Mumps Infectivity Studies in Hamsters. (20470)

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The transmission of mumps to experimental animals other than monkeys(1), cats(2), guinea pigs(3), suckling mice(4), and suckling hamsters(5) has not proved successful in the past. Attempts to infect adult hamsters with this agent by various routes so far have met with failure(6).

In the course of producing specific antisera in adult hamsters, it was observed that these animals, after intranasal instillation of the virus, yielded sera with high levels of complement fixing antibodies against the soluble and viral mumps antigens. These findings led to the investigation of the pathogenicity of mumps for hamsters and to attempts at serial passage of the virus in hamster lungs. The serological and tissue responses in these animals to the intranasal inoculation of both egg-adapted and current epidemic strains of mumps virus will be described in this report, together with results of attempts to adapt the virus to lung tissues of adult hamsters.

Materials and methods. The Enders strain of mumps, which had been received from the Children's Hospital, Philadelphia, in 1949, and had undergone 20 allantoic passages in chick embryos in this laboratory, was used as the standard strain throughout these experiments.

Golden hamsters of 7-10 weeks of age were inoculated intranasally under ether anesthesia with 0.3 ml amounts of allantoic fluid containing $10^{8.5}$ EID_{50}/ml of mumps virus. A batch of 3-4 animals was anesthetized and sacrificed by exsanguination from the brachial artery each day for a period of 38 days, commencing 24 hours after inoculation and the sera of each batch were pooled. Lungs, spleens, and parotid glands of each batch were similarly pooled and 25% suspensions of each pool of organs were prepared by grinding with quartz in a mortar in physiological saline. The suspensions were centrifuged at 1500 r.p.m. for 5 minutes and the supernates were removed, shell-frozen and stored at —32°C until used. The tissue suspensions, to which were added 1000 units of penicillin and 1000 μg of dihydrostreptomycin per ml, were titrated in the amniotic sac of 9-day-old chick embryos and the amount of virus present in these suspensions was estimated by testing the amniotic fluids for presence of specific hemagglutinins, using Salks' method(7). The 50% infectivity titer (EID_{50}) was calculated by Reed and Muench's method(8).

Attempts at virus isolation from 4 throat washings of patients acutely ill with mumps were carried out in parallel series, one part of the washings being inoculated intranasally into hamsters, another part being inoculated amniotically into developing chick embryos.

Mumps antisera used in the complement fixation (C.F.) and hemagglutination inhibition (H.I.) tests of this work were prepared in hamsters by a single intranasal inoculation of 0.3 ml of allantoic fluid containing $10^{8.5}$ EID_{50}/ml of mumps virus. The animals were bled 14 days after inoculation and their sera were pooled. Antisera against the newly isolated strains were prepared in a similar manner. Details of serological procedures used in this work are described earlier(9).

Results. The serological responses of hamsters to a single intranasal inoculation of the egg-adapted Enders strain of mumps virus are summarized in Table I. The tests were carried out on pools of serum collected daily from 3-4 animals from the first day after inoculation. For simplification of tabulation, the results of these tests have been grouped together over 4-day periods and their mean values are recorded in Table I. The C.F. titers are expressed as reciprocal values of the original dilution of serum giving complete fixation in the presence of 2 units of complement. Hemagglutination titers are expressed as reciprocal values of the highest final dilution of serum causing complete inhibition of 4 hemagglutinating units of a mumps virus suspension. It can be seen from Table I that

TABLE I. Serological Response of Hamsters to Experimental Infection with Mumps Virus.

Animals No.	Days after infection	Mean values of C.F. titer with		Haemagglutination-inhibition titer
		Soluble antigen	Viral antigen	
24	1- 6	0	0	0
16	7-10	88	56	160
16	11-14	128	112	300
16	15-18	112	128	360
12	19-22	64	85	213
12	23-26	43	64	240
8	27-30	32	64	160
12	31-34	37	43	133
16	35-38	22	40	160

TABLE II. Mumps Virus Titers in Lungs of Hamsters after Inoculation with $10^{7.3}$ EID_{50} of Infected Allantoic Fluid.

Days after inoculation	Virus recovered per g of lung*
1	4.53
2	3.46
3	3.01
4	3.82
5	2.09
6	.99
7	<.57
8	<.57

* Figures represent logs of reciprocals of the EID_{50} based on haemagglutination titers.

TABLE III. Recovery of Mumps Virus from Hamster Lungs on Serial Lung Passage.

Passage No.	Virus recovered per g of lung*	
	Exp. series 1	Exp. series 2
1	3.66	4.4
2	3.4	4.1
3	4.05	4.8
4	3.7	5.2
5	4.59	4.4
6	4.79	4.3
7	5.06	4.1
8	3.8	3.3
9	3.5	3.3
10	4.4	2.3
11	0	0

* Figures represent logs of reciprocals of the EID_{50} based on haemagglutination titers.

C.F. antibodies against the soluble antigen do not appear in the serum of hamsters before the 7th day following inoculation. They reach a maximum level between the 11th and 14th day after inoculation. The antibodies against the viral antigen appear also at the 7th day after inoculation and they reach a maximum level between the 15th and 18th day. The decrease in antibody levels to the soluble antigen precedes the decrease in antibody levels to the viral antigen by approximately 5 days. Antibodies against both antigens, however, are still present in significant titers as late as the 38th day after experimental infection. Anti-hemagglutinins also appear on the 7th day, reach a maximum level between the 15th and 18th day and decrease in a manner similar to the C.F. titers. They are still present in significant titer on the 38th day after infection.

Results of attempts at isolation of virus from the lung tissues after a single intranasal inoculation of allantoic fluid containing $10^{8.5}$ EID_{50}/ml of mumps virus are presented in Table II. The results indicate that the virus was present in the lungs in significant titer up to the 4th day after inoculation. It decreased considerably from the 4th to the 6th day and could not be recovered from the 7th day on.

Results of attempts to transmit the virus by serial passage through lungs of hamsters are given in Table III. In the first series of experiments, transmission of the virus was successful in 10 consecutive passages with significant amounts of virus demonstrable in the lung tissues of animals sacrificed 3 days after inoculation. No virus could be detected in the lungs at the 11th-13th passages and no antibody response could be shown in surviving animals of these groups. A second series of experiments in which hamsters were sacrificed on the 3rd, 4th, and 5th day after inoculation gave very similar results to those of the first series of experiments (Table III). Judging by these results, the decrease in virus content of the lung tissues on passage apparently did not follow a simple dilution factor. Hence, attempts are being made at

present to adapt the virus to these tissues by alternating passages through hamster lungs and the amniotic sac of chick embryos.

Attempts at isolation of mumps virus from throat washings by parallel passages through chick embryos and hamsters were carried out successfully in 4 instances. In one of these attempts, the virus was carried through 12 continuous passages in hamster lungs, where it attained a titer of $10^{4.3}$ EID_{50}/g of lung during the first 2 passages. However, as in the case of the egg-adapted Enders strain, the virus was lost in the following passages and no antibodies developed in the serum of surviving animals of these groups. With the 3 other attempts, virus was carried only through a limited number of hamster passages, with titers attained similar to that above. It was found that virus isolated from throat washings by intranasal inoculation into hamsters was readily adaptable to chick embryos.

Histopathology. Examination of animals sacrificed on the 4th and 5th day after inoculation with allantoic suspensions of virus revealed macroscopic focal lesions in the lungs. The histopathological examination of these lungs showed edema of the bronchial walls and fibrous tissues of larger blood vessels, with an infiltration of lymphocytes and large mononuclear cells into these areas. The alveolar walls over wide areas were much thickened due to congestion of alveolar capillaries and an infiltration of mononuclear cells. The alveolar spaces in such areas were empty or contained coagulated fluid of high protein content. In some areas there was a sparse accumulation of mononuclear phagocytes within the alveoli and occasional red blood cells were seen in these regions. The picture represented that of an interstitial pneumonitis.

Lungs of control animals, which had been inoculated similarly with a mixture of equal volumes of mumps virus and undiluted specific antiserum, showed complete absence of evidence of interstitial or alveolar exudate and their microscopic appearance was essentially that of normal lung tissues. Lungs of hamsters inoculated with a mixture of mumps virus and Influenza A (PR8) antiserum represented a microscopic picture very much the same as that of lungs of the animals inoculated with the virus suspension only.

Spleens and parotid glands of these hamsters appeared normal on macroscopic and microscopic examination. This finding confirmed results of several unsuccessful attempts at isolation of virus from portions of these organs. Histological examination of lung sections of hamsters inoculated with the 4th lung passage of the freshly isolated strain of mumps virus revealed round cell infiltration of the smaller bronchial walls and surrounding interstitial tissue. The picture represented that of a bronchitis with a focal interstitial pneumonitis.

Summary. Adult hamsters inoculated intranasally with the egg-adapted Enders strain of mumps virus produced complement fixing antibodies against both the soluble and viral antigens. These antibodies were present in measurable amounts from the 7th day after inoculation until the 38th day when the experiment was terminated. Antibodies against specific hemagglutinins also appeared on the 7th day, reached a maximum level and decreased in a similar manner to the complement fixing antibodies. The production of antibodies against the soluble antigen was considered to be indicative of virus multiplication in the tissues of the animals. Multiplication was then demonstrated by recovery of the virus after serial passage through hamster lungs. Mumps virus has been isolated from hamster lungs following intranasal inoculation of throat washings from 4 patients with epidemic parotitis. Serial passage in hamsters carried out with one of these strains gave results similar to those obtained using the egg-adapted Enders strain. Histopathological examination of lungs of hamsters inoculated either with the egg-adapted Enders strain or with a lung passage of a freshly isolated strain of mumps revealed the presence of bronchitis and interstitial pneumonitis. Specific antiserum when mixed with the standard virus suspension and similarly inoculated prevented such pathological changes, while non-specific serum had no such effect.

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Reactive Sulfhydryl and Disulfide Groups of Protyrosinase and Tyrosinase.* (20471)

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The methods of conversion of protyrosinase, the inactive form of tyrosinase, to tyrosinase have been the object of extensive studies(1). In general, it may be said that amphipathic anions, carbamates, and temperatures between 60° and 70°C for 5 minutes will bring about this change. The object of this and subsequent studies is an attempt to ascertain what physical or chemical changes occur in such a conversion.

Materials and methods. Protyrosinase was obtained from diapause grasshopper eggs. These eggs were triturated with a mortar and pestle and diluted with 0.9% NaCl to a volume of 10 cc per g of eggs. This was centrifuged and the lipoidal layer removed. The remaining supernatant was recentrifuged, decanted and 1 g of KH_2PO_4 per 250 cc of supernatant added. After standing 2 hours at 0°C, this was centrifuged and the supernatant decanted. Enough $(NH_4)_2SO_4$ was added to make the supernatant 19.3% saturated (12% solution). Two hours later the precipitate was centrifuged away and the supernatant brought to 31.1% saturation with $(NH_4)_2SO_4$ (18% solution). After 2 hours at 0°C, this was centrifuged, the supernatant discarded and the precipitate resuspended in a volume of 0.9% NaCl equal to one-half that of the solution centrifuged. After one hour this was centrifuged, the precipitate discarded and the supernatant brought to 31.1% saturation with $(NH_4)_2SO_4$. After 2 hours, this was centrifuged and the precipitate resuspended in a volume of NaCl equal to that used in the previous step. This was dialyzed against 3 changes of NaCl for at least 24 hours. The resulting preparation showed no tyrosinase activity and contained on an average 0.5 mg of nondialyzable material per cc, which in turn had in it 0.21% copper and 14% nitrogen.

A modification of the methods employed by Mirsky and Anson(2) was used to determine the reactive sulfhydryl and disulfide groups. The limitations of this method have been adequately discussed by these authors. In the determination of the sulfhydryl groups, one cc of proenzyme or enzyme plus 2 cc of 0.9% NaCl plus 0.1 cc of phosphate buffer (pH 7.5) plus 0.1 cc of 0.1 M $K_3Fe(CN)_6$ were allowed to stand at 35°C for one hour. At the end of this period, the protein was precipitated with 0.1 cc of 0.05 N H_2SO_4 and 3.3 cc of a saturated solution of $(NH_4)_2SO_4$, filtered out and one cc of Folin and Malmros(3) ferric sulfate solution added to 3 cc of the supernatant. This was allowed to

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