity was complement-dependent is shown in Table 2. Antiserum, which had been absorbed four times with the live organisms as previously described, was reacted in the usual test system with fresh and heat-inactivated (56 C for 0.5 hr) complement. The portion of the antiserum reacted with unheated complement had bactericidal activity, whereas the portion reacted with the heated complement had no detectable activity. In all three experiments, the initial drop in bactericidal activity occurred in the first two

TABLE 1. *Effect of repeated absorptions with homologous bacteria upon the bactericidal and agglutinating activity of hyperimmune anti-Salmonella typhimurium serum*

Expt no.	Antibody source	Absorbing agent (per ml of serum per absorption	No. of absorptions	ED_{50}	Per cent activity	Agglutination titer
				ml		
A	Whole serum "Herz"	10^{11} HK cells*	0	3.4×10^{-5}	100	1:640
			1^0	ND†	ND	1:160
			2^0	6.6×10^{-4}	5.1	1:80
			3^0	7.4×10^{-4}	4.6	1:10
			4^0	7.8×10^{-4}	4.4	<1:10
B	Whole serum "Rob"	10^{11} HK cells	0	4.0×10^{-5}	100	1:160
			1^0	5.6×10^{-4}	7.1	1:40
			2^0	4.4×10^{-3}	0.9	<1:10
			3^0	5.2×10^{-3}	0.8	<1:10
			4^0	4.2×10^{-3}	0.95	<1:10
			5^0	5.2×10^{-3}	0.8	<1:10
C	Whole serum, "Herz"	10^{10} live cells	0	3.4×10^{-5}	100	1:160
			1^0	7.6×10^{-5}	44.7	1:80
			2^0	9.4×10^{-5}	36.2	1:40
			3^0	8.4×10^{-5}	40.5	1:10
			4^0	8.4×10^{-5}	40.5	<1:10
D	Purified γ-M antibody (0.05 mg/ml)	10^{11} HK cells	0	6.6×10^{-4}	100	1:40
			1^0	5.3×10^{-3}	12.0	<1:10
			2^0	7.4×10^{-3}	8.9	<1:10
			3^0	8.9×10^{-3}	7.4	<1:10
			4^0	9.6×10^{-3}	6.9	<1:10
E	Purified γ-G antibody (0.066 mg/ml)		0	8.0×10^{-3}	100	1:40
		10^{10} HK cells	1	$<10^{-1}$	0	ND
		10^{11} HK cells	1	$<10^{-1}$	0	ND
		10^{12} HK cells	1	$<10^{-1}$	0	ND
F	Mixture of purified γ-M (0.05 mg/ml) and purified γ-G (1.0 mg/ml)	10^{11} HK cells	0	4.2×10^{-4}	100	1:40
			1^0	7.0×10^{-4}	60	1:10
			2^0	4.0×10^{-3}	10.5	<1:10
			3^0	8.0×10^{-3}	5.2	<1:10

* HK = heat-killed.
† No data.

TABLE 2. *Effect of heating complement on the residual bactericidal activity of rabbit anti-Salmonella typhimurium serum after four successive absorptions*

Antiserum* dilution	Per cent survival in the presence of unheated complement†
10^{-1}	ND‡
10^{-2}	25
10^{-3}	15
10^{-4}	70
10^{-5}	100

* Antiserum "Herz."
† Survival rate was 100% in the presence of heated complement.
‡ No data.

absorptions, after which a plateau in activity occurred in all subsequent absorptions. The slight differences detected between determinations were considered to be within experimental error.

A further attempt was made to absorb the activity from the antiserum with larger numbers of bacteria. Further absorption of the antiserum with a highly concentrated suspension of heat-killed cells (2.5×10^{11} bacteria per milliliter of serum), a 25-fold increase in absorbing dose, did not remove the bactericidal activity which had been left after one or four absorptions with 10^{10} bacteria per milliliter (Table 3). Clearly, the residual bactericidal activity observed after repeated absorptions was not absorbable under the conditions used, and was complement-dependent.

Absorption of whole antiserum with subsequent gradient analysis. To determine the effect upon the concentrations of the two types of immunoglobulins during the absorption of the whole antiserum, the residual agglutinating activity present in the absorbed sera was studied by density-gradient ultracentrifugation. After each absorption, samples were removed, sterilized by filtration, studied by bactericidal assay and agglutination titers, and subsequently ultracentrifuged

TABLE 3. *Bactericidal activity of rabbit anti-Salmonella typhimurium serum after extensive absorption*

Absorption* procedure	ED_{50}
	ml
None	4.6×10^{-5}
1 × with 10^{10} per ml	1.4×10^{-4}
1 × with 10^{10} per ml, plus 1 × with 2.5×10^{11} per ml	1.9×10^{-4}
4 × with 10^{10} per ml	1.6×10^{-4}
4 × with 10^{10} per ml, plus 1 × with 2.5×10^{11} per ml	1.7×10^{-4}

* Antiserum "Herz" was absorbed with heat-killed cells.

in a sucrose gradient. Protein analysis and agglutination titers were determined on the various fractions obtained from the gradient.

Figure 1 represents the results of one such experiment. Antiserum was absorbed repeatedly with 10^{11} cells per milliliter of serum for each absorption. All γ-G antibody (top of the gradient) detectable by agglutination had been removed after the second absorption along with a portion of γ-M antibody (bottom of the gradient). However, the bactericidal titer of the antiserum at this stage of absorption had been reduced only by 1.5 logs (from 3.4×10^{-5} to 6.6×10^{-4}). The level of γ-M antibody, as detected by agglutination and bactericidal activity, remained unchanged through the third and fourth absorptions. After the fifth absorption, γ-M antibody was no longer detectable by agglutination, but the bactericidal activity had not changed significantly. It was not possible to test gradient fractions directly for bactericidal activity, because of the small amounts of antiserum employed and the dilution of antibody during the course of fractionation.

Thus, it appeared that the γ-G antibody was removed preferentially from the serum by absorption. The γ-M persisted after all detectable γ-G antibody had been removed. In addition, when γ-M antibody, detectable by agglutination, was absorbed, the serum still retained its bactericidal activity. The remaining activity was probably due to γ-M globulin, since it was absorbed at a slower rate than γ-G, which was expected from the demonstration by Robbins et al. (24*a*) that γ-M antibodies have a lower avidity than γ-G antibodies. This remaining γ-M antibody, responsible for bactericidal activity, must be a molecule with very low avidity, but with high activity. This property would explain our failure to detect its presence by agglutination, and our inability to absorb it from antisera.

Absorption of normal rabbit sera. The possibility

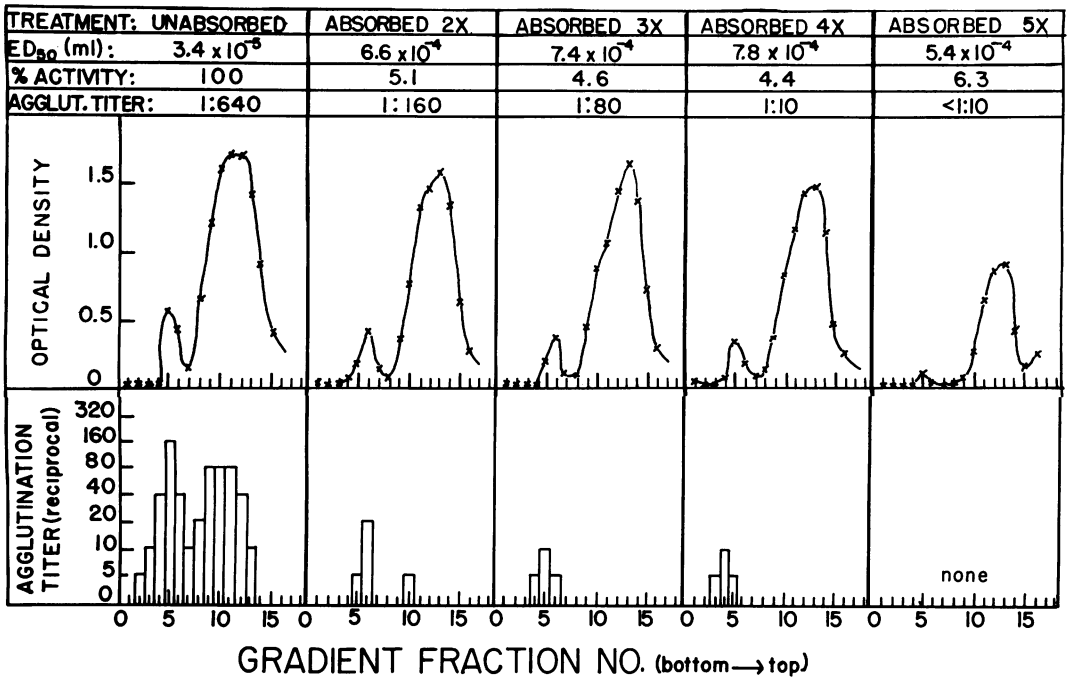

FIG. 1. *Analysis of fractions of hyperimmune rabbit anti-Salmonella typhimurium serum obtained by density gradient ultracentrifugation after serial absorptions with homologous antigen.*

existed that the residual activity was due to a "normal" serum component which was not an immunoglobulin. Tests of normal antisera of rabbits of the same stock revealed that the degree of activity was low ($ED_{50} \sim 10^{-1}$) and that it was readily absorbable by a single dose of 10^{10} bacteria per milliliter. This relative ease of absorption of normal antibody was as expected (18).

Absorption of purified γ-M and γ-G antibodies. Highly purified anti-*S. typhimurium* immunoglobulins were tested for absorbability. These experiments (Table 1, experiments D and E) demonstrated the relative ease with which one can absorb γ-G antibody and the inability to completely absorb pure γ-M. On the basis of these experiments and the data obtained by gradient centrifugation of hyperimmune whole serum, it is reasonable to ascribe nonabsorbable activity as antibody of the γ-M class of immunoglobulins.

A mixture of the purified γ-M and γ-G antibodies was also tested (Table 1, experiment F) to see if the γ-G either interfered with the absorption of γ-M by virtue of its greater avidity or if it enhanced the absorbability of γ-M antibody by a process akin to coprecipitation. The results indicate that no demonstrable inferference or enhancement of absorbability occurred; in fact, the residual activity (5.2%) was comparable with the results obtained in experiment D (6.9%), where γ-M globulin alone was involved.

Absorption in the presence of complement. The possibility was considered that antibody of low avidity can be absorbed by antigen only in the presence of complement, as shown by Sterzl et al. (26). The serial absorptions were carried out in the presence of a volume of calf complement, known to be active in the bactericidal reaction, equal to the volume of the serum to be absorbed. As a control, saline was substituted for complement in another series of absorptions with the same antiserum and antigen. A second control series involved absorption in the absence of a diluting fluid, with a subsequent dilution with saline made after the absorption step. The results (Table 4) indicate, conclusively, no effect of complement in enhancing absorption and no effect of allowing the absorption to take place in a more dilute milieu of either complement or saline.

To check on the effect of hydrogen ion concentration in the system, since it had not been controlled in the absorption procedure, the *p*H of the absorbed sera at each stage of the experiment was measured. The results (Table 4) indicate that at no time did the *p*H drop below 6.4 and that it was usually in the range of neutrality (*p*H 6.8 to 7.2), except in the cases where complement was present when it was as high as 8.0. This finding obviated the possibility that a drop in *p*H was responsible for an elution of antibody from the absorbing antigen which would have accounted for "lack of avidity," since Robbins et al. (24*a*) showed that a very minimal dissociation of the bacterium-antibody complex occurs at *p*H values above 6.0 with either γ-G or γ-M immunoglobulin.

DISCUSSION

The results of these experiments indicated that a small fraction of γ-M antibody remains in the hyperimmune rabbit sera after repeated serial absorption with either heat-killed or live organisms. The residual 1 to 5% bactericidal activity of hyperimmune serum always remained, whereas agglutinating activity was consistently removed with ease. Osawa and Muschel (22) obtained similar results with a rabbit anti-*S. typhosa* serum which before absorption yielded an ED_{50} value of 5.2×10^{-4} ml, but nevertheless retained an activity of 3.9×10^{-3} ml (7.0%) after two absorptions. The activity removed by absorption is con-

TABLE 4. *Bactericidal activity of rabbit anti-Salmonella typhimurium serum serially absorbed with homologous antigen in the presence or absence of complement*

No. of absorptions	Dilution*	Absorbed in the presence of				Absorbed in the absence of added complement of or saline but diluted accordingly with saline in the assay	
		Complement		Saline			
		ED_{50}†	*p*H‡	ED_{50}	*p*H	ED_{50}	*p*H
0	1:2	5.5×10^{-5}	8.0	5.2×10^{-5}	7.2	5.4×10^{-5}	7.2
1	1:2	3.0×10^{-4}	8.0	1.4×10^{-4}	7.2	3.2×10^{-4}	7.2
2	1:4	1.9×10^{-4}	7.6	2.5×10^{-4}	7.0	7.6×10^{-4}	7.0
3	1:8	1.9×10^{-4}	7.6	1.4×10^{-4}	6.8	1.8×10^{-4}	6.8
4	1:16	9.9×10^{-5}	7.6	1.1×10^{-4}	6.4	3.8×10^{-4}	6.8

* Dilution due to an equal volume of complement or saline added at each stage of absorption.
† The ED_{50} values were calculated for a 1:2 dilution of serum.
‡ The *p*H of the absorbed serum.

siderable (99 to 95%); however, the residual unabsorbable antibody is quite significant in terms of activity (ED_{50} = 10^{-3} to 10^{-4} ml). A reasonable estimate of the amount of antibody can be made on the basis of the studies of Robbins et al. (24*a*), in which the molarity of γ-M antibody required for the ED_{50} dose in this system, with the same bacterial stain used in the present study, is 2.78×10^{-11} M. Thus, residual ED_{50} values in the range of 10^{-3} to 10^{-4} would yield values for the absorbed fraction of whole antiserum of 2.78×10^{-8} to 2.78×10^{-7} M or 27.8 to 278 μg/ml of serum based on a molecular weight of 10^6. These amounts of antibody would be difficult to detect by the classical precipitin reaction, but might be detectable by gel diffusion (17) if their avidity in the precipitin reaction was great enough. Repeated attempts to detect them by immunodiffusion failed to yield visible lines of precipitate which would lead one to the conclusion that they were "nonprecipitating." The ease of detection of these quantities by the bactericidal reaction is an index of its greater sensitivity.

After an initial drop in bactericidal titer as the result of one or two absorptions, the antisera retained a certain level of activity which remained constant through at least three further absorptions. Sucrose-gradient ultracentrifugation analysis of whole antisera, which had been absorbed repeatedly, showed that all γ-G detectable by agglutination was removed before all detectable γ-M agglutinating antibody. Further evidence implicating γ-M antibody as being responsible for the nonabsorbable fraction was seen in the experiments involving the purified antibodies.

Several studies have indicated that γ-M antibody is less "avid" than γ-G antibody. Since γ-M antibodies were more efficient in sensitizing erythrocytes for complement-dependent lysis, it was concluded that this was a result of the high rate of dissociation of the γ-M antibody from the erythrocyte and its ability to transfer to other erythrocytes (26*a*). Studies concerned with purified γ-M and γ-G antibodies against the cell surface of *S. typhimurium* demonstrated that both antibodies had bactericidal activity, but that the specific activity of γ-M antibody was much greater in the bactericidal reaction (24*a*). They also showed that γ-G was the more "avid" immunoglobulin as measured by the greater stability of its complexes with *S. typhimurium* to increasing hydrogen ion concentration.

The residual activity observed after repeated absorption can be explained in terms of "avidity" of the two immunoglobulins for the bacterial cell surface. Absorption therefore removed antibodies of high avidity (primarily γ-G, and, to a lesser extent, γ-M). The remaining bactericidal activity was the result of the action of antibodies (γ-M) of low avidity for the cell surface. Even though this antibody was in low concentration, it could still exert considerable activity through its ability to transfer from cell to cell. This concept is consistent with the experiments which have shown that the biological activity of immunoglobulins is mediated by that portion of the molecule not directly involved in the antigen-antibody interaction (5). This idea that complement-fixing antibody is not consumed in the process of bacteriolysis was demonstrated with cholera vibrios by Pfeiffer and Friedberger in 1903 (19). They found, according to Muir, that bound antibody split off from the bacteria when lysis occurred. Cholera vibrios (sensitized with antibody and washed) set free, when introduced into the peritoneal cavity of a normal guinea pig, antibody sufficient to cause lysis of fresh vibrios in amounts much greater than those which had been sensitized. Such antibody would of course give great advantage to the host, since it is not inactivated by binding to antigen in the process of killing bacteria.

Under these experimental conditions, this residual antibody can be also described as "nonavid" rather than of "low" avidity, since, even if the binding constant of these antibodies was very low, we would still expect some removal of activity after each step of the serial absorption. This was not the case, since a plateau of activity was reached after the first or second absorption. A puzzling feature was the fact that purified γ-M antibody exhibited this same plateau of activity in spite of the fact that it had been prepared by absorption onto and subsequent elution from bacteria. This meant that it had to have a considerable degree of avidity to have survived the purification process. One possibility is that a fraction of the antibody was altered, during the subsequent purification steps, to convert it to nonavid antibody. In hyperimmune serum, a certain portion of γ-M antibody would also be "nonavid," and would not be expected to be extracted by the purification procedure. Thus, the similarity of effects with whole serum and purified γ-M may not be ascribable to the same molecular species.

Another hypothesis to explain the results would be that the residual nonabsorbable activity is due to an antibody directed against a site on the bacterial surface which is present in very small amounts. This site would be highly immunogenic, i.e., inducing large amounts of antibody in proportion to its own mass. The mass of this site would be insufficient (on either live or heat-killed bacteria) to absorb out the activity except by heroic efforts, but the site would be extremely vulnerable to the complement-mediated bacteri-

cidal reaction. Some evidence to support such an hypothesis is the finding that some strains of gram-negative organisms are insusceptible to the bactericidal reaction in spite of being smooth strains. Such strains are indistinguishable either chemically or antigenically from susceptible strains (20). Such insusceptible strains would lack this highly vulnerable site. The activity directed by antibody against this site would be in addition to the much larger amount of activity directed against the usual somatic antigens which would account for 95 to 99% absorbability of bactericidal activity by the early absorptions, and for 5 to 1% residual unabsorbable activity remaining.

Several reports in the literature have dealt with undefined factors in serum which appear to have bacteriostatic or bactericidal activity, but are not able to be specifically absorbed from the serum: an induced tuberculostatic substance described by Kochan and Raffel (13), a normal tuberculostatic factor, difficult to absorb, by Kochan et al. (12), and a normal antibody against *S. typhimurium* detected by Sterzl et al. (26). In the latter investigation, a factor was demonstrated in precolostral calf serum which could be absorbed only in the presence of complement. They concluded that the factor was probably not antibody, because, normally, antibody does not require complement for binding with the antigen. However, they also proposed that this possibility could not be excluded, since antibodies of low avidity need complement for binding with the antigen; it prevents rapid dissociation of the antibodies from the antigen. Our attempts to enhance absorption of antibody by allowing each absorption to take place in the presence of liberal amounts of complement showed that the presence of complement had no effect. Thus, the possible absence of complement in the absorption mechanism did not explain the phenomenon. This study emphasizes the caution with which one should regard lack of specific absorption as a criterion for designating an activity as not being dependent on antibody.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-01663 from the National Institute of Allergy and Infectious Diseases for M. H. and K. V. K., Career Development Award HD-053-00384 and Public Health Service grant 6-K3-HD-22 for J. B. R., and grant 5T1-GM-869 from the Division of General Medical Sciences for K. V. K.

LITERATURE CITED

1. ADLER, F. L. 1952. Bactericidal action mediated by antibodies specific for heterologous antigens adsorbed to bacterial cells. Proc. Soc. Exptl. Biol. Med. **79**:590–602.
2. ADLER, F. L. 1953. Studies on the bactericidal reaction. I. Bactericidal action of normal sera against a strain of *Salmonella typhosa*. J. Immunol. **70**:69–78.
3. ADLER, F. L. 1953. Studies on the bactericidal reaction. II. Inhibition by antibody and antibody requirements of the reaction. J. Immunol. **70**:79–88.
4. BENACERRAF, B., AND E. A. KABAT. 1950. A quantitative study of the Arthus phenomenon induced passively in the guinea pig. J. Immunol. **64**:1–19.
5. COHEN, S., AND R. R. PORTER. 1964. Structure and biological activity of immunoglobulins. Advan. Immunol. **4**:287–349.
6. HEIDELBERGER, M., H. P. TREFFERS, AND M. MAYER. 1940. A quantitative theory of the precipitin reaction. VII. The egg albumin-antibody reaction in antisera from the rabbit and the horse. J. Exptl. Med. **71**:271–282.
7. HERZBERG, M. 1962. Living organisms as immunizing agents against experimental salmonellosis in mice. J. Infect. Diseases **111**:192–203.
8. HERZBERG, M., M. J. JAWAD, AND D. PRATT. 1965. Correlation of succinate metabolism and virulence in *Salmonella typhimurium*. J. Bacteriol. **89**:185–192.
9. KABAT, E. A., AND B. BENACERRAF. 1949. A quantitative study of passive anaphylaxis in the guinea pig. Passive sensitization with non-precipitable or "univalent" rabbit antiovalbumin. J. Immunol. **62**:97–101.
10. KABAT, E. A., AND M. HEIDELBERGER. 1937. A quantitative theory of the precipitin reaction. V. The reaction between crystalline horse serum albumin and antibody formed in the rabbit. J. Exptl. Med. **66**:229–250.
11. KAROLCEK, J., M. RESUNKO, M. DROSHOVIOVA, I. ODLER, AND L. BATOROVA. 1964. Immune reactions in human beings vaccinated with typhoid O, Vi, and O^+ Vi^+ H vaccines, with special reference to the specific bactericidal activity of the serum. J. Hyg. Epidemiol. Microbiol. Immunol. (Prague) **8**:179–189.
12. KOCHAN, I., C. PATTON, AND K. ISHAK. 1963. Tuberculostatic activity of normal human sera. J. Immunol. **90**:711–719.
13. KOCHAN, I., AND S. RAFFEL. 1960. A property of immune sera inhibitory for the growth of the tubercule bacillus. J. Immunol. **84**:375–383.
14. KUHNS, W. J. 1953. Immunological studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. III. Studies of the passive arthus reaction in guinea pigs using human precipitating and non-precipitating diphtheria antitoxin. J. Exptl. Med. **97**:903–910.
15. KUHNS, W. J., AND A. M. PAPPENHEIMER. 1952. Immunological studies of antitoxin produced in normal and allergic individuals hyperimmunized with diphtheria toxoid. J. Exptl. Med. **95**:363–392.

16. KUNKEL, H. G., J. H. ROCKY, AND T. TOMASI. 1960. Methods of separation and properties of antibodies of high molecular weight, p. 30. *In* Problems in microbiology. Rutgers Univ. Press, New Brunswick, N.J.
17. MARRACK, J. R. 1963. Sensitivity and specificity of methods of detecting antibodies. Brit. Med. Bull. **19**:178–182.
18. MICHAEL, J. G., J. L. WHITBY, AND M. LANDY. 1962. Studies on natural antibodies to Gram-negative bacteria. J. Exptl. Med. **115**:131–146.
19. MUIR, R. 1931. Bactericidal action, p. 267–286. *In* P. Fildes and J. C. G. Ledingham [ed.], A system of bacteriology in relation to medicine. H. M. Stationery Office, London.
20. MUSCHEL, L. H. 1960. Serum bactericidal reactions. Ann. N.Y. Acad. Sci. **88**:1265–1272.
21. MUSCHEL, L. H., AND H. P. TREFFERS. 1956. Quantitative studies on the bactericidal actions of serum and complement. I. A rapid photometric growth assay for bactericidal activity. J. Immunol. **76**:1–10.
22. OSAWA, E., AND L. H. MUSCHEL. 1964. The bactericidal actions of O and Vi antibodies against *Salmonella typhosa*. J. Immunol. **92**:281–285.
23. OVARY, Z., AND G. BIOZZI. 1954. Passive sensitization of the skin of the guinea pig with human antibody. Intern. Arch. Allergy Appl. Immunol. **5**:241–245.
24. PAPPENHEIMER, A. M. 1940. Anti-egg albumin antibody in the horse. J. Exptl. Med. **71**:263–269.
24*a*. ROBBINS, J. B., K. KENNY, AND E. SUTER. 1965. The isolation and biological activities of rabbit γM- and γG-anti-*Salmonella typhimurium* antibodies. J. Exptl. Med. **122**:385–402.
25. STAUB, A. M., AND C. DAVARPANAH. 1956. Etude immunochemique sur les Sallmonelles. II. Analyse des precipitines de quelques serums anti-*S. gallinarum* et anti-*S. typhi*. Ann. Inst. Pasteur **91**:338–354.
26. STERZL, J. V., V. PESAK, J. KOSTKA, AND M. JILEK. 1964. The relation between the bactericidal activity of complement and the character of the bacterial surfaces. Folia Microbiol. **9**:284–298.
26*a*. TALIAFERRO, W. H., AND L. G. TALIAFERRO. 1961. Intracellular transfer of gamma-1 and gamma-2 Forssman hemolysins. Proc. Natl. Acad. Sci. **47**:713–724.
27. TREFFERS, H. P., M. HEIDELBERGER, AND J. FREUND. 1947. Antiporteins in horse sera. III. Antibodies to rabbit serum albumin and their reaction with antigen. J. Exptl. Med. **86**:95–105