IMMUNITY IN MUMPS

VI. Experiments on the Vaccination of Human Beings with Formolized Mumps Virus*

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A previous communication (1) outlined the prophylactic effect in *M. mulatta* of formol-inactivated suspensions of mumps virus prepared from the infected parotid gland. It was shown that the injection of such material led, in the majority of animals, to the production of specific complement-fixing antibody. Moreover in about 60 per cent of the vaccinated animals evidence of increased resistance on challenge with active mumps virus was obtained.

In view of the results in monkeys, similar experiments were carried out in human beings employing the complement fixation test and the skin test, which have been recently described (2–4), as means of selecting individuals presumptively susceptible to mumps. The present report reviews these investigations.

Methods and Materials

Procedures Employed in Selecting Individuals and in Testing the Effect of Vaccination.—With consent of their parents or guardians, four groups of children were studied who were inmates of 3 institutions. Subsequently these groups will be referred to as 1, 2, 3, and 4. In addition a number of adults and children who were naturally exposed to mumps within their families were vaccinated and their subsequent behavior observed. The results so obtained will also be briefly summarized.

A negative complement fixation test (3) was the only criterion of susceptibility in the selection of groups 1 and 2. In choosing the individuals for group 3, not only were the results of this test used, but also as a further check on susceptibility, skin tests (4) were carried out after the challenging inoculation had been administered. Group 4 consisted of those who, at the beginning of the experiment, were found to be negative both by complement fixation and skin test.

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The procedures were varied because of the following considerations. It has been shown that certain persons may respond to the skin test by the formation of complement-fixing antibody (4). It is possible, therefore, that the skin test also might alone give rise to some increase in resistance. In the first 2 experiments it seemed desirable to avoid such a response by omitting this test, although it was realized that by so doing a few resistant individuals might be included, since not all persons previously infected with the virus indefinitely retain demonstrable antibody in their serum (2). In the third experiment skin tests were done at the end of the period of incubation when it seemed that an immunizing effect would not occur as a result of such tests. The skin test, however, was used at the outset in the 4th experiment because at this point it appeared to be of greater importance to obtain every assurance possible that only susceptible persons were originally included than it was to eliminate any adjuvant immunizing effect of the skin test.

In a few instances negative histories of mumps were also accepted as corroborative evidence of susceptibility. But on the whole a history of mumps in institutionalized children has often in our experience been unreliable, and so has usually been disregarded.

In groups 1, 2, and 4 approximately half the children were given suspensions of mumps virus inactivated by the addition of formalin (1). The others were reserved as controls. In group 3 about two-thirds of the children were vaccinated. On the 14th to the 17th day after the first dose of vaccine was injected, the resistance of all the children in each group was challenged by the inoculation of active monkey virus. A suspension of the virus was introduced directly into one Stensen's duct of each of the children in groups 1 and 2. The material was sprayed over the surface of the buccal mucosa of those in groups 3 and 4.

Preparation of Formolized Vaccine.—The method of preparing the vaccine has been previously described (1). A 10 per cent suspension of the infected gland in physiologic salt solution containing 0.3 per cent commercial formalin was used in groups 1 and 2; a 2.5 per cent suspension containing the same concentration of formalin was employed in groups 3 and 4. The vaccines were kept in rubber-capped vials at about 4°C. until injected. In the first experiment the period of storage before use was 47 days, in the second 11 days, in the third 60 days, and in the fourth 7 days.

Dosage and Technique of Vaccination and Challenge.—Formolized vaccine was administered in groups 1, 2, and 4 by the subcutaneous route in 2 doses each of 0.3 ml. of the 10 per cent or 0.5 ml. of the 2.5 per cent suspension at an interval of 5 days. In group 3, 3 doses of 2.5 per cent vaccine were given subcutaneously at intervals of 5 days.

To afford the optimal practical method for the control of mumps in the field, a vaccine should lead to the induction of immunity within the incubation period. Since this period is approximately 15 to 21 days, the time selected for challenging the vaccinated groups was 14 to 17 days from the date of the first injection of vaccine. It has been demonstrated previously in susceptible monkeys (1) and, it will be shown in this communication that in susceptible human beings also the mumps experimentally produced by injection of virus directly into Stensen's duct, is characterized by an incubation period of about 6 to 7 days. Thus in the cases of those vaccinated persons who were inoculated *via* the duct and subsequently developed the disease, the interval from the time of the first injection of vaccine to that of the appearance of fully developed mumps was about 19 to 22 days; *i.e.*, the usual incubation period of the natural disease. Accordingly these conditions of vaccination and of challenge inoculation, although they did not precisely reproduce those which might occur in epidemics, nevertheless seemed reasonably exacting. When the challenging virus was sprayed, the incubation period which varied from 17 to 22 days was comparable to the range exhibited by the natural disease. Here, then, the interval elapsing between the first injection of vaccine and the appearance of symptoms, when they occurred, was from 31 to 39 days. In the group of adults and children who were naturally exposed, the vaccine, of course, was administered after the exposure had occurred.

The amount of active virus used for intraparotid challenge was 0.4 ml. of a 5 per cent suspension in infusion broth or physiologic salt solution of infected monkey parotid. When the virus was sprayed, the inoculum consisted in group 3 of 0.75 ml. of a 4 per cent suspension of infected monkey gland, and in group 4 of 1 ml. of a 6 per cent suspension of gland. The titer of complement-fixing antigen of the inocula used in groups 1 and 2 was between 1–300 and 1–600; that of the inoculum employed in group 3 was 1–375, in group 4 it was 1–150. Materials of the 10th, 11th, 12th, 13th, and 14th monkey passages were employed. The infective capacity of the virus was demonstrated in susceptible monkeys both immediately before and shortly after its inoculation into human beings. Suspensions of active virus were kept frozen in solid CO_2 until just before they were used.

That the virus employed for challenge was similar in all essential respects to the agent responsible for the natural disease was shown by the behavior of a child exposed by chance to mumps which had developed in a boy who had previously received active monkey virus in the form of a spray. This accidental exposure to the experimental disease gave rise, after the average period of incubation, to a typical attack of mumps.

Clinical Criteria Employed in the Diagnosis of Experimental Mumps.—Increase in temperature, enlargement of the parotid glands, enlargement of the other salivary glands, redness about Stensen's duct, and symptoms of discomfort, anorexia, headache, and nausea, when they occurred, were recorded daily by a group of nurses in constant attendance and who took care of no other patients. The children were also visited daily by one or two of the authors who confirmed and likewise recorded the significant findings.

Although all these signs and symptoms were noted, many were so irregular in occurrence and in intensity that in the analysis of the data we have depended mainly upon the enlargement of the parotid glands as a criterion of clinically apparent disease. In this communication, therefore, the details only of parotid swelling will be presented and merely general reference, when relevant, made to other possible manifestations of infection. The degree of parotid swelling observed each day has been recorded by a simple system of arbitrary units:

1 = unmistakable but minimal enlargement
2 = moderate enlargement
3 = marked enlargement
4, 5, 6, = increasing degrees of marked enlargement

Complement Fixation Tests.—Before injection of the vaccine and repeatedly thereafter the serum of each child was tested for the presence of complement-fixing antibody. The technique employed in such tests has been described (1).

Skin Tests.—The preparation of the materials used for this test and the manner in which they are employed have been described in previous communications (2, 4).

The Effect of Vaccination as Revealed by Clinical Observation

Groups 1 and 2.—These two experiments in which active virus was introduced *via* the parotid duct were carried out essentially in the same manner. The results, therefore, can be regarded as fairly comparable. Group 1 consisted of 6 vaccinated and 6 control children; group 2 of 9 vaccinated[1] and 8 controls.

[1] One of the vaccinated subgroup in group 2, J. H., was not challenged by intraparotid inoculation but received active virus in the form of a spray. He is therefore not mentioned in Table II but his immunologic responses are recorded in Table VI. It is of interest that among a number of children who were inadvertently exposed to J. H., 6 cases of mumps occurred. The first of these came down 17 days after the onset of J. H.'s case and the others at various intervals thereafter.

In group 2 were also included 3 unvaccinated children who gave serologic evidence of previous attacks of mumps as indicated by the presence of complement-fixing antibody in their sera at the beginning of the experiment. Presumably, therefore, these individuals would be resistant to inoculation because of a previously acquired natural immunity (2, 3).

TABLE I

Group 1. Swelling of Inoculated Parotid Gland Following Introduction of Active Virus via Stensen's Duct

Subgroup	Name	Accumulated units of swelling				Duration of swelling after 4th day	Estimate of resistance§
		First 4 days		After 4th day			
		Total*	Moderate or marked‡	Total	Moderate or marked		
						days	
1 Vaccinated	E. N.	2	0	4	2	3	R
	C. Q.	6	5	8	7	4	R
	A. R.	3	2	7	4	4	R
	S. R.	6	6	3	2	2	R
	A. S.	3	2	8	4	6	R
	J. S.	0	0	17	14	7	S
Mean		3.3	2.5	8	6	4.3	
2 Controls	W. B.	0	0	18	14	10	S
	P. D.	0	0	18	15	9	S
	L. K.	0	0	13	11	6	S
	P. M.	2	2	14	12	8	S
	N. P.	0	0	17	13	10	S
	F. R.	3	2	16	13	9	S
Mean		0.8	0.7	16	13	8.5	

* Figures indicate accumulated daily units of all swelling recorded as "1+" or greater according to the notation described on p. 409.

‡ Figures indicate accumulated daily swelling of moderate or marked degree (see p. 409).

§ R indicates that the individual presented evidence of increased resistance according to the criteria mentioned in the text; S indicates no evidence of increased resistance.

Difference in Behavior of the Inoculated Parotid Gland Exhibited by Naturally Resistant and Susceptible Vaccinated and Unvaccinated Individuals.—A variable degree of swelling of the inoculated gland appeared after the challenge in all who received the virus by this route. But the interval between the challenge and the time at which the maximal enlargement of the gland was noted varied considerably in the vaccinated and control groups. In Tables I and II the accumulated degree of all swelling as well as of moderate or marked enlargement of the inoculated gland recorded for each individual in groups 1 and 2 before

and after the 4th day is presented. It is clear that many of the vaccinated children (subgroups 1) exhibited considerable enlargement before the 4th day

TABLE II

Group 2. Swelling of Inoculated Parotid Gland Following Introduction of Active Virus via Stensen's Duct

Subgroup	Name	Accumulated units of swelling				Duration of swelling after 4th day	Estimate of resistance§
		First 4 days		After 4th day			
		Total*	Moderate or marked‡	Total	Moderate or marked		
						days	
1	C. B.	0	0	2	0	2	R
Vaccinated	R. B.	4	2	8	8	5	S
	J. C.	8	6	1	0	1	R
	G. H.	2	2	9	5	6	S
	C. H.	11	11	3	2	3	R
	L. K.	2	2	3	0	4	R
	E. O.	9	9	7	4	6	R
	F. O.	12	12	0	0	0	R
Mean.....		6.0	5.5	4.1	2.3	3.3	
2	M. G.	5	2	11	10	4	S
Controls	W. L.	11	9	4	0	4	R
	L. M.	0	0	10	9	4	S
	M. N.	2	2	5	4	3	R
	C. N.	3	0	20	18	9	S
	G. P.	1	0	16	15	7	S
	R. V.	10	9	11	11	3	R
Mean.....		4.5	3.1	11	9.5	4.8	
3‖	G. diC.	3	0	1	0	1	R
Positive complement fixation	W. H.	3	2	0	0	0	R
	G. L.	8	7	9	5	6	R
Mean.....		4.6	3.0	3.3	1.6	2.3	

* See footnote *, Table I.

‡ See footnote ‡, Table I.

§ See footnote §, Table I.

‖ The individuals in this group were not vaccinated but had positive complement fixation tests at the beginning of the experiment.

which tended to disappear relatively soon thereafter. In contrast, the majority of those in the unvaccinated groups (subgroups 2) showed little or no swelling before the 4th day. That which appeared subsequently in most of the controls,

as compared with the majority of vaccinated children, was more marked and of longer duration. These differences are reflected in the differences between the means of the total swelling before and after the 4th day in the vaccinated and control groups. It can be shown by a "t-test" that these means differ significanlty in the first experiment ($P = <0.01$) and are on the borderline of significance in the second experiment ($P = 0.05$).[2]

There is experimental evidence which indicates that this accelerated response to intraparotid inoculation of the virus is characteristic of the previously infected and consequently resistant organism. As Johnson and Goodpasture (5) observed, and as we have repeatedly noted (1), the monkey convalescent from mumps, when reinoculated, frequently responds by an enlargement of the gland which occurs within 24 to 48 hours and thereafter gradually subsides. In the normal monkey, on the other hand, a definite increase in the size of the gland is not usually discernible before the 6th postinoculative day. That the same phenomenon may occur in man is demonstrated by the results obtained in the 3 unvaccinated children with initially positive complement fixation tests who are mentioned in subgroup 3 of Table II. It will be seen that these individuals all responded to the challenge with active virus by accelerated enlargement of the gland which in only one persisted longer than the 3rd day after inoculation. These facts, then, would appear to warrant the conclusion that the significantly higher incidence of accelerated reactions in the vaccinated groups as contrasted with the controls indicated in the former an enhanced resistance.

Although it is possible, as we have done, to compare numerically each group with the other, it is impossible, because of the small numbers involved, to obtain an exact quantitative value indicative of resistance or susceptibility in the case of any one child. Nevertheless it would seem fair to assume that a child in whom a very large proportion of the total swelling occurred after the 4th day was susceptible and conversely that a child who exhibited a relatively small proportion of swelling after this time was resistant or partially resistant. On the basis of these criteria, we have indicated in Tables I and II those individuals whom we consider to have been susceptible or resistant to the challenge. These estimates may be summarized as follows:—

	Vaccinated		Controls	
	Resistant	Susceptible	Resistant	Susceptible
Group I	5	1	0	6
Group II	6	2	3	4
Total	11	3	3	10

[2] The differences were taken as the total degree of swelling occurring before the 4th day minus the total degree of swelling occurring after the 4th day.

Apparently about four-fifths of the vaccinated may be considered to have exhibited evidence of resistance as compared with about one-fourth of the controls. If so, it may be concluded that in about one-half the children resistance was increased as a result of vaccination.

Extension of Infection to the Other Salivary Glands.—Only one of all those included in both of these experiments developed swelling of the uninoculated parotid gland. On the other hand, some enlargement of the submaxillary and sublingual glands was recorded in many instances. The degree of such enlargement on the whole was considerably greater in the controls. Although, as already stated, we are not inclined to stress the significance of such observations, in these cases the difference in swelling of these other salivary glands probably affords further evidence for the protective effectiveness of vaccination.

Groups 3 and 4.—To avoid the development of an accelerated reaction which, when it tends to persist, may render it difficult to determine whether true parotitis of the inoculated gland has subsequently ensued and also to secure more information concerning the value of the vaccine, two additional experiments were carried out in which the children were challenged by copiously spraying the oral cavity with active virus. Following this procedure no definite accelerated reactions were observed. The disease which ensued after incubation periods ranging from 17 to 22 days closely resembled that encountered in nature. In some cases both parotids were enlarged, in others the parotitis was unilateral. The accumulated units of swelling of both glands recorded for each subject are presented in Tables III and IV.

With the exception of the change in the route of challenge inoculation, the design of the experiments in general was the same as that formerly followed. In group 3, however, the children who were to receive the vaccine were divided into 2 subgroups, one of which received 2 doses and the other 3 doses. This was done to determine whether an increase in the amount of vaccine, as well as repetition of antigenic stimulation, would prove more effective.

To supplement the information obtained by means of complement fixation tests concerning the susceptibility of these children, it will be recalled that skin tests were also carried out at the time symptoms of mumps appeared in group 3 and at the beginning of the experiment in group 4. In attempting to evaluate the data recorded in Table III, it should be borne in mind that positive skin tests were obtained in 4 of the children in group 3 (R. S. and C. C., subgroup 1; C. Bu., subgroup 2; T. McG., subgroup 3, see Table VII). We have, therefore, excluded them from the discussion of the prophylactic effect of the vaccine which follows. In group 4, besides the vaccinated and control subgroups, several other children were included (subgroup 3, Tables IV and VIII). They consisted of 4 unvaccinated individuals who originally showed doubtful or negative skin and complement fixation tests but who later developed appreciable antibody titers, presumably as a result of the skin tests (4), and a fifth child who had a persistently negative complement fixation test but exhibited a dermal reaction at 24 hours which, however, faded at 48 hours (Table VIII). These children were offered the challenge in an attempt to learn something in regard to the significance of these ambiguous immunologic responses in relation to resistance.

Incidence of Parotitis in Vaccinated and Control Groups.—We may first analyze the results obtained in group 3 (Table III). It is clear that neither

TABLE III

Group 3. Swelling of Both Parotid Glands Following Introduction of Active Virus as an Oral Spray

Subgroup	Name	Accumulated units of swelling		Duration of swelling	Estimate of resistance§
		Total*	Moderate or marked‡		
				days	
1	S. B.	0	0	0	R
Vaccinated twice	A. B.	2	0	2	R
	R. B.	3	0	2	R
	C. C.	0	0	0	R
	J. E.	80	75	12	S
	R. H.	0	0	0	R
	E. M.	10	9	4	S
	F. M.	2	0	2	R
	F. O.	0	0	0	R
	R. S.	0	0	0	R
Mean		9.7	8.4	2.2	
2	C. Bl.	4	2	2	S
Vaccinated three	C. Bu.	0	0	0	R
times	R. C.	34	33	7	S
	C. H.	0	0	0	R
	E. R.	0	0	0	R
	R. S.	2	0	1	R
	M. S.	9	6	4	S
	J. We.	0	0	0	R
	C. Wh.	2	0	2	R
	J. Wi.	22	16	7	S
Mean		7.3	5.7	2.3	
3	R. B.	5	2	2	S
Controls	R. F.	0	0	0	R
	T. F.	15	13	4	S
	L. J.	6	0	4	S
	C. K.	17	14	5	S
	T. McG.	0	0	0	R
	W. P.	6	4	4	S
	J. S.	11	6	5	S
	M. W.	20	15	5	S
	E. W.	12	9	4	S
Mean		9.2	6.3	3.3	

* Figures indicate accumulated daily units of swelling of both parotid glands.

‡ Figures indicate accumulated daily units of swelling of both parotid glands recorded as moderate or marked (see p. 409).

§ See footnote §, Table I.

TABLE IV

Group 4. Swelling of Both Parotid Glands Following Introduction of Active Virus as an Oral Spray

Subgroup	Name	Accumulated units of swelling		Duration of swelling	Estimate of resistance§
		Total*	Moderate or marked‡		
				days	
1	J. B.	2	0	1	R
Vaccinated	W. B.	0	0	0	R
	J. C.	0	0	0	R
	J. F.	1	0	1	R
	A. K.	15	14	6	S
	E. M.	4?	4?	1	R
	J. N.	0	0	0	R
	J. R.	0	0	0	R
	G. T.	0	0	0	R
	A. Y.	0	0	0	R
Mean		2.2	1.8	0.9	
2	R. Gre.	22	22	6	S
Controls	R. Gri.	15	15	5	S
	E. J.	0	0	0	R
	T. J.	8	0	6	S
	R. K.	35	33	6	S
	E. L.	0	0	0	R
	J. P.	0	0	0	R
	W. P.	0	0	0	R
	L. P.	12	10	5	S
	M. W.	13	12	4	S
Mean		10.5	9.2	3.2	
3‖	A. G.	0	0	0	R
Original immunologic	M. M.	0	0	0	R
status doubtful.	T. P.	5	0	4	S
No vaccine	H. S.	0	0	0	R
	J. T.	0	0	0	R
Mean		1.0	0	0.8	

* See footnote *, Table III.
‡ See footnote ‡, Table III.
§ See footnote §, Table I.
‖ See text and Table VIII for details concerning these individuals.

the means of total accumulated swelling nor the average duration of the parotitis were significantly different in the vaccinated and control subgroups. This is evidently due to the fact that a few vaccinated children developed

marked involvement of the gland. Nevertheless, an examination of the individual results strongly suggests that the vaccine acted prophylactically in certain instances. Thus, excluding the children in each subgroup who gave positive skin tests, it is apparent that all the controls exhibited a moderate or marked parotitis. In contrast, in a considerable number of the vaccinated children the swelling was minimal or absent.

To obtain a basis for comparison, we have assumed that any vaccinated individual showed evidence of increased resistance whose total accumulated parotid swelling was approximately one-half (or less) that of the control child (R. B) who revealed in his subgroup the least degree of enlargement among those who presented any signs of parotitis. According to this criterion, in Table III we have indicated the immunologic status of each child. Omitting the individuals definitely positive by the first skin test, these estimates for group 3 may be summarized as follows:—

Vaccinated		Controls	
Resistant	Susceptible	Resistant	Susceptible
11	6	1	8

The difference between the vaccinated and controls is still apparent if one considers only those children who did not react in any way to the first skin test:—

Vaccinated		Controls	
Resistant	Susceptible	Resistant	Susceptible
7	3	0	5

Further evidence in support of this criterion of resistance may be derived from a comparison of Tables III and VII which shows that none of the 7 vaccinated children considered to be resistant on the basis of total accumulated swelling exhibited sufficient enlargement of the glands to be recorded as moderate or marked (2+ or greater). Such responses, on the other hand, were noted in all the skin-test-negative controls. As in groups 1 and 2, then, there is indication here that the vaccine enhanced the resistance in at least one-half of those to whom it was given and who were considered to be susceptible on the basis of skin and complement fixation tests.

The data, however, show that the prophylactic effect of the vaccine was not increased when 3 instead of 2 doses were administered. Indeed less protection seems to have been induced by the greater quantity of antigen. If not due to chance variation, this somewhat paradoxical result may possibly be attributable to the fact that the interval between the last dose of vaccine and the challenge

inoculation was only 5 days in the case of the subgroup which received 3 doses as compared with 10 days in the subgroup which received 2 doses. A period so short may not have been sufficient to permit the occurrence of maximal antigenic stimulation, especially if any resistance induced by the first 2 doses of vaccine were temporarily depressed by the third injection of inactivated virus.

The fourth experiment yielded similar results. From Table IV, it is apparent that the incidence of parotid enlargement of any significant degree among the vaccinated is less than in the control group. Indeed only one of the 10 vaccinated children developed swelling comparable to that shown by 6 of the controls. As before we may tabulate the results as follows:—

Vaccinated		Controls	
Resistant	Susceptible	Resistant	Susceptible
9	1	4	6

The difference in the means of the total accumulated swelling, which is here statistically significant, reflects the behavior of the individuals. It is accordingly again possible to attribute a prophylactic effect to the vaccine in about one-half of those who received it.

Compared with those in group 3, a relatively large proportion of the controls in this experiment in spite of preliminary negative skin and complement fixation tests proved resistant on challenge. It is possible that in group 4, the inoculation of the skin test material, which itself has been shown to have some antigenic capacity (4), may have increased resistance by affording an additional stimulus which was not received by the children of group 3.

Only 1 of the 5 unvaccinated children (subgroup 3, Table IV) who developed antibody as a result of skin-testing or presented doubtful skin reactions before challenge showed unequivocal signs of mumps. The results obtained in this group will be subsequently discussed when the immunologic data obtained in the 4 experiments are reviewed.

Swelling of the Submaxillary and Sublingual Glands.—The majority of children both among vaccinated and controls in groups 3 and 4 presented varying degrees of enlargement of the submaxillary and sublingual glands. But in many instances some increase in the size of these organs or in the tissues adjacent to them was recorded within a week after the challenge had been given; *i.e.*, a week or 10 days before parotid involvement, when it occurred, was discerned. Accordingly considerable doubt existed in the minds of those who carried out the examinations as to whether in any given case such signs were to be attributed to infection with the virus of mumps or to other causes such as perhaps a response to the introduction of an irritative or allergenic factor existing in the monkey gland emulsion. That these reactions, in certain instances at least, may well have been of a non-specific character is supported by the fact that the children in group 3 whose skin tests were found to be positive and who failed to develop parotitis nevertheless did present enlargement of either the submaxillary or sublingual glands. Since, as we have seen (2, 4), it is probable that the majority of these children had been rendered immune by previous inapparent disease, in them at least it would seem reasonable to interpret the swelling of these glands as not indicative of primary viral infection. The cause, therefore, of such swelling in any individual among the vaccinated and control children must remain in doubt in both of these experiments.

Results of Vaccination Following Natural Exposure to Mumps

As occasion offered, both adults and children who were exposed to cases of mumps which had occurred in nearly all instances within their own families were given 2 injections of 2.5 per cent formolized vaccine at an interval of 5 days. In all these cases the history of mumps was negative or doubtful. Only those whose skin tests were considered to be negative or questionably negative, *i.e.*, erythema less than 11 × 11 mm. (average diameter) were selected for vaccination. Whenever possible other members of the family who were similarly exposed and whose history and skin tests were negative were not vaccinated, and so served as controls.

Of a total of 98 individuals, 50 gave negative or questionably negative skin tests. Twenty-seven of these negative reactors were vaccinated. Among them 10 cases of mumps subsequently appeared. Five of the 23 negative reactors who did not receive the vaccine developed mumps. Three other unvaccinated individuals who reacted to the skin test (13 × 13 mm. in 2 cases, 23 × 23 in 1 case) also came down with the disease.

Although it was recognized that the intensity of exposure varied considerably, and although the number of those who became ill was small, these results appear to be unequivocal in showing that under the conditions described the vaccine when administered *after* infection does not prevent the development of parotitis. This result is not surprising in view of many unsuccessful attempts to establish active immunity in viral diseases after infection has occurred.

It cannot be, however, positively asserted that the vaccination was entirely without effect. Inspection of the clinical data shows that 3 cases among the unvaccinated were characterized by extensive involvement of the salivary glands with marked enlargement. In none of the vaccinated persons were these signs more than moderately developed. Moreover the disease in one of the unvaccinated cases was complicated by orchitis and in another by meningoencephalitis. These observations, then, suggest without being in any sense conclusive, that vaccination after exposure may tend to reduce the severity of the simple disease and prevent or diminish its complications.

The Effects of Vaccination and Experimental Infection as Revealed by Immunologic Procedures

An examination of the immunologic data summarized in Tables V, VI, VII, and VIII reveals several facts of interest.

Negative Complement Fixation and Skin Tests as Indices of Susceptibility.— In the first place, they show that the sera of all the experimental subjects selected for vaccination contained no detectable antibody. Its absence in most instances was confirmed by repeated tests on specimens obtained just before the first or second dose of vaccine was administered. Negative tests were also characteristic of the children selected as controls. These facts, as

previously pointed out, afford reasonable assurance that presumably about two-thirds at least of the individuals selected were susceptible (3). In the third

TABLE V

Group 1. Results of Immunologic Tests

Subgroup	Name	Serial complement fixation tests									Skin test
		Original test 1943	Prior to 1st dose of vaccine 1943*	Prior to 2nd dose of vaccine 1943*	Prior to challenge 1943*	Following challenge					1944
						1943				1944	
		1/26	4/2	4/7	4/19	4/26	5/3	5/10	5/17	5/22	7/26
1‡	E. N.	0§	0	0	24	6	192	1536	1536	24	Sens.‖
Received 2	C. Q.	0	0	0	0?	0	192	384	>384	24	?¶
doses of	A. R.	0	0	0	0	0	384	768	1536	6?	? sens.
vaccine	S. R.	0	0	0	384	768	192	1536	1536	>24	? sens.
	A. S.	0	0	0	0	0	192	384	1536	24	+
	J. S.	0	0	0	6(wk)	6?	192	192	>384	192	nd
2‡	W. B.	0	0	nd**	0	0	0	96	1536	16	+
Received no	P. D.	0	0	nd	0	0	192	384	1536	48	0
vaccine	L. K.	0	0	nd	0	0	24	96	384	24	+
	P. M.	0	0	nd	0	0	96	96	768	16	nd
	N. P.	0	0	nd	0	0	0?	384	1536	nd	nd
	F. R.	0	0	nd	0	0	24	192	1536	24	+

* Blood samples for complement fixation tests were taken a few minutes before vaccine or challenge materials were given.

‡ Subgroup 1 received subcutaneously on April 2 and April 7, 1943, respectively 0.5 ml. of a 10 per cent suspension of formolized infected parotid gland of the 9th monkey passage. On April 19, 1943, all the individuals in subgroups 1 and 2 were inoculated unilaterally in Stensen's duct with 0.4 ml. of active virus of the 11th monkey passage diluted 1–15 in infusion broth.

§ Titer of complement fixation test is recorded as the reciprocal of the highest final dilution of serum giving fixation denoted by "1+." In certain instances where this endpoint was not actually observed, it was calculated as the geometrical mean of the dilutions which showed fixation greater than "1+" and less than "1+" respectively.

‖ Sens. indicated that the individual reacted to the control material containing normal monkey parotid.

¶ ? indicates that either an erythematous reaction less than 10 × 10 mm. was observed at 48 hours or that a reaction occurred at 24 hours but disappeared by 48 hours.

** nd, not done.

series of experiments the results of skin testing were used after challenge to increase the chances of including only susceptible children. On the basis of the results so obtained, 4 individuals in these experiments who had been

originally chosen on the basis of negative complement fixation tests were revealed as presumptively resistant and so have been excluded from considera-

TABLE VI

Group 2. Results of Immunologic Tests

Subgroup	Name	Serial complement fixation tests							Skin test
		Original test 1943*	Prior to challenge 1943*	Following challenge					1944
				1943				1944	
		5/18	6/15	6/21	6/28	7/5	7/13	7/25	7/25
1‡	C. B.	0§	0	0	192	384	1536	96	+
Received 2	R. B.	0	0	0	96	384	1536	48	?‖
doses of vaccine	J. C.	0	96	384	384	384	1536	24	+
	J. H.	0	0	6?	6?	96	768	48	+
	G. H.	0	0	0	96	192	768	48	+ sl. sens.¶
	C. H.	0	1536	>1536	1536	1536	1536	48	0
	L. K.	0	3072	>1536	1536	1536	1536	96	+
	E. O.	0	48	96	384	1536	1536	48	+
	F. O.	0	1536	>1536	1536	768	1536	192	Sens.
2‡	M. G.	0	nd**	0	96	192	1536	48	+
Controls received no vaccine	W. L.	0	nd	0	384	192	192	24	+
	L. M.	0	nd	0	384	192	1536	48	+
	M. N.	0	nd	0	96	192	384	48	?
	C. N.	0	nd	0	96?	192	384	48	?
	G. P.	0	nd	0	6?	192	384	12	+
	R. V.	0	nd	0	384	384	768	96	+
3‡	G. DiC.	>24	nd	24	384	384	1536	192	+
Immune by complement fixation test	W. H.	>24	nd	96	48	192	192	48	+
	G. L.	>24	nd	96	384	384	ac‡‡	24	0

* Blood samples for complement fixation tests were taken before vaccination was begun and a few minutes before challenge material was given.

‡ Subgroup 1 received subcutaneously on June 1 and June 6, 1943, 0.3 ml. of a pooled 10 per cent formolized suspension of the infected parotid glands of the 10th and 12th monkey passages. On June 15, 1943, all the individuals (with the exception of J. H. who was sprayed) in subgroups 1, 2, and 3 were inoculated unilaterally into Stensen's duct with 0.4 ml. of active mumps virus of the 10th monkey passage diluted 1–20 in infusion broth.

§ See footnote §, Table V.

‖ See footnote ¶, Table V.

¶ See footnote ‖, Table V.

** nd, not done.

‡‡ ac, serum was anticomplementary.

tion in the evaluation of the clinical results. Skin tests were performed before the fourth experiment on a large group and only those showing negative tests were selected for vaccination (Table VIII). There is an additional reason,

TABLE VII

Group 3. Results of Immunologic Studies

Subgroup	Name	Serial complement fixation tests												Serial skin tests*				
		Original test	Prior to 1st dose of vaccine§	Prior to 2nd dose of vaccine§	5 days prior to challenge§	Immediately prior to challenge§	Following challenge											
		7/27‡	9/14	9/19	9/24	9/29	10/6	10/13	10/20	10/27	11/3	11/10		1st	2nd	3d	4th	5th
1‖ Received 2 doses of vaccine	S. B.	0¶	0	0	24	48	24	12	24	48	24	12		0	Sens.	nd	nd	nd
	A. B.	0	0	0	0	0	0	0	96	192	96	96		0	0	+	nd	nd
	R. B.	0	0	0	48	48	48	24	24	12	24	24		0	0	0	+	nd
	C. C.	0	0	0	12	12	24	6	6	12	96?	12		+	nd	nd	nd	nd
	J. E.	0	0	0	0	0	0	0	192	384	1536	768		0	0	?	nd	nd
	R. H.	nd**	0	0	48	24	48	24	48	48	48	24		?‡‡	+	nd	nd	nd
	E. M.	0	0	0	0	0	0	0	96	96	192	192		?	?	?	nd	nd
	F. M.	0	0	0	48	24	48	24	24	24	24	24		?	+	nd	nd	nd
	F. O.	0	0	0	96	48	24	24	24	48	48	48		0	+	nd	nd	nd
	R. S.	0	0	0	>96	192	192	96	96	48	48	48		+	nd	nd	nd	nd
2‖ Received 3 doses of vaccine	C. Bl.	0	0	0	0	0	0	6	384	384	384	384		?	0	0	?	nd
	C. Bu.	0	0	0	96	48	96	24	24	12	12	6		+	nd	nd	nd	nd
	R. C.	0	0	0	0	0	0	6	192	384	768	192		0	?	nd	nd	nd
	C. H.	0	0	0	48	48	48	24	12	48	48	24		?	Sens.	nd	nd	nd
	E. R.	0	0	0	0	0	0	0	0?	6	6	6		Sens.	nd	nd	nd	nd
	R. S.	0	0	0	24	24	12	0	0	6	0	0		0	0	Sens.	nd	nd
	M. S.	0	0	0	0	0	12	24	192	768	768	768		Sens.	Sens.	nd	nd	nd
	J. We.	0	0	0	0	0	0	0	0	12	12	12		0	0	0	?	?
	C. Wh.	0	0	0	0	0	0	0?	96	48	48	48		0	0	0	0	?
	J. Wi.	0	0	0	0	0	0	0	384	192	384	192		0	0	?	nd	nd
3‖ Controls received no vaccine	R. B.	0	nd	nd	0	nd	0	0	48	96	96	96		0	?	0	0	0
	R. F.	0	nd	nd	0	nd	0	0	6	0?	24	24		?	?	nd	nd	nd
	T. F.	0	nd	nd	0	nd	0	0	192	48	24	48		0	?	nd	nd	nd
	L. J.	0	nd	nd	0	nd	0	0	24	48	96	48		?	0	+	nd	nd
	C. K.	0	nd	nd	0	nd	0	0	6	6	48	48		?	?	nd	nd	nd
	T. McG.	0	nd	nd	0	nd	0	0	0	96	192	96		+	nd	nd	nd	nd
	W. P.	0	nd	nd	0	nd	0	0	192	48	96	96		0	?	?	?	nd
	J. S.	0	nd	nd	0	nd	0	0	0	0?	96	96		?	?	+	nd	nd
	M. W.	0	nd	nd	0	nd	0	0	192	96	192	192		0	?	nd	nd	nd
	E. W.	0	nd	nd	0	nd	0	6	192	192	192	192		0	?	?	nd	Sens

* First tests performed 8 to 16 days following the challenge. Subsequent tests done at weekly intervals thereafter until reactions appeared or sensitivity (denoted by "sens.") developed. The fifth series of tests were done on November 15th.

‡ All dates recorded in the table refer to the year 1944.

§ See footnote *, Table V.

‖ Subgroup 1 received subcutaneously on September 14 and September 19, 1944, 0.5 ml. of a pooled 2.5 per cent formolized suspension of the infected parotid glands of the 12th and 14th monkey passages. Subgroup 2 received 3 doses of the same material by the same route on September 14th, September 19th, and September 24th. On September 29th the buccal mucosae of each individual in subgroups 1, 2, and 3 were sprayed with 1 ml. of pooled active virus of the 12th and 13th monkey passages diluted 1-25 in a medium consisting of 1 part infusion broth and 3 parts of physiologic salt solution.

¶ See footnote §, Table V.

** nd, not done.

‡‡ See footnote ¶, Table V.

TABLE VIII

Group 4. Results of Immunologic Studies

Subgroup	Name	Serial complement fixation tests												Serial skin tests			
		Original test		After original skin test		Prior to 2nd dose of vaccine*	Prior to challenge*	Following challenge						Original test		Following challenge	
								1945						1944		1945	
		Date 1944	Results	Date 1944–45	Results	12/27/44	1/5/45	1/12	1/19	1/26	2/2	2/9	2/16	Date	Results	2/13	3/8
1‡ Received 2 doses of vaccine	J. B.	7/27	0§	12/13	0	0	0	6	6?	6	48	96	>192	11/8	0	0	+
	W. B.	7/27	0	12/13	0	0	0	0	0	768	192	192	192	11/8	0	0	?
	J. C.	7/27	0	12/13	0	0	0	0	0	24	48	192	96	11/8	0	0	0
	J. F.	7/27	0	12/13	0	0	0	0	0	48	384	384	192	11/8	0	0	0
	A. K.	7/27	0	12/13	0	0	0?	6	6	96	192ac‖	ac	ac	11/15	0	0	+
	E. M.	7/27	0	12/13	0	0	0	0	0	48	192	384	384	11/15	0	0	+
	J. N.	11/14	0	12/13	0	0	0	0	0	12	24	<24NP	>24	11/15	0	0	0
	J. R.	7/27	0	12/13	0	0	0	0	0	0	48	192	192	11/15	0	?	+
	G. T.	7/27	0	12/13	0	0	0	0	0	48	192	192	192	11/15	0	0	?
	A. Y.	7/27	0	12/13	0	0	0	0	0	96	192	192	384	11/15	0	?	?
2‡ Controls received no vaccine	R. Gre.	7/27	0	12/13	0	nd¶	0	0	0	48	192	384	384	11/15	0	0	0
	R. Gri.	12/22	0	1/2	0	nd	0	0	0	48	192	96	96	12/22	0	0	+
	E. J.	12/22	0	12/22	0	nd	0?	0	0	384	192	<96	48	12/22	0	0	0
	T. J.	12/22	NP **	1/2	NP	nd	0?	ac	0	0?	6?NP	6	ac	12/22	0	0	0
	R. K.	7/27	0	12/22	0	nd	0	0	0	0	0	24	96	12/19	0	+	nd
	E. L.	7/27	0	12/13	0	nd	0?	0	6?	12	96	96	96	11/15	0	?	0
	J. P.	12/22	0	1/2	0	nd	0	6	0	0?	0?	12	24	12/22	0	?	0
	W. P.	7/27	0	12/13	0	nd	0	0	0	48	192	96	192	11/15	0	0	0
	L. P.	12/22	0?	1/2	0	nd	0?	0	0	192	192	768	384	12/22	0	+	nd
	M. W.	7/27	0	12/13	0	nd	0	0	0	96	192	192	96	11/15	0	0	Sens. ‡‡
3‡ Possibly immune by complement fixation or skin test. No vaccine	A. G.	12/22	0	12/13	>24	nd	6?	12	0	0?	12	6	12	11/15	?§§	0	0
	M. M.	12/22	0	12/13	>24	nd	6	12	6?	12	12	12	12	11/15	0	0	0
	T. P.	12/22	0	1/2	6	nd	0	0	0	96	192	96	96	12/22	0	0	?
	H. S.	12/22	0	12/13	>24	nd	12	12	12	12	12	6	6	11/15	?	0	0
	J. T.	11/14	0	12/13	0	nd	0	0	0	0	0	<6NP	48	11/15	?	0	0

* See footnote *, Table V.

‡ Subgroup 1 received subcutaneously on December 22nd and December 27th respectively 0.5 ml. of a 2.5 per cent formolized infected parotid gland of the 15th monkey passage. On January 5, 1945, the buccal mucosae of all the individuals in this subgroup as well as those in subgroups 2 and 3 were sprayed with 1 ml. of active virus of the 14th monkey passage diluted 1–15 in physiologic salt solution.

§ See footnote § of Table V.

‖ ac, serum was anticomplementary.

¶ nd, not done.

** Serum fixed complement in presence of normal parotid suspension.

‡‡ See footnote ‖, Table V.

§§ See footnote ¶, Table V.

then, to believe that the resistance which was demonstrated among the skin-negative vaccinated children in groups 3 and 4 was not due to previous inapparent infection but to the inoculation of inactivated virus.

The Development of Complement-Fixing Antibody Following Vaccination.—Certain of the children in the first 3 experiments developed complement-fixing antibody after vaccination. From Tables V and VII, it is apparent that antibody formation was recognizable only following the second dose of vaccine. Furthermore, the results included in Table VII serve to define more exactly the time at which this antibody may be expected to emerge. It was not present 5 days but had appeared in some cases in moderate titer 10 days after the first dose of vaccine; *i.e.*, at least 5 days before the challenge was administered.

Although vaccination in certain individuals stimulated the formation of antibody, it is obvious that not all those who were vaccinated so responded. Indeed this occurred in only 18 out of a total of 45, an incidence which was less than that recorded in analogous experiments in monkeys (1).

Furthermore an increase in the amount of vaccine and the number of doses administered did not appear to increase the number of those who responded. This is evident from a comparison of the titers obtained after vaccination and prior to challenge in the vaccinated subgroups of the third experiment (Table VII), in one of which 2 doses and in the other 3 doses were given of the same vaccine. It is possible, however, that had a longer period intervened before challenge after the third dose had been given, antibody response would have been more frequent.

Lack of Correlation between the Development after Vaccination of Complement-Fixing Antibody and Resistance.—If the number in the vaccinated groups who developed antibody be compared with the number of those who were considered to be resistant or partially resistant as a result of vaccination, it at once becomes evident that there is no constant association between the emergence of antibody and absence or modification of the disease. Of 31 vaccinated individuals who were considered to be resistant, only 14 developed complement-fixing antibody. The lack of constant relationship is particularly well shown by a comparison of the clinical observations and complement fixation tests in group 4 (Tables IV and VIII). Only one case of moderately severe mumps was observed among the 10 vaccinated children. In spite of this high level of resistance, no complement-fixing antibody was detected in any of these individuals prior to challenge. Previously, results of vaccination experiments in monkeys likewise had failed to reveal a constant relationship between resistance and the presence of antibody at the time of challenge (1). In these experiments with human beings, on the other hand, it should be pointed out that with one possible exception (J. S., group 1), all those in whom antibody did appear subsequently proved refractory on challenge. Although its presence is usually associated with resistance, it would seem, then, that demonstrable complement-fixing antibody following vaccination is not an indispensable concomitant of immunity either in the monkey or the human being. A similar independence of complement-fixing as well as of other antibodies and resistance has been shown in the case of other viral antigens (6, 7). It should be clearly understood, how-

ever, that these observations do not in any way impair the significance of the presence of complement-fixing antibody as an index of immunity when it occurs naturally in the serum.

Appearance or Increase of Complement-Fixing Antibody Following Inoculation of Active Virus.—All the controls originally regarded as being susceptible developed antibody following the challenge with active virus. This response suggests strongly, particularly in the case of those who were inoculated by spraying, that infection had taken place in every control, whether or not there were accompanying symptoms. But the appearance of antibody under these conditions cannot be accepted as absolute evidence for infection, at least when the virus is introduced directly into the parotid, as will be presently pointed out.

The approximate time after challenge at which antibody first appeared in the controls can be determined from the data presented in Tables V, VI, VII, and VIII. Following the intraparotid inoculations in groups 1 and 2, antibody could not be demonstrated in the sera of the controls on the 7th day but was present in nearly all instances by the 14th day. In contrast, after inoculation by spray antibody could not be revealed on the 14th day but was demonstrable on the 21st day. These findings are consistent with the difference in incubation times following the two routes of inoculation.

In most instances the maximal titers were in the range usually encountered in convalescence from the natural disease. On the whole, however, the highest levels reached in the controls of groups 3 and 4 were lower than those observed in the controls of groups 1 and 2. Possibly the direct inoculation of the parotid gland may tend to give rise to a more pronounced antigenic stimulation.

Fourfold or greater increases in the titers were also noted in the group of 3 individuals who had naturally occurring antibody in their blood and were challenged *via* Stensen's duct (Table VI). It is impossible to determine whether this phenomenon depended on a response to the establishment of an inapparent infection or whether the active virus introduced into the gland without causing infection acted merely as an antigen, not endowed with the capacity of multiplication, to stimulate a rise in the level of circulating antibody. Considerable support for the latter hypothesis is to be found in the results of unpublished experiments in which it has been shown that formol-inactivated virus introduced into the parotid gland of normal monkeys was antigenic. Because of the foregoing considerations, it is impossible to state categorically that antibody response following intraparotid challenge is alone conclusive evidence of infection.

With the serologic behavior of the non-vaccinated, we may compare the results following the challenge in the vaccinated groups. In 27 children who failed to develop antibody as a result of vaccination, it appeared after the active virus had been administered. In such children—at least in those who received the virus as a spray—the agent presumably entered the tissues and

gave rise to an infection. Whether or not overt disease ensued, this infection would almost certainly lead to permanent immunity. In this connection it will be recalled that for the immunization of dogs against distemper, Laidlaw and Dunkin (8) employed a procedure whereby the incomplete and transitory immunity induced by formol-inactivated virus was reinforced by the subsequent inoculation of active material.

Quite otherwise were the results in those who did develop antibody in response to vaccination. Only 4 of a total of 18 yielded evidence of further increase in titer after challenge. In none of those who had developed antibody and who were inoculated by spraying (Table VII) did the antibody subsequently increase in concentration. Such behavior might be interpreted unequivocally as indicative of complete immunity were it not for the fact that the antibody persisted for a long time as will be immediately pointed out.

The Persistence of Antibody after Challenge.—About 13 months after challenge, complement fixation tests were done on specimens of serum from 31 of the 32 individuals who were originally included in groups 1 and 2 (Tables V and VI). At that time antibody was found in the sera of 30 children. One child (A. R., Table V) gave a doubtful test. The antibody levels had declined in all save 3 instances to those characteristic of persons who have undergone natural attacks of mumps at some time in the more or less remote past (2, 3). The 3 exceptions (F. O. and G. DiC., Table VI, and A. R., Table V) exhibited titers slightly above the highest normal level (1–96) usually encountered (3). The persistence of antibody in the vaccinated as well as in the controls strongly suggests that the former had experienced an infection as a result of the challenge whether or not symptoms or increase in antibody titer upon challenge had been observed, since it is unlikely that antibody induced as a result of vaccination with inactive virus would endure so long.

The Development of Dermal Hypersensitivity Following Challenge.—In groups 1 and 2 skin tests were first carried out about 14 months after challenge (Tables V and VI). Among the 31 children included in these experiments, 17 were definitely positive and 6 were doubtful, 3 were negative, 2 reacted to normal monkey protein, and 3 were no longer available for test. Because skin tests were not done at the outset, it is impossible to be certain of the exact number who became sensitive as a result of the experimental procedures. However, on the basis of the correlation which has been shown to exist between the results of the skin and complement fixation test in normal individuals (4), it is probable that a large proportion of the children who originally were negative by complement fixation test were also skin-test–negative at the outset and so became hypersensitive to the virus or its products following the challenge.

The third experiment (Table VII) in general yielded more data of the same sort, although the incidence of those who developed definitely positive skin tests after the challenge was lower. Tests on 30 children done 8 to 16 days

after challenge were definitely positive in 4 and so are to be interpreted in these cases as probable indications of previous exposure to the virus. The results of subsequent tests performed at weekly intervals during the following month showed that 6 of those who were negative or doubtful by the first test became positive and 10 others originally negative became doubtful. Sensitivity to monkey protein in 3 children made it impossible to determine whether specific hypersensitivity was present. One individual showed no evidence whatever of becoming sensitive to the virus during the period of study. Many of those in group 4 also failed to become skin-positive. Inspection of Table VIII will show that only 7 of a total of 25 individuals became definitely positive and only 3 doubtful within 2 months after the challenge.

We are unable to give an entirely satisfactory explanation for the failure of so many individuals in the third and fourth experiments to become frankly hypersensitive within the periods of observation. But it is probable that certain of those who had not developed hypersensitivity would have done so at a later date, since it has been shown that in some persons hypersensitivity is established only after the lapse of 2 months following the onset of symptoms (2).

The Possibility of Increased Resistance as a Result of Skin Testing.—A correlation of the immunologic behavior of the 5 children in subgroup 3 of group 4 (Table VIII) with the clinical observations is instructive.

It is evident that 4 of the children, in contrast to those in subgroups 1 and 2 responded to the intradermal test by the development of complement-fixing antibody, although none of them originally exhibited definitely positive skin reactions. To test the significance of this antibody response in relation to resistance, vaccination with formolized material was omitted. Following the challenge, 3 of them failed to show any increase in antibody titer and did not develop skin sensitivity during the period of observation. In contrast, the fourth child (T. P.), who exhibited only a low titer of antibody after the skin test, responded after the challenge by producing antibody in considerable amount and presented a doubtful indication of dermal hypersensitivity. Of these 4 children he was the only one who developed unmistakable signs of mumps. The fifth child in this subgroup (J. T.) showed a skin reaction to the first test at 24 hours which disappeared by 48 hours; but antibody did not appear in his serum as a result of skin testing. This boy did not present signs of mumps following challenge nor on subsequent skin tests was any dermal reaction elicited. Nevertheless antibody emerged in moderate titer which suggests that infection had occurred in an inapparent form.

These findings afford additional evidence to show that in certain skin-test–negative individuals, most of whom presumably have not had previous contact with the virus, the intradermal inoculation of virus heated at 65°C. for 20 minutes may occasionally induce the formation of antibody; *i.e.*, act as a primary antigenic stimulus (4). They suggest, moreover, that increased resistance may on occasion also follow such intradermal inoculations whether or not antibody is evident and so afford some support for the hypothesis already presented to account for the failure of a relatively large proportion of the

controls in group 4 to develop overt disease. That resistance is not invariably increased by skin testing, however, is shown by the development of mumps in patient T. P.

COMMENT

It remains here to consider how much the results which have already been critically analyzed contribute to the problem of the development of a practical method for the induction of active artificial immunity against mumps.

Vaccination with formol-inactivated virus apparently led to increased resistance in about 50 per cent of the children in each of 4 groups whose immunologic status was subsequently tested by experimental inoculation of pathogenic material. This degree of protection in human beings is of the same magnitude as that observed in monkeys vaccinated in the same manner (1). Evidently, then, the vaccine as employed in these experiments did not induce that level of immunity which would be desirable from the ideal standpoint. Moreover, when administered to persons who had previously been exposed in the ordinary manner to mumps, it failed to prevent parotitis.

Notwithstanding these limitations, we are inclined to regard the findings as definitely encouraging because of the following considerations. It has been shown that it is possible to achieve in man an immune response by the parenteral inoculation of inactivated virus. That this could be done at all might have been seriously doubted because Johnson and Goodpasture failed to demonstrate in monkeys increased resistance following the injection of active virus by any route other than the parotid duct or the oral cavity (5). Furthermore, the protective effect of the vaccine might be apparent in a larger proportion of persons under conditions of less severe subsequent exposure. Thus it would seem probable that the amount of virus used for challenge in our experiments was much greater than that which usually would be received through natural contact with the disease. The experimental conditions were also exacting in that a fairly short period of 10 to 12 days or less was allowed to elapse between the last dose of vaccine and the application of the challenge. It is true that in certain individuals this was sufficient to provide for the development of complement-fixing antibody. Nevertheless it may have been too brief to permit the factors of resistance—whatever these may be—to attain in every person complete mobilization. It is scarcely necessary to point out here that the maximal effect of vaccination against tetanus, diphtheria, and pertussis cannot be expected within the interval we have employed.

The results of serial complement fixation tests indicated that in many of the individuals who showed no definite signs of mumps vaccination did not entirely prevent infection since antibody appeared in their blood after the challenge inoculation. This further evidence against the capacity of the vaccine to induce complete immunity need not necessarily be interpreted in a pejorative

sense. Indeed, these observations suggest that the vaccine could be employed to provide a partial immunity which in turn might permit inapparent or much modified infection to occur upon subsequent natural exposure to the virus—an event which would be expected to induce a solid and enduring immunity.

Furthermore, vaccination with formolized material might prove of value in reducing the incidence of complications such as orchitis or encephalitis. In the experiments which have been described, each of these conditions has been occasionally observed. None of these patients had been vaccinated. No conclusion, however, in this respect can be drawn on the basis of the available data.

CONCLUSION

The results observed after experimental inoculation of active mumps virus into 41 vaccinated and 32 unvaccinated children,—with the consent of their parents or guardians,—indicated that formol-inactivated mumps virus obtained from the parotid gland of the infected monkey and employed as a vaccine in the manner which has been described increased the resistance of about half of those to whom it was administered.

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