THE REACTIVITY OF VARIOUS HUMAN SERA WITH MUMPS COMPLEMENT FIXATION ANTIGENS*

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(Received for publication, March 13, 1948)

A complement fixation test for mumps was first described by Enders and his coworkers (1, 2). This test, which employed suspensions of parotid glands from monkeys infected with mumps virus, was found very useful since antibodies could be detected in all patients following an attack of the disease. It proved particularly valuable as an aid in the diagnosis of some of the more unusual manifestations of infection with the virus of mumps, such as meningoencephalitis in the absence of preceding or concurrent parotitis (3). Extensive studies on the correlation between the results of the complement fixation test and resistance to mumps (4) showed that the disease occurred practically always in individuals whose sera gave negative reactions prior to infection. A positive test was obtained in 72 per cent of the subjects admitting parotitis at some time in the past. It also was found positive in 42 per cent of individuals who failed to reveal previous attacks of mumps. This finding, indicating the frequency of inapparent infections with mumps virus, was substantiated by the demonstration in some exposed individuals of the formation of complement-fixing antibodies in the absence of clinical signs of disease.

The adaptation of mumps virus to the chick embryo led to a more readily available source of antigen (5, 6). An analysis of various preparations of tissues and fluids derived from chick embryos infected with this agent revealed that they contained at least two serologically distinct complement fixation antigens (7). One of these was found to be closely linked with the virus and present predominantly in the amniotic and allantoic fluids; the other was smaller in size and demonstrable mainly in the infected tissues; *i. e.*, in the amniotic and allantoic membranes. The virus-bound or "V" antigen could be differentiated from the smaller soluble or "S" antigen by serum cross-absorption technique, in that antibodies against V could be removed from human convalescent sera by absorption with this antigen, leaving most of anti-S in solution and, conversely, absorption with S left antibodies against V in the serum. This observation of antigenic differences was confirmed by the fact that human sera reacted to a varying extent with S and V preparations. The antigen

* The work described in this paper was aided by the Office of Naval Research and the U. S. Public Health Service.

derived from the infected monkey parotid gland, which had been used by the earlier workers, has not been compared as yet with the chick embryo materials. It is impossible, therefore, to state definitely at present whether it contained measurable quantities of both the V and S antigens or only one of them. Certain of the observations to be described point to dominance of the S antigen in suspensions of monkey parotid glands.

It is the purpose of this paper to summarize the results of an extensive analysis of the serological reactivity of human sera taken at various stages of infection. The results show that antibodies against S antigen appear earlier, as a rule, than those against V, and that anti-V remains measurable usually for a longer period than anti-S. Thus, it will be shown that both antigens have their place in the early diagnosis of mumps, whereas in the determination of susceptibility use of the V antigen only appears sufficient.

Materials and Methods

Preparation of Complement-Fixing Antigens.—The egg-adapted strain of mumps virus used for the preparation of antigens was obtained from Dr. John F. Enders in the 5th amniotic passage (6). It has been adapted to the allantoic sac of the chick embryo yielding allantoic fluids of high infectivity (10^8 ID_{50}/ml.). The antigens were prepared from allantoic fluids and chorioallantoic sacs of the 14th to 16th allantoic passages. Suitable numbers of 8-day-old chick embryos were inoculated with 0.5 ml. of infected allantoic fluid diluted in broth to 10^{-2} to 10^{-4}. The technic of inoculation has been described previously (7). After 5 days of further incubation of the eggs at 36–37°C. the allantoic fluids as well as the allantoic sacs were harvested aseptically. The fluids containing the virus-bound antigen (V) were dialyzed in sterile cellophane bags against 20 volumes of M/100 phosphate-buffered saline of pH 7.0 in order to remove most of the urates prior to irradiation with ultraviolet light by a technic previously described (8).

The allantoic sacs were thoroughly washed in sterile buffered saline solution, drained on sterile filter paper, and weighed. A 20 per cent suspension in buffered saline solution was made by emulsifying the tissue in a Waring blendor for 3 minutes. After preliminary centrifugation of the suspension at 2,000 R.P.M. for 10 minutes, the supernatant fluid was subjected to high speed centrifugation at 20,000 R.P.M. for 20 minutes. The supernatant fluid obtained after this centrifugation served as soluble (S) antigen.

Control antigens were prepared according to the methods described above from uninfected chick embryos of the same age and usually from the same batch of eggs supplying the mumps preparations. The normal allantoic fluid gave positive complement fixation tests with human sera only very rarely. In these cases, the control antigens prepared from normal allantoic membranes, likewise, gave positive results. The normal allantoic fluid was omitted, therefore, in later tests.

The dialyzed infected allantoic fluid was irradiated in order to inactivate the virus, and the membrane antigen was treated in the same way. To all antigens 1:10,000 merthiolate was added as a preservative. They proved to be stable at 4°C. for at least 3 months.

Human Sera.—Blood was collected from patients[1] at varying stages of infection with mumps virus and permitted to clot. The serum was separated and inactivated at 60°C. for 20 minutes. In case reactions with the normal control antigen were encountered, a second

[1] The sera of 63 cases were studied in collaboration with Dr. Vera Oldfeld of the Epidemisjukhuset, Stockholm, Sweden.

heating of the sera to 60°C. for 20 minutes frequently decreased this reactivity (2) but rarely removed it completely. The sera were stored in the frozen state at −10°C. until used for the tests.

Complement Fixation Test.—Twofold dilutions of the sera were prepared beginning with undiluted serum or 1:2 in cases where low or negative reactions were expected, and with correspondingly higher dilutions in convalescent specimens. Of each dilution 0.1 ml. was transferred to 4 tubes each. Thus, 4 series of increasing dilutions were obtained, the first set to receive saline solution (serum control); the second, mumps allantoic fluid (V antigen); the third, the supernatant fluid of normal allantoic sac (N antigen); and the fourth, the supernatant fluid of mumps allantoic sac (S antigen). The V and S antigens were used in optimal dilution; *i.e.*, the dilution giving the highest titer with a standard serum according to preliminary titrations. Such a standardization test is shown in Table I. The N antigen was diluted to the same extent as the S preparation, both showing approximately the same amount of nitrogen and phosphorus. Each antigen dilution was mixed with an equal volume of

TABLE I

Optimal Titration of S and V Antigens and Convalescent Serum

Serum 112	S antigen											V antigen						Saline
	Und.	1:2	1:3	1:4	1:6	1:8	1:12	1:16	1:24	1:32	1:48	Und.	1:2	1:3	1:4	1:6	1:8	
1:32	0	0	0	0	0	0	0	0	0	0	wk	0	0	0	0	(0)	tr	c
1:64	0	0	0	0	0	0	0	0	0	0	st	0	0	0	0	(0)	tr	
1:128	0	0	0	0	0	0	0	0	(0)	wk	ac	0	0	0	0	(0)	wk	
1:256	ac	wk	(0)	(0)	0	0	(0)	tr	wk	st	c	(0)	0	0	(0)	wk	ac	
1:512	c	c	c	(c)	(c)	(c)	(c)	c	c	c	c	tr	0	0	tr	st	(c)	
1:1024	c	c	c	c	c	c	c	c	c	c	c	ac	ac	ac	c	c	c	
Saline	c											c						

0 = no hemolysis; tr = trace; wk = weak; st = strong; ac = almost complete; c = complete hemolysis.

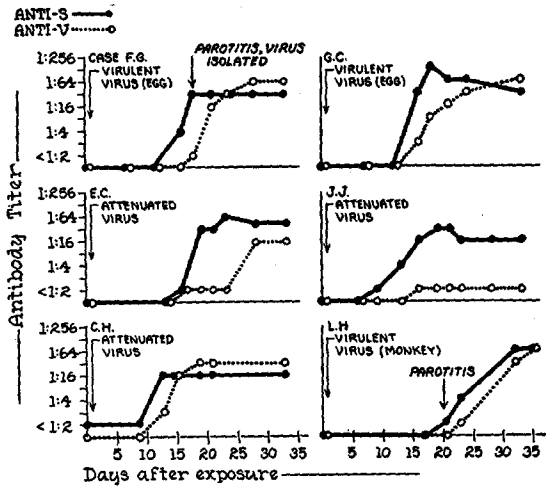
suitably diluted guinea pig complement and the mixture was then added to the corresponding tubes, in 0.2 ml. amounts. In large tests the antigen-complement mixtures were added by means of an automatic pipette. Sharp and Dohme "Lyovac" complement was used throughout.[2] It was adjusted to contain 1.5 minimal hemolytic units per 0.1 ml. After the primary incubation of the test at 37°C. for 1 hour, 0.2 ml. of sensitized sheep cells (2.5 per cent) was added by automatic pipette. The test was further incubated at 37°C. for 1 hour when readings were recorded. The last dilution of serum giving complete fixation of complement (no hemolysis) was considered to be the end point. All antibody titers are recorded as the initial dilution of serum. Known positive sera were included in all tests.

EXPERIMENTAL

The Antibody Response Following Experimental Exposure to Mumps Virus.—In order to study the development of antibodies to the two antigens in the course of infection with mumps virus, it was felt that experimental exposure of man to this agent would yield more dependable data, since the time of expo-

[2] We are indebted to Sharp and Dohme, Inc., for a generous supply of this material.

sure would be accurately known. Sera of such experimental cases were available from studies for other purposes which will be reported separately (9). Fig. 1 demonstrates graphically the results of complement fixation tests with the V and S antigens and sera taken from a number of representative cases at varying intervals after experimental exposure to an oral spray of three different virus preparations. Cases F.G. and G.C. had been exposed, with other individuals, to 5th passage amniotic fluid infected with a strain of mumps virus (J.P.), which had been isolated directly in eggs by the amniotic route from the spinal fluid of a patient with meningoencephalitis (10). F.G. and others not shown in the figure developed parotitis on about the 18th day after exposure; G.C. failed to do so and remained apparently well. On comparing the sero-



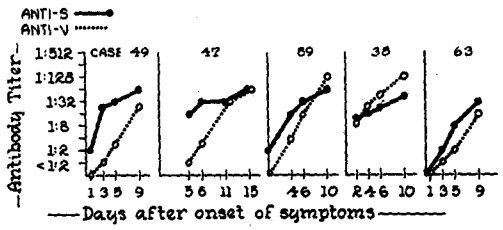
TEXT-FIG. 1. Development of antibodies following experimental exposure to mumps virus.

logical results, it can be seen that both individuals developed antibodies to about the same extent, although only one became clinically ill. This result appears comparable to subclinical infections encountered under epidemic conditions (2, 4, 11). In both cases, antibodies to S antigen appeared earlier and reached high levels before anti-V commenced to rise. It should also be noted that anti-S had already reached a high titer at the time parotitis became manifest.

Cases E.C., J.J., and C.H. were exposed to 16th passage allantoic fluid of the strain of virus received from Dr. Enders; *i. e.*, the same agent which was used for the preparation of the antigens. These subjects, as well as several others not shown in the figure, failed to develop clinical signs of mumps. This strain, therefore, may be considered attentuated in regard to its pathogenicity for man. It can be seen that anti-S developed after about the same interval as in the

cases exposed to the virulent strain of virus. The lag in the development of anti-V was quite marked in case E.C., and patient J.J. failed altogether to develop a significant level of this antibody. The serological response of patient C.H., on the other hand, was practically indistinguishable from that of the cases F.G., and G.C., exposed to the virulent virus. Finally, the sixth patient, L.H., serves as an example of several cases exposed to infected monkey parotid gland, supplied by Dr. J. F. Enders. In this patient, parotitis developed on the 20th day after exposure, at a time when both these antibodies were as yet low in titer. Again anti-S appeared somewhat before anti-V. The difference between these two antibodies might have been more strikingly demonstrable if sera had been available between the 22nd and 32nd days after exposure.

The Antibody Response Following Natural Infection.—Sera from cases of the natural disease during the epidemic of 1946–47 could be collected only after appearance of symptoms. The date of onset was supplied by the parents and its accuracy was possibly influenced by their vigilance. The serological data



TEXT-FIG. 2. Development of antibodies in epidemic cases of mumps.

obtained in many of these cases demonstrate that the antibody response in the natural disease is similar to that encountered after experimental exposure to the virus, as shown in Fig. 2 (cases 47, 49, and 89). The antibodies against S usually rise prior to those against V. In some of the cases, the first serum may have been taken too late to show this difference clearly, as in case 38, but the slope of the respective antibody curves indicates that anti-S may have been exceeding anti-V prior to the date of the first bleeding. Finally, case 63 is included to show that in some patients anti-V may develop simultaneously with anti-S or even prior to the latter.

These data demonstrate that the reactivity of sera taken in the first few days of illness frequently is restricted to interaction with the S antigen. As a rule, this reaction leads to complete fixation of complement in all tubes up to the second last effective dilution of serum, the last giving only partial fixation. On occasions, however, the reactions with S antigen show marked zoning, and only partial fixation of complement, over a wide range of serum dilutions. This phenomenon has been observed previously by Enders, Cohen, and Kane (2), with antigens prepared from infected parotid glands of monkeys. Examples of

this activity are listed in Table II. Sera taken later in the disease always show the usual complete fixation of complement over a series of dilutions and partial fixation in only one or two further tubes. This reactivity of early sera is considered significant but its interpretation is not yet clear.

The frequency with which these antibody relationships are encountered may be gleaned from Fig. 3. In this figure results are collected of complement

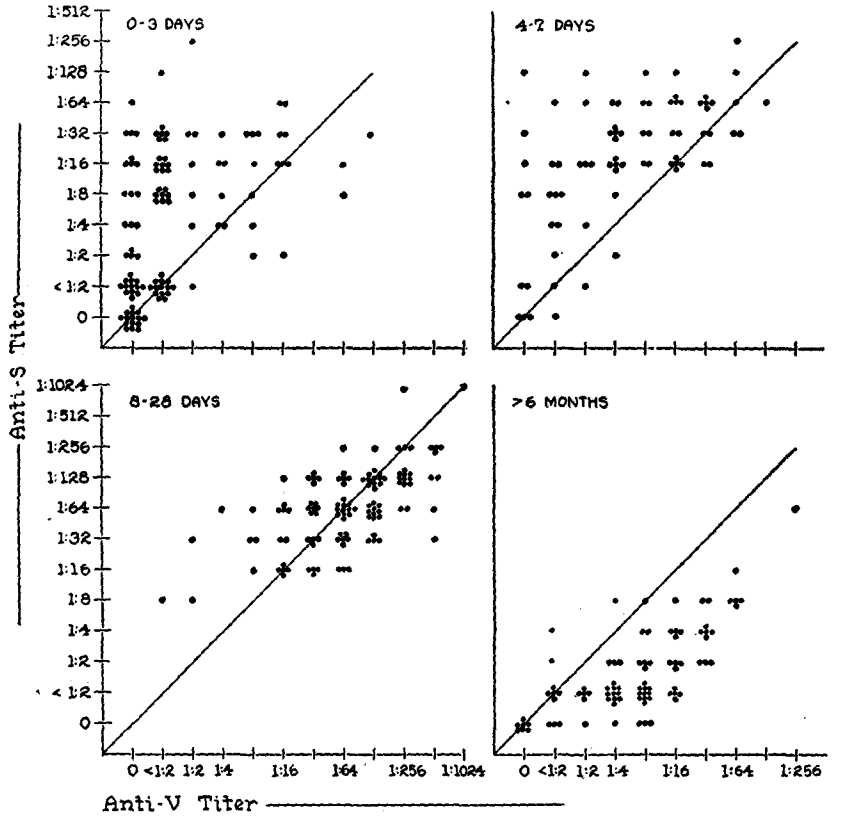
TABLE II

Cases of Mumps Showing Zoning and Partial Fixation of Complement with S Antigen in the First Days after Onset

Institution	Patient No.	Onset of disease	Time after onset	S antigen								V antigen
				Dilution of serum								
				1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	Serum titer
		1947	*days*									
D	77	3/9	2		st	wk	wk	ac	(c)			0
			19		0	0	0	(0)	wk			32
D	55	2/17	1		c	st	tr	wk	c			0
			28		0	0	0	0	(0)	c		256
H	131	4/11	4	wk	tr	0	0	0	0			8
			7	0	0	0	0	0	0			32
H	154	4/26	2	ac	st	wk	ac	(c)	c			0
			4	st	wk	tr	(0)	tr	ac			0
			6		(0)	0	0	(0)	wk			4
H	153	4/25	1	wk	tr	(0)	(0)	(0)	st			0
			3	0	0	0	0	0	0			<2
S	189	4/12	0	wk	wk	st	st	ac				<2
			11			0	0	0	tr	wk		64
S	168	3/30	4		tr	tr	tr	tr	wk	ac		8
			19				0	0	0	0	ac	256

fixation tests with several hundred sera. Each dot represents one serum showing the titer of antibodies against S on the ordinate and that against V on the abscissa. The sera are grouped according to the time they were taken after the reported day of onset of mumps. It can be seen that in the 1st week of illness, most of the dots lie above the diagonal line, indicating that anti-S exceeds anti-V. In the first 3 days some of the sera have no antibodies against either S or V; many have no or low anti-V but already high anti-S antibodies; some are high in both, and in only a few the anti-V antibodies exceed the anti-S.

From 4 to 7 days after onset, only very few of the patients fail to reveal significant levels of antibodies against both antigens. The incidence of cases with high titers against S but low levels of antibodies against V has decreased. From 8 to 28 days after onset practically all cases possess high levels of antibodies against V and S. Of the sera charted almost all had reached titers against the



TEXT-FIG. 3. Correlation between antibody levels against S and V antigens at various times after onset of mumps.

S antigen of 1:16 or higher, and most of them attained similar levels when tested with the V antigen. Thereafter the antibodies begin to decrease in titer, the S antibodies usually at a somewhat faster rate than those reacting with V. If sera are analyzed, which were collected 6 months to several years after infection with mumps virus, it can be seen that in practically all of them anti-V exceeds anti-S. The latter antibody may be low or absent in sera which show relatively high levels of anti-V. The sera of certain patients analyzed several years after an established infection with mumps virus show no detectable antibodies against either antigen.

In a comparison of results from uncomplicated cases of mumps with those of patients showing complications, such as meningoencephalitis, orchitis, pancreatitis, and others, no significant qualitative differences became apparent but the levels of antibodies tended to be higher in such patients. This difference was about twofold when the geometric mean antibody levels were calculated for the two groups. Since only few complications were encountered during the mumps epidemic of 1946–47, further observations are required to ascertain whether or not there is a significant relationship between the time of appearance of antibodies and their concentration on the one hand, and the development of complications on the other.

The Rôle of the V and S Antigens in the Diagnosis of Mumps.—A summary of the data presented, thus far, provides a diagnostic scheme as shown in Table III. If the serum of a patient has a high titer against the S antigen (1:16 or higher) and few or no antibodies against V, the present illness is probably

TABLE III

Tentative Interpretation of Serological Results

Anti-S titer	Anti-V titer	Interpretation
High	Low or absent	First days of disease
High	High	Recent apparent or inapparent infection, or current mumps of several days' duration
Low or absent	Positive to varying extent	Long past apparent or inapparent infection

caused by mumps virus. High levels of both antibodies indicate a recent or a current attack of several days' standing. Little or no anti-S, and distinct levels of anti-V (1:2 or higher) are considered signs of long past infection. The table provides certain criteria for diagnosis which must be interpreted in the light of the individual patient.

It has already been pointed out that the order of events in the first days of illness does not always conform to the presented picture, but it does so sufficiently often to permit the early diagnosis of certain clinical manifestations, such as mumps meningoencephalitis in the absence of preceding or concurrent parotitis. Table IV shows serological data obtained in 12 cases of mumps meningoencephalitis in which parotitis was absent or developed at a later date (case L.H.).[3] In 8 of the 12 cases, the finding of high anti-S and low anti-V levels provided a serological diagnosis of mumps early after onset. Six of these 8 cases from whom a second serum specimen was obtained at a later date showed

[3] The sera of 2 of these cases (L.M., and M.D.) were submitted by Dr. A. J. Rhodes, of the Connaught Medical Research Laboratories, Toronto, Canada.

TABLE IV

Early Diagnosis of Mumps MeningoEncephalitis without Parotitis by Complement Fixation Tests with S and V Antigens

Patient	Time after onset	Serum titer *vs.*		Remarks
		S antigen	V antigen	
	days			
H.C.	8	256	8	
	15	512	32	
L.H.	6	64	4	Parotitis on 14th day
	13	64	64	
C.B.	4	32	<4	
	18	64	64	
M.D.	2	64	4	
	14	32	128	
J.P.	2	16	<2	No second serum, virus isolated from spinal fluid
D.L.	2	16	<2	
	15	64	256	
Nys.	2	256	2	No second serum
J.S.	6	64	<4	
	19	128	64	
Ahr.	4	4	<2	
	18	16	16	
Mar.	3	4	2	
	15	16	64	
A.P.	2	4	4	Virus isolated from spinal fluid
	6	4	8	
	10	8	32	
	17	16	128	
Wri.	2	<2	4	
	12	128+	128+	

subsequent rises particularly in anti-V. From the spinal fluid of one of the 2 patients from whom a second serum could not be obtained, mumps virus was isolated. This confirmed the result of the complement fixation test (10). The

serological diagnosis of the remaining 4 cases listed in Table IV depended, as in the studies of Kane and Enders (3), on the demonstration of rises in antibodies over a period of 1 week or longer. Significant rises in antibodies were measured with both antigens. In several additional patients with meningoencephalitis, the first available sera were taken rather late in the disease and yielded high titers against both V and S antigens. In these, no rises in antibodies could be measured and thus definite proof was not obtained that the neurological signs were a result of infection with mumps virus.

TABLE V

Antibody Response Following Subcutaneous Vaccination or Skin Testing

Case No.	Vaccination				Case No.	Skin testing			
	Anti-V		Anti-S			Anti-V		Anti-S	
	A	B	A	B		A	B	A	B
1	0	0	0	0	1	0	0	0	0
2	0	0	0	0	2	0	0	0	0
3	0	6	0	3	3	0	8	0	0
4	<2	48	<2	12	4	0	32	0	0
5	<2	48	0	32	5	0	32	0	8
6	<2	24	<2	4	6	<2	4	<2	4
7	<2	24	<2	2	7	<2	16	<2	2
8	<2	128	0	8	8	<2	32	4	8
9	2	8	0	<2	9	<2	32	<2	4
10	2	96	<2	6	10	<2	64	0	4
11	2	128	0	24	11	2	32	<2	8
12	4	48	<2	16	12	2	16	0	0
13	16	96	3	8	13	4	16	0	0
					14	4	32	0	0

A = serum taken before vaccination or skin test.

B = serum taken 2 weeks after vaccination or skin test.

The Antibody Response Following Vaccination and Skin Testing.—It has been shown by others that both vaccination of monkeys and man with inactivated mumps virus (12–14) and skin tests in man (2, 15) performed with mumps antigen, caused the formation of complement fixing antibodies. These observations were confirmed in a study of a number of cases of each type. Centrifugally concentrated virus served as vaccine and infected allantoic fluid as skin test material. The virus in both instances was inactivated by ultraviolet irradiation. Table V summarizes the data selected from representative groups which show the variability of the response. The results obtained following the two types of stimuli were very similar. As a rule, the antibodies against V developed to a distinctly higher titer than those against S. In the majority of

these cases a restimulation of antibodies is indicated by the fact that low levels of anti-V were found in many of the sera taken before the skin test or before vaccination, a finding compatible with past apparent or inapparent infection. Among those cases whose sera did not react with V antigen prior to vaccination or skin testing, some responded with the formation of antibody, others did not. Whether those who responded had lost their antibodies in the years following infection, and whether their reaction must therefore be classified as restimulation, or whether the response represents antibody formation *de novo*, cannot be stated at present.

Non-Specific Reactions.—The reactions obtained with these antigens in the majority of the sera studied appeared specific in that the sera failed to react with control antigens. However, sera were encountered, on occasion, which gave positive complement fixation tests with suspensions of normal allantoic membrane. These occurred in spite of heating to 60°C. for 20 minutes. A second heating to 60°C. (2) removed some, but not all, of the non-specific reaction. This reactivity with control antigen affected particularly the interpretation of the reactions with S antigen and not so much those with V antigen. Absorption of such sera with particulate components of normal allantoic sacs sedimentable at 20,000 R.P.M., removed this reactivity and uncovered the specific reaction with the S preparation. Table VI demonstrates several such experiments. As can be seen, the three serum specimens obtained from case 64 all reacted to about the same extent with N antigen. After absorption this reactivity was lost, whereas the reaction with the S preparation, although reduced by the absorption, showed the expected characteristic rise in titer during the first few days of parotitis. The reaction with V was not measurably affected by the absorption, nor was the Wassermann reaction. The latter was apparently a false positive, inasmuch as it became negative during convalescence (16). Case 67 gave essentially similar results except that a true positive Wassermann test was encountered in this patient. Case 46 is included to show that absorption with normal membrane particles does not affect the titer against S antigen in sera which fail to show a reaction with N.

The absorption experiments seemed to indicate that antibodies to normal chick components may occur in man on occasion and that Wassermann antigen is not involved in this reaction. To elucidate this question further, sera were obtained from 6 luetic patients with no recent history of mumps. None of these reacted with the normal membrane antigen and 4 were free of specific mumps antibodies. These data suggested, on the other hand, that heterophile antibodies, possibly related to Forssman antibodies, might be responsible for some of the non-specific reactions. This possibility was further supported by the observations that the sensitized sheep cells in the complement fixation test were agglutinated by some of the sera in the lower dilutions. Absorption of such sera with washed sheep erythrocytes frequently removed the non-specific

TABLE VI

Removal of Non-Specific Reactions by Absorption with Particulate Components Derived from Normal Allantoic Membranes

Case No.	History	Date of bleeding	Treatment of serum	Antibody titers *vs.*			Wassermann reaction
				N antigen	S antigen	V antigen	
64	Pansinusitis 3/9 Parotitis 3/13	*1947* 3/13	— Absorbed	1:32 <1:4	1:32 1:8	1:16 1:16	+ +
		3/15	— Absorbed	1:32 —	1:32 1:8	1:64 1:64	
		3/18	— Absorbed	1:32 —	1:64 1:32	1:128 1:128	
67	Lues. Skin test for mumps 3/15	3/15	— Absorbed	1:16 1:2	1:16 1:4	1:4 1:4	+ +
		3/31	— Absorbed	1:16 <1:4	1:32 1:16	1:128 1:128	+ +
46	Parotitis 3/3	3/4	— Absorbed	<1:2 —	1:32 1:32	<1:2 <1:2	
		3/8	— Absorbed	— —	1:128 1:128	1:24 1:32	— —
		3/12	— Absorbed	— —	1:128 1:128	1:128 1:128	

TABLE VII

Removal of Non-Specific Reactions by Absorption with Sheep Erythrocytes

Case No.	History	Date of bleeding	Treatment of serum	Antibody titers *vs.*		
				N antigen	S antigen	V antigen
D4	Positive (parotitis 3/7/47)	*1947* 3/7	— Absorbed	1:64 <1:4	1:64 <1:4	1:4 0
		3/21	— Absorbed	1:32 <1:2	1:64 1:32	1:64 1:64
D7	Negative	3/3	— Absorbed	1:8 0	1:8 0	
51	Positive (parotitis 2/28/47)	3/17	— Absorbed	0 0	1:64 1:64	1:32 1:32

reactivity, whereas absorption of specific mumps convalescent sera revealed that the mumps antibodies were not affected by such procedures. Table VII shows some examples of this kind.

DISCUSSION

The data presented show that sera obtained from human beings at various stages of infection with the virus of mumps may differ distinctly in their content of antibodies against the two antigens, the virus particle (V) and the soluble antigen (S). In the early days of disease, anti-S antibody may reach high titers, whereas anti-V may still be low or absent. This has permitted the serological recognition of an infection with mumps virus, on occasion, on the 1st or 2nd day of illness. This presumptive test has been confirmed in all instances studied by the demonstration of rises in antibodies in subsequent sera. It was found of great assistance in the early diagnosis of several cases of mumps meningoencephalitis in the absence of parotitis.

For determination of resistance to mumps, use of the V antigen alone appears sufficient. Many more individuals, who experienced either apparent or inapparent infections at some time in the past, will react with V than with S. The rôle played by anti-V in the resistance of an individual to mumps has not been definitely established. However, as has been shown (11), in a small group of children, no cases of mumps occurred among those giving significant reactions with V prior to an epidemic. On the other hand, not all of the children whose sera failed to react with V developed clinical or subclinical infections. This latter observation may depend upon several possible factors: (*a*) the intimacy of exposure may vary; (*b*) amounts of anti-V too small to measure may, nevertheless, give protection, and (*c*) an antibody different from that measured by the described complement fixation technic may be responsible for resistance.

A comparison of the data published by Enders and his coworkers (1–4), with those presented above, indicates that the antigen in suspensions of infected parotid glands of the monkey is dominantly of the S type. This suggestion is based particularly upon the fact that the marked zoning and the incomplete fixation of complement with occasional sera of the early acute phase which have been observed using monkey antigen were also found to occur employing the S antigen of the chick embryo, but not the V preparation. This suggestion is in line too with the finding of high antibody levels against monkey parotid antigen in some other patients on the first few days of disease. The monkey antigen, as prepared, contains virus particles. Whether the amount of it is sufficient to be reactive in the complement fixation reaction cannot be stated until strict comparisons are made of the various preparations of antigen with suitable immune sera.

The serological response obtained under conditions of experimental infection with a human pathogenic strain of mumps virus appeared to be similar to that

of the epidemic illness in every respect studied. Furthermore, the exposure of human beings to "attentuated" mumps virus gave, in most instances, antibody levels against V and S antigens comparable to those seen in uncomplicated cases of the natural disease. These data seem to indicate, therefore, that such a procedure may lead to an enduring immunity, a suggestion which has already been explored (17) and which will be investigated further.

Intradermal or subcutaneous injection of inactivated mumps virus has been shown in the past to stimulate formation of complement-fixing antibodies (2, 12–15). The present studies revealed that frequently only anti-V was formed. If anti-S also developed, its titer constituted, as a rule, only a fraction of that measured against V. Antibody formation in these cases was noted particularly if the treated individual already possessed some antibodies, whereas only part of those with negative complement fixation tests responded to such stimuli. It is possible that in these instances too the rise in antibodies was the result of restimulation by small quantities of antigen, whereas for the *de novo* formation of antibodies, possibly more concentrated preparations of virus may be needed. These relationships call for further analysis.

SUMMARY

Human sera taken at various stages of mumps have been analyzed in regard to their reactivity with two serologically distinct complement fixation antigens which were derived from the infected chick embryo. Antibodies to the soluble or S antigen appear earlier in the disease and, as a rule, reach high levels before antibodies against the virus-bound or V antigen commence to rise. In early convalescence, both antibodies reach high levels. Subsequently antibodies against the S antigen decrease usually at a faster rate than those against V, so that after a period of several years, frequently only anti-V may be left.

These findings were found helpful in diagnostic procedures. The use of both the V and S antigens has permitted the early diagnosis of manifestations of mumps in the absence of parotitis, such as meningoencephalitis, since the finding of high levels of anti-S and of low titers of, or no, anti-V is considered diagnostically significant for the first few days of illness. For the determination of resistance the use of the V antigen appears more useful.

Following vaccination or skin testing, antibodies against both antigens may develop; those against V increase more regularly and to higher titers than those against S.

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