SERUM AND TISSUE INHIBITORS OF VIRUS

HAROLD S. GINSBERG

Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University and the University Hospitals, Cleveland, Ohio

INTRODUCTION

That so-called "natural immunity" is dependent upon a series of complex factors is agreed by most, and is certainly attested to by the scope of papers presented in the present symposium. It is tempting, as one reviews published reports and personal research experiences, to consider those natural substances of the host which inhibit biological properties of viruses as being important participants in this constant contest between parasites and their potential prey. Materials isolated from tissues, body secretions, or serum, which can inhibit viruses under experimental conditions, have received particular emphasis in the investigation of nonspecific resistance to viral infections. Theoretically it is possible to increase resistance to viral infection at various steps in the infectious process: (a) the virus particle itself may be inactivated; (b) the host cell may be altered so that attachment of virus cannot be accomplished; (c) the susceptible cells may be affected so that the essential infectious viral materials cannot enter the cell; (d) the host cells' metabolic apparatus may be sufficiently influenced to prevent the synthesis of essential viral materials; (e) egress of newly formed virus from the host cells may be prevented; and (f) host reaction to the infectious process may be sufficiently diminished so that many of the injurious effects of viral invasion are eliminated.

It should be possible to affect one or more of these stages in viral infection and thus increase nonspecific resistance, but in actuality the known host materials of tissues and serum which inhibit viral infections act directly only upon the viral particle. The inactivation of various biological properties of viruses by combination of inhibitor with viral particle will therefore be considered. This paper will not attempt to review the literature completely on all inhibitors of viral activity, but rather will discuss those substances in tissues or serum which can serve as models for a consideration of the role these materials may play in "natural resistance" to viral infections. The evidence will be considered which bears upon the function these substances may have in the host.

INHIBITORS FROM TISSUES

Soluble inhibitors of viral activity obtained from tissues possess a variety of chemical compositions, identified as proteins, mucoproteins, glycoproteins, mucopolysaccharides, and lipoproteins. However, a relatively specific chemical structure is identified with the inhibitor of each virus. Thus, although the inhibitor does not have the specificity of an antibody, its reaction with virus is dependent upon complementariness of its chemical groupings in relation to the viral particle. The data available clearly demonstrate that the known inhibitors all exert their effects by combining with viral particles and thus preventing normal activity. None of the inhibitors described has been shown to alter other steps in the infectious process. The inhibitors which have most general interest are those which can prevent viral particles from infecting cells. Because of certain theoretical considerations, inhibitors which reduce biological functions, such as agglutination of red blood bells by influenza viruses, have also aroused considerable interest and require discussion. In table 1 are listed the inhibitors to be considered, the tissue from which each can be obtained, the chemical composition of the inhibitor, the virus inhibited, and the viral property inactivated.

Soluble inhibitors of the myxoviruses (influenza, mumps, and Newcastle disease viruses) have been obtained from a wide variety of tissues and body secretions (8, 26). The materials obtained appear to be of similar if not identical chemical composition, although the inhibitors from urine (24, 33, 55), mucinous secretions (25), and brain (6) are the ones which have received the most detailed chemical analysis. These inhibitors appear to be conjugated proteins with oligosaccharides as prosthetic groups. Acetylated neuraminic acid residues (*N*-acetylneuraminic acid) are terminal units joined by a glycosidic linkage to an adjacent sugar residue (25, 26).

TABLE 1

Viral inhibitors from tissues

Virus	Inhibitor's Chemical Nature	Tissue	Viral Property Inhibited
Myxoviruses	Mucoprotein	Numerous	Hemagglutination
Myxoviruses	Glycoprotein (brain glycoside)	Brain	Hemagglutination
PVM	Protein	Mouse lung	Hemagglutination
Theiler's GDVII	Mucopolysaccharide	Mouse stomach and intestine	Hemagglutination and infectivity
Theiler's TO*	Mucopolysaccharide	Mouse stomach and intestine	Hemagglutination
Russian Far East	Lipid	Brain	Infectivity
Poliovirus	Lipoprotein	Susceptible primate cells	Infectivity

* Only strain propagated in intestinal tract.

The inhibitory compounds prevent hemagglutination, but not infection, by myxoviruses. Nevertheless, they have received extensive investigation because it was shown that the inhibitors are related to the so-called receptor substance on host cells susceptible to infection (3, 8, 26) and on erythrocytes responsive to agglutination by myxoviruses (8, 31, 34). It had been postulated that the reaction between virus and inhibitor simulates the initial step of virus infection, namely, virus-host cell combination (29); the foregoing data support this hypothesis.

Hemagglutination, but not infectivity, is inhibited by the mucoprotein (or glycoprotein). The failure of virus to be inactivated completely results from dissociation of the virus-inhibitor complex as a consequence of hydrolysis of the inhibitor by the enzyme which is an integral part of the viral particle. The enzyme, now termed a neuraminidase (5), by cleavage of the inhibitor's neuraminic acid-hexosamine bond, hydrolyzes the mucoprotein, dissociates the virus-inhibitor complex, and permits the viral particle to retain its property of infectivity (7, 26). It was originally speculated that the viral enzymes served to permit viral penetration of host cells. Another postulate which should be considered is that the neuraminidase is essential for influenza virus particles to escape inactivation by the large quantity of inhibitor normally present in nasopharyngeal secretions (17). Without its neuraminidase, influenza virus, as well as other myxoviruses, would be rapidly inactivated upon entrance into the nasopharynx and could never effect infection. It may therefore be proposed that the presence of neuraminidase as an intimate portion of the surface of the viral particle is a function of evolutionary changes which have permitted myxoviruses to survive in nature.

A protein obtained from susceptible tissues combines with pneumonia virus of mice (PVM) and inhibits hemagglutination but not infectivity of the virus (14). In this instance virus-inhibitor combination is dissociated not by an enzyme possessed by viral particles, but rather by a proteolytic enzyme in tissue juices (60). The protein inhibitor is considered to be a component of susceptible host cells, probably the receptor substance, and is not detectable in other mouse tissues or in secretions of the respiratory tract.

Attention may now be turned to a discussion of inhibitors which not only combine with viruses but also inactivate their capacity to infect susceptible cells.

The first of these inhibitors to be considered is perhaps intermediate between those which only prevent hemagglutination and those which completely inactivate virus. A mucopolysaccharide obtained from the wall of the mouse stomach and small intestine can combine with and inhibit hemagglutination and infectivity of the GDVII strain of Theiler's encephalomyelitis virus (41, 42). The TO strain of Theiler's virus propagated in the mouse intestinal tract was inhibited as measured by hemagglutination but not by infectivity (41). This result was dependent upon the presence of an enzyme in the mouse's intestinal tract which dissociated virus from inhibitor, as

described for PVM (60). The inhibitor-destroying enzyme was not present in the central nervous tissue and consequently inactivation of viral infectivity could be accomplished by the inhibitor when virus-inhibitor mixtures were inoculated intracerebrally. It will be recalled that the encephalomyelitis viruses, the TO strain as well as the FA strain, which also was not inactivated by the intestinal mucopolysaccharide, are natural parasites of the mouse's intestinal tract, producing latent infections, whereas the GDVII strain is not found in the intestinal tract but instigates a fatal infection of the central nervous system, a tissue that does not contain a mucopolysaccharide which can inactivate the GDVII virus.

The remaining tissue inhibitors to be discussed inactivate viral infectivity. A lipid extracted from mouse brains inactivated infectivity of Russian Far East virus (10). This lipid inhibitor protected mice when injected intravenously 24 hr before or 2 and 24 hr after 10 to 30 LD_{50} of virus (10). Although it belongs in the general class of lipids, the chemical nature of the inhibitor has not been further defined, nor is its mechanism of action known.

Infectivity of poliomyelitis viruses can be inhibited by a lipoprotein extracted from susceptible primate cells grown in tissue culture (30). A similar inhibitor could not be obtained from cultured nonprimate cells which were not susceptible to infection and with which poliovirus could not combine. The evidence presented strongly implied that the lipoprotein inhibitor was the receptor material to which virus attached to establish the initial phase of infection (30).

The available data suggest that in general viral inhibitors extracted from tissues represent the receptor materials with which viruses combine. The variation in chemical composition of each inhibitor is a reflection of the specific nature of the host cell-virus interaction. There is reasonably sound evidence to indicate that host cell susceptibility to a specific viral infection is primarily dependent upon the presence of receptor material which permits virus association with and subsequent invasion of the host cell (30). There are no data, however, which indicate that the inhibitory substances which have been described play any role in protection against viral infection. Indeed, resistance to infection with influenza is not even afforded by the presence of large quantities of the mucoprotein inhibitor of influenza virus in nasopharyngeal secretions through which virus must penetrate to reach susceptible cells.

INHIBITORS FROM SERUM

Let us now turn attention to viral inhibitors *other than specific antibodies* which have been demonstrated to be present in sera of animals which are susceptible to infection by the virus discussed. Detailed reviews of viral inhibitors in animal sera and the possible relationship of these inhibitors to specific antibody have been made recently (2, 32, 52) and therefore this aspect of viral inhibitors will not be discussed. The inhibitory substances may simply be divided into those which are stable to heat, that is, are not inactivated at temperatures of 50 to 60 C, and those which are heat labile within this temperature range.

Heat-stable Inhibitors

The heat-stable inhibitors to be discussed, although not as numerous or as complex as those obtained from tissues, are in general similar in nature (table 2). Indeed, if one considers the blood merely a mirror of the tissues, it would be reasonable to assume that inhibitors found in serum are identical to those obtained from cells. The best example to support this contention is the heat-stable inhibitor in serum which prevents hemagglutination but not infection by myxoviruses (8). The evidence available indicates that the mechanism of action and chemical structure of this inhibitor is closely similar if not identical to that obtained from tissues and other body fluids (8, 26). A protein or protein complex present in sera from horses and rabbits has been shown to neutralize infectivity as well as inhibit hemagglutination of some strains of Asian influenza viruses (12, 38). These inhibitors have not been demonstrated in sera from humans or other susceptible hosts, and therefore their role in resistance to infection cannot be assessed.

A lecithin-like compound in serum inactivates infectivity of influenza and Newcastle disease viruses but does not inhibit hemagglutination by these agents (57). This same lipid inhibitor also can inactivate the infectivity of psittacosis virus, a virus considerably different from myxoviruses (58). The origin and role of this lipid inhibitor is not known, but it is tempting to speculate that it combines, *in vitro* at least, with and inactivates the above listed viruses by virtue of their lipo-

TABLE 2

Heat-stable inhibitors from serum

Virus	Inhibitor's Chemical Nature	Viral Property Inhibited
Myxoviruses	Mucoprotein	Hemagglutination
Asian influenza	Protein	Hemagglutination and infectivity
Myxoviruses	Lipid (lecithin-like)	Infectivity
Psittacosis	Lipid (lecithin-like)	Infectivity
Arbor B*	Lipid	Infectivity
Yellow fever; Jap B	?	Infectivity

* St. Louis, Russian Far East, and Japanese B encephalitis viruses.

philic properties, as recently pointed out by Noll and Youngner (47).

A lipid inhibitor of St. Louis, Russian Far East, and Japanese B encephalitis viruses has been obtained from sera of mice, hamsters, rabbits, and horses (9). It is not known whether it is similar to the lecithin-like substance just described or is an inhibitor of different chemical composition. Sera from rodents and marsupials have also been shown to contain an inhibitor which inactivates yellow fever, Japanese B, St. Louis, and West Nile encephalitis viruses (35). The chemical nature of the inhibitor was not studied and it is not possible to determine whether it is similar to the lipid inhibitor of other Arbor B viruses. A number of other viruses have been shown to be inhibited by sera obtained from a variety of animals. It is not clear whether the viral inactivating substances are specific antibodies or nonspecific inhibitors, and therefore they will not be considered in this discussion which relates to nonspecific resistance.

From available data a function in nonspecific host resistance cannot be readily ascribed to the inhibitors discussed. It appears from evidence on the pathogenesis of neurotropic viral infections that lipid inhibitors in serum probably play no great role in resistance, in view of the fact that a stage of viremia occurs regularly and virus can be isolated from blood. The inhibitors of influenza viruses likewise appear to have no role in resistance to infection, since viremia is not an essential stage in the pathogenesis of infection and the inhibitors are readily demonstrated in usual quantities in sera of patients with influenza. Probably these compounds are chiefly viral receptor substances or other cell components which upon normal cell breakdown have been solubilized and escaped into the blood.

Heat-labile Inhibitors

Potentiation of neutralization of viruses by addition of fresh unheated serum to serum containing specific antibodies, or inactivation of viruses by unheated serum not containing antibodies, has been described by a number of investigators (4, 7, 11, 15, 20, 22, 23, 39, 40, 43–45, 51, 53, 65). The viruses known to be neutralized in this manner are vaccinia (7, 15, 23, 43), variola (43), Rous sarcoma (45), Western equine encephalomyelitis (16, 44, 65), mumps (22, 39, 40), influenza A and B (11, 22, 53), Newcastle disease virus (4, 22), herpes simplex (20), dengue (51), and measles (1). In the few instances (19, 64) in which adequate experiments were done, the heat-labile material involved in neutralization was shown to be related to the properdin system (49). Rather than describe the details of inactivation of various viruses by a heat-labile serum factor with or without antibody, for purposes of convenience it will be assumed that in all the above instances the properdin system is important. It is agreed at the outset that, although it is appealing, this assumption may be unwarranted. Data are not available, however, to confirm or deny it. Nevertheless, the discussion will be confined to the so-called properdin system: its components, properties, and possible significance. It should also be recognized at this point that there is some contention that properdin actually is not a single substance and that therefore properdin and the properdin system as such do not exist. In order to present the evidence obtained and the concepts evolved, however, properdin and the properdin system will be considered to exist as described by Pillemer and coworkers (49). The main arguments against this viewpoint will be presented subsequently.

PROPERDIN SYSTEM

The properdin system has at least 6 separate and identifiable components (49). All of the components are essential for any of the varied activ-

ities ascribed to this most complex system, and because the function of the whole is completely dependent upon the integrity of its parts, it may be properly called a "system." It should also be pointed out that properdin and the 4 components of complement are relatively unstable, and each is readily affected by a number of variables which make rigid control of all experimental conditions mandatory. That the properdin system has a variety of activities has been amply demonstrated. The major categories of these activities are hemolytic (28), bactericidal (61), virus inactivation (64), and protozoa inactivation (18, 27). The capacity of the properdin system to inactivate certain viruses will be discussed here.

Viruses Affected by Properdin System

Table 3 lists in the left hand column those viruses which have been proved to be inactivated by the properdin system. Newcastle disease virus was the initial virus shown to be neutralized (64). Using children's sera which did not contain specific antibodies it was possible to demonstrate that the properdin system inactivated influenza A and B viruses (R. J. Wedgwood and H. S. Ginsberg, *unpublished data*) as suggested by earlier studies of the heat-labile serum inhibitor in human and animal sera (22). Finkelstein and co-workers have presented evidence to indicate that herpes simplex virus is inactivated by the properdin system (19). The studies of Van Vunakis and her colleagues imply that T2 bacteriophage is inactivated by the properdin system (59). Wedgwood, using T7, confirmed the effect of the properdin system on some bacterial viruses (R. J. Wedgwood, *unpublished data*). Cowan (13), employing T2, T4, and T6, demonstrated a similar neutralization of bacteriophage with "normal" sera but ascribed the phenomenon to antibody rather than properdin *per se* because of striking variations in neutralization titers with each virus.

TABLE 3

Inactivation of viruses by properdin system

Viruses Inactivated	Viruses Probably Inactivated	Viruses Not Inactivated
Newcastle disease	Mumps	Poliomyelitis
Influenza A	Vaccinia	Coxsackie
Influenza B	Variola	ECHO
Herpes simplex	Western equine	Adenoviruses
T2 bacteriophage	Rous sarcoma	
T7 bacteriophage	Dengue	
	Measles	

TABLE 4

Inactivation of Newcastle disease virus by properdin system

(1) Quantity of virus inactivated linear function of properdin concentration
(2) Quantity of virus inactivated linear function of magnesium concentration
(3) Temperature dependent: 10 to 37 C
(4) Maximum virus inhibition at ionic strength 0.1
(5) Maximum virus inhibition at pH 6.5 to 7.0
(6) Properdin and Mg^{++} combined with virus; not complement
(7) Virus-properdin complex dissociable

Listed in the middle column are several viruses which are neutralized by heat-labile factors in human or animal sera (7, 15, 16, 22, 23, 39, 43–45, 51, 65). The properdin system may contain the heat-labile materials involved. The heat-labile substances may act alone on some viruses or may work to potentiate the neutralization of others by antibody. In fact, neutralization of certain strains of dengue virus by specific antibody appears to require a heat-labile serum factor (51). The neutralization of dengue virus suggests a situation similar to the neutralization of *Toxoplasma gondii* which requires both specific antibody and the properdin system (18, 27).

In the third column are listed some important viruses known not to be inactivated by the properdin system either alone or with antibodies (2, 21, 36). It would be intriguing to understand the basic differences in those agents so sensitive to inactivation by the properdin system from those resistant to neutralization by these serum components. It seems reasonable to predict that the differences may lie in the surface structure of the individual agents.

Inactivation of Newcastle Disease Virus (NDV) by Properdin System

Inactivation of a susceptible virus by the properdin system has been investigated in greatest detail employing Newcastle disease virus (NDV) (64). The data will be summarized to elucidate at least one of the mechanisms by which this complex system can act.

The properdin system accomplishes inhibition of hemagglutination and infectivity. It must be emphasized again that to inhibit virus not only is properdin required but also all 4 components of complement and the divalent cation, magnesium. An insight may be gained into the complexity of this system and the multiplicity of reactions which possibly occur to inactivate virus when it is noted (table 4) that the amount of virus inhibited is directly related to the quantity of properdin (between 0.1 and 5 units) and the concentration of magnesium (between 0.02 and 2.5 mM per L); that viral inhibition is temperature dependent as a straight-line function between 10 and 37 C; and that maximum viral inactivation is obtained at an ionic strength of 0.1 and a pH between 6.5 and 7.0. Further insight into the mechanism of inactivation of NDV by the properdin system is obtained from the fact that properdin combines with the viral particles but none of the components of complement can be shown to attach to virus; that the virus-properdin complex is probably accomplished through a magnesium bridge; and that chelation of magnesium at least partially dissociates the virus-properdin complex and results in partial reactivation of virus. These data suggest that the components of complement act as cofactors, but that properdin, mediated through magnesium, actually accomplishes the viral inhibition.

Properties of Properdin

Listed in table 5 are the known properties of this material, properdin, which has such diverse and striking actions. Properdin is a protein which by earlier studies of its physical properties was considered to be a β-2-globulin (63). However, the electrophoretic characteristics and other physical properties suggest that a preferable terminology by current convention is that of a γ-1-globulin (37). Properdin comprises only a small fraction (less than 0.02 per cent) of the total serum proteins (34). By ultracentrifugation, what was considered to be purified properdin was not homogenous, and contained components having sedimentation constants of 6S, 9S, 12S, and 18S. However, properdin activity as measured either by zymosan or bactericidal assays was primarily associated with the 18S component (19S at infinite dilution) (37). The isoelectric point of the protein was 5.6. Purified properdin was antigenic in rabbits, and all biological activities (viral inactivation, bactericidal, and hemolytic) were neutralized by the specific antibody (50). Further immunological studies by agar diffusion techniques, using antiserum absorbed with a serum from which properdin had been removed (RP), suggest that the antibody is directed against a single antigenic component, evidence which strongly implies that properdin is a single molecular species (C. F. Hinz, Jr., *et al.*, *unpublished data* and (62)).

TABLE 5

Properties of properdin

1. γ-1-Globulin electrophoretically
2. <0.02% of serum protein
3. 18S sedimentation constant of active component
4. Isolectric point pH 5.6
5. Antigenic
6. Activity neutralized by antibody

Whether properdin is a single substance or a number of substances, possibly of the nature of classical antibodies, is today a point of vigorous contention (13, 46, 48, 56). A simple statement cannot as yet be made as there are considerable conflicting data. However, some critical evidence is available which bears upon this important concept. Based in large part upon the finding that so-called "purified properdin" agglutinated zymosan and fixed complement as in an antigen-antibody reaction, Nelson (46) proposed that properdin is not a single substance but rather a number of low avidity natural antibodies for different polysaccharide antigens which require complement to strengthen the bond between antibody and antigen. These findings have been confirmed, but the confirmatory studies were extended to demonstrate clearly that the so-called "purified properdin" was a heterogeneous mixture; that it contained many substances found in blood; that these blood components were distinct from properdin; that it did indeed contain an agglutinin for zymosan particles; and that the zymosan agglutinin (perhaps an antibody) could be adsorbed from the properdin preparation without reduction in properdin concentration (37). These latter studies tend to corroborate the immunological data which imply that properdin is a single substance, *i.e.*, a single molecular species.

Cowan (13), in an investigation of the inactivation of T2, T6, and T7 bacteriophages by human

sera, demonstrated that different sera neutralized each phage to varying degrees, and therefore suggested that neutralization of bacteriophage was not an adequate technique for properdin assay. These data were further interpreted to imply that either there are several different properdins, or that properdin is not a single substance, but a family of cross-reacting antibodies which combine with zymosan.

Toussaint and Muschel (56) also studied bacteriophage neutralization by so-called "normal serum" and demonstrated that a phage type-specific substance in serum other than properdin was essential for bacteriophage inactivation. Neither the exact role of properdin *per se* in phage neutralization nor the role of antibody in the absence of properdin was studied. These experiments were interpreted to indicate that specific antibody, not properdin, was the essential factor for bacteriophage neutralization.

There are as yet no data available to satisfy these objections to the postulate that properdin is a single substance and not a classical antibody. One hypothesis which might be proposed to explain the above data (13, 37, 56), as well as those of Van Vunakis and colleagues (59), is that so-called normal sera do contain type-specific antibodies as well as the components of the properdin system, both of which are directed against the bacteriophages employed. Thus, with bacteriophages, as with *Toxoplasma gondii* (18, 27) and possibly dengue virus (51), both antibody and the properdin system may be necessary for neutralization.

Clearly, considerable data are required before one can describe the nature of properdin and the properdin system with confidence. It appears, however, that to explain the conflicting evidence and to elucidate the nature of properdin, if it does indeed exist as a single substance, information must be obtained with highly purified preparations of all components of the properdin system and experiments must be carried out in the absence of other serum products. Furthermore, to study the role of other serum factors, possibly antibody, which may be important in the reactions described, the experiments must be done in the complete absence, as well as in the presence, of properdin and other components of the so-called "system."

Finally, brief attention should be paid to the possible role which the properdin system may play in natural resistance to infection. Despite the extensive studies on the actions of the properdin system *in vitro* and on correlation of levels of properdin with resistance or susceptibility to infection, there is no unambiguous evidence to demonstrate that this complex system does indeed play an important role in resistance to disease. It must be noted, however, that a system with 6 variables, each of which is essential for its function, is not simply controlled, and therefore the rigid proof required is not attained easily. It may also be candidly pointed out that even the role of complement *per se* is not well understood, and that many specific antibodies exist, are measured almost daily, and yet cannot be assigned a biological function.

SUMMARY

It seems rather disheartening to be forced to state in conclusion that of the number of substances in tissues and blood which react with and inhibit viral activities, there is no indisputable evidence that any are responsible for resistance to infection. It is suggested that inhibitors detected in tissues are host-cell receptor substances with which virus attaches to cells; heat-stable inhibitors in serum are similar in composition and may arise from the tissue inhibitors or products of degenerated cells. Properdin and its cofactors, which are essential for inactivation of some viruses, may play a role in nonspecific resistance to infection, but data to support this contention must still be obtained.

REFERENCES

1. Adams, J. A. and Imagawa, D. T. 1957 Immunological relationship between measles and distemper viruses. Proc. Soc. Exptl. Biol. Med., **96**, 240–244.
2. Allen, R., Finkelstein, R. A., and Sulkin, S. E. 1958 Viral inhibitors in normal animal sera. Texas Repts. Biol. and Med., **16**, 391–421.
3. Anderson, S. G., Burnet, F. M., Fazekas de St. Groth, S., McCrea, J. F., and Stone, J. D. 1948 Mucins and mucoids in relation to influenza virus action. VI. General discussion. Australian J. Exptl. Biol. Med. Sci., **26**, 403–411.
4. Bang, F. B., Foard, M., and Karzon, D. T. 1951 Mode of action of heat-labile serum inactivating substance on Newcastle disease virus. Bull. Johns Hopkins Hosp., **88**, 83–100.

5. BLIX, F. G., GOTTSCHALK, A., AND KLENK, E. 1957 Proposed nomenclature in the field of neuraminic and sialic acids. Nature, **179**, 1088.
6. BOGACH, S. 1957 Inhibition of viral hemagglutination by brain ganglioside. Virology, **4**, 458–466.
7. BRIODY, B. A., LEDINKO, N., AND STANNARD, C. 1951 Studies on vaccinia virus. II. Neutralization of vaccinia virus by normal guinea pig serum. J. Immunol., **67**, 413–422.
8. BURNET, F. M. 1951 Mucoproteins in relation to virus action. Physiol. Revs., **31**, 131–150.
9. CASALS, J. AND OLITSKY, P. K. 1947 Inactivation of certain neurotropic viruses *in vitro* by serum lipids. Science, **106**, 267–268.
10. CASALS, J. AND OLITSKY, P. K. 1948 Protection of mice against encephalitis virus by means of organic solvent extracts of brain tumor. Science, **108**, 690–691.
11. CHU, C. M. 1951 The action of normal mouse serum on influenza virus. J. Gen. Microbiol., **5**, 739–757.
12. COHEN, A. AND BELYAVIN, G. 1959 Hemagglutination inhibition of Asian influenza viruses. A new pattern of response. Virology, **7**, 59–74.
13. COWAN, K. M. 1958 Interpretation of properdin levels determined by phage neutralization technique. Science, **128**, 778–779.
14. CURNEN, E. C. AND HORSFALL, F. L., JR. 1946 Studies on pneumonia virus of mice (PVM). III. Hemagglutination by the virus; the occurrence of combination between the virus and a tissue substance. J. Exptl. Med., **83**, 105–132.
15. DOUGLAS, S. R. AND SMITH, W. 1930 A study of vaccinal immunity in rabbits by means of *in vitro* methods. Brit. J. Exptl. Pathol., **11**, 96–111.
16. DOZOIS, T. F., WAGNER, J. C., CHEMERDA, C. M., AND ANDREW, V. M. 1949 The influence of certain serum factors on the neutralization of Western equine encephalitis virus. J. Immunol., **62**, 319–331.
17. FAZEKAS DE ST. GROTH, S. 1952 Nasal mucus and influenza virus. I. The haemagglutination inhibitor in nasal secretions. J. Hyg., **50**, 471–490.
18. FELDMAN, H. A. 1956 The relationship of toxoplasma antibody activator to the serum properdin system. Ann. N. Y. Acad. Sci., **66**, 263–267.
19. FINKELSTEIN, R. A., ALLEN, R., AND SULKIN, S. E. 1958 Properdin sensitivity of herpes simplex virus. Virology, **5**, 567–568.
20. GELLER, P., COLEMAN, V. R., AND JAWETZ, E. 1953 Studies on herpes simplex virus. V. The fate of viable herpes simplex virus administered intravenously to man. J. Immunol., **71**, 410–418.
21. GINSBERG, H. S. 1956 Characteristics of the new respiratory viruses (adenoviruses). I. Qualitative and quantitative aspects of the neutralization reaction. J. Immunol., **77**, 271–278.
22. GINSBERG, H. S. AND HORSFALL, F. L., JR. 1949 A labile component of normal serum which combines with various viruses. Neutralization of infectivity and inhibition of hemagglutination by the component. J. Exptl. Med., **90**, 475–495.
23. GORDON, M. H. 1925 Studies of the viruses of vaccinia and variola. Med. Research Council (Brit.), Spec. Rep. Seri., No. **98**.
24. GOTTSCHALK, A. 1951 *N*-Substituted isoglucosamine released from mucoproteins by the influenza virus enzyme. Nature, **167**, 845–847.
25. GOTTSCHALK, A. 1958 Chemical biology of mucopolysaccharides. Ciba Foundation Symposium, **1958**, 287.
26. GOTTSCHALK, A. 1959 Chemistry of virus receptors. In *The Viruses*, vol. III. Edited by F. M. Burnet and W. M. Stanley. Academic Press, Inc., New York.
27. GRÖNROOS, P. 1955 The action of properdin in *Toxoplasma gondii*. Ann. Med. Exptl. et Biol. Fenniae, **33**, 310.
28. HINZ, C. F., JR., JORDAN, W. S., JR., AND PILLEMER, L. 1956 The properdin system and immunity. IV. The hemolysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria. J. Clin. Invest., **35**, 453–457.
29. HIRST, G. K. 1943 Adsorption of influenza virus on cells of the respiratory tract. J. Exptl. Med., **78**, 99–109.
30. HOLLAND, J. J. AND MCLAREN, L. C. 1959 The mammalian cell-virus relationship. II. Adsorption, reception and eclipse of poliovirus by HeLa cells. J. Exptl. Med., **109**, 487–504.
31. HOWE, C., ROSE, H. M., AND SCHNEIDER, L. 1957 Enzymic action of influenza virus on human erythrocyte stroma components. Proc. Soc. Exptl. Biol. Med., **96**, 88–94.
32. KLEIN, M. 1958 The significance of human antiviral neutralizing substances in animal sera. Ann. N. Y. Acad. Sci., **70**, 362–368.
33. KLENK, E., FAILLARD, H., AND LEMPFRID, H. 1955 Über die enzymatische Wirkung von

Influenzavirus. Z. physiol. Chem., **301**, 235–246.

34. KLENK, E. AND LEMPFRID, H. 1957 Über die Natur der Zellreceptoren für das Influenzavirus. Z. physiol. Chem., **307**, 279–283.
35. KOPROWSKI, H. 1946 Occurrence of nonspecific virus neutralizing properties in sera of some neurotropic mammals. J. Immunol., **54**, 387–394.
36. LEDINKO, N. AND MELNICK, J. L. 1953 Poliomyelitis viruses in tissue culture. V. Reaction of virus and antibody; variables of the quantitative neutralization test. Am. J. Hyg., **58**, 223–247.
37. LEPOW, I. H., PILLEMER, L., SCHOENBERG, M. D., TODD, E. W., AND WEDGWOOD, R. J. 1960 The properdin system and immunity. X. Characterization of partially purified human properdin. J. Immunol., **83**, 428–436.
38. LEVY, A. H., NORMAN, P. S., AND WAGNER, R. R. 1959 Electrophoresis of serum inhibitors of influenza viruses. Federation Proc., **18**, 580.
39. LEYMASTER, G. R. AND WARD, T. G. 1948 Titration of mumps neutralizing antibody in chick embryos. Am. J. Hyg., **48**, 45–52.
40. LEYMASTER, G. R. AND WARD, T. G. 1949 The effect of complement in the neutralization of mumps virus. J. Immunol., **61**, 95–105.
41. MANDEL, B. AND RACKER, E. 1953 Inhibition of Theiler's encephalomyelitis virus (GDVII strain) of mice by an intestinal mucopolysaccharide. I. Biological properties and mechanism of action. J. Exptl. Med., **98**, 399–415.
42. MANDEL, B. AND RACKER, E. 1953 Inhibition of Theiler's encephalomyelitis virus (GDVII strain) of mice by an intestinal mucopolysaccharide. II. Purification and properties of the mucopolysaccharide. J. Exptl. Med., **98**, 417–420.
43. MCCARTY, K. AND GERMER, W. D. 1952 Two heat-labile factors in normal sera which neutralize variola virus. Brit. J. Exptl. Pathol., **33**, 529–536.
44. MORGAN, I. M. 1945 A quantitative study of the neutralization of WEE virus by its antiserum and the effect of complement. J. Immunol., **50**, 359–371.
45. MUELLER, J. H. 1931 The effect of alexin in virus-antivirus mixtures. J. Immunol., **20**, 17–23.
46. NELSON, R. A. 1958 An alternative mechanism for the properdin system. J. Exptl. Med., **108**, 515–535.
47. NOLL, H. AND YOUNGNER, J. S. 1959 Virus-lipid interactions. II. The mechanism of adsorption of lipophilic viruses to water-insoluble polar lipids. Virology, **8**, 319–343.
48. OSAWA, E. AND MUSCHEL, L. H. 1959 Bactericidal action of normal serum and the properdin system. Federation Proc., **18**, 589.
49. PILLEMER, L., BLUM, L., LEPOW, I. H., ROSS, O. A., TODD, E. W., AND WARDLAW, A. C. 1954 The properdin system and immunity: I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. Science, **120**, 279–285.
50. PILLEMER, L., HINZ, C. F., JR., AND WURZ, L. 1957 Preparation and properties of anti-human properdin rabbit serum. Science, **125**, 1244–1245.
51. SABIN, A. B. 1950 The dengue group of viruses and its family relationships. Bacteriol. Revs., **14**, 225–232.
52. SKARNES, R. C. AND WATSON, D. W. 1957 Antimicrobial factors of normal tissues and fluids. Bacteriol. Revs., **21**, 273–294.
53. SMITH, W. AND WESTWOOD, M. A. 1949 Factors involved in influenza hemagglutination reactions. Brit. J. Exptl. Pathol., **30**, 48–60.
54. STONE, J. D. 1948 Prevention of virus infection with enzyme of *V. cholerae*. I. Studies with viruses of mumps-influenza group in chick embryos. Australian J. Exptl. Biol. Med. Sci., **26**, 49–64.
55. TAMM, I. AND HORSFALL, F. L., JR. 1952 A mucoprotein derived from human urine which reacts with influenza, mumps, and Newcastle disease viruses. J. Exptl. Med., **95**, 71–97.
56. TOUSSAINT, A. J. AND MUSCHEL, L. H. 1959 Neutralization of bacteriophage by normal serum. Nature, **183**, 1825–1827.
57. UTZ, J. P. 1949 Studies on the inactivation of influenza and NDV by a specific lipid fraction of normal sera. J. Immunol., **63**, 273–279.
58. UTZ, J. P. 1948 Effect *in vitro* of specific lipid fractions of normal animal sera on psittacosis virus. Proc. Soc. Exptl. Biol. Med., **69**, 186–189.
59. VAN VUNAKIS, H., BARLOW, J. L., AND LEVINE, L. 1956 Neutralization of bacteriophage by the properdin system. Proc. Natl. Acad. Sci. U. S., **42**, 391–394.
60. VOLKERT, M. AND HORSFALL, F. L., JR. 1947 Studies on a lung tissue component which combines with pneumonia virus of mice (PVM). J. Exptl. Med., **86**, 393–407.
61. WARDLAW, A. C. AND PILLEMER, L. 1956

The bactericidal activity of the properdin system. J. Exptl. Med., **103**, 553–575.

62. WEDGWOOD, R. J., TODD, E. W., AND SCHOENBERG, M. D. 1959 Biological characterization of properdin. Federation Proc., **18**, 603.
63. WEDGWOOD, R. J. AND PILLEMER, L. 1959 The nature and interactions of the properdin system. Acta Haematol., **20**, 253–259.
64. WEDGWOOD, R. J., GINSBERG, H. S., AND PILLEMER, L. 1956 The properdin system and immunity. VI. The inactivation of Newcastle disease virus by the properdin system. J. Exptl. Med., **104**, 707–725.
65. WHITMAN, L. 1947 The neutralization of WEE virus by human convalescent serum. The influence of heat-labile substances in serum on neutralization index. J. Immunol., **56**, 97–108.

DISCUSSION

It was suggested that properdin does not neutralize bacteriophage in the absence of antibody. This was illustrated by absorption of antiserum with T2 bacteriophage, after which the serum lost its activity against T2 bacteriophage but retained its activity against T7 bacteriophage. In the light of such evidence it may be inaccurate to refer to properdin as a virucidal substance, as originally described. Properdin has therefore been referred to as a family of antibodies (Muschel, Washington). On the other hand, with Newcastle disease virus the reaction to properdin in the presence of antibody is quite different from the reaction to properdin alone. In the presence of antibody, it is difficult to demonstrate the action of the properdin system. In the absence of antibody, however, the requirement for properdin can be readily demonstrated, and neutralization of the virus may be observed. In interpreting the action or reality of properdin, therefore, it seems important to study a pure system. Addition of antibody to the system may so complicate the observations as to make it difficult to detect the action of properdin alone. Before it will be possible to define clearly the role of properdin against viruses or bacteria it is necessary that highly purified properdin and components of complement be available. To determine the role of antibody in this system it is also important to work with antibodies separated from properdin and complement (Ginsberg, Cleveland).

It is not necessarily correct to think of heat-labile serum inhibitors of viruses as inactive in interfering with virus infectivity. Recent work in several laboratories has shown that serum inhibitor of Asian influenza virus can interfere with infectivity. This inhibitor is associated with the α-1-globulin of horse and other animal species (Takatsy, G. and Barb, K., On the normal serum inhibitors for the avid Asian strains of influenza virus, Acta Virologica, **3** (Suppl.), 71–77, 1959. Cohen, A. and Belyavin, G. (1959) Hemagglutination inhibition of Asian influenza viruses: a new pattern of response, Virology, **7**, 59–74. Zhdanov, V. M., Hamburg, V. P. and Svet-Moldavsky, G. J., Antigenicity of the inhibitor of influenza virus strain A/Asian/57, J. Immunol., **82**, 9–11, 1959) (Wagner, Baltimore).