IMMUNITY IN MUMPS

IV. The Correlation of the Presence of Complement-Fixing Antibody and Resistance to Mumps in Human Beings*

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The epidemiologic pattern of mumps suggests that inapparent infection leading to the establishment of permanent immunity is frequent. Thus only about 60 per cent of the adult population in the United States have experienced a recognizable attack of this disease (1) whereas about 90 per cent of the same individuals have, during childhood or adolescence, contracted measles. Since there is no reason to believe that the virus of mumps is less widely disseminated than that of measles, it would seem that one factor which could account, in part at least, for the lower incidence of overt mumps might be specific immunity induced by subclinical infection. Direct evidence that this sort of infection by the virus of mumps did take place in one individual following exposure in the laboratory was obtained by means of the complement fixation test and the test for dermal hypersensitivity described previously (2, 3). The development of methods for the identification of individuals who have experienced such infections, should they prove to be numerous, would clearly be desirable. If, in the future, a procedure of immunization were to become available, such tests would be essential in its practical application.

Accordingly, studies to be described in this and the following communication (4) were undertaken among groups of adults and children in which the two techniques mentioned above were employed. The results were also correlated, when possible, with the record in respect to a known attack of mumps and, in certain cases, with the events which followed adequate exposure to the disease. Such correlations not only afford direct evidence in support of the epidemiologic deduction that the incidence of subclinical infection is significantly large

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but are helpful in the evaluation of these two tests as indices of susceptibility or resistance.

Procedures

Personnel Selected for Study.—Fifty groups of adults and children have been studied either by means of the complement fixation test, the skin test, or by both techniques. To save repeated reference to the characteristics of each group, the pertinent information in regard to their composition and recent exposure to mumps is presented in Table I. It should be pointed out that when a subsequent reference is made either in this or the following paper to a group, the number of individuals may not then correspond, for the following reasons, to that of the same group mentioned in Table I: (*a*) some individuals were not tested by one or the other technique; (*b*) those yielding doubtful or unsatisfactory tests may have been omitted; children may have been considered separately from the adults in those groups consisting of both young and old persons.

Persons whose ages lay between 2 and 17 years inclusive have been arbitrarily classified as children. The majority, however, were between 4 and 15 years of age inclusive. Similarly among the adults, although the ages ranged from 18 to 88 years, the very large majority were from 18 to 30 years old.

Since one of the principal objectives in these investigations was to determine the incidence and significance of positive and negative complement fixation and skin tests in persons who had never had mumps or who had the disease at some time in the relatively remote past, *i.e.* at least 6 months to a year previously, it is obvious that ideally all the individuals included in this part of the study should have had precise knowledge concerning a previous attack, and should not have been exposed during the recent past. Only in a comparatively few groups was this ideal situation approximated. Therefore the available information briefly indicated in Table I in respect to the dependability of the history and to recent exposure is of importance in the interpretation of the results to be presented. It is evident that only in the groups of medical students and a few others could much confidence be placed in the statements concerning previous attacks. With the exceptions of groups XXXVI and XXXVIII where epidemics had been in progress for some time, in those which had been recently exposed to mumps the tests were carried out a few days after exposure was known to have occurred. But within this short interval it is unlikely that such exposure could have significantly altered the preexisting immunologic responses of the individuals. Accordingly insofar as recent infection with the virus of mumps is concerned, they have been considered as "normal."

Complement Fixation Tests.—The technique employed for the complement fixation tests has been described in detail (2, 5). Dilutions of sera and antigens are recorded in terms of their *final* dilution before the addition of sensitized cells. The sera removed from the clots were in most instances heated twice at 60°C. with an interval of cooling at 4°C. or at room temperature to inactivate the complement and eliminate anticomplementary activity. Usually a "2 tube test" was carried out in which only serum dilutions of 1–6 and 1–24 were included. This range was sufficient to include the endpoint of over one-half of the sera from "normal" persons. When the endpoint was higher than the 1–24 dilution, it was in certain instances determined afterwards.

As one would expect, titers of antibody in most individuals who experienced mumps at some time in the more or less remote past were found to be low as compared with those recorded for persons recently convalescent (3). Weak reactions denoted by 1+ or 2+ in the 1–6 dilution of the serum were often observed. The results of experiments, in which 1.5 and 1.0 units of complement were employed to increase the sensitivity of the test have indicated that such reactions in the routine test, where 2 units of complement are used, are of significance

TABLE I

Group	Status	Composition*		Children		Recent exposure to mumps‡	History of mumps
		Adults	Children	Mean age	Age range		
				yrs.	*yrs.*		
I	Temporarily institutionalized homeless children	2	34	7.3	1–16	Yes	Unreliable
II	Institutionalized crippled children	3	60			Yes	Not given
III	Preparatory school for boys	0	62			Yes	Reliable
IV	Male mental defectives	24	97	14.7	9–17	Yes	Not given
V	Male mental defectives	36	15	11.9	6–17	Yes	Not given
VI	Female mental defectives	50	94	13.8	8–17	Yes	Not given
VII	Female mental defectives	27	58	12.4	8–17	Yes	Not given
VIII	Female mental defectives	84	4	15.7	13–17	Yes	Not given
IX	Female mental defectives	130	4	15.7	14–17	Yes	Not given
X	Normal families	36	6			Yes	Reliable
XI	Normal families	38	12			Yes	Reliable
XII	Male mental defectives	57	6	14.1	9–17	Yes	Not given
XIII	Hospital nurses	49	0			Yes	Fairly reliable
XIV	Female mental defectives	55	30	12.8	6–17	Yes	Not given
XV	Male and female medical students	100	0			No	Reliable
XVI	Male dental students	66	0			No	Fairly reliable
XVII	Male medical students	124	0			No	Reliable
XVIII	Male medical students	59	0			No	Reliable
XIX	Male medical students	88	0			No	Reliable
XX	Hospital nurses	18	0			Yes	Fairly reliable
XXI	Children in day nursery	0	16	4.3	3–5	No	Fairly reliable
XXII	Children in day nursery	0	19	4.2	3–5	No	Fairly reliable
XXIII	Patients in a children's hospital	0	19	7.4	4–16	Yes	Fairly reliable
XXIV	Institutionalized crippled children	0	36			No	Unreliable
XXV	Hospital nurses	24	0			Yes	Fairly reliable
XXVI	Male medical students	119	0			No	Reliable
XXVII	Male medical students	141	0			No	Reliable
XXVIII	Male medical students	39	0			No	Reliable
XXIX	Male medical students	7	0			No	Reliable
XXX	Laboratory workers	46	0			No	Reliable
XXXI	Public health students	25	0			No	Unreliable
XXXII	Institutionalized aged men	10	0			No	Unreliable

* Individuals from 1 to 17 years of age inclusive classed as "children;" those older than 17 years as adults.

‡ As far as could be ascertained, all exposures occurred within a week or 10 days before complement fixation or skin tests were done with the exception of groups XXXVI and XXXVIII.

TABLE I—*Concluded*

Group	Status	Composition*		Children		Recent exposure to mumps‡	History of mumps
		Adults	Children	Mean age	Age range		
				yrs.	*yrs.*		
XXXIII	Hospital nurses and doctors	15	0			No	Fairly reliable
XXXIV	House officers	12	0			Yes	Reliable
XXXV	Laboratory workers	13	0			Yes	Unreliable
XXXVI	White Army personnel	101	0			Yes	Unreliable
XXXVII	Colored Army personnel	180	0			Yes	Unreliable
XXXVIII	Colored Army personnel	453	0			Yes	Unreliable
XXXIX	Institutionalized deaf children and adults	65	90	15.8	7–17	No	Fairly reliable
XL	Male mental defectives	42	0			No	Not given
XLI	Patients in a children's hospital	0	22			Some cases	Fairly reliable
XLII	Hospital patients	0	17			Uncertain	Not given
XLIII	Institutionalized deaf children	0	144	9.9	6–16	Yes	Fairly reliable
XLIV	Institutionalized deaf children	0	53	8.1	1–17	No	Fairly reliable
XLV	Institutionalized deaf children	0	34	6.3	4–10	No	Fairly reliable
XLVI	Institutionalized deaf children	0	154	13.7	4–17	No	Fairly reliable
XLVII	Male mental defectives	0	33	10.7	8–14	No	Unreliable
XLVIII	Male mental defectives	0	100	10.6	6–15	No	Not given
XLIX	Male mental defectives	4	61	13.8	7–17	No	Not given
L	Hospital nurses	21	0			Yes	Fairly reliable

in a large proportion of cases provided the controls reveal no evidence of fixation. Thus of 10 sera which gave in a dilution of 1–6 reactions of "2+," "1+," or "tr," 9 were definitely positive when complement was reduced. In contrast only one of 7 sera yielding negative reactions in the routine test gave evidence of fixing any complement when 1 unit was employed. We have therefore regarded a reaction of "1+" as the endpoint in serum titrations. It is freely admitted, however, that the designation of such weak reactions as positive occasionally may be erroneous. When the degree of fixation was minimal in the lowest dilution tested as denoted by "tr" (trace), the result has been recorded as doubtful, although there is reason to believe that the majority of "trace" reactions are indicative of a low concentration of specific antibody.

EXPERIMENTAL

Correlation between the Presence of Complement-Fixing Antibody and Resistance to Infection.—The data obtained in outbreaks of mumps among the groups of

institutionalized personnel mentioned in Table II enabled us to form an estimate of the value of the test as an index of resistance or susceptibility. There the results of 517 tests in groups of exposed children and adults are recorded. In addition the number of cases of overt disease is given which occurred *afterwards* among the negative and positive reactors as well as among those whose tests were doubtful or unsatisfactory.

It is clear that 56 of the 61 cases of parotitis which were noted in these groups appeared among the negative reactors. A single case was seen among those yielding positive reactions. This was in an individual whose serum gave only incomplete fixation in the lowest dilution (1–6) tested. One case was observed

TABLE II

Correlation of Attack Rate of Mumps and Results of Complement Fixation Tests

Group	Total No. tested	No. positive	No. negative	No. doubtful	No. unsatisfactory	Total cases	No. cases in positive	No. cases in negative	No. cases in unsatisfactory	No. cases in doubtful
I	24	10	13	1	0	3	0	3	0	0
IV	125	15	92	15	3	9	0	9	0	0
VI*	133	51	67	9	6	15	0	14	0	1
VII‡	75	22	38	14	1	1	0	1	0	0
XXXIV	12	4	5	3	0	1	0	1	0	0
XLIII	148	61	70	4	13	32	1§	28	3	0
Combined...	517	163	285	46	23	61	1	56	3	1

* In this group 13 cases had appeared before complement fixation tests were done.

‡ In this group 7 cases had appeared before complement fixation tests were done.

§ Incomplete fixation 1–6 dilution of serum.

among the "doubtful" reactors and 3 among those whose sera were anticomplementary.

Although the number of persons giving negative tests was greater than that of those giving positive tests, the difference in the attack rates of the two types of reactors is statistically significant. These results are indeed quite unequivocal and leave no doubt that with very rare exceptions a positive complement fixation test can be interpreted as an index of resistance.

Appearance of Complement-Fixing Antibody in Persons Exposed to Mumps Who Failed to Develop the Disease.—Conclusive serologic evidence of the occurrence of subclinical infections was afforded during two outbreaks of mumps which occurred in two different institutions (groups VI and VII and group XLIII). As soon as the first cases had been observed, complement fixation tests were done on the sera of all the individuals who were exposed. Furthermore in one of the institutions such tests were done not only on the sera of the inmates of the building where the disease had appeared, but also on spec-

imens from individuals quartered in two other buildings who were not exposed and of whom none came down subsequently with mumps. The results in these last two groups (XXXIX and XLVI) may be therefore regarded as control for those obtained in the groups subjected to exposure. Approximately 1 month after the first series of tests had been completed and many secondary cases of mumps had appeared in the exposed groups, a second series of tests was performed on sera obtained from as many as possible of those whose first tests had been negative and who failed to develop signs of mumps. The results of both series of tests are summarized in Table III together with the number of cases of mumps which were observed in the groups. In one institution about one-third of those who were exposed and who did not become ill developed antibody during the interval between the two series of tests; in the other institution two thirds of the exposed responded in this manner. On the other hand,

TABLE III

Increase in Complement-Fixing Antibody in Persons Exposed to Mumps Who Failed to Develop Clinical Signs of Infection

Group	No. retested Negative by 1st complement fixation test	No. showing titer > 1-24 by 2nd test*	Per cent showing titer > 1-24 by 2nd test	Cases of mumps in same dormitory
VI and VII	52	15	29	40
XLIII	26	17	65	32
XXXIX and XLVI (controls—not exposed)	28	0	0	0

* Titers of 1-24 and greater included.

none developed antibody during the same period who were living in the buildings where out-breaks failed to occur.

Correlation of History of Mumps with Results of Complement Fixation Tests.—(*a*) *In Adults.*—In Table IV the results of 951 complement fixation tests are correlated with the statements in respect to a previous attack of mumps given by the individuals from whom the sera were obtained. Simple calculations on the basis of the combined data show that (*a*) the result of the test corresponded to the history in 68 per cent of the cases, (*b*) the test was positive in about 77 per cent of the cases in which the history was positive, (*c*) the test was positive in about 42 per cent of the cases in which the history was negative. Even when allowance is made for a considerable percentage error in the histories which no doubt was present in certain of the groups, these results provide definite evidence that the majority of persons who have had mumps maintain antibody in low concentration for an indefinite period in their blood. Indeed among the 592 medical students who are mentioned in Table IV, the correlation between positive test and positive history is 0.8. Since their histories are regarded as

the most reliable which we have been able to obtain and the numbers are fairly large, it would seem most likely that about four-fifths of adults, at least of this age, who have during childhood or youth been infected with the virus retain complement-fixing antibody in their blood. Conversely this calculation indicates that in as many as 20 per cent of this age group the antibody may fall to levels not detectable by the technique employed. It is possible, however, as

TABLE IV

Correlation of History and Complement Fixation Test in Adults

Group	H+ CF+*		H+ CF−		H− CF+		H− CF−		Total‡
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	
XIII	4	25	4	25	1	6	7	44	16
XV	36	44	17	21	13	16	16	19	82
XVII	59	51	13	11	19	17	25	22	116
XVIII	22	44	5	10	8	16	15	30	50
XIX	35	48	4	6	18	25	16	22	73
XXVI	40	38	18	17	18	17	30	28	106
XXVII	70	56	12	10	12	10	30	24	124
XXVIII	20	57	1	3	6	17	8	23	35
XXIX	4	67	0	0	1	17	1	17	6
XXX	16	41	4	10	6	15	13	33	39
XXXI	8	33	4	17	6	25	6	25	24
XXXII	3	33	0	0	4	44	2	22	9
XXXIII	1	7	0	0	7	47	7	47	15
XXIV	2	20	1	10	3	30	4	40	10
XXXV	0	0	0	0	8	73	3	27	11
XXXVI	45	58	8	10	19	24	6	8	78
XXXVII	59	42	40	28	14	10	29	20	142
L	9	60	1	7	0	0	5	33	15
Combined...	433	45	132	14	163	17	223	23	951

* H = history of mumps.
CF = complement fixation test.
‡ Doubtful and unsatisfactory tests omitted.

pointed out below, that the use of allantoic or amniotic fluids as antigens may further reduce the proportion of those acknowledging a previous infection who fail to give a positive test.

Although the number of reactors among those with negative histories was much smaller, nevertheless they comprised about two-fifths of this class. This proportion remains approximately the same whether one considers all the results included in Table IV or only those obtained in the various groups of medical students (all groups, 42 per cent; medical students, 40 per cent). Again, error in respect to the history may account in some degree for the fre-

quent association of negative history with the presence of antibody. In the majority of cases, however, another explanation must be sought. The evidence so far presented concerning the origin of the antibody would make it appear most probable that previous inapparent infection with the virus was responsible for most of the positive reactions which have been observed.

(*b*) *Children.*—In Table V the results of complement fixation tests on the sera of 116 children have been arranged according to the history of mumps as recorded for each child. The numbers are small in comparison with the adult groups. This was due to the fact that in most of the children's groups the histories were considered to be unreliable. Even in the case of the groups mentioned in the table, it is recognized that the statements concerning previous

TABLE V

Correlation of History and Complement Fixation Test in Children

Group	H+ CF+*		H+ CF−		H− CF+		H− CF−		Total‡
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	
I	3	14	4	18	7	32	8	37	22
XXI	2	14	0	0	4	29	8	57	14
XXII	1	7	0	0	9	64	4	29	14
XXIII	4	22	1	6	0	0	13	72	18
XXIV	6	19	0	0	11	35	14	45	31
XLI	8	47	2	12	2	12	5	30	17
Combined....	24	21	7	6	33	28	52	45	116

* H = history of mumps.
CF = complement fixation test.
‡ Doubtful and unsatisfactory tests omitted.

attacks may be often erroneous. Nevertheless it will be seen by making simple computations that as far as they go the correlations correspond closely to those for the adult groups. Thus about 78 per cent of children with positive histories and about 38 per cent of those with negative histories gave positive tests.

Here, too, we have a further indication that inapparent infection is common in children as well as in adults; but the incidence of such infection as indicated by the proportion of those with positive tests and negative histories (28 per cent) which is greater than that in most of the adult groups, is probably somewhat exaggerated. Had it been possible to obtain accurate histories in all cases, this proportion might have been smaller.

The incidence of Positive and Negative Reactors among Adults and Children.—

(*a*) *Adults*—In Table VI are summarized, without regard to history, the

results of 2021 complement fixation tests on the sera of the same number of individuals. Of the latter, 1665 (the first 13 groups) were healthy persons

TABLE VI

Results of Complement Fixation Tests in Adults

Group	No. tested	Tests doubtful		Tests unsatisfactory		No. definite tests	Tests positive		Tests negative	
		No.	Per cent*	No.	Per cent*		No.	Per cent‡	No.	Per cent‡
Medical§ students	672	32	5	13	2	627	398	63	229	37
XIII	52	4	8	2	4	46	20	44	26	57
XXV	21	1	5	1	5	19	13	68	6	32
XXX	46	3	7	1	2	42	25	60	17	41
XXXI	25	1	4	0	0	24	14	58	10	42
XXXII	10	3	33	0	0	7	5	71	2	29
XXXIII	15	0	0	0	0	15	8	53	7	47
XXXIV	12	3	25	0	0	9	4	45	5	56
XXXV	13	0	0	0	0	13	8	62	5	39
XXXVI	101	16	16	7	7	78	64	82	14	18
XXXVII	180	9	5	12	7	159	83	52	76	48
XXXVIII	453	53	12	51	11	349	217	62	132	38
XXXIX	65	5	8	10	15	50	39	78	11	22
Combined.....	1665	130	8	97	6	1438	898	63	540	38
IV	24	3	13	1	5	20	1	5	19	95
VI	49	3	6	5	10	41	17	41	24	59
VII	27	7	26	1	4	19	9	47	10	53
VIII	82	4	5	0	0	78	36	46	42	54
IX	132	19	14	1	1	112	45	40	67	60
XL	42	7	17	0	0	35	8	23	27	77
Combined.....	356	43	12	8	2	305	116	38	189	62
All groups combined.....	2021	173	9	105	5	1743	1014	58	729	42

* Percentage of all tests.

‡ Percentage of definite tests.

§ Included as medical students are groups XV, XVII, XVIII, XIX, XXVI, XXVII, XXVIII, and XXIX.

who had maintained essentially normal contact with their environment; the remaining 356 (the last 6 groups) were mental defectives most of whom had been confined in the same institutions since early childhood. It will be seen that the mean proportion of positive tests among the mentally normal persons was 63 per cent. It contrast among the mentally defective it was 38 per cent.

This large difference can most logically be attributed to the greater opportunity of the normal groups for previous contact with cases of mumps. If this be the correct explanation, this significant difference can be regarded as further evidence for interpreting the presence of complement-fixing antibody as an index of past infection.

Excepting groups XIII, XXXVI, and XXXIX, deviation from the over-all mean of 63 per cent positive tests among the groups of mentally normal adults in which the numbers are reasonably large is not great. No reason for the low incidence in group XIII is apparent, but in the Army camp where group XXXVI with an incidence of 82 per cent was stationed an epidemic of mumps had been in progress during the previous 5 months.[1] A widespread epidemic of this disease had also occurred 4 years before among group XXXIX where the incidence of positive tests was 78 per cent. In view, therefore, of the general uniformity of the results in the rather considerable number of normal individuals tested whose origin throughout the United States was widely diverse, it can be predicted with some assurance that when the sera of an adequate random sampling of adults are tested according to the technique employed in these studies, about three-fifths will be found to be positive. This proportion should not, however, be taken as representing only those who may have been infected either overtly or subclinically with the virus. Thus, as already noted, an appreciable number of sera which gave "tr" reactions and hence have been excluded in calculating the percentage of definitely negative and positive tests would probably have been shown to contain antibody had the amount of complement employed in the test been reduced. Moreover the results of skin tests (4) have afforded evidence of previous inapparent infection in certain persons whose complement fixation tests were negative.

It will be observed that the proportion of positive reactors corresponds closely to that reported for adults in general throughout the United States who have undergone known attacks of mumps (1). This agreement, however, is apparently fortuitous since, as has been demonstrated above, many persons who gave reliable statements of not having had mumps nevertheless exhibited titers of antibody.

It is also pertinent to note in connection with the results presented in Table VI that the majority of a small group of aged men had antibody in their sera (group XXXII, age range 72 to 88 years). Although its presence may have been due to repeated exposures, it is equally possible that the antibody may represent the result of the original infection, presumably contracted in early life. If so, an attack of mumps, like that of yellow fever (7), would leave immunologic evidence of its passage which endures for an amazingly long time.

From Table VI, furthermore, a conception of the reliability of the technique may be obtained. In about 9 per cent of all tests "tr" reactions were observed, although in certain

[1] For a detailed account of this epidemic, see McGuinness and Gall (6).

groups the proportion of such results was only 4 to 6 per cent. These very weak reactions present difficulty in interpretation as we have indicated above. One cannot dismiss them as negative in character and therefore modifications in the technique should be explored which might further limit their occurrence. It is possible that the use of allantoic or amniotic fluid of embryonated hen's eggs infected with mumps virus may serve to diminish their number. For recently it has been found that many sera yielding doubtful or even negative reactions with infected monkey gland give unequivocal fixation of complement in the presence of these antigens (8). In this connection it should also be noted that doubtful reactions were usually more frequent in lots of sera which had been shipped to Boston as compared with those collected locally and brought immediately to the laboratory. Accordingly prolonged transportation, especially of serum associated with clotted blood, should be avoided if possible.

The proportion of unsatisfactory tests, *i.e.* those in which the anticomplementary effect of the serum or non-specific fixation with normal parotid gland obscured the results, was about 5 per cent. This relatively low figure is attributable in no inconsiderable degree to the procedure of "double heating" which has been described (2, 5). Anticomplementary specimens were also more frequently encountered in those which had been sent by mail or express to the laboratory.

(*b*) *Children.*—Similar data which are summarized in Table VII have been accumulated for a total of 1154 children. As in the case of the adults, a fairly large proportion were mental defectives who had been maintained in institutions for the feeble-minded during most of their lives. The proportion of positive tests was significantly higher among the groups of mentally normal children (first 14 groups, Table VII). For these the mean incidence of positive sera was 57 per cent and for the mentally defective groups (last 8 groups, Table IV), 32 per cent. It would seem reasonable to assume that the disparity here is also due to differences in the chance of exposure to mumps.

Although somewhat lower, the proportion of positive tests in all the mentally normal children approached that of the corresponding group of adults (57 and 63 per cent respectively). If the presence of antibody is indeed an index of past infection, one might anticipate on epidemiologic grounds that this difference would be greater. Inspection of the results for the individual groups, however, will show that in two of them (groups XXXIX and XLVI) in which the numbers were large, the incidence of positive tests was exceptionally high. In both these instances it is known that about 4 years before extensive epidemics of mumps had occurred. If, then, these two groups be omitted, the mean incidence of positive tests in the remaining groups of mentally normal children is about 49 per cent. For the purpose of comparison, if the two intensely exposed groups (XXVI and XXIX) of mentally normal adults (Table VI) be eliminated, the mean incidence of positive tests of the remainder is 61 per cent, or 11 per cent greater than that of the children. Although the number of adults is about twice as great as that of children this difference is significant. It will also be observed that even among the mentally defective groups the proportion of positive reactors is somewhat higher in the adults.

The results, then, of the complement fixation tests in children as compared with adults in general conform to the fact that the previous attack rate in-

creases with age, and so they may be accepted as providing additional evidence in support of the interpretation of a positive test as an index of past infection. It is relevant here to point out that an even larger difference between the in-

TABLE VII

Results of Complement Fixation Tests in Children

Group	No. tested	Tests doubtful		Tests unsatisfactory		No. definite tests	Tests positive		Tests negative	
		No.	Per cent*	No.	Per cent*		No.	Per cent‡	No.	Per cent‡
I	24	1	4	0	0	23	10	44	13	57
XIII	25	3	12	1	4	21	8	38	13	62
XXI and XXII	30	0	0	1	3	29	15	52	14	48
XXIII	19	1	5	0	0	18	4	22	14	78
XXIV	35	3	9	0	0	32	18	56	14	44
XXV	32	5	16	1	3	26	11	42	15	58
XXXIX	90	10	11	7	8	73	50	69	23	32
XLI	22	3	14	2	9	17	10	59	7	41
XLII	17	0	0	0	0	17	12	71	5	30
XLIII	144	4	3	12	8	128	61	48	67	52
XLIV	53	1	2	1	2	51	30	59	21	41
XLV	34	2	6	0	0	32	9	28	23	72
XLVI	154	7	5	13	8	134	102	76	32	24
Combined.....	679	40	6	38	6	601	340	57	261	43
IV	101	12	12	2	2	87	14	16	73	84
V	23	11	48	1	4	11	3	27	8	73
VI	84	6	7	1	1	77	34	44	43	56
VII	48	7	15	0	0	41	13	32	28	68
XIV	25	6	24	2	8	17	7	42	10	58
XLVII	33	1	3	0	0	32	18	56	14	44
XLVIII	100	8	8	2	2	90	9	10	81	90
XLIX	61	2	3	11	18	48	29	60	19	40
Combined.....	475	53	11	19	4	403	127	32	276	69
All groups combined.....	1154	93	9	57	5	1004	467	47	535	54

* Percentage of all tests.

‡ Percentage of definite tests.

cidence of positive skin reactions in certain groups of children and adults has been found (4).

The results presented in Table VII also provided further data for estimating the reliability of the test. It will be seen that the relative numbers of doubtful and unsatisfactory tests are quite comparable to those obtained in the adult groups.

The Concentration of Complement-Fixing Antibody in Normal Persons[2].—(*a*) *Adults.*—Compared with the individual recently convalescent from mumps (3), one might expect that the titer of complement-fixing antibody should be low in the person who had experienced the disease several months or years before the test was carried out. To verify this hypothesis as well as to obtain information of practical value in interpreting the results of the complement fixation test as applied to the diagnosis of meningoencephalitis (9), the range of titers in 197 adults whose tests were positive and who were not known to have had mumps in the recent past were determined and are presented in Table VIII. Inspection of the table will show that about 90 per cent of these titers were 1–48 or less. Furthermore only 2.5 per cent of them were 1–192

TABLE VIII

Range of Titers of Complement-Fixing Antibody in Adults

Group	No. of individuals giving titer* of										Total
	1–6	1–12	1–24	1–36	1–48	1–72	1–96	1–144	1–192	>1–24 ‡	
XVII	17	15	9	4	7	4	3	0	0	0	59
XVIII	1	2	6	6	4	3	0	0	0	0	22
XIX	3	10	12	5	15	1	0	0	0	3	49
XXVIII	3	5	5	2	5	5	0	0	0	1	26
XXIX	0	2	0	0	0	2	0	0	0	0	4
XXX	2	5	4	2	9	0	0	0	1	0	23
XXXII	1	3	1	1	0	0	0	0	0	0	6
XXXV	3	1	1	1	1	1	0	0	0	0	8
Combined.....	30	43	38	21	41	16	3	0	1	4	197
Per cent.....	15	22	19	11	21	8	2	0	0.5	2	

* Titers given as final dilutions of serum before addition of sensitized cells.

‡ Endpoints not determined.

or possibly higher. Probably this small proportion of relatively high titers would have proved to be even smaller if the precise endpoints of 4 sera showing titers of 1–24 or higher which occurred in groups XIX and XXVIII had been found. However this may be, it is evident on the basis of these data that a very large majority of normal adults do not exhibit titers higher than 1–192. We have therefore regarded titers exceeding this level as presumptive evidence of a recent infection.

[2] In a previous communication (9) reference was made to the present paper in regard to the titer considered to be presumptive evidence of recent infection. At that time only 197 determinations of endpoints on the sera of both adults and children were available. Subsequently additional data of the same sort were obtained which are now included in Tables VIII and IX.

(*b*) *Children.*—Among 113 children giving positive tests and no history of recent infection, a similar distribution of titers, which is presented in Table IX, occurred. But it will be seen that there was a tendency for the titers of more of these individuals to be higher than among the older groups. Thus 8 per cent of the adults gave titers of 1–72 as contrasted with 16 per cent of the children. That more children should yield higher titers might be deduced from the fact that in them the disease must have been in most instances more recently experienced. But such comparatively recent infections did not apparently lead to a significantly greater number of titers exceeding 1–192. Accordingly, when found, this level may also be regarded as presumptive of a recent infection in this age group.

TABLE IX

Range of Titers of Complement-Fixing Antibody in Children

Group	No. of individuals giving titer* of									Total
	1–6	1–12	1–24	1–36	1–48	1–72	1–96	1–144	1–192	
XXI and XXII	0	3	0	3	5	6	0	0	0	17
XXIV	1	2	4	0	7	3	0	2	0	19
XLIII‡	12	12	9	3	1	1	3	4	2	47
XLIX	1	6	4	4	7	8	0	0	0	30
Combined.....	14	23	17	10	20	18	3	6	2	113
Per cent.....	13	20	15	9	18	16	3	5	2	

* Titers given as final dilucions of serum before addition of sensitized cells.

‡ The children in this group had been exposed to mumps a few days before the tests were done.

Complement-Fixing Antibody in the Blood of Mothers and Their Infants.—An attempt was made to determine whether transplacental transmission of antibody from mother to infant occurred. Although the sera of 15 mothers and their offspring have been tested, in only 5 such pairs of specimens were the tests satisfactory. This large number of failures was due to the fact that cord bloods were employed as a source of infant's serum, most of which proved to be strongly anticomplementary. Among these 5 pairs, however, the results of complement fixation tests on each closely agreed. In 3 of them in which antibody was demonstrated in the mothers' sera, the cord blood of their respective infants also contained the factor in approximately the same concentration. In 2 pairs both the sera of the mothers and their respective infants were negative. Although the number of tests is very small, these results indicate that in mumps the complement-fixing antibody is transferred across the placenta. This evidence is also given some support by the fact that in the sera of 5 infants and young children beween the ages of 4 months and 1 year

who have been tested no antibody was found. On the other hand, of 3 children 2 years of age, 2 gave positive tests. These observations suggest that the antibody which may occur in newborn children is lost during the months following their birth.

DISCUSSION

The conclusions listed below which we have drawn from the results presented in this paper are fairly obvious and so require little discussion. But it is perhaps pertinent to emphasize here those which we regard as more significant, as well as to suggest certain other deductions which seem to be implicit in the data.

The results taken as a whole show that the antibody which brings about fixation of complement whenever found in the sera of normal human beings has arisen in response to previous infection with the virus of mumps and not as a product of some unknown process of physiological maturation or through antigenic stimulation by a biologically unrelated but chemically similar agent. Of perhaps equal importance is the demonstration that the mumps antibody may frequently appear not only as a result of overt disease but also from inapparent or subclinical infection.

In view of these findings, it might, then, be predicted that individuals in whom this antibody is present would prove resistant to exposure since it is common knowledge that an attack of mumps in nearly all cases confers a solid and lasting immunity. Experiment has confirmed this expectation. It should, however, by no means be inferred from this reasoning that the complement-fixing antibody is itself the essential factor in the mechanism of immunity. On the contrary there are several indications which strongly suggest that the antibody may be distinct from that upon which resistance depends.[3] The complement-fixing antibody, as far as available knowledge goes, can therefore only be regarded as an index of immunity—immunity in which it may well play no rôle.

It has been known for some time that strains of certain viruses may occur which are antigenically distinct, for example influenza A and B or the three strains of foot-and-mouth disease. Accordingly we have been alert to note any phenomena that would suggest the existence of a similar diversity among strains of mumps virus. So far no indication of antigenic multiplicity has been encountered. In fact the study of several strains obtained from the Boston area indicated a complete homogeneity (2). The results of over 3,000 complement fixation tests reported in this paper on the sera of persons coming

[3] See references 2 and 10. Moreover, it has been recently observed in unpublished experiments employing embryonated eggs as indicators of infection that the concentration of the neutralizing factor in convalescent mumps sera does not necessarily parallel their concentration of complement-fixing antibody.

from various sections of the United States afford indirect evidence for the essential unity of mumps virus as an antigen. For were there a variety of distinct strains existing in this country, it would seem highly improbable that with the single strain of virus employed as antigen, the incidence of positive reactions observed in the various groups which have been studied would have shown such uniformity.

Regarded from a practical standpoint, the findings described in this paper indicate that the complement fixation test might be employed to distinguish from the potentially susceptible a large portion (75 to 80 per cent) of the resistant individuals in any group. But in most instances skin testing as described in the following paper (4) would be far more convenient and less expensive. Indeed, except in young children and a small proportion of adults, the skin test, as will be shown, appears to be a somewhat more sensitive indicator of the resistant individual. However, since the correlation between the results obtained by the two methods is not complete, the complement fixation test might be employed to provide as much information as possible in those cases for which the greatest accuracy is sought.

SUMMARY

Of 163 persons giving positive complement fixation tests who were exposed to mumps, 1 afterwards developed the disease; of 285 negative reactors similarly exposed, 56 afterwards came down with mumps.

Of 78 individuals subjected to intimate exposure to mumps whose tests were originally negative and who failed to develop the disease, 41 per cent gave positive reactions when tested 1 month later.

Seventy-seven per cent of complement fixation tests done on the sera of 565 normal adults who admitted a previous attack of mumps were positive. A similar correlation was recorded in tests on the sera of a small group of children with positive histories. Of 356 medical students admitting previous attacks, 80 per cent gave positive tests. Of 386 normal adults who denied previous attacks, 42 per cent gave positive tests; of 85 children giving negative histories, 38 per cent reacted positively.

The results of complement fixation tests on the sera of 1665 normal adults (over 17 years) and 679 children (1 to 17 years) are recorded. It has been shown that 63 per cent of the adults and 57 per cent of the children had antibody in their blood which reacted with the virus of mumps. In groups in which exceptionally intense exposure was not known to have occurred in the past, the proportions of positive reactors were: adults, 61 per cent; children, 49 per cent. In contrast to these normal persons, the incidence of positive reactors among permanently institutionalized mental defectives was 38 per cent of 356 adults and 32 per cent of 475 children.

In only 2 per cent of 320 normal adults and children did the titer of com-

plement-fixing antibody reach 1–192. In no instance in which the endpoint was determined was a higher titer recorded.

The results of complement fixation tests on the sera of mother and newborn infant were essentially the same in 5 instances.

CONCLUSIONS

1. With very rare exceptions, individuals giving positive complement fixation tests for mumps are resistant to infection by natural exposure.

2. Inapparent infections with the virus of mumps occur and, on the basis of the serologic evidence, may represent on the average about one-third of all infections.

3. The presence in the blood of complement-fixing antibody capable of reacting to antigen containing the virus of mumps is indicative of previous infection with this virus.

4. Titers of complement-fixing antibody exceeding 1–192 are very rarely found in the sera of normal individuals. Titers higher than this can, therefore, be regarded as presumptive evidence of recent infection.

5. Transplacental passage of mumps complement-fixing antibody probably occurs.

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