BACTERIAL HEMAGGLUTINATION AND HEMOLYSIS

ERWIN NETER

Statler Research Laboratories and Department of Pediatrics, Children's Hospital, Laboratory of Bacteriology, Roswell Park Memorial Institute, and Departments of Pediatrics and Bacteriology, University of Buffalo, School of Medicine, Buffalo, New York

CONTENTS

"Every part is disposed to unite with the whole, that it may thereby escape from its own incompleteness."—*Leonardo Da Vinci.*

I. INTRODUCTION

Investigations carried out during the past fifteen years have revealed that the red blood cell is a valuable tool for microbiologic studies. In 1941, Hirst (1) as well as McClelland and Hare (2) independently described viral hemagglutination, when these authors observed clumping of erythrocytes in the presence of influenza virus. This hemagglutination is specifically inhibited by homologous viral antiserum. Since that time numerous other viruses have been found to possess hemagglutinating properties. The viral hemagglutination test has been used extensively both for identification and titration of hemagglutinating viruses and for detection and titration of the corresponding antibodies. Also, this reaction has been studied intensively as a model of virus infection of the host cell, including the mechanism of virus attachment and the identity of cell receptors. Viral hemagglutination is discussed in modern textbooks of bacteriology, virology, and microbiology and was reviewed in detail by Burnet (3). Therefore, this subject is not included here, although reference will be made to differences and similarities between viral and bacterial hemagglutination.

Bacteria, too, cause agglutination of erythrocytes. Eight types of bacterial hemagglutination are known at this time: (1) The first type (direct bacterial hemagglutination) was described more than half a century ago by Kraus and Ludwig (4), who observed clumping of erythrocytes in the presence of staphylococci and vibrios. Although the list of hemagglutinating bacteria has been enlarged since that time, the mechanism of this reaction remains to be elucidated. (2) A second, fundamentally different type of bacterial hemagglutination was reported in 1947 by Keogh, North, and Warburton (5). These authors found that antigen obtained from *Hemophilus influenzae* is readily adsorbed to the surface of red blood cells and that agglutination takes place on addition of homologous bacterial antiserum. This indirect, conditioned, or passive hemagglutination reaction and its hemolytic modification since have been demonstrated with numerous other bacterial antigens, and the tests have proved to be extraordinarily sensitive for the detection and titration of bacterial antibodies. Generally speaking, it is the polysaccharide rather than the protein antigens which are adsorbed by untreated red blood cells. However, even protein antigens can be attached to erythrocytes by several methods, representing types 3, 4, and 5 of bacterial hemagglutination. (3) Specific hemagglutination follows exposure of erythrocytes to antigen-antibody mixture. (4) In the fourth type, the erythrocytes are pretreated with tannic acid, exposed to protein antigen, and thus become specifically agglutinable in the presence of homologous protein antibodies. (5) Similarly, antigens may be attached to the surface of erythrocytes by chemical linkages. (6) Modification of erythrocytes with proteins may be accomplished, too, through the agency of incomplete red blood cell antibodies, the latter serving as *Schleppers* (carriers) (6). (7) Bacteria may produce hemagglutination in yet another way: they cause changes of the erythrocytes which result in the appearance on the surface of the cell of an otherwise undetectable antigenic component. Agglutination ensues upon addition of antibodies reacting specifically with this newly exposed antigen. (8) Finally, certain bacteria produce changes in normal serum which result in a newly acquired hemagglutinating capacity. Table 1 presents a summary of these eight types of microbial hemagglutination.

Certain fundamental similarities and differences between these types of bacterial hemagglutination should be pointed out. In type 1 hemagglutination is effected by a bacterial

TABLE 1

Types of microbial hemagglutination

1. Bacterium (bacterial antigen) or virus	+	RBC	———			→ HA	Direct microbial hemagglutination
2. Bacterial or viral antigen	+	RBC	→ Modified RBC	+ Bacterial or viral antibody		→ HA	Indirect, conditioned, or passive microbial hemagglutination
3. Microbial antigen and antibody	+	RBC	———			→ HA	
4. Microbial antigen	+	Tannic acid treated RBC	→ Modified RBC	+ Microbial antibody		→ HA	
5. Microbial antigen and complexing chemical	+	RBC or pre-treated RBC	→ Modified RBC	+ Microbial antibody		→ HA	
6. Microbial antigen-incomplete RBC antibody complex	+	RBC	→ Modified RBC	+ Homologous antibody		→ HA	Red cell linked antigen test
7. Bacterium (enzyme)	+	RBC	→ Altered RBC	+ RBC antibody		→ HA	Thomsen-Friedenreich phenomenon
8. Bacterium	+	serum	→ Altered serum	+ RBC		→ HA	Bacteriogenic hemagglutination

RBC = red blood cells.
HA = hemagglutination.

product and does not depend on the presence of microbial antibodies; in fact, these antibodies inhibit the reaction. On the other hand, antibodies bring forth hemagglutination in types 2 to 7. In types 2 to 6 the antibodies are directed against microbial and other antigens adsorbed to the surface of the red blood cells, whereas in type 7 the hemagglutinating antibody reacts with antigenic components of the erythrocytes themselves, unmasked by bacterial action. Finally, hemagglutination of type 8 is due to bacterial alteration of the serum, which endows the latter with a newly acquired hemagglutinating activity.

It is the purpose of this paper to review critically for the first time the pertinent data, so widely distributed in the literature, on bacterial hemagglutination and hemolysis and to point out areas in need of future research.

II. DIRECT BACTERIAL HEMAGGLUTINATION

Hemagglutinating Bacteria and the Hemagglutination Reaction

A list of hemagglutinating bacteria together with selected references are presented in table 2. Perusal of this table shows that a fairly large number of unrelated bacterial species possess this activity. Although all bacterial species have not yet been tested, it is clear that certain species, *e. g.*, shigellae, do not cause hemagglutination. Obviously, there is no relationship in the taxonomic position between active and inactive bacteria. For example, most strains of *Hemophilus influenzae* are inactive, whereas many strains of *Hemophilus aegyptius* are active. Similarly, in the family *Enterobacteriaceae*, some members, such as the AD (alkalescens-dispar) group are active, and members of the genus *Shigella* are inactive. Further, even within species and serogroups (*Escherichia coli*) there are hemagglutinating and non-hemagglutinating strains [Kauffmann (10)]. It should be stressed that negative findings may be due to the selection of the donor species of the red blood cells. It has been shown that, with a given strain, erythrocytes from one animal species are agglutinated, whereas those from others are not. To illustrate: *H. aegyptius* agglutinates human red blood cells readily, chicken cells to a lesser degree, and monkey, rabbit, and rat cells not at all. Most strains of the AD group are highly active with human cells, and only a few strains agglutinate erythrocytes from rabbit, guinea pig, and sheep. On the other hand, certain strains of *E. coli*, *Hemophilus pertussis*, and *Clostridium septicum* agglutinate red cells from all animal species tested, although not necessarily to the same degree. Attention should be called to Guyot's (8) observation to the effect that erythrocytes from different individuals belonging to the same animal species revealed identical reactivity with strongly, weakly, or non-reacting strains of *E. coli*. The reason for the above mentioned differences in activity of different bacteria toward erythrocytes from various animal species remains to be elucidated.

TABLE 2

Bacteria causing direct hemagglutination

Bacteria*	References
Micrococcus pyogenes var. *aureus*	4
Sarcina sp	7
Escherichia coli	7, 8, 9, 10, 11
Salmonella sp	7
AD (alkalescens-dispar) Group	12, 13
Vibrio comma	7, 12
Vibrio sp	4
Pseudomonas sp	14
Hemophilus aegyptius	15
Hemophilus influenzae	15, 16
Hemophilus pertussis	5, 12, 17, 18
Bacillus parapertussis	5
Brucella bronchiseptica	5, 12
Corynebacterium diphtheriae	7, 19
Corynebacterium pseudodiphtheriticum	19
Clostridium botulinum	20, 21
Clostridium septicum	22

* The present scientific names and not necessarily those of the authors are used in tables 2, 3 and 4.

At this time it seems unwise to this reviewer, because of inadequate data, to offer generalizations on the identity of the hemagglutinating principles and the mechanism of the direct hemagglutination reaction. It is stated by Dafaalla and Soltys (22) that the hemagglutinating factor of *C. septicum* cannot be separated from the bacterial cell. If this were so, one should expect that bacterial cells are attached selectively to red blood cells. So far as this reviewer is aware, this has not been demonstrated. Indeed, with several species separation of the hemagglutinating factors from the bacteria has been accomplished.

Extensively studied is the hemagglutinating

antigen of *H. pertussis* (17, 18, 23, 24). It is heat labile, being destroyed at 56 C for 30 minutes. It is inhibited by lipid components obtained from red blood cells. It has been clearly demonstrated that the erythrocyte receptor, which may be identical with, or at least related to, the inhibitory lipid, differs from the receptor for influenza virus. *H. pertussis* contains another antigen which is readily adsorbed on red blood cells and does not cause direct hemagglutination (*cf.* p 170). The heat lability of hemagglutinating principles has been established also for those obtained from *E. coli*, *H. aegyptius*, and *C. septicum*.

So far as the hemagglutinating factor of *Clostridium botulinum* is concerned, it is distinct from the toxin, as evidenced by the results of precipitation studies, utilizing the Oudin serum-agar technique, and by the observation that red blood cells remove from a filtrate only the hemagglutinating factor but not the toxin. Lowenthal and Lamanna (25, 26) presented evidence to the effect that, depending upon the pH, the toxin and hemagglutinin either form a complex or are dissociated. The botulinal hemagglutinin is adsorbed by numerous animal tissue elements other than erythrocytes. Its uptake by red blood cells appears to be a true adsorption reaction, because the reaction has a negative temperature coefficient and the combination does not appear to follow the law of simple multiple proportions. Electrolytes are required. It is of interest to note also that botulinal hemagglutinin is reversibly attached to erythrocytes, and that the receptor is different from that for influenza virus. In this connection attention should be called to another observation of the reversibility of bacterial hemagglutination. Upon addition of homologous bacterial antiserum to a red blood cell suspension which had been agglutinated by a pseudomonas strain the erythrocytes became disagglutinated and the bacteria agglutinated (14).

The hemagglutinating factor obtained from *Corynebacterium diphtheriae* is said to be an antigenic lipid (19). The antigenicity of many of the hemagglutinating principles has been established by the observation of the specific inhibitory effects of homologous antisera on hemagglutination by *Micrococcus pyogenes*, *H. aegyptius*, *H. pertussis*, *C. diphtheriae*, and *C. septicum*. A notable exception appears to be *E. coli*; however, before the active hemagglutinating factor can be considered to be non-antigenic, confirmation of the single report (7) is required, particularly in view of the antigenic complexicity of this species.

Only limited information is available regarding the component (receptor) of the red blood cell which interacts with the hemagglutinating principle. That it is not identical with the influenza virus receptor is clearly evident from the facts that neither the receptor destroying enzyme (RDE) nor influenza virus interfere with hemagglutination by *H. pertussis* and *H. aegyptius*. The observation that certain lipids inhibit this reaction suggests that lipid components of the red blood cell serve as receptors.

It is obvious that much additional work needs to be done, particularly with respect to the characterization of many bacterial hemagglutinating principles, the corresponding red blood cell receptors, and the process of the hemagglutination reaction itself.

Related Reactions

The hemagglutinating capacity is not restricted to bacteria nor are erythrocytes the only agglutinable cells. Since the early days of modern microbiology it has been known that certain plant products, referred to as phytoagglutinins, agglutinate red blood cells. Agglutinating substances are found also in higher fungi (27). *Rickettsia orientalis*, too, agglutinates certain red blood cells from individual fowl; this reaction is specifically inhibited by homologous antiserum (28). Reference to viral hemagglutination has been made in the introduction. Even pleuropneumonia-like organisms (PPLO) can cause agglutination of erythrocytes (29).

In connection with the foregoing data on bacterial hemagglutination it is interesting that the agglutinability of erythrocytes from different animal species varies characteristically with different plant agglutinins. For example, Landsteiner (30) found that lentil extract was considerably more active toward rabbit than pigeon red blood cells; in contrast, bean extracts were far more active toward pigeon than rabbit erythrocytes. Similar observations were made with ricin and abrin. More recently, efforts have been made to discover seed extracts for the differentiation of blood groups of man. For example, Boyd (31), who refers to these antibody-like substances from plants as lectins, isolated a substance which agglutinates A and AB, but not O cells, and which

precipitates blood group A substance. A plant agglutinin for human erythrocytes containing the N antigen has been described (32). Nungester and Halsema (32a) observed that Flexner-Jobling carcinoma cells, in contrast to erythrocytes, were not agglutinated by certain phytoagglutinins, although the latter were absorbed by both cell suspensions.

Bacteria agglutinate cells other than erythrocytes. Rosenthal (9) found that *E. coli* causes clumping of leukocytes, thrombocytes, spermatozoa, spores of molds, and pollens. More recently, Rolle and Kalich (33) reported that the ability or inability of *E. coli* strains to agglutinate spermatozoa is related to pathogenicity or lack of pathogenicity. However, before this conclusion can be accepted, confirmatory evidence is required.

III. INDIRECT, CONDITIONED, OR PASSIVE HEMAGGLUTINATION AND HEMOLYSIS

Hemagglutinating Bacteria and the Hemagglutination and Hemolysis Reactions

A large variety of bacterial species contain antigens which are readily adsorbed by untreated red blood cells and thereby render these modified[1] erythrocytes specifically agglutinable by homologous bacterial antibodies. Table 3 gives data on the bacterial species, the antigens involved in the reaction, and selected references. It is likely that other bacterial species, not yet tested, also produce erythrocyte modifying antigens.

In all but two instances, chemical analysis of the modifying antigens has revealed that they are polysaccharides or contain polysaccharides as the serologically active determinant. However, the active material of *Pasteurella pestis* was tentatively considered to be a protein, although full chemical and physical analyses were not undertaken and no claim regarding the purity of the antigen was made (69, 70). The more recent observation of Chen (72) suggests that the alcohol-precipitated envelope antigen contains both protein and polysaccharide. It is conceivable, therefore, that the hemagglutination reaction involves the latter material. Alternatively, it is possible that the polysaccharide may have facilitated the adsorption of the protein (S. J. Silverman, personal communication, February 10, 1956). The second apparent exception pertains to the flagellar antigen of *Salmonella typhosa*. Since only a single experiment was carried out, suggesting that the flagellar antigen was adsorbed on untreated red blood cells (41), corroborative evidence is required. However, it should be kept in mind that, as will be pointed out (p 177), Boyden and Anderson (87) demonstrated adsorption of a tuberculo-protein to untreated red blood cells. Further studies on the interaction of various bacterial proteins and untreated erythrocytes are needed.

Unheated fresh *Escherichia coli* cultures fail to modify red blood cells or do so only to a minimal degree. Upon standing for several days at 37 C or following heating at 100 C for 1 hour, the cultures and their supernatants become highly active. This result is explained by two independent effects of heat, namely, release from the bacterial cells of somatic antigen into solution and activation of the soluble antigen (46). The latter effect can be shown also with purified lipopolysaccharides. It is interesting to note, however, that the extent of heat activation varies with different antigen preparations. For example, two unheated salmonella lipopolysaccharides failed to modify red blood cells in concentration of up to 1000 μg per ml, while another, prepared by the identical method, was active in a concentration of 6 μg per ml. Following heating, the two former preparations were effective in a concentration of 12 μg per ml and the latter in a concentration of 1.5 μg per ml (59). The activation from a non-modifying to a modifying antigen can be accomplished also by treatment with sodium hydroxide (51, 59, 88). However, it must be pointed out that not all bacterial polysaccharides become erythrocyte-modifying upon treatment with sodium hydroxide (51). Furthermore, the mode of action of both heat and sodium hydroxide treatment remains to be determined. It is clear, however, that these treatments do not solely cause the appearance of an increased number of antigen molecules, since they do not effect the antibody neutralizing capacity of the materials (89). Suggestive evidence indicates that these treatments of *S. typhosa* O lipopolysaccharide result in liberation of a large portion of lipid and thus remove a component which conceivably interferes with the adsorption of the antigen by red blood cells (55). This concept finds support in the

[1] The term modification, as used in this review, connotes antigenic alteration of erythrocytes by attachment of bacterial antigens.

TABLE 3

Indirect, conditioned, or passive bacterial hemagglutination

Bacteria	Antigens	References
Micrococcus sp.	Polysaccharide	34, 35, 36
Diplococcus pneumoniae	Polysaccharide (acidic polymer)	34, 36, 37, 38
Streptococcus sp.	Crude, polysaccharide	34, 39
Gram-positive bacteria	Crude, common antigen	40
Neisseria meningitidis	Polysaccharide	34
Neisseria gonorrheae	Polysaccharide	41, 41a
Escherichia coli	Crude, lipopolysaccharide	42, 43, 44
	Vi (crude, acidic polymer)	37, 45
Enteropathogenic *E. coli*	Crude, lipopolysaccharide	44, 46, 47, 48, 48a
Ballerup*	Crude	49
	Vi (crude)	47, 50
Aerobacter aerogenes	Crude, polysaccharide	43, 51, 52
Aerobacter cloacae	Polysaccharide	51
Paracolobactrum sp.	Crude	42, 43
Salmonella typhosa	O (crude, polysaccharide, lipopolysaccharide)	37, 38, 47, 53, 54, 55, 56
	Vi (crude)	38, 47, 50, 54, 56, 57, 58
	H (protein) (?)	41
Salmonella sp.	Crude, polysaccharide, lipopolysaccharide	42, 52, 53, 59, 60, 61
Shigella sp.	Crude, polysaccharide	36, 62, 63, 64
Serratia marcescens	Lipopolysaccharide	37
Proteus sp.	Crude	42, 43
	Lipopolysaccharide	34, 37
X_{19}, X_K	Crude, polysaccharide	65, 66
Pseudomonas aeruginosa	Crude	43
	Lipopolysaccharide	37, 67
Pseudomonas sp.	Crude	43
Vibrio comma	Crude	68
Pasteurella pestis	Protein (?)	69, 70
	Polysaccharide	71, 72
Pasteurella multocida	Capsular antigen	73
Pasteurella tularensis	Lipopolysaccharide, polysaccharide	37, 74, 75
Brucella sp.	Crude	42, 76, 77, 78
	Lipopolysaccharide-polypeptide	78
Malleomyces mallei	Crude	79
Hemophilus influenzae	Crude, polysaccharide	5, 34
Hemophilus pertussis	Crude	18
Corynebacterium diphtheriae	Polysaccharide	36
Mycobacterium tuberculosis	Crude	*cf.* 80
	Fractions, polysaccharide	81, 82, 83
Mycobacterium paratuberculosis	Crude	84
Mycobacterium phlei	Crude	84
Leptospira sp.	Crude	85, 86

* Now included in *Escherichia freundii*.

inhibitory activity of various lipids, mentioned on page 172. It will be interesting to learn whether the above *Salmonella* lipopolysaccharides, which differ so markedly in erythrocyte modifying capacity, contain different amounts of firmly bound lipid. In this connection attention should be called to the observation of Middlebrook (90) to the effect that two polysaccharides were present in a tuberculin fraction, one active, the other inactive, in erythrocyte modification, and yet both inhibiting hemagglutination specifically.

Modification of erythrocytes, demonstrable by

subsequent agglutination in the presence of homologous antibodies, has been accomplished with approximately 2000 molecules of *S. typhosa* antigen per red blood cell (88). This conclusion is based on the observation that 25×10^9 erythrocytes adsorb 100 μg of the antigen.

Until very recently, all bacterial antigens operative in the hemagglutination test were found to be characteristic of the species, common to closely related species, or characteristic of serogroups or serotypes. Rantz and associates (40) demonstrated, by means of this technique, an antigen that appears to be common to many unrelated gram-positive bacteria (among others, staphylococcus and various species of streptococci).

Surprisingly few studies have been reported on the process of attachment of bacterial antigen to untreated red blood cells. It has been shown (59, 91) with crude *E. coli* cultures as well as with purified *Salmonella abortus-equi* and *E. coli* lipopolysaccharides that electrolytes are required, inasmuch as modification does not occur in 5 per cent glucose and 5 per cent sucrose solutions. In contrast, these antigens become attached to the erythrocytes in the presence of isotonic sodium chloride, potassium chloride, or sodium citrate solutions. The failure of calcium chloride solution to effect erythrocyte modification (59) remains unexplained. Interestingly, electrolytes are likewise required for the attachment of certain viruses to red blood cells and of bacteriophage to bacteria. Mention may be made of the fact that modification takes place very rapidly (within a few minutes) at 37 C and only to a minimal or slight extent at 4 C. For these reasons, the term "adsorption", as used in this review and many papers on the subject, does not connote a particular mode of attachment of the bacterial antigens.

Red blood cells from man, horse, sheep, rabbit, ox, and goat are equally well modified by *S. typhosa* Vi antigen (57), as are erythrocytes from man, dog, rabbit, sheep, guinea pig, rat, and chicken by *E. coli* antigen (44). On the other hand, Boyden (79) found that different amounts of mallein were required for modification of erythrocytes from various animal species; he noted furthermore that ox red blood cells adsorb the antigen without being agglutinated by homologous antiserum. Human erythrocytes of blood group O are more suitable for the adsorption of *Pasteurella tularense* polysaccharide than red blood cells of blood groups A and B (74). No information is presently available to explain these differences.

As yet little is known regarding the receptors on the surface of the red blood cell to which bacterial antigens can be attached. Such specific receptors do in fact exist. This is evident from the findings that neither the A, B, or Rh antigens are blocked in *E. coli* and *P. tularense* modified erythrocytes (44, 75) nor does treatment of red blood cells with receptor destroying enzyme (RDE) and periodate interfere with modification by bacterial polysaccharide (36, 75). It is conceivable that lipid structures serve as receptors. This is suggested by the observation that certain lipids, such as lecithin, cephalin, lipid extract from red blood cell stromata, and certain serum fractions rich in lipids inhibit the attachment of certain bacterial antigens to erythrocytes (49, 79, 92, 93, 94). A non-specific factor inhibiting the adsorption of tuberculin antigen by sheep cells has been described by Fine and associates (95). This factor has not yet been identified. It is of interest that it was present more frequently in patients with various diseases than in healthy individuals.

Of particular importance is the fact that several bacterial antigens, either simultaneously or consecutively, modify erythrocytes and that one antigen apparently does not block the adsorption of another (37, 44, 59, 60). Whether different receptors are operative with different bacterial antigens remains to be determined in the future. In contrast to the foregoing observations, an apparent interference between two *S. typhosa* antigens has been demonstrated by Spaun (56). This author found that when a standard amount of O antigen was mixed with a relatively large amount of Vi antigen for modification of erythrocytes, agglutination occurred only in the presence of Vi antibodies; however, red blood cells became agglutinable by either O or Vi antibodies when smaller amounts of Vi antigen were used together with the same amount of O antigen. Spaun's observations have been confirmed recently by Landy and Ceppellini (95a), and it is clear therefore that there exist interfering and non-interfering antigen combinations.

With this modification red blood cells acquire a new serological specificity. Homologous bacterial antibodies cause specific agglutination. In the presence of complement and antibodies cer-

tain modified erythrocytes are *lysed*. However, it should be noted that erythrocytes from different sources are not equally susceptible to lysis. For example, sheep red blood cells modified with crude or purified *E. coli* antigens are readily lysed, whereas human erythrocytes are almost entirely resistant to lysis. This latter finding is not due to lack of adsorption of the bacterial antigen, since the treated red blood cells are as readily agglutinated by homologous bacterial antiserum as are sheep red blood cells. The question arises as to whether lysis of modified human red blood cells may occur in the presence of antibodies and complement of human origin. This was found not to be so (59). Similarly, Skillman and co-workers (96) obtained lysis with sheep cells but not with human cells in a streptococcal hemolysis test; hemagglutination was obtained with erythrocytes from both species. Middlebrook (97), however, observed lysis of human red blood cells modified by tuberculin, although hemolysis occurred only with larger amounts of antiserum than were required for hemagglutination. Further studies are needed with a large variety of bacterial antigens and erythrocytes from many animal species to determine the conditions for, or refractoriness to, lysis.

Attention should be called to the failure of some serum specimens to cause hemagglutination of tuberculin modified erythrocytes, when a fraction of the same sera showed antibody activity (98). As yet, the interfering serum component(s) have not been identified. A number of reactions interfering with antibody activity have been discovered and may be briefly mentioned here. Serum albumin inhibits agglutination of red blood cells exposed to a mixture of tuberculoprotein and homologous antibody (87). The *S. typhosa* R antibody interferes with the O antibody in causing death of the bacterial cell (99). Also, *in vivo* interference of the tuberculo-polysaccharide antibody with the antibody present in a newly discovered plasma fraction (IV-10) responsible for the delayed type of tuberculin (PPD) skin sensitivity has been reported (100). The latter findings require confirmation.

The observation of Staub (88) to the effect that non-precipitating antibody can be demonstrated in the *S. tyhposa* hemagglutination test is of interest. Whether this antibody is in fact totally devoid of precipitating activity, remains to be determined. Also, reference should be made to the observation indicating that modification and subsequent hemagglutination may be enhanced if papain or trypsin treated red blood cells are used (96). Finally, attempts have been made to increase the sensitivity of the hemagglutination reaction by the use of anti-globulin serum (Coombs test) (38, 54) and by replacement of the hemolytic with the conglutination system (38).

Related Reactions

The attachment of antigenic material, resulting in the acquisition of a new serologic specificity, is restricted neither to bacterial antigens nor to erythrocytes. Modification of red blood cells and subsequent hemagglutination in the presence of homologous microbial antibodies has been demonstrated with antigens obtained from rickettsiae (101, 102), trypanosome (103), schistosome (104), candida (105), histoplasma (106), and trichomonas (107).

De Gregorio (108) made the interesting observation that leukocytes, splenic, hepatic, and renal cells adsorb salmonella antigen *in vitro* and thus become specifically agglutinable in the presence of homologous bacterial antibodies. Working with a polysaccharide preparation made from tuberculin, Boyden (109) showed that modified leukocytes and lymphocytes become attached to modified erythrocytes upon addition of homologous antibodies, indicating that these white cells, too, had adsorbed the antigen. Further studies on the possible modification of other tissue cells are needed. Such studies may reveal information as to which cells have receptors for one antigen or another. Since leukocytes play a significant role in the generalized Shwartzman reaction (110), the question may be raised as to whether the bacterial antigen, responsible for this reaction, is adsorbed to white blood cells; adsorption may lead to injury of the leukocytes and to subsequent formation of "fibrinoid" material. In this connection one recalls that particles other than cells, *e. g.*, collodion particles and ion exchange resins (111), are capable of adsorbing various antigens and thus become agglutinable in the presence of the corresponding antibodies. Also, it is interesting to note that Adler (112) succeeded in attaching bacterial antigens to heterologous bacteria and rendering them susceptible to the bactericidal activity of the antibody reacting with the ar-

tificially attached antigen. It has been known for many years that bacteria may adsorb to their surface components of the culture or suspending media.

An interesting reaction between bacteria and erythrocytes was described by Nelson (113) under the name of the immune-adherence phenomenon. This author observed the attachment of spirochetes and pneumococci to red blood cells, provided that homologous antimicrobial antibodies and complement were present. Under the conditions of these experiments hemagglutination did not occur. Following adherence of the microorganisms to erythrocytes enhanced phagocytosis was observed. It may be pointed out that Rieckenberg (114) as early as 1917 described a similar immunologic reaction; namely, the adherence of trypanosomes to blood platelets in the presence of homologous antibody. The relationship between these two reactions is not entirely clear because of inadequate data in Rieckenberg's paper.

Since completion of this review a report on this subject has been published by Lamanna and Hollander (114a).

Biologic Significance

The bacterial hemagglutination and hemolysis reactions are of interest to the bacteriologist, virologist, immunologist, hematologist, and to the clinician. Of particular importance is the question whether bacterial modification also occurs *in vivo*. To date, only limited information is available. Ceppellini and De Gregorio (115) failed to induce *in vivo* modification by injection of a bacterial polysaccharide from a ballerup strain. However, they made the interesting observation that erythrocytes modified *in vitro* survived for 28 hours in non-immunized animals, whereas in immunized rabbits clinical evidence of *in vivo* hemolysis occurred within a few hours. In addition, by means of the Coombs test, the reaction between bacterial antibodies and modified red blood cells was demonstrated. In a later report (108) De Gregorio stated that when large amounts (10 mg) of Vi antigen were injected intravenously into guinea pigs, antigen became attached to erythrocytes *in vivo* but not to tissue cells. Similarly, Boyden (109), working with a polysaccharide fraction made from tuberculin, showed that erythrocyte modification takes place *in vivo* in guinea pigs, provided that relatively large amounts (10–20 mg) of antigen are injected. Further work needs to be done to determine whether *in vivo* modification occurs following injection of other antigens as well. Of particular interest will be information as to whether adsorption of bacterial antigen to erythrocytes and other cells occurs in natural infections of man and animals. Skillman and co-workers (96) claimed to have demonstrated *in vivo* modification of red blood cells with streptococcal antigen in patients with active rheumatic fever. This important observation requires confirmation from other laboratories. If, indeed, modification takes place during natural infections, it is conceivable that hemolysis and anemia occur with the appearance of homologous bacterial antibodies, and the question arises as to whether this modification contributes to sludging of blood. In this connection attention should be called to the reports of Nungester and his associates (115a, 115b). These authors showed that pneumococcus polysaccharide affects red blood cells, resulting in enhanced sedimentation *in vitro* and altered flow *in vivo*.

It is established that the somatic antigens of enterobacteriaceae are made up of polysaccharide, lipid, and protein (or a protein-like substance) (*cf.* 116). It is the polysaccharide which endows this complex with its serological specificity, demonstrable by means of the hemagglutination test and other methods. This somatic antigen also has both toxic and pyrogenic properties. Renewed interest in the pathogenesis of fever following injection of bacterial pyrogens has been shown (117, 118). For these reasons, study of the interaction between these and similar antigens and red blood cells may eventually throw light on the modes of action of these materials as toxins and pyrogens. It has been shown (89) that, to some extent, treatment with heat, sodium hydroxide, and periodate has different effects on toxicity, pyrogenicity, erythrocyte modifying capacity, and antibody neutralizing ability of these lipopolysaccharides, suggesting that different reactive groupings are operative in these activities.

Attention should be called to the properdin-binding capacity of zymosan and certain bacterial polysaccharide antigens (119). Properdin, as is well known (120), is one of the non-specific factors contributing to resistance to infection. To what extent, if any, various polysaccharide

antigens combine with plasma factors, such as properdin, and with circulating and sessile cells *in vivo* during experimental and natural infection remains to be determined.

In 1947 Keogh and associates (5) made the significant observation that red blood cells which had adsorbed *Hemophilus influenzae* antigen became refractory to agglutination by influenza virus and by the hemagglutinin of *Hemophilus pertussis*. A detailed study of the effects of bacterial polysaccharides on viral hemagglutination has been carried out by MacPherson and associates (51). These authors observed that polysaccharide antigens prepared from *Aerobacter aerogenes* and *A. cloacae* inhibit red blood cell agglutination by influenza, mumps, and Newcastle disease viruses. Furthermore, they noticed that modified red blood cells adsorb PR 8 virus as readily as untreated erythrocytes; mumps virus, on the other hand, was poorly adsorbed by polysaccharide modified red blood cells. Therefore, two mechanisms account for the inhibition of virus hemagglutination. In the former instance, the increased charge on the surface of the red blood cells inhibits clumping by, but not adsorption of, influenza virus. In the second instance, inhibition of virus adsorption readily explains the failure of hemagglutination. The complexity of the subject is also evident from the fact that one particular polysaccharide antigen of MacPherson *et al.* is adsorbed to red blood cells, but does not inhibit virus hemagglutination.

Hemolysis of erythrocytes modified by bacterial antigens is of interest also to the student of antibody-mediated hemolysis, since it shows that anti-erythrocyte antibodies are not needed for this reaction. This observation parallels studies on hemolysis of chemically modified azo-erythrocytes in the presence of complement and antibodies to the attached groups (121). Similarly, antigenically modified bacteria are killed upon addition of complement and of antibodies directed against an artificially attached antigen (112). These data may eventually aid in the elucidation of the mode of action of antibody and complement in lysis of erythrocytes and killing of bacteria.

Applications

The indirect, conditioned, or passive bacterial hemagglutination and hemolysis tests have been applied to the study of many problems. It is not the purpose of this review, nor does space permit, to appraise critically all these applications. Rather, a few representative examples will be cited to indicate the potential usefulness of these techniques.

Demonstration and titration of bacterial antibodies. In many instances the hemagglutination and hemolysis tests proved to be considerably more sensitive than other serological methods used for comparison, for example, the conventional bacterial agglutination test. Differences in sensitivity of these methods may be due, in part at least, to differences in the amount of antigen present on the surface of bacteria and of modified red blood cells. At times, the superiority of the hemagglutination method is of such a magnitude as to appear qualitative rather than quantitative. For example, Gaines and Landy (67) found antibodies to *Pseudomonas aeruginosa* in the sera of normal individuals exclusively by means of the hemagglutination test. In one instance the hemagglutinin titer was as high as 1:960, and yet bacterial agglutinins could not be detected. Similar observations have been recorded by Neter and co-workers (122) with respect to antibodies to enteropathogenic *E. coli* in the serum of healthy adults. On the other hand, the hemagglutinin titers in the sera of volunteers who had ingested *E. coli* were from 5 to 20 times higher than the corresponding titers of bacterial agglutinins, although the same serogroup of *E. coli* was used (123). An explanation of these differences must await the results of future research. It is evident, however, that in the search for antibodies against bacterial polysaccharides the hemagglutination and hemolysis tests may be used to advantage, particularly should other methods yield negative results. In passing, mention should be made of the fact that preliminary absorption with untreated red blood cells is a necessity, when serum specimens are tested with heterologous red blood cells. This difficulty can be circumvented by the use of human erythrocytes (blood group O, Rh negative) in the examination of human serum specimens.

The polyvalent hemagglutination and hemolysis tests. A particularly welcome feature of the bacterial hemagglutination test, at least so far as enterobacterial antigens are concerned, is the fact that several antigens can be simultaneously adsorbed on erythrocytes. Such a red blood cell

suspension thus becomes a useful tool as a polyvalent antigen for the detection of any homologous antibody. This polyvalent hemagglutination test has been used in the study of the antibody response of patients with infections due to enteropathogenic *E. coli*, salmonellae, and shigellae (59, 60, 122). Further studies on the diagnostic potentialities of this method are indicated.

Demonstration and titration of bacterial antigens. The hemagglutination or hemolysis inhibition tests provide useful tools for identification and determination of minute amounts of certain bacterial antigens. To this end, the antigen under investigation is first mixed with a suitable amount of a known bacterial antiserum; then, erythrocytes modified by the homologous antigen are added, and the inhibition of specific hemagglutination or hemolysis is noted. The sensitivity of this procedure can be illustrated by the fact that as little as 0.006 μg of *Shigella dysenteriae* polysaccharide could be measured, although no efforts had been made to increase the sensitivity of the method (64). Similarly, Wright and Feinberg (75) determined *P. tularense* antigen in amounts of 0.001 μg. Demonstration of microbial antigens by means of the hemagglutination inhibition test in specimens from patients may eventually become a suitable method for early and specific diagnosis of certain infectious diseases. This approach has been used by Warburton and co-workers (124) for the detection of antigen in cerebrospinal fluid from patients with *H. influenzae* meningitis and by O'Connor and MacDonald (65) for the diagnosis of scrub typhus. It is conceivable that this method may be applicable also to the early diagnosis of enteric infections (59).

Differentiation of bacterial antigens. The hemagglutination and hemagglutination inhibition tests have been used with success for the study of closely related antigens. For example, such differences were noted among Vi antigens of *S. typhosa* and a ballerup strain (50). Similarly, two different modifying substances could be demonstrated in tuberculin by means of hemagglutination and hemagglutination inhibition tests (81).

Serologic identification of unstable bacterial suspensions. Use of soluble bacterial antigen in the hemagglutination reaction obtained from strains in unstable suspension, which are unsuitable for agglutination tests, may make possible their identification. This approach has been fruitful in studies on *P. pestis* (69) and certain strains of *E. coli* (48).

Antigenic composition of microorganisms. The hemagglutination inhibition test has been used successfully for the determination of the location of bacterial antigens in bacterial cells (83), and the hemagglutination test for the existence of cross-reacting antigens in unrelated bacterial species (80, 125). In view of the well known antigenic relationship between *Mycobacterium tuberculosis* and *Mycobacterium leprae* it is not surprising to find that patients with Hansen's disease give a positive tuberculin hemagglutination test (126, 127).

Removal of an antigen from a mixture. If one is dealing with a mixture of two antigens, only one of which is adsorbable on untreated erythrocytes, the latter may then be used for the separation of these antigens. It appears likely that this absorption technique will lend itself for this purpose, just as erythrocytes have been used for the separation of botulinal hemagglutinin and toxin (26).

Immunization with modified erythrocytes. Erythrocytes modified by a bacterial antigen may be employed for immunization purposes. Indeed, a pure Vi antiserum was produced in rabbits by the injection of appropriately modified rabbit erythrocytes (50). Similarly, antibodies developed following the injection of erythrocytes coated with *E. coli* (44) antigen, and *P. tularense* (75) hapten. A non-immunizing hapten thus becomes an immunizing antigen in the rabbit (75).

Aid in determination of pathogenicity. It has been suspected on epidemiological grounds that certain serogroups of *E. coli* cause epidemic and sporadic diarrhea of the newborn and young infant. Feeding studies have contributed supporting evidence. However, in the majority of infants antibody formation could not be demonstrated by the conventional methods. The hemagglutination test, however, yielded positive results and thus provided additional evidence of the pathogenicity of these microorganisms, now referred to as enteropathogenic *E. coli* (46, 48a, 59).

Clinical applications for diagnosis of infections. Before any new serologic technique, such as the bacterial hemagglutination and hemolysis tests, are introduced into clinical practice, it is of utmost importance that a full understanding of their usefulness and limitations be available. Particularly, it is necessary to know whether the

presence of antibodies or antigens thus demonstrated is correlated with activity of the infectious process. With the introduction of the Middlebrook-Dubos tuberculo-polysaccharide hemagglutination test, many clinicians hoped that this method will give them the long awaited serologic test for the specific diagnosis of active tuberculosis, be it mild or severe, recent or of long standing, in infants or adults. As is well known today and emphasized by Middlebrook (80) himself this test does not deserve widespread routine clinical application at this time. However, suggestive evidence of the potential clinical usefulness of a variety of bacterial hemagglutination tests is at hand, although only adequate trials in the field will give the final answers. For example, it is conceivable that the hemagglutination technic may provide information of diagnostic significance with regard to infantile diarrhea due to enteropathogenic *E. coli* (59), salmonellosis (60), shigellosis (63), and other bacterial infections. The test has been applied also to the detection of typhoid carriers (45, 128). Recently, Skillman and associates (96) reported that a hemagglutination test for the demonstration of streptococcal antibodies as well as demonstration of *in vivo* modification of patients' red blood cells with streptococcal antigen have proved to be of considerable aid in ascertaining activity of rheumatic fever. Confirmation of these results is needed, before the conclusions can be accepted.

IV. BACTERIAL HEMAGGLUTINATION AND HEMOLYSIS OF ERYTHROCYTES TREATED WITH ANTIGEN-ANTIBODY MIXTURE

Boyden and Anderson (87) made the significant observation that exposure of untreated sheep red blood cells to a mixture of tuberculoprotein and homologous antibody results in hemagglutination and, in the presence of complement, in hemolysis. This reaction differs in certain essential features from the polysaccharide hemagglutination and hemolysis tests discussed in the previous section. In the first place, the antigen is a protein. Secondly, it becomes dissociated from erythrocytes upon repeated washings. Thirdly, sensitization of erythrocytes with the antigen-antibody mixture and subsequent hemagglutination takes place readily at both 37 and 4 C. Apparently, Middlebrook (90) also observed agglutination of red blood cells exposed to a mixture of antigen and antibody. Boyden and Anderson made another significant observation; namely, that the antibody operative in this particular hemagglutination test is inhibited by a human serum fraction and that seemingly negative immune sera obtained from immunized rabbits produce hemagglutination if the albumin fraction is first removed. Clearly, a new type of hemagglutination reaction has been discovered, warranting further research.

V. BACTERIAL HEMAGGLUTINATION OF TANNIC ACID PRETREATED ERYTHROCYTES

On the assumption that modification of erythrocytes by polysaccharide antigen (*cf.* section III) takes place in two stages, namely, alteration of the surface of the red blood cell followed by attachment of the polysaccharide antigen, Boyden (129) investigated a variety of substances, in order to determine whether any of these materials produce the first effect and render red blood cells capable of adsorbing protein antigens. These attempts proved to be highly successful. Certain inulin preparations were shown to be effective, but far superior results were obtained with tannic acid. Both materials in higher concentration agglutinate erythrocytes. In the procedure, which has to be carried out with great care, erythrocytes are first treated with tannic acid. These pretreated red blood cells are then exposed to the protein antigen and thus acquire a new serologic specificity, inasmuch as they are specifically agglutinated in the presence of homologous protein antibodies. In this way Boyden could demonstrate hemagglutination of tuberculo-protein modified red blood cells by tuberculin antibodies. Similarly, streptococcal protein antibodies could be detected. Boyden also devised a hemagglutination inhibition test for the titration of protein antigens. Boyden's contribution represents a significant advance and served as a valuable stimulus.

Table 4 summarizes data on various bacterial protein antigens which can be adsorbed on tannic acid treated erythrocytes. The homologous antibodies can be titrated with modified erythrocytes, the antigens by means of the hemagglutination inhibition test.

To illustrate the sensitivity of this procedure, the following observations are cited. Stavitsky (130) showed that as little as 0.001 μg of diphtheria toxoid can be measured accurately. Erythrocyte modification was accomplished with 0.125

TABLE 4
Bacterial hemagglutination of tannic acid treated erythrocytes

Bacteria	Antigen	References
Streptococcus pyogenes	Protein	129
Corynebacterium diphtheriae	Toxin, toxoid	37, 130, 131, 132, 133
Pasteurella pestis	Protein	37, 71, 134, 135, 136
	Toxin, toxoid	37, 137
Clostridium perfringens	Alpha toxin lecithinase	131
Clostridium tetani	Toxoid	131
Mycobacterium tuberculosis	Protein	82, 100, 129, 138, 139

μg per ml of diphtheria toxoid (130) and with 0.3 μg per ml of purified capsular protein of *Pasteurella pestis* (136).

The sensitivity of the tannic acid hemagglutination test for the titration of bacterial protein antibodies becomes even more apparent on comparison with other available methods. For example, the hemagglutination test proved to be 20 to 50 times more sensitive for the titration of antibodies to the capsular antigen of the plague bacillus than the complement fixation test (136).

Modification of erythrocytes is inhibited by certain cations, particularly Mn. The presence of these and other inhibiting substances in crude diphtheria toxoid probably accounts for the failure to demonstrate erythrocyte modification with some of these preparations (133). Studies on interfering materials may eventually aid in our understanding of the precise changes caused by tannic acid treatment which result in the newly acquired capacity for protein adsorption.

Tannic acid treated erythrocytes adsorb two protein antigens (diphtheria and tetanus toxoids) simultaneously (131), just as untreated erythrocytes adsorb several polysaccharide antigens. Mention should be made that tannic acid treated erythrocytes can adsorb polysaccharide antigens as well. However, there are differences in the temperature and time dependence of these adsorptions. Protein is adsorbed readily at lower temperature and at a greater speed than polysaccharide antigen. For these reasons Chen and Meyer (134) use a temperature of 37 C for 1 hour for polysaccharide adsorption and room temperature for a maximum of 15 minutes for protein adsorption.

The tannic acid hemagglutination test lends itself readily to the study of many bacteriologic, serologic, and immunologic reactions. Nonflocculating antitoxin has been detected (131). The method has been applied with success to the study of the role of the protein antigen of the plague bacillus in immunity and to the diagnosis of this infection (134). Several protein antigens have been detected in the tubercle bacillus (139), and information has been gained on the location of various antigenic components on the surface and in the depth of the bacterial cell (83). Finally, attention should be called to the observation of Hinz and Pillemer (140) that lysis of tannic acid treated cells, noted by several investigators, is mediated by the properdin system.

VI. BACTERIAL HEMAGGLUTINATION BY ANTIBODIES AGAINST CHEMICALLY ATTACHED PROTEIN ANTIGENS

In addition to the aforementioned tannic acid hemagglutination test, protein antigens may be attached to erythrocytes also by chemical linkages. Such a method, devised by Pressman, Campbell, and Pauling (140a), was used by Stavitsky and Arquilla (141). The method employs bis-diazotized benzidine as the protein-conjugating material. The authors showed that, in addition to several non-bacterial proteins, diphtheria toxoid can thus be attached to erythrocytes; the latter become agglutinated in the presence of homologous diphtheria antitoxin. It is feasible to use the method also as a hemolysis test, provided that complement is added to the system. Titration of the antigens is possible by the use of the hemagglutination or hemolysis inhibition procedures. Cole and Farrell (142) succeeded in attaching to formalinized erythrocytes tuberculin PPD via tetrazotized benzidine.

VII. RED CELL LINKED ANTIGEN HEMAGGLUTINATION TEST

Coombs and his associates have made an important contribution by devising a new approach for attachment of antigens to red blood cells. They have shown that non-bacterial protein

antigens may be chemically coupled to incomplete Rh (143), ox red cell (144) and Forssman (6) antibodies. These erythrocyte antibodies by themselves do not cause hemagglutination. They serve as *Schleppers* (carriers) for the protein. Erythrocytes treated with such an antigen-antibody complex then become agglutinable in the presence of homologous antibodies to the artificially attached antigen. Reference to these methods is made, because it seems plausible to this reviewer that this ingenious approach of Coombs and associates may lend itself to the study of bacterial protein antigens as well.

VIII. BACTERIAL PANAGGLUTINATION

The Thomsen-Friedenreich Hemagglutination Phenomenon and its Biologic Significance

In 1927, Thomsen (145) observed the apparent change of blood group of a blood specimen, inasmuch as the erythrocytes were agglutinated in the presence of any normal human serum. The study of this accidentally made observation revealed that it was due to a propagating agent, affecting all human erythrocytes, and rendering them panagglutinable. The agglutination reaction was due to an apparent change of a latent red blood cell receptor, referred to as L (latent) receptor and its reactivity with a third blood group agglutinin. An extensive investigation of the Thomsen hemagglutination phenomenon was carried out by Friedenreich (146), and the results were published in monograph form (in English) in 1930. Reading this treatise a quarter of a century later, this reviewer is impressed with the excellence of Friedenreich's experimental studies and his interpretation of the results. This hemagglutination reaction is frequently and justifiably referred to as Thomsen-Friedenreich hemagglutination phenomenon.

The following facts have emerged from these fundamental studies.

1. The transformation of the red blood cell is due to enzymatic activity of certain bacteria and uncovers a substrate on the surface of the erythrocyte. This altered substrate, referred to as T receptor or T antigen, has the ability to react with the corresponding T antibody. (The abbreviation T was introduced by Friedenreich in recognition of Thomsen's discovery.)

2. T agglutinins are present in normal human sera, although conspicuously absent in cord blood and blood from young infants.

3. The bacterial enzyme does not by itself cause agglutination of red blood cells. The enzyme is heat labile and filterable.

4. The T antibody is not a bacterial agglutinin, in as much as it does not cause agglutination of the very cultures which produce a transformation of red blood cells.

5. The active principle of the bacterial culture is rapidly fixed by red blood cells and, as its activity progresses, eluted in an unaltered state from the bacterial cell.

6. Transformed red blood cells no longer adsorb the transforming principle.

7. The T agglutinin reacts optimally at 15–20 C.

8. The T agglutinin differs from the normally occurring cold agglutinins, as evidenced by the results of absorption studies.

9. Bacterial transformation can be accomplished with red blood cells from animal species other than man. With guinea pig erythrocytes hemolysis can be demonstrated in addition to hemagglutination, provided that T antibodies and complement are present.

10. The panagglutinating activity has been demonstrated in two strains of diphtheroids, in several strains of vibrios, and one strain of a luminescent cocco-bacillus.

11. One of the active bacteria also has the capacity of destroying the T antigen by means of the "receptor destroying principle".

12. This hemagglutination reaction may lead to erroneous results in blood grouping. Transfusion into guinea pigs of altered erythrocytes leads to shock associated with hemolysis. (The similarity of this reaction to paroxysmal hemoglobinuria is mentioned.)

All basic observations of Thomsen and of Friedenreich have been amply confirmed during the ensuing 25 years. Friedenreich's concept regarding the mode of action of transforming bacteria has certain striking resemblances to the action of influenza virus on erythrocytes, as elucidated by Hirst (1) in 1941. In both instances, the evidence strongly suggests that adsorption of the microbial enzyme or the virus on the red blood cells represents the first step of microbe-erythrocyte interaction; that a specific chemical compound on the surface of the red blood cell serves as receptor; that enzymatic change of the receptor takes place; that elution of the microbial enzyme or virus follows this enzymatic modification of the red blood cell receptor; and that the altered erythrocyte no longer adsorbs the enzyme or virus.

It should be mentioned that various bacteria cause somewhat different changes on erythrocytes. *Micrococcus pyogenes* var. *aureus* unmasks an antigen different from the T receptor (147). It is noteworthy also that periodate treated erythrocytes are agglutinated by a panagglutinin other than the T antibody (147).

Important studies have been carried out with the enzyme of the cholera vibrio, usually referred to as RDE (receptor destroying enzyme) (148). The following changes are effected by this enzyme in addition to panagglutinability.

1. Inagglutinability of red blood cells by influenza virus due to lack of virus adsorption.

2. Increased agglutinability in the presence of "incomplete" Rh antibodies, provided that the red blood cells contain the Rh antigen.

3. Altered immunogenic characteristics of the erythrocytes.

Among other panagglutinating bacteria are *Clostridium perfringens*, *Vibrio proteus*, and *Diplococcus pneumoniae* (149, 150). A partially purified enzyme preparation obtained from *C. perfringens* type B showed identical or, at least, similar activities on erythrocytes as the cholera vibrio enzyme. According to Chu (149), many other bacterial species cause panagglutinability without some of the other changes associated with RDE action. It should be emphasized that some bacteria produce panagglutinability without viral inagglutinability, and others produce viral inagglutinability without panagglutinability.

It is remarkable that influenza virus causes almost identical changes on the red blood cell as RDE, presumably because it contains a similar enzyme. Furthermore, it has been shown that certain of these changes can be effected by other well known enzymes (trypsin, ficin, papain) and by periodate.

So far as the biologic significance and applications of this hemagglutination reaction are concerned, it is obvious that contamination of a blood specimen with panagglutinating bacteria leads to erroneous results in blood grouping. This is because such cells are agglutinated by blood grouping serum, even if the corresponding blood group antigens (A, B, etc.) are not present on the surface of the red blood cells. Study of the activity of bacterial enzymes causing panagglutinability have revealed important informaton on the chemical identity of the receptors serving for the attachment of influenza and other viruses and on the antigenic makeup of the erythrocyte. Enzyme (trypsin) treatment of red blood cells is now used routinely for the detection of "incomplete" Rh antibodies.

Recently, it was shown by Stewart, Petenyi, and Rose (151) that erythrocytes after treatment with influenza virus have a greatly shortened survival time and that the panagglutinin may be responsible for their rapid destruction. That bacterial infections can cause an abrupt decrease in the titer of T agglutinins has been shown to occur in experimental anthrax infection (152). Friedenreich's observation that transfusion of altered erythrocytes causes *in vivo* hemolysis has not attracted the attention it deserves of students of "auto-immune" (auto-antibody) hemolysis and other disease states with a similar pathogenesis. So far as the clinical significance of the bacterial and viral panagglutination reactions is concerned, the following questions present themselves: (1) Are natural bacterial and viral infections associated with enzymatic changes of red blood cells? (2) If so, are these enzymatically altered cells agglutinated or lysed by panagglutinins or specific immune bodies acting on unmasked antigenic components and do they engender auto-antibodies? It is conceivable that these or similar events account for certain manifestations (enhanced erythrophagocytosis, hemolytic anemia, sludging of blood) of various disease processes. These reactions, related to the Thomsen-Friedenreich phenomenon, cannot be discussed here in detail, however provocative the data and their implications are.

IX. BACTERIOGENIC HEMAGGLUTINATION

Another type of bacterial hemagglutination, which is closely related to the Thomsen-Friedenreich reaction, was discovered by Davidsohn and Toharsky (153, 154) and was described as bacteriogenic hemagglutination. These authors observed that a special bacterial strain belonging to the genus *Corynebacterium* produced changes in human plasma and serum which endow these materials with panagglutinating capacity. Of importance is that even cord serum, which does not contain T antibody, can be rendered agglutinating by the microorganism. The strain was named *Corynebacterium* H in honor of Hektoen. The active principle can be separated

from the bacterial culture by filtration. Among a large number of other bacterial strains tested, only a strain of *Pseudomonas aeruginosa* was found to be active. The filtrate also alters red blood cells themselves, rendering them panagglutinable. By means of absorption studies it was shown that the antigen-antibody system involved in bacteriogenic hemagglutination is different from that of the Thomsen-Friedenreich reaction and entirely independent of blood groups. In conformity with the nomenclature of the Thomsen-Friedenreich reaction (T antigen and T antibody), Davidsohn and Toharsky gave their antigen-antibody system the designation of H. The identity of the agglutinating principle appearing in plasma and serum remains to be established, and no claim has been made as to whether it is an antibody in the strict sense of the word. It has been shown by Spielmann (155) that certain bacteria render human serum specimens containing "incomplete" Rh antibodies capable of causing Rh hemagglutination. From a practical point of view it is obvious that contamination of blood grouping serum with a strain causing bacteriogenic hemagglutination renders this reagent unsuitable. No information is at hand as to the possible *in vivo* occurrence of transformation of plasma and bacteriogenic hemagglutination.

X. NOMENCLATURE

It is only natural that, with the rapid development in a new field, such as bacterial hemagglutination and hemolysis, a number of terms are being introduced, some of them being synonyms. In a recent editorial (156) the present author has pointed out that a logical, generally acceptable nomenclature should be devised. Such a task should not be undertaken by a reviewer of the subject, and recommendations, therefore, will not be offered here. Rather, some of the terms used (but not necessarily coined) by investigators in this field are herewith presented with some comments.

1. Bacterial products or components which, without participation of antibodies, cause agglutination of red blood cells are referred to as "hemagglutinins" (4). It should be kept in mind that this term is used also for antibodies acting on antigenic components of red blood cells as well as for antibodies causing agglutination of modified erythrocytes. Antibodies against the bacterial product responsible for bacterial hemagglutination have been called "antihemagglutinins" (4).

2. Agglutination of red blood cells resulting from the action of bacteria or bacterial products has been referred to as "direct bacterial hemagglutination" (46) in order to differentiate this reaction from that requiring bacterial antibodies ("indirect bacterial hemagglutination").

3. Antigens which become attached to untreated red blood cells have been referred to as "erythrocyte modifying antigen" (94), "hemosensitin" (81), "erythrocyte sensitizing substance" (ESS) (85), and "erythrocyte coating antigen" (35). The term "erythrocyte sensitization" has been used also for the reaction between erythrocytes and antibodies (complete and incomplete) directed against the former's components. The term "coating" must not imply coverage of the entire surface, since the attached antigens leave certain surface antigens uncovered.

4. Antibodies causing agglutination of modified erythrocytes have been called "hemagglutinins" (43).

5. The following terms have been used with regard to hemagglutination or hemolysis of modified erythrocytes: "hemagglutination", "passive hemagglutination" (38), "conditioned hemagglutination" (103), "indirect hemagglutination" (46), "antigen-coated red cell technic" (6), "polysaccharide specific hemagglutination" (134), "Keogh antigen adsorption test" (69), "Keogh method" (83), and "polysaccharide lysis test" (41). The term "indirect bacterial hemagglutination" was coined to differentiate it from that due to direct bacterial action in the absence of antibody (46).

6. The hemagglutination method, employing tannic acid treated erythrocytes, is referred to as "Boyden method" (83), "protein-specific hemagglutination test" (134), and "hemagglutination test". It may be pointed out that the term "protein-specific hemagglutination test" may be misleading, since tannic acid treated erythrocytes also adsorb polysaccharides.

XI. SUMMARY AND OUTLOOK

Perusal of the numerous papers dealing directly or indirectly with bacterial hemagglutination and hemolysis clearly reveals that striking advances have been made, particularly during the

last decade. Many related reactions are of interest to bacteriologists, virologists, hematologists, and immunologists alike. It is also obvious, as mentioned during the foregoing discussion, that numerous problems await elucidation by competent and interested workers. It is noteworthy that far more is known regarding indirect than direct bacterial hemagglutination, whereas the reverse is true regarding viral hemagglutination. The study of the interaction of bacteria and their antigens with red blood cells has yielded valuable information on the antigenic composition of various microorganisms and of erythrocytes, and the hemagglutination and hemolysis reactions have been useful as tools for the detection and titration of numerous bacterial antigens and antibodies. If some years from now another review be written on bacterial hemagglutination and hemolysis, it can be confidently expected that many of the gaps in our knowledge, now so clearly apparent, will have been filled.

REFERENCES

1. HIRST, G. K. 1941 The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. Science, **94**, 22–23.
2. MCCLELLAND, L., AND HARE, R. 1941 The adsorption of influenza virus by red cells and a new *in vitro* method of measuring antibodies for influenza virus. Can. Public Health J., **32**, 530–538.
3. BURNET, F. M. 1952 Haemagglutination in relation to host cell–virus interaction. In *Ann. Rev. Microbiol.*, **6**, pp 229–246. Edited by C. E. Clifton, S. Raffel, and H. A. Barker. Annual Reviews, Stanford, Calif.
4. KRAUS, R., AND LUDWIG, S. 1902 Über Bacteriohämagglutinine und Antihämagglutinine. Wien. klin. Wochschr., **15**, 120–121.
5. KEOGH, E. V., NORTH, E. A., AND WARBURTON, M. F. 1947 Haemagglutinins of the *Haemophilus* group. Nature, **160**, 63.
6. COOMBS, R. R. A., AND FISET, M. L. 1954 Detection of complete and incomplete antibodies to egg albumin by means of a sheep red cell-egg albumin antigen unit. Brit. J. Exptl. Pathol., **35**, 472–477.
7. FUKUHARA, Y. 1909 Über Hämagglutinierende Eigenschaften der Bakterien. Z. Immunitätsforsch., **2**, 313–322.
8. GUYOT, G. 1908 Über die bakterielle Hämagglutination (Bakterio-Haemoagglutination). Centr. Bakteriol. Parasitenk., **47**, 640–653.
9. ROSENTHAL, L. 1943 Agglutinating properties of *Escherichia coli*. J. Bacteriol., **45**, 545–550.
10. KAUFFMANN, F. 1948 On haemagglutination by *Escherichia coli*. Acta Pathol. Microbiol. Scand., **25**, 502–506.
11. COLLIER, W. A., AND JACOEB, M. 1955 Experiments with a hemagglutinating strain of *E. coli*. Antonie van Leeuwenhoek. J. Microbiol. Serol., **21**, 113–123.
12. GRIFFITTS, J. J. 1948 Hemagglutination by bacterial suspensions with special reference to *Shigella alkalescens*. Proc. Soc. Exptl. Biol. Med., **67**, 358–362.
13. GUPTA, N. P. 1950 A note on the haemagglutination by organisms of the alkalescens-dispar group. Acta Pathol. Microbiol. Scand., **27**, 300–303.
14. DRIMMER-HERRNHEISER, H. 1953 Haemagglutination by a pseudomonas of faecal origin. Bull. Research Council Israel, **2**, 445.
15. DAVIS, D. J., PITTMAN, M., AND GRIFFITTS, J. J. 1950 Hemagglutination by the Koch-Weeks bacillus (*Hemophilus aegyptius*). J. Bacteriol., **59**, 427–431.
16. SMITH, C. H. 1954 Relationship between haemagglutination and pathogenicity in strains of *Haemophilus* isolated from the eye. J. Pathol. Bacteriol., **68**, 284–287.
17. WARBURTON, M. F., AND FISHER, S. 1951 The haemagglutinin of *Haemophilus pertussis*. Australian J. Exptl. Biol. Med. Sci., **29**, 265–272.
18. FISHER, S. 1950 The haemagglutinin of *Haemophilus pertussis*. Australian J. Exptl. Biol. Med. Sci., **28**, 509–516.
19. LEARNED, G. R., AND METCALF, T. G. 1952 A study of the hemagglutinative behaviour of the lipid antigens of *Corynebacterium diphtheriae*. Trans. Kansas Acad. Sci., **55**, 431–438.
20. LAMANNA, C., AND LOWENTHAL, J. P. 1951 The lack of identity between hemagglutinin and the toxin of type A botulinal organism. J. Bacteriol., **61**, 751–752.
21. STERNE, M. 1954 Hemagglutination by *Clostridium botulinum* type D. Science, **119**, 440–441.
22. DAFAALLA, E. N., AND SOLTYS, M. A. 1951 Studies on agglutination of red cells by clostridia. Brit. J. Exptl. Pathol., **32**, 510–515.
23. FISHER, S. 1948 The inhibition of per-

tussis haemagglutinin by extracts of erythrocytes. Brit. J. Exptl. Pathol., **29**, 357–363.

24. Fisher, S. 1949 The erythrocyte receptor for pertussis haemagglutinin. Brit. J. Exptl. Pathol., **30**, 185–189.
25. Lowenthal, J. P., and Lamanna, C. 1951 Factors affecting the botulinal hemagglutination reaction, and the relationship between hemagglutinating activity and toxicity of toxin preparations. Am. J. Hyg., **54**, 342–353.
26. Lowenthal, J. P., and Lamanna, C. 1953 Characterization of botulinal hemagglutination. Am. J. Hyg., **57**, 46–59.
27. Bernheimer, A. W., and Farkas, M. E. 1953 Hemagglutinins among higher fungi. J. Immunol., **70**, 197–198.
28. O'Connor, J. L. 1945 Hirst's haemagglutination phenomenon exhibited by *Rickettsia orientalis* (Syn. Tsutsugamushi). Med. J. Australia, **2**, 459–460.
29. Fahey, J. E. 1954 A hemagglutination inhibition test for infectious sinusitis of turkeys. Proc. Soc. Exptl. Biol. Med., **86**, 38–40.
30. Landsteiner, K. 1945 *The specificity of serological reactions*, p. 5. Revised ed. Harvard University Press, Cambridge, Mass.
31. Boyd, W. C., Shapleigh, E., and McMaster, M. 1955 Immunochemical behavior of a plant agglutinin (lectin). Arch. Biochem. Biophys., **55**, 226–234.
32. Ottensooser, F., and Silberschmidt, K. 1953 Haemagglutinin anti-N in plant seeds. Nature, **172**, 914.

32a. Nungester, W. J., and Halsema, G. 1953 Reaction of certain phytoagglutinins with Flexner-Jobling carcinoma cells of the rat. Proc. Soc. Exptl. Biol. Med., **83**, 863–866.

33. Rolle, M., and Kalich, J. 1954 Zur Diagnose der toxischen Kolikeime durch Agglutination der Spermazellen. Münch. med. Wochschr., **96**, 579–580.
34. Keogh, E. V., North, E. A., and Warburton, M. F. 1948 Adsorption of bacterial polysaccharides to erythrocytes. Nature, **161**, 687–688.
35. Rountree, P. M., and Barbour, R. G. H. 1952 Antibody to the erythrocyte-coating polysaccharide of staphylococci: Its occurrence in human sera. Australasian Ann. Med., **1**, 80–83.
36. Hayes, L. 1951 Specific serum agglutination of sheep erythrocytes sensitized with bacterial polysaccharides. Australian J. Exptl. Biol. Med. Sci., **29**, 51–62.
37. Landy, M. 1954 On hemagglutination procedures utilizing isolated polysaccharide and protein antigens. Am. J. Public Health, **44**, 1059–1064.
38. Bier, O. 1951 Observations préliminaires sur l'hémagglutination, l'hémolyse et la conglutination "passives". Ann. inst. Pasteur, **81**, 650–656.
39. Kirby, W. M. M. 1951 Hemagglutination reaction in streptococcal infections and acute rheumatic fever. Proc. Soc. Exptl. Biol. Med., **78**, 519–522.
40. Rantz, L. A., Randall, E., and Zuckerman, A. 1956 Hemolysis and hemagglutination by normal and immune sera of erythrocytes treated with a nonspecies specific bacterial substance. J. Infectious Diseases, **98**, 211–222.
41. Thomas, J. C., and Mennie, A. T. 1950 Bacterial polysaccharides in the diagnosis of infections. The polysaccharide lysis test. Lancet, **II**, 745–746.

41a. Chanarin, I. 1954 An investigation of *Neisseria gonorrhoeae* by a red cell sensitization technique. J. Hyg., **52**, 425–443.

42. Freeman, N. L., Felsenfeld, O., and Eveland, W. C. 1955 Slide hemagglutination tests with O antigens of enteric organisms and *Brucella*. Am. J. Clin. Pathol., **25**, 332–335.
43. Needell, M. H., Neter, E., Staubitz, W. J., and Bingham, W. A. 1955 The antibody (hemagglutinin) response of patients with infections of the urinary tract. J. Urol., **74**, 674–682.
44. Neter, E., Bertram, L. F., Zak, D. A., Murdock, M. R., and Arbesman, C. E. 1952 Studies on hemagglutination and hemolysis by *Escherichia coli* antisera. J. Exptl. Med., **96**, 1–15.
45. Landy, M., and Lamb, E. 1953 Estimation of Vi antibody employing erythrocytes treated with purified Vi antigen. Proc. Soc. Exptl. Biol. Med., **82**, 593–598.
46. Neter, E., Gorzynski, E. A., Zalewski, N. J., Rachman, R., and Gino, R. M. 1954 Studies on bacterial hemagglutination. Am. J. Public Health, **44**, 49–54.
47. Le Minor, L., Le Minor, S., and Grabar, J. 1952 Réaction d'hémagglutination passive et d'hémolyse directe au moyen de globules rouges sensibilisés par des substances solubles 0 et Vi d'entérobacteriacées. Ann. inst. Pasteur, **83**, 62–70.
48. Ogawa, T., Onaka, N., Miyao, K., Ogawa,

J., MIZUNO, Y., AND KONDO, M. 1954 Investigation on the hemagglutination of *Escherichia coli* O_{111}, O_{55}, and O_{26} from the view point of a practical value. Nagoya Med. J., **2**, 101–109.

48a. STULBERG, C. S., ZUELZER, W. W., AND PAGE, R. H. 1956 *Escherichia coli* O127: B8, a serotype causing infantile diarrhea. J. Immunol., **76**, 281–287.

49. DE GREGORIO, M., AND DI NARDO, A. 1955 Emoagglutinazione condizionata (*S. ballerup*). Bull. ist. sieroterap. milan., **34**, 123–130.

50. CHU, D. C.-Y., AND HOYT, R. E. 1954 Reactions of T Vi and ballerup Vi haptenes with antisera against Vi-coated erythrocytes. J. Hyg., **52**, 100–104.

51. MACPHERSON, I. A., WILKINSON, J. F., AND SWAIN, R. H. A. 1953 The effect of *Klebsiella aerogenes* and *Klebsiella cloacae* polysaccharides on haemagglutination by and multiplication of the influenza group of viruses. Brit. J. Exptl. Pathol., **34**, 603–615.

52. RABE, E. F., AND PLONKO, M. 1954 The antibody response to gram negative organisms: An explanation of the differences between bacterial and hemagglutinating antibody titers. Pediatrics, **14**, 351–356.

53. TAKEDA, Y., WATANABE, T., KURIBAYASHI, K., AND KIUCHI, K. 1952 Studies on the hemagglutination of erythrocytes sensitized with endotoxins. Japan. J. Exptl. Med., **22**, 273–284.

54. CEPPELLINI, R., AND DE GREGORIO, M. 1953 Emagglutinazione ed emolisi condizionate mediante gli antigeni della *Salmonella typhi*. Boll. ist. sieroterap. milan., **32**, 429–444.

55. LANDY, M., TRAPANI, R-J., AND CLARK, W. R. 1955 Studies on the O antigen of *Salmonella typhosa*. Am. J. Hyg., **62**, 54–65.

56. SPAUN, J. 1952 Determination of *Salmonella typhi* O and Vi antibodies by hemagglutination. Acta Pathol. Microbiol. Scand., **31**, 462–469.

57. CORVAZIER, P. 1952 Etude de l'antigène Vi a l'aide d'une technique d'hémagglutination passive. Ann. inst. Pasteur, **83**, 173–179.

58. SPAUN, J. 1951 On the determination of Vi - antibodies by haemagglutination. Acta Pathol. Microbiol. Scand., **29**, 416–418.

59. NETER, E., WESTPHAL, O., LÜDERITZ, O., AND GORZYNSKI, E. A. 1956 The bacterial hemagglutination test for the demonstration of antibodies to *Enterobacteriaceae*. Ann. N. Y. Acad. Sci., *in press*.

60. NETER, E., GORZYNSKI, E. A., GINO, R. M., WESTPHAL, O., AND LÜDERITZ, O. 1956 The enterobacterial hemagglutination test and its diagnostic potentialities. Can. J. Microbiol., **2**, 232–244.

61. FULTHORPE, A. J. 1954 Agglutination of sheep erythrocytes sensitized with *Salmonella* polysaccharides. J. Pathol. Bacteriol., **68**, 315–325.

62. CHUN, D., AND PARK, B. 1956 Demonstration of *Shigella flexneri* antigens by means of hemagglutination test. J. Infectious Diseases, **98**, 82–87.

63. NETER, E., AND WALKER, J. 1954 Hemagglutination test for specific antibodies in dysentery caused by *Shigella sonnei*. Am. J. Clin. Pathol., **24**, 1424–1429.

64. NETER, E., AND GORZYNSKI, E. A. 1954 Erythrocyte-modifying capacity of *Shigella dysenteriae* (Shiga) antigen and its polysaccharide component. Proc. Soc. Exptl. Biol. Med., **85**, 503–506.

65. O'CONNOR, J. L., AND MACDONALD, J. M. 1950 Excretion of specific antigen in the urine in Tsutsugamushi disease (scrub typhus). Brit. J. Exptl. Pathol., **31**, 51–64.

66. HAN, E. S. 1951 Hemagglutination test for epidemic and murine typhus fever using sheep erythrocytes sensitized with proteus OX19 extracts. Am. J. Trop. Med., **31**, 243–251.

67. GAINES, S., AND LANDY, M. 1955 Prevalence of antibody to pseudomonas in normal human sera. J. Bacteriol., **69**, 628–633.

68. FELSENFELD, O., FREEMAN, N. L., AND MOORING, V. L. 1955 Tube and slide technic in the hemagglutination of *Vibrio comma*. Am. J. Trop. Med. Hyg., **4**, 318–320.

69. AMIES, C. R. 1951 The envelope substance of *Pasteurella pestis*. Brit. J. Exptl. Pathol., **32**, 259–273.

70. SILVERMAN, S. J. 1954 The isolation of fractions from *Pasteurella pestis* for use in a hemagglutination test. J. Lab. Clin. Med., **44**, 185–193.

71. NEEL, R., TASLIMI, H., AND EFTEKHARI, M. 1955 Valeur pratique compareé des reactions d'agglutination de conglutination directe d'hémagglutination polyosidique et protéinique pour le diagnostic de la peste. Arch. inst. d'Hessarek., **9**, 85–107.

72. CHEN, T. H. 1952 The method of the

hemagglutination test and some observations on the antigen. J. Immunol., **69**, 587–596.

73. CARTER, G. R. 1955 A hemagglutination test for the identification of serological types. Am. J. Vet. Research., **16**, 481–484.
74. ALEXANDER, M. M., WRIGHT, G. G., AND BALDWIN, A. C. 1950 Observations on the agglutination of polysaccharide-treated erythrocytes by tularemia antisera. J. Exptl. Med., **91**, 561–566.
75. WRIGHT, G. G., AND FEINBERG, R. J. 1952 Hemagglutination by tularemia antisera. J. Immunol., **68**, 65–71.
76. BRODHAGE, H., AND FEY, H. 1955 Beobachtungen bei der Hämagglutinationreaktion auf Bang und Tuberkulose mit menschlichen und tierischen Sera. Z. Hyg. Infectionskrankh., **141**, 76–81.
77. HIRSCHBERG, N., AND YARBROUGH, M. E. 1952 Fractions of *Brucella* for adsorbed antigens for collodion agglutination and hemagglutination tests. J. Infectious Diseases, **91**, 238–245.
78. CARRERE, L., AND ROUX, J. 1952 Hémagglutination passive d'hématies sensibilisées par antigènes brucelliques ou des substances solubles spécifiques. Ann. inst. Pasteur, **83**, 810–813.
79. BOYDEN, S. V. 1950 Adsorption by erythrocytes of antigens of *Pfeifferella mallei* and *Pfeifferella whitmori*. Proc. Soc. Exptl. Biol. Med., **73**, 289–291.
80. MIDDLEBROOK, G. 1954 Laboratory aids to diagnosis and therapy. Ann. Rev. Med., **5**, 339–348.
81. SORKIN, E., AND BOYDEN, S. V. 1955 A study of antigens active in the Middlebrook-Dubos hemagglutination test present in filtrates of culture of *Mycobacterium tuberculosis*. J. Immunol., **75**, 22–27.
82. GRABAR, P., BOYDEN, S., TAQUET, A., AND BORDUAS, A. 1952 Mise en évidence de deux anticorps différents dans les sérums tuberculeux par hémagglutination passive. Compt. rend., **234**, 899–901.
83. MEYNELL, G. G. 1954 The antigenic structure of *Mycobacterium tuberculosis*, var. *hominis*. J. Pathol. Bacteriol., **67**, 137–150.
84. FISHER, S. 1951 Antigenic relationships of erythrocyte adsorbable fractions of some mycobacteria. Australian J. Exptl. Biol. Med. Sci., **29**, 1–8.
85. CHANG, R. S., AND MCCOOMB, D. E. 1954 Erythrocyte sensitizing substances from five strains of leptospirae. Am. J. Trop. Med., **3**, 481–489.
86. COX, C. D. 1955 Hemolysis of sheep erythrocytes sensitized with leptospiral extracts. Proc. Soc. Exptl. Biol. Med., **90**, 610–615.
87. BOYDEN, S. V., AND ANDERSEN, M. E. 1955 Agglutination of normal erythrocytes in mixtures of antibody and antigen, and haemolysis in the presence of complement. Brit. J. Exptl. Pathol., **36**, 162–170.
88. STAUB, A.-M. 1954 Role des anticorps antipolyosidiques dans l'agglutination des bacilles typhiques. Ann. inst. Pasteur, **86**, 618–635.
89. NETER, E., WESTPHAL, O., LÜDERITZ, O., GORZYNSKI, E. A., AND EICHENBERGER, E. 1956 Studies of enterobacterial lipopolysaccharides: Effects of heat and chemicals on erythrocyte-modifying, antigenic, toxic, and pyrogenic properties. J. Immunol., **76**, 377–385.
90. MIDDLEBROOK, G. 1952 Antigens of tubercle bacillus involved in hemagglutination and hemolysis reactions. Bull. N. Y. Acad. Med., **28**, 474–475.
91. NETER, E., AND ZALEWSKI, N. J. 1953 The requirement of electrolytes for the adsorption of *Escherichia coli* antigen by red blood cells. J. Bacteriol., **66**, 424–428.
92. NETER, E., WESTPHAL, O., AND LÜDERITZ, O. 1955 Effects of lecithin, cholesterol, and serum on erythrocyte modification and antibody neutralization by enterobacterial lipopolysaccharides. Proc. Soc. Exptl. Biol. Med., **88**, 339–341.
93. BOYDEN, S. V., AND GRABAR, P. 1954 Rôle des lipides dans la sensibilisation des érythrocytes par les constituants de la tuberculine. Ann. inst. Pasteur, **87**, 257–267.
94. NETER, E., ZALEWSKI, N. J., AND ZAK, D. A. 1953 Inhibition by lecithin and cholesterol of bacterial (*Escherichia coli*) hemagglutination and hemolysis. J. Immunol., **71**, 145–151.
95. FINE, M., FLOCKS, M., AND FEICHTMEIR, T. V. 1955 Nonspecific hemagglutination inhibiting factor in human serum. Am. J. Med. Sci., **229**, 670–675.

95a. LANDY, M., AND CEPPELLINI, R. 1955 Production of 'O inagglutinability' in erythrocytes coated with typhoid Vi and O antigens. Nature, **176**, 1266–1267.

96. SKILLMAN, R. K., SPURRIER, W., FRIEDMAN, I. A., AND SCHWARTZ, S. O. 1955 Rheumatic fever activity determination by

two correlative methods. Arch. Internal Med., **96**, 51–60.

97. MIDDLEBROOK, G. 1950 A hemolytic modification of the hemagglutination test for antibodies against tubercle bacillus antigens. J. Clin. Invest., **29**, 1480–1485.
98. GERSTL, B., DAVIS, W. E., JR., KIRSH, D., HOLLANDER, A. G., BARBIERI, M., AND WEINSTEIN, S. B. 1955 Detection of apparently absent circulating antibodies in tuberculous sera. Am. Rev. Tuberc., **72**, 345–355.
99. ADLER, F. L. 1953 Studies on the bactericidal reaction. J. Immunol., **70**, 79–88.
100. COLE, L. R., AND FAVOUR, C. B. 1955 Correlations between plasma protein fractions, antibody titers, and the passive transfer of delayed and immediate cutaneous reactivity to tuberculin PPD and tuberculopolysaccharides. J. Exptl. Med., **101**, 391–420.
101. CHANG, S. 1953 A serologically-active erythrocyte-sensitizing substance from typhus rickettsiae. J. Immunol., **70**, 212–214.
102. CHANG, R. S., MURRAY, E. S., AND SNYDER, J. C. 1954 Erythrocyte-sensitizing substances from rickettsiae of the Rocky Mountain spotted fever group. J. Immunol., **73**, 8–15.
103. MUNIZ, J. 1950 On the value of "conditioned hemólysis" for the diagnosis of American trypanosomiasis. "O Hospital", **38**, 685–691.
104. KAGAN, I. G. 1955 Hemagglutination after immunization with schistosome antigens. Science, **122**, 376–377.
105. VOGEL, R. A., AND COLLINS, M. E. 1955 Hemagglutination test for detection of *Candida albicans* antibodies in rabbit antiserum. Proc. Soc. Exptl. Biol. Med., **89**, 138–140.
106. NORDEN, A. 1949 Agglutination of sheep's erythrocytes sensitized with histoplasmin. Proc. Soc. Exptl. Biol. Med., **70**, 218–220.
107. MCENTEGART, M. G. 1952 The application of a haemagglutination technique to the study of *Trichomonas vaginalis* infections. J. Clin. Pathol., **5**, 275–280.
108. DE GREGORIO, M. 1955 Fissazione di antigeni sulla superficie cellulare. Boll. ist. sieroterap. milan., **34**, 118–122.
109. BOYDEN, S. V. 1953 Fixation of bacterial products by erythrocytes in vivo and by leucocytes. Nature, **171**, 402–403.
110. THOMAS, L., AND GOOD, R. A. 1952 Studies on the generalized Shwartzman reaction. J. Exptl. Med., **96**, 605–624.
111. EVANS, E. E., AND HAINES, R. F. 1954 The agglutination of ion exchange resin particles coated with polysaccharide. J. Bacteriol., **68**, 130–131.
112. ADLER, F. L. 1952 Bactericidal action mediated by antibodies specific for heterologous antigens adsorbed to bacterial cells. Proc. Soc. Exptl. Biol. Med., **79**, 590–593.
113. NELSON, R. A., JR. 1953 The immune-adherence phenomenon. Science, **118**, 733–737.
114. RIECKENBERG, H. 1917 Eine neue Immunitätsreaktion bei experimenteller Trypanosomen-Infektion: die Blutplättchenprobe. Z. Immunitätsforsch., **26**, 53–64.
114a. LAMANNA, C., AND HOLLANDER, D. H. 1956 Demonstration of particulate adhesion of the Rieckenberg type with the spirochete of syphilis. Science, **123**, 989–990.
115. CEPPELLINI, R., AND DE GREGORIO, M. 1953 Crisi emolitica in animali batterio-immuni trasfusi con sangue omologo sensibilizzato in vitro mediante l'antigene batterico specifico. Boll. ist. sieroterap. milan., **32**, 445–453.
115a. NUNGESTER, W. J., AND KLEIN, L. F. 1937 Effect of pneumococcus type III specific polysaccharide on sedimentation of blood cells. Proc. Soc. Exptl. Biol. Med., **36**, 315–317.
115b. YOUNGNER, J. S., AND NUNGESTER, W. J. 1944 The effect of type III pneumococcus polysaccharide and gelatin on the circulation and sedimentation rate of erythrocytes in mice. J. Infectious Diseases, **74**, 247–253.
116. WESTPHAL, O., AND LÜDERITZ, O. 1954 Chemische Erforschung von Lipopolysacchariden gramnegativer Bakterien. Angew. Chem., **66**, 407–417.
117. WOOD, W. B., JR. 1955 The pathogenesis of fever. Am. J. Med., **18**, 351–353.
118. TODD, J. P. 1955 Bacterial pyrogens. J. Pharm. and Pharmacol., **7**, 625–641.
119. PILLEMER, L., SCHOENBERG, M. D., BLUM, L., AND WURZ, L. 1955 Interaction of the properdin system with polysaccharides. Science, **122**, 545–549.
120. PILLEMER, L., BLUM, L., LEPOW, I. H., ROSS, O. A., TODD, E. W., AND WARDLAW, A. C. 1954 The properdin system and immunity. Science, **120**, 279–285.
121. SILVERSTEIN, A. M., AND MALTANER, F. 1952 Hemolysis with complement of

intact azo-erythrocytes sensitized with antisera homologous to the attached azo-grouping. J. Immunol., **69**, 197–200.

122. NETER, E., WESTPHAL, O., LÜDERITZ, O., GINO, R. M., AND GORZYNSKI, E. A. 1955 Demonstration of antibodies against enteropathogenic *Escherichia coli* in sera of children of various ages. Pediatrics, **16**, 801–808.
123. NETER, E., ZALEWSKI, N. J., AND FERGUSON, W. W. 1953 *Escherichia coli* hemagglutinin response of adult volunteers to ingested *E. coli* O55 B_5. Proc. Soc. Exptl. Biol. Med., **82**, 215–219.
124. WARBURTON, M. F., KEOGH, E. V., AND WILLIAMS, S. W. 1949 A haemagglutination test for the diagnosis of influenzal meningitis. Med. J. Australia, **1**, 135–137.
125. MAILLARD, E. R., AND GAGLIARDO, F. J. 1952 The value of absorption in serologic tests for tuberculosis. Am. Rev. Tuberc., **66**, 762–764.
126. GERNEZ-RIEUX, C., MONTESTRUC, E., AND TACQUET, A. 1951 Les réactions d'hémagglutination et d'hémolyse conditionnée dans les différentes formes de la lèpre. Ann. inst. Pasteur Lille, **4**, 3–15.
127. LEVINE, M. 1951 Hemagglutination of tuberculin sensitized sheep cells in Hansen's disease (leprosy). Proc. Soc. Exptl. Biol. Med., **76**, 171–173.
128. STAACK, H. H., AND SPAUN, J. 1953 Serological diagnosis of chronic typhoid carriers by Vi haemagglutination. Acta Pathol. Microbiol. Scand., **32**, 420–423.
129. BOYDEN, S. V. 1951 The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J. Exptl. Med., **93**, 107–120.
130. STAVITSKY, A. B. 1954 Procedure and general applications of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells. J. Immunol., **72**, 360–367.
131. STAVITSKY, A. B. 1954 Specific applications of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells. J. Immunol., **72**, 368–375.
132. FISHER, S. 1952 The estimation in vitro of small amounts of diphtheria antitoxin by means of a haemagglutination technique. J. Hyg., **50**, 445–456.
133. LANDY, M., TRAPANI, R-J., FORMAL, R., AND KLUGLER, I. 1955 Comparison of a hemagglutination procedure and the rabbit intradermal neutralization test for the assay of diphtheria antitoxin in human sera. Am. J. Hyg., **61**, 143–154.
134. CHEN, T. H., AND MEYER, K. F. 1954 A hemagglutination test with the protein fraction of *Pasteurella pestis*. J. Immunol., **72**, 282–298.
135. MCCRUMB, F. R., JR., MERCIER, S., CHEN, T. H., MEYER, K. F., AND GOODNER, K. 1955 Studies on the antibody patterns in pneumonic plague patients. J. Infectious Diseases, **96**, 88–94.
136. LANDY, M., AND TRAPANI, R. J. 1954 A hemagglutination test for plague antibody with purified capsular antigen of *Pasteurella pestis*. Am. J. Hyg., **59**, 150–156.
137. WARREN, J., WALZ, U., REEDAL, J. S., AND AJL, S. J. 1955 Immunological properties of purified *Pasteurella pestis* toxin. J. Bacteriol., **70**, 170–176.
138. MEYNELL, G. G. 1954 The antigenic structure of *Mycobacterium tuberculosis*, var. *hominis*. J. Pathol. Bacteriol., **67**, 137–150.
139. BOYDEN, S. V., AND SORKIN, E. 1955 A study of antigens active in the tannic acid hemagglutination test present in filtrates of culture of *Mycobacterium tuberculosis*. J. Immunol., **75**, 15–21.
140. HINZ, C. F., JR., AND PILLEMER, L. 1955 The requirement for the properdin system in the hemolysis of human erythrocytes treated with tannic acid. J. Clin. Invest., **34**, 912.
140a. PRESSMAN, D., CAMPBELL, D. H., AND PAULING, L. 1942 The agglutination of intact azo-erythrocytes by antisera homologous to the attached groups. J. Immunol., **44**, 101–105.
141. STAVITSKY, A. B., AND ARQUILLA, E. R. 1955 Procedure and applications of hemagglutination and hemagglutination-inhibition reactions with bis-diazotized benzidine and protein-conjugated red blood cells. J. Immunol., **74**, 306–312.
142. COLE, L. R., AND FARRELL, V. R. 1955 A method for coupling protein antigens to erythrocytes. J. Exptl. Med., **102**, 631–645.
143. COOMBS, R. R. A., HOWARD, A. N., AND WILD, F. 1952 Titration of antisera to soluble proteins on the basis of an agglutination reaction. Brit. J. Exptl. Pathol., **33**, 390–397.
144. COOMBS, R. R. A., HOWARD, A. N., AND MYNORS, L. S. 1953 A serological procedure theoretically capable of detecting

incomplete or non-precipitating antibodies to soluble protein antigens. Brit. J. Exptl. Pathol., **34**, 525–534.

145. THOMSEN, O. 1927 Ein vermehrungsfähiges Agens als Veränderer des isoagglutinatorischen Verhaltens der roten Blutkörperchen, eine bisher unbekannte Quelle der Fehlbestimmung. Z. Immunitätsforsch., **52**, 85–107.
146. FRIEDENREICH, V. 1930 *The Thomsen hemagglutination phenomenon*. Levin and Munksgaard, Copenhagen, Denmark.
147. MOSKOWITZ, M., AND TREFFERS, H. P. 1950 An agglutinin in normal sera for periodate-treated red cells. Science, **111**, 717–719.
148. BURNET, F. M., AND ANDERSON, S. G. 1947 The "T" antigen of guinea-pig and human red cells. Australian J. Exptl. Biol. Med. Sci., **25**, 213–217.
149. CHU, C. M. 1948 Enzymic action of viruses and bacterial products on human red cells. Nature, **161**, 606–607.
150. MCCREA, J. F. 1947 Modification of red-cell agglutinability by *Cl. welchii* toxins. Australian J. Exptl. Biol. Med. Sci., **25**, 127–136.
151. STEWART, W. B., PETENYI, C. W., AND ROSE, H. M. 1955 The survival time of canine erythrocytes modified by influenza virus. Blood, **10**, 228–234.
152. WRIGHT, G. G., AND SLEIN, J. B. 1951 Variation in the serum T-agglutinin during anthrax-infection in the rabbit. J. Exptl. Med., **93**, 99–106.
153. DAVIDSOHN, I., AND TOHARSKY, B. 1940 The production of bacteriogenic hemagglutination. J. Infectious Diseases, **67**, 25–41.
154. DAVIDSOHN, I., AND TOHARSKY, B. 1942 Bacteriogenic hemagglutination. J. Immunol., **43**, 213–225.
155. SPIELMANN, W. 1954 Versuche zur Modifizierung von anti-Rh-testseren durch Bakterien und deren Kulturfiltrate, Z. Immunitätsforsch., **111**, 460–470.
156. NETER, E. 1956 Microbial hemagglutination as an immunologic technique. (Editorial) Am. J. Clin. Pathol., **26**, 165–168.