ISOLATION OF MUMPS VIRUS FROM HUMAN BEINGS WITH INDUCED APPARENT OR INAPPARENT INFECTIONS*

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Many deductions concerning the spread of mumps virus were made from clinical and epidemiologic observations before confirmation by laboratory tests was possible. The evidence presented by various authors in regard to this subject has been critically reviewed by Gordon (1). The following conclusions appeared justified on epidemiologic grounds: (*a*) The average incubation period of typical mumps was shown to be 18 days. The disease was found to become communicable about 48 hours prior to the onset of glandular enlargement and the period of communicability to last for several days following the appearance of signs of illness. (*b*) Cases of unusual manifestations of mumps, such as primary meningoencephalitis or primary orchitis in the absence of recognized involvement of the salivary glands, could be properly classified only if they occurred in institutions or families where typical mumps was prevalent. It was shown, on occasion, that these patients were able to transmit mumps to contacts, possibly because of the presence of virus in their saliva at some stage of the infection. (*c*) Inapparent infections with mumps virus were thought to be frequent, since in spite of the prevalence of the disease only about 60 per cent of the adult population of the United States had suffered clinical attacks. It was also inferred that such inapparent cases of infection might spread the virus to susceptible individuals, thus maintaining the chain of infection in an epidemic.

With the development of laboratory tests for the diagnosis of infections with mumps virus (2, 3) and of technics for the isolation of the agent in chick embryos (4, 5), it has become possible to confirm many of the earlier observations by more direct proof. Thus, the existence of inapparent infections and their relative frequency has been demonstrated by application of complement fixation tests (2, 6, 7). By the same means, the diagnosis of unusual manifestations of mumps has become a routine matter (8, 3). Virus has been isolated from the saliva of cases of parotitis as late as 6 days after onset of disease (9). It has also been demonstrated in the spinal fluid of patients with meningoencephalitis without salivary gland involvement (10, 11).

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The present paper is concerned with the isolation of mumps virus from the saliva of human beings at various stages of infection. It was considered difficult to approach these problems under epidemic conditions since frequently the time and intensity of exposure would not be accurately known, whereas the study of cases experimentally exposed to mumps virus offered the advantage of controlling both these factors. The results obtained under such experimental conditions afford evidence that the period of infectivity of cases with involvement of the salivary glands may begin as early as 6 days prior to onset of recognizable glandular swelling, and may last for more than one week. It will also be shown that a patient with orchitis without salivary gland involvement harbored mumps virus in his saliva at some time prior to the development of symptoms. Finally, it will be demonstrated that cases of inapparent infection may excrete virus in saliva for considerable periods of time and that they may be responsible for spreading the disease in an epidemic.

Methods and Materials

Selection of Susceptible Subjects.—Institutionalized children in good physical condition and without known histories of mumps were bled and their sera tested for antibodies against mumps complement fixation antigens. Those children whose sera failed to react with the soluble and the virus antigens (12) were considered susceptible to mumps and, therefore, chosen for these experiments with the permission of parents and guardians.

Virus.—Two strains of mumps virus (F and B) were used. They had been isolated from the saliva of cases of parotitis by amniotic inoculation of chick embryos (13). Amniotic fluid of the fifth passage was quick frozen and stored in glass-sealed ampules in a dry-ice cabinet until used. The preparations of both strains contained 10^7 50 per cent infectivity doses for chick embryos per ml. and 5000 (F) and 7500 (B) hemagglutinating units.

Isolation of Virus from Saliva.—Saliva or mouth washings were obtained in the first experiment, depending on the ability of the children to furnish saliva. In the second experiment saliva was mechanically aspirated by means of a device consisting of a thin bent copper tube leading into a glass trap which was in turn connected with an electrically driven suction pump. Individual equipment was used for each child. The specimens were immediately transferred into ampules which were sealed off in an oxygen flame. After quick freezing the ampules were transported to the laboratory packed in dry-ice. They were stored at −70°C. until inoculation of chick embryos was possible.

Before injection the specimens were thawed and centrifuged at 2000 R.P.M. for 10 minutes. The supernatant fluids were incubated at 37°C. for 1 hour with penicillin and streptomycin, using 1000 units of each per ml. The specimens were injected in 0.2 ml. amounts into the amniotic cavity of ten 8-day-old chick embryos, under direct observation as described previously (12). After further incubation for 5 days at 36–37°C. the eggs were chilled for 1 to 3 hours at 4°C. and the amniotic fluids were harvested aseptically. These were tested individually on slides for their ability to agglutinate chicken red cells. Further transfers were made by injecting undiluted amniotic fluids into new groups of eggs. A specimen of saliva was considered negative if three amniotic passages failed to indicate the presence of virus.

For the identification of the isolated strains of virus pools of amniotic fluids of the respective groups of eggs were used as antigens for complement fixation tests with known acute and convalescent sera of patients with mumps. In all cases tested the agent isolated was identified as mumps virus.

Serologic Tests.—Blood was drawn from all individuals at intervals to study the development of complement-fixing antibodies. The technics and the antigens employed have been fully described in previous communications (3, 12).

EXPERIMENTAL

Two experiments were conducted with a total of 15 children. The experiments differed in regard to the amount of virus to which the subjects were exposed and the method by which it was applied. In all other respects the two tests were essentially alike. Before exposure to the virus the children were isolated in a hospital ward where they remained for the period of observation, under the care of trained nurses. Their temperatures were recorded twice daily, and 4 times daily in cases in which fever was observed. Beginning 2 weeks after exposure the children were examined daily for signs and symptoms of disease by one of the authors, except for a few occasions when adverse weather conditions rendered the institution inaccessible.

Experiment 1 (November, 1947).—Seven children were exposed to active mumps virus which was deposited by means of a coarse spray on the mucous membrane of the oral cavity, particularly close to the orifices of Stensen's duct. Four of the children received 2.0 ml. of amniotic fluid containing strain F, and three children were infected with the same amount of strain B. The amount of virus sprayed for each child corresponded to 2×10^7 ID_{50} for chick embryos in the case of both strains.

Experiment 2 (January, 1948).—In the second experiment, eight subjects were exposed to finely dispersed virus, by means of an atomizer,[1] operated by compressed air. Thus the children inhaled small droplets and droplet nuclei through their mouths. A mixture of equal parts of strains F and B was employed. Four of the subjects were exposed to a spray of 1.0 ml. of a 1:100 dilution of infected amniotic fluid, containing 10^5 ID_{50} for chick embryos, and the remaining children inhaled 1.0 ml. of undiluted fluid containing 10^7 ID_{50}.

Since the clinical observations and the laboratory findings of the two experiments were similar, they will be discussed together. A summary of all the data is presented in Figs. 1 and 2.

Clinical Observations.—As can be seen from the figures, four of the fifteen children came down with a clinically well defined parotitis (cases 2, 7, 11, and 12). Two additional cases (Nos. 1 and 5) showed signs of involvement of the submaxillary glands. Case 6 developed orchitis without parotitis. One case (No. 15) came down with tonsillitis and palpable lymph nodes 17 days after exposure to mumps virus. The purulent exudate and the high temperature suggested an intercurrent bacterial infection, which was promptly relieved by chemotherapy. The additional seven children (cases 3, 4, 8, 9, 10, 13, and 14) remained well during the period of observation except for minor febrile responses. The significance of these elevations of temperature is questionable.

The incubation periods fell into the range encountered in the epidemic disease. The first case of parotitis (No. 7) was observed on the 14th day after

[1] Kindly supplied by the Vaponefrin Company, Upper Darby, Pennsylvania.

exposure; the remaining three subjects showed signs of parotid swelling on the 15th, 16th, and 19th day after infection (cases 11, 12, and 2). Submaxillary

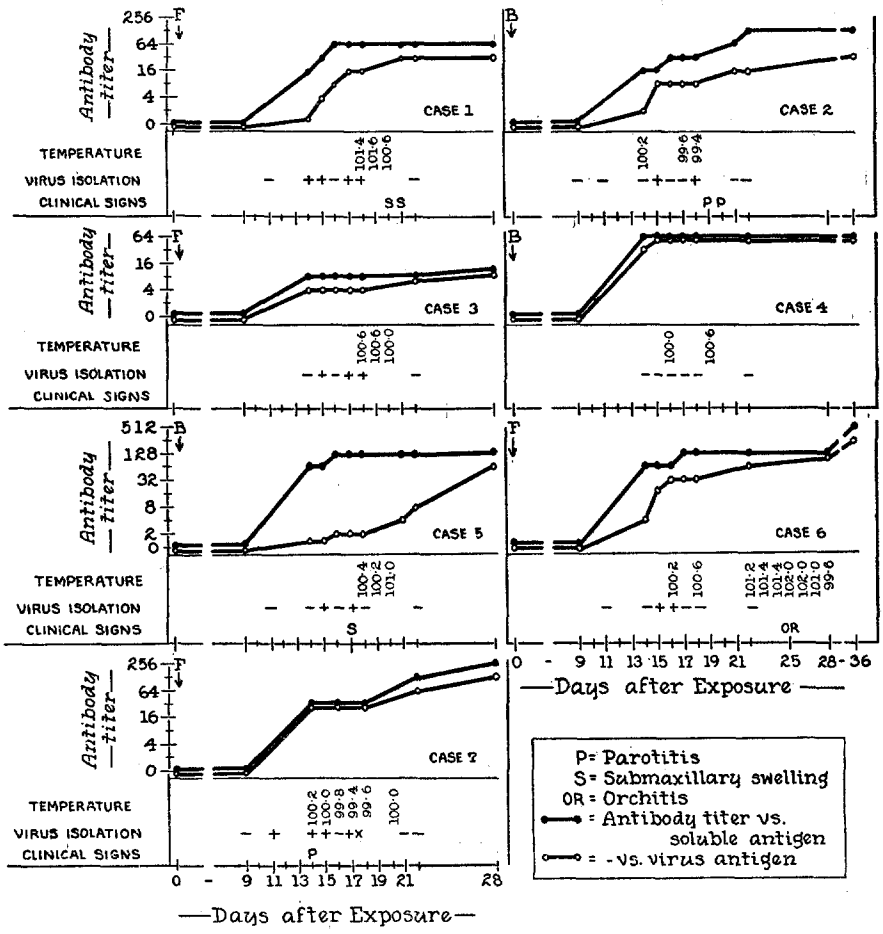

FIG. 1. Experiment 1. Summary of results

involvement was noted on the 17th and 20th day in cases 1 and 5. Case 6 developed orchitis on the 25th day after spraying of the virus.

The clinical course in all patients with involvement of the salivary glands was mild and the swellings subsided within 4 to 5 days. The orchitis affected only one of the testicles. This case, likewise, was very mild and lasted for 4 days. It responded to conservative treatment and the pain was rapidly relieved by analgesics. The general mildness of the illnesses produced is reflected in the moderate febrile responses recorded in Figs. 1 and 2.

Serologic Results.—Complement fixation tests were performed with sera taken from all children at intervals during the course of the experiments. All cases

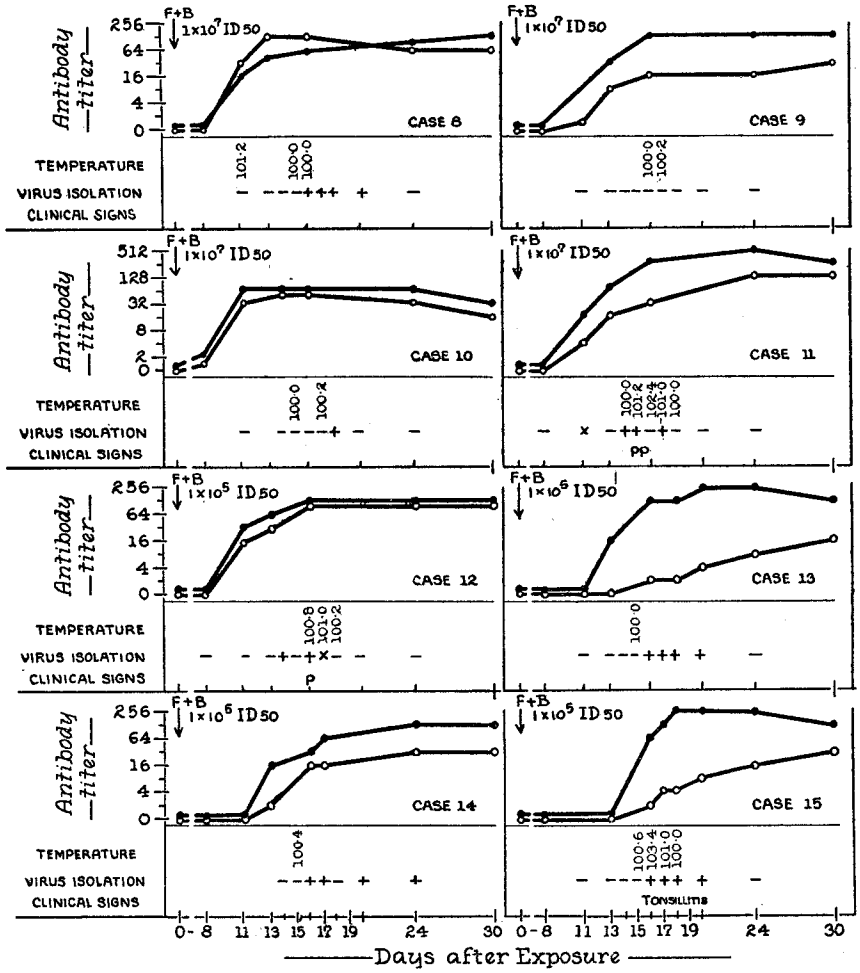

FIG. 2. Experiment 2. Summary of results

who showed signs of infection developed antibodies to the soluble (S) as well as to the virus (V) antigen in a manner described for the epidemic disease (3). The antibodies to the soluble antigen commenced to rise in less than 2 weeks after exposure. Some of the cases showed also appreciable titers to the virus antigen at that date (cases 7, 11, and 12). Thus, both anti-V and anti-S antibodies had reached high levels before signs of disease were noted. All cases showed further increases in antibodies in the following 2 weeks.

The eight children who failed to exhibit signs of mumps showed antibody responses similar to those of the children who developed clinical illness. These cases must be classified, then, as inapparent infections. The rate of antibody formation against the V antigen was somewhat delayed in three of the children (cases 5, 13, and 15).

Isolation of Virus.—Series of specimens of saliva were thus available from (*a*) six patients with involvement of the salivary glands; (*b*) one patient with a manifestation of mumps without salivary gland involvement (orchitis); and (*c*) eight subjects who were classified as having inapparent infections according to their serological response. This group includes the patient with tonsillitis.

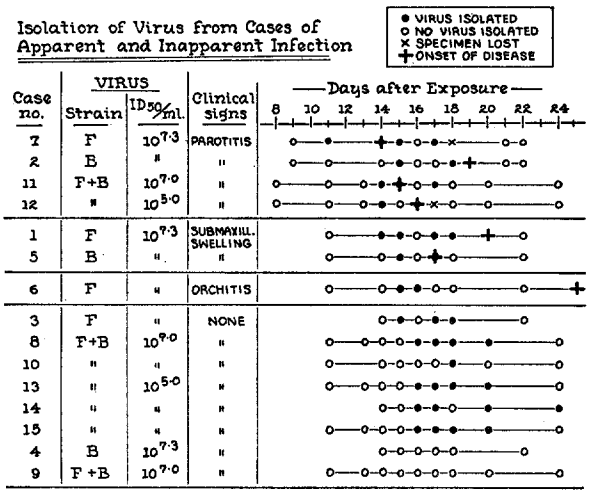

FIG. 3

Figs. 1 and 2 show the attempts and successful isolations obtained from each of the subjects in relation to clinical and serological findings. Fig. 3 summarizes the result of the isolation procedures, grouped according to the clinical signs of infection seen in the children. The positive findings require no comment. The negative data, on the other hand, do not necessarily indicate that no virus was excreted on the day the specimen was obtained. As can be seen, negative results were recorded in some instances in which the specimens of the preceding and following days were positive. It is possible that the technic of sampling of the saliva was inadequate or that the excretion of virus occurred intermittently. The collection of specimens was unfortunately less regular in the later days of the two experiments because of snow storms, which prevented traveling to the institution on several occasions. Attempts to isolate virus on the 1st and 2nd days after exposure failed. Later in the experimental period, as can be seen in Fig. 3, all children who showed clinical signs of parotitis or of

submaxillary swelling excreted virus for several days. In all these cases, virus was isolated 2 to 6 days prior to the appearance of swelling, beginning at the 11th to 15th day after spraying. Virus was also demonstrated in some of the samples of saliva taken after onset of the disease. Thus in case 7 virus was still present on the 4th day of illness.

Isolation of virus was not only successful in the cases just described but also from the saliva of the case of orchitis, who showed no involvement of the salivary glands. Virus was found on the 15th and 16th days after exposure to mumps virus, *i.e.*, 10 and 9 days prior to the onset of orchitis. In addition, virus was isolated from the saliva of six of the eight children who experienced

TABLE I

Isolation of Virus from the Saliva of Representative Cases

Case No.	Passage No.	Time after exposure, *days*										Period of excretion
		11	13	14	15	16	17	1	20	22	24	
												days
	1	0/8*		0/9	**3/5**	0/8	**3/6**	**6/6**		0/8		
1	2	0/5		0/8	**5/6**	0/6	**6/7**			0/7		
	3	0/8		**8/8**		0/6						At least 5
	1	0/8	0/6	0/6	0/6	0/6	‡	0/3	0/6		0/5	
12	2	0/7	0/6	**1/5**	0/7	**5/5**		0/6	0/7		0/8	
	3	0/8	0/4	**6/6**	0/7			0/8	0/8		0/8	3–4

Bold-faced type is used to emphasize the positive isolations.

* None of eight eggs showed positive hemagglutination on 5th day after inoculation. Two of the ten originally injected embryos died of non-specific causes during the incubation period.

‡ All embryos died on account of bacterial contamination.

inapparent infections (cases 3, 8, 10, 13, 14, and 15) at about the same time as from the clinical cases of mumps. As shown, virus was isolated from these children for periods of 1 to 9 days. Only children 4 and 9 failed to reveal virus in any of the available specimens.

These data show then that virus could be isolated from thirteen out of fifteen children exposed to mumps virus under experimental conditions. Table I gives the results of the isolation of virus in two of the children. As can be seen, some of the specimens of saliva yielded, on first passage in the majority of the injected eggs, sufficient concentrations of virus to cause hemagglutination. With other samples two, or in one instance even three, passages were required before positive results were obtained. A specimen which failed to yield hemagglutination by the third passage was considered free of virus. The allantoic fluids of these eggs never showed hemagglutinating properties. The

death rate in chick embryos inoculated with bacteriologically sterile saliva was frequently high. In other instances the antibiotic failed to inhibit bacterial multiplication and a few entire groups of eggs were lost because of such contamination.

DISCUSSION

It is obvious that no quantitative and statistically valid conclusions can be drawn from the data presented, since these were derived from only a small number of cases of induced infection with mumps virus. However, the data confirm experimentally some of the earlier conclusions deduced from epidemiologic observations (1). Thus, the incidence of inapparent infections in mumps has been estimated to be of the order of 30 to 40 per cent. Serological analysis of epidemics in recent years has confirmed this contention in that a high percentage of exposed subjects developed complement-fixing antibodies for mumps in the absence of clinical signs of infection (2, 6, 7). The experimental exposure leads to similar results. In the present series, eight of the fifteen exposed children must be classified as having had inapparent infections.

The period of communicability of the experimental cases showing involvement of the salivary glands began, according to the results of isolation of virus, on the 11th to the 15th day after exposure and extended to the 18th to 19th day. Thus, virus appeared in the saliva 2 to 6 days prior to the appearance of glandular swelling, and it was found as late as 4 days after onset of the clinical signs. Leymaster and Ward (9) obtained virus from one case of epidemic mumps on the 6th day of disease. It is possible that the experimental results are strongly influenced by the intensity of exposure. A smaller dose of virus ($<10^5$ ID_{50}) might conceivably delay the onset of viral excretion for a few days, as well as prolong the incubation period.

Most of the children who failed to show clinical signs of involvement of the salivary glands nevertheless excreted virus in their saliva at certain times after exposure. One of these developed orchitis at a later stage. It had been shown in the past on epidemiological grounds that such cases of unusual manifestations of mumps, including meningoencephalitis without parotitis, may spread the infections to susceptible individuals (14, 15).

The isolation of virus from the cases of inapparent infection was successful over a period of from 15 to 24 days after exposure. They became "transitory carriers" for from 1 to 9 days or longer. Unfortunately, no specimens of saliva were collected after the 24th day. However, there are strong indications that one of the children of the second experiment, classified as having inapparent infection, was still harboring the virus on the 30th day after exposure, when the group were released from the hospital and returned to their cottage. Between 15 and 18 days thereafter, four cases of parotitis occurred in that cottage in children not belonging to the group. Although there is no definite proof that

the virus was derived from one of the returning children, the possibility cannot be denied, because of the time relationships encountered. Whether the virus, if introduced by one of the children, was spread through the saliva, or whether it was present on contaminated clothing cannot be determined in this case. However, transmission by contaminated apparel has generally been disregarded as a source of infection (1).

It appears, then, that the virus used for experimental exposure closely resembled the epidemic agent and had not become attenuated to any large extent as a result of five passages in the chick embryo. The data, in addition, seem in good agreement with the earlier epidemiologic observations. However, certain differences were noted in the rate of production of antibodies in the experimentally exposed subjects in comparison with cases observed under epidemic conditions. In the natural disease antibodies to the soluble antigen may be present at the onset of swelling, or they rise very shortly thereafter; antibodies to the virus antigen usually appear in measurable quantities several days later, when antibodies to the soluble antigen have reached high levels. Only in the case of complications, such as orchitis, may high titers to both antigens be found at the time of their onset. On the other hand, in the experimental groups, distinct antibody levels for both antigens were found in the cases with involvement of the salivary glands, several days before clinical signs of the disease, with the exception of one patient who showed a low titer with the virus antigen at the time of the submaxillary swelling. The patient with orchitis had high antibody levels 10 days before he showed signs of illness, and subsequently a second rise in antibodies to both antigens was found. It is possible that the findings described are the result of the rather intensive exposure under experimental conditions since most of the children were sprayed with 10^7 ID_{50} (for chick embryos). When the dose was decreased to 10^5 ID_{50} the antibodies to the V antigen appeared at a slower rate in two of the three cases who were classified as inapparent infections (cases 13 and 15).

There does not seem to be a relationship between the development of complement-fixing antibodies and the excretion of virus in saliva in the cases described. With the exception of case 3, whose antibody levels were the lowest in the two groups, antibody titers to the soluble antigen were high and, in some cases antibodies to the virus antigen were measurable in large amounts when virus was isolated from the saliva. The relationship between complement-fixing and neutralizing antibodies has not been fully established. However, preliminary experiments with sera of some of the cases revealed that neutralizing antibodies were circulating in the blood stream at a time when virus was found in the saliva. It seems possible that after the virus has invaded the salivary glands it is no longer accessible to humoral antibodies unless they reach such high concentrations in the blood as to be reflected in the secretions. Such a situation would not be unlike that observed in the case of infections of man with influenza virus. These questions will be the subject of further studies.

SUMMARY

Exposure of fifteen children to mumps virus of fifth amniotic passage in chick embryos led to involvement of the salivary glands in six, orchitis in the absence of other manifestations of mumps in one, and to no signs of illness in eight. Attempts to isolate virus from the saliva of these individuals gave the following results:

1. All patients with involvement of the salivary glands excreted virus beginning on the 11th to 15th day after exposure, 2 to 6 days prior to onset of clinical signs of disease and extending up to the 4th day of illness.

2. The patient with primary orchitis without any recognized involvement of the salivary glands excreted virus for 2 days, beginning on the 15th day after exposure and 10 days prior to his illness.

3. Six of the eight children classified as having inapparent infections because of their serologic response in the absence of clinical signs of illness, began to excrete virus on the 15th to 16th day after exposure for from 1 to 9 or more days.

The epidemiologic significance of these data is discussed.

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