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Propagation of Mumps Virus in Suckling Mice and in Mouse Embryo Tissue Cultures. (19668)

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Adaptation of mumps to mice, undertaken to facilitate investigation of this disease, has been accomplished in 2 different ways. In one series of experiments 20 preliminary passages in mouse embryo tissue culture were carried out before virus grew consistently in brains of suckling mice. Later it was found that mumps virus could be more readily adapted to suckling mice by prior passage in suckling hamsters(1). Illness and death occurred only in the latter series of mice, infections being inapparent in those originally inoculated with tissue culture material. It is felt that these investigations have practical interest, as they may pave the way for use of mice in mumps neutralization tests. In addition, mumps infections of mice give some indication of the varied potentialities of this virus as an agent of disease.

Materials and methods. Tissure culture. With modifications, methods employed in tissue culture were ones developed by Earle and associates(2-6). The nutrient fluid consisted of horse serum 40%, balanced saline (Earle's) 40%, and 1:1 extract from 9-day chick embryos, 20%. Antibiotics added to the saline gave it a concentration of 5 mg of streptomycin and 500 units of penicillin per ml. Supporting tissue consisted of mouse embryos in an advanced stage of development. These were comminuted by forcing them through an 18-8 stainless steel sieve of 24 mesh, .014 inch wire diameter(6). Virus containing inocula were mixed directly with tissue pulp and the mixture pipetted into T-60 flasks,* prior to addition of 8 ml of nutrient fluid. Once the cell suspension was evenly distributed over the floor of the flask a sheet of perforated cellophane was dropped on it. Supernatant fluids were drained off and fresh fluid added so as to give a total of 3 fluid changes a week. In later passages, mouse embryo juice was used in the last 2 of these changes. At weekly intervals, the cellular material was scraped from the cellophane, ground, and frozen before using as a virus inoculum for the next tissue culture passage. Roughly 0.5 ml of cellular debris plus fluid were carried over from one passage to another. Precise determination of total dilutions of virus included in the mixture of cells and fluid, was not attempted. Serum ultrafiltrates were assayed in a few alternative passages, but multiplication of virus did not appear to be sustained in this medium. *Virus.* The strain of mumps virus employed for both animal and tissue culture experiments was one originally isolated from human milk(7) and carried through 6 to 7 passages in the amniotic cavity of embryonated eggs. *Mouse passages.* Almost all passages were carried out with A strain mice, although a few regular Swiss mice were employed initially. Suckling mice were inoculated intracerebrally at 1 or 2 days of age and brains were harvested a week later, being ground without abrasive and made up as extracts diluted 1:10 in meat infusion broth.

Identification of virus. Supernatant fluid from each weekly tissue culture passage was inoculated into 7-day embryonated eggs by the amniotic route. Amniotic fluids, harvested separately a week later, were used in hemagglutination tests to determine the presence of

* The design of this flask will be published later.

mumps virus. Brains harvested from each weekly passage in mice were inoculated in eggs in similar fashion, using a 1:10 dilution. All positive amniotic fluids were pooled and titered for hemagglutinin content. Identification of virus, done periodically in the course of tissue culture and animal passages, was by anti-hemagglutination and complement-fixation tests run with specific immune sera(8). Routine cultures of all materials in whole meat broth and on blood agar slants were used to check on bacterial contamination.

Growth of virus in tissue culture. Mumps virus was carried through 20 weekly passages in mouse embryo tissue culture. These passages were continuous except for the third and sixth in which amniotic fluids, from eggs infected with material from the preceding tissue culture passage, were used as inocula. No cellular changes ascribable to virus were observed. Hemagglutinins were not demonstrable in supernatant fluids, although these fluids were infectious for eggs in dilutions as high as 10^{-4}. Attempts were made to grow mumps virus in mouse tissues other than those from embryos. Mammary gland, placenta, liver, and suckling mouse brain were all assayed. In addition a mixture of cells from various adult organs including uterus, spleen, liver, and kidneys was cultured in mouse embryo juice. In these tissues, mumps virus had either disappeared or was only barely demonstrable after 7 days, failing to appear in subsequent passage. Cultures of a mammary adenocarcinoma and a hepatoma of mice were tried with similar results, except that virus seemed to survive particularly well in these tissues. To check the thermo-resistance of mumps virus, sealed ampules of infected amniotic fluids, freshly harvested, were placed in an incubator at 36°C. On 2 separate trials, mumps virus was found to remain infectious after 9 days at this temperature, indicating that virus might passively survive a single tissue culture passage.

Inoculation of mice with tissue culture virus. In the course of the first 10 passages in mouse embryo tissue culture, repeated attempts were made to transmit the virus to suckling and to adult mice, none of which developed signs of illness. Mumps virus as grown in tissue culture did, however, show an unexpected ability to survive in mouse brain, particularly in young suckling mice. Amniotic fluid, from eggs inoculated with 10th passage mouse embryo tissue culture, was inoculated intracerebrally into one-day-old mice in amounts of 0.03 ml. Brains of 3 of these mice, harvested and pooled on the following day, infected eggs when diluted 10^{-5}. Similar brain pools had titers of 10^{-3} and 10^{-2} at 7 and 12 days respectively after inoculation. The original fluid infected eggs in a dilution of 10^{-8}. Continued passage was not successful. After 15 passages in mouse embryo tissue culture, mumps virus was carried through 5 successive transfers in brains of suckling mice. For 3 transfers, brain extracts were readily infectious for eggs, but extracts of the 4th and 5th transfers were markedly less so and those from the 6th passage contained no demonstrable virus. Repetition of this experiment led to similar results. Fifth passage brain extract infected eggs in a dilution of 10^{-2}. Mumps virus carried through 20 tissue culture passages was better adapted to growth in suckling mice, as indicated by 13 successive (*in vivo*) passages. Some loss of egg-adaptation appeared to result from growth in mice. Thus brain extracts from the first 7 mouse passages infected most eggs inoculated, positive amniotic fluids having hemagglutinin titers of 1:320 or better. From the 8th passage on, fewer eggs became infected, the pattern of the hemagglutination was less firm, and positive fluids had low hemagglutination titers. When such fluids were reinoculated into eggs, high hemagglutinin titers were again obtained. No signs of illness were noted in any mice receiving tissue culture virus.

Growth of mumps virus in mouse brain following passage in brains of suckling hamsters. When mumps virus, freshly isolated from a patient in amniotic fluid was used to inoculate suckling hamsters intracerebrally, illness and death usually followed as elsewhere described (1). After 6 hamster passages, the virus was inoculated into suckling mice. For the first 5 mouse brain passages, there was no sign of illness. On 6th passage, however, a majority of mice inoculated developed a striking illness. No 2 mice presented the same clinical pic-

ture, but several had spastic convulsions, one dying immediately afterward. Some had arched backs, and others were hyperexcitable, leaping and running around their jars. Although illness was absent in the next 2 passages, it reappeared and persisted in the 9th and following passages. It has been possible to isolate mumps virus from all 19 passages made to date. At present, by harvesting only mice having definite signs of illness, which is usually possible by the 11th day after inoculation, it is hoped to develop a more uniformly fatal strain of virus. Pathological studies, now in progress(9), indicate that, as in suckling hamsters, there are marked histologic changes in the brains of suckling mice having clinical signs as a result of infection with mumps virus.

Discussion. Experiments described above demonstrate that mumps virus will grow readily in mouse embryonic tissue. Weller and Enders(10), who were first to propagate this virus in tissue culture, used amniotic membranes from chicken embryos and a strain of virus which had previously gone through 42 egg passages. They demonstrated production of hemagglutinins in their tissue cultures, whereas hemagglutinins were at no time demonstrable in cultures of mouse embryo tissue. Apparently, this lack of hemagglutinins was not due to the presence of inhibitors, for when supernatant fluids were tested against mumps infected amniotic fluid, no inhibition of hemagglutination occurred. Although supernatant tissue culture fluid infected eggs in a dilution of 10^{-4} this relatively low titer of virus might account for lack of hemagglutination. To our knowledge, however, hemagglutinins have not been demonstrated with mumps virus propagated in mammalian tissue.

In initial attempts at adaptation, passage of mumps virus in mouse brain was not initially successful, either with infected amniotic fluid or with supernatant fluid from the first 10 tissue culture passages. Ability of mumps virus to survive for 12 days in suckling mouse brain suggested, however, that with further propagation in mouse embryo tissue culture, mouse adaptation might be achieved.

Adaptation to growth in suckling mice developed progressively as virus from the 10th, 15th and 20th passage tissue cultures was assayed. Although tissue culture led to a strain of mumps virus which would multiply consistently in suckling mouse brain, this strain has failed so far to develop obvious pathogenicity. This lack of virulence may reflect the fact that the mouse embryos, cultivated in their entirety, consisted of many tissues. Mumps virus propagated in such media would not, necessarily, develop specific affinity for brain tissue, as encountered in *in vivo* transfers. When a strain of mumps which had caused illness and death in suckling hamsters was used in mice, pathogenicity, not encountered initially, appeared suddenly in the 6th passage. It became permanently established when mice were carefully selected for harvesting. In reviewing these experiments, it seems possible that tissue culture actually attenuated the intracerebral virulence of mumps virus for mice. Mumps virus as freshly isolated from its human host is naturally virulent for suckling hamsters(1) and apparently this natural virulence can be carried over into mice. Another point of interest is that ability of virus to multiply in brain tissue seemed to have no relation to its pathogenic effects. Thus in suckling mice originally inoculated with hamster brain containing virus and developing severe illness, the amount of virus recoverable in eggs appeared no greater than in those mice of the tissue culture series which never became ill.

As mumps virus from any source was progressively passed in mouse brain it appeared to lose much of its adaptation to embryonated eggs, as indicated by the lesser number of eggs becoming infected and the lowered hemagglutinin titer of infected amniotic fluids. Second egg passage, however, would fully restore high hemagglutinin titers. Although factors may have been involved other than loss of egg adaptation, these results suggest that mumps virus, when propagated for a sufficient time in mouse brain, resembles more closely the virus as found in man. Thus in the course of making virus isolation from human saliva and spinal fluids(11), it was observed that although little or no hemagglutination appeared in amniotic fluids on first egg passage, such fluids would become

strongly positive on 2nd and 3rd egg passages.

In continuing experiments, efforts are being made to develop a variant of mumps virus which will be more uniformly fatal for suckling, and if possible, for adult mice. A development of such virulence, if it could be achieved, should make it possible to perform neutralization tests in mice. As described above such tests are already under study.

Summary. 1. Mumps virus has been carried through 20 passages in mouse embryo tissue culture in the course of which it developed a progressive ability to grow in brains of suckling mice, without apparent illness of the animals. 2. Another strain of mumps, after passage in brains of suckling hamsters, also multiplied readily in the brains of suckling mice, leading to illness and, in a small proportion, to death on continued passage. 3. Practical and theoretical implications of this adaptation of mumps virus to mice have been discussed.

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Occurrence of Coxsackie Virus Complement Fixing Antibodies in Sera of Normal Monkeys.* (19669)

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In the course of investigations regarding Coxsackie virus complement fixing (cif.) antibodies in monkeys, it was found that a homologous hyperimmune Leon type poliomyelitis monkey serum manifested a high titer (1:64) for one of the Coxsackie virus (C virus) prototype antibodies (Easton-10)(1). The possible reasons for its presence in a specific poliomyelitis monkey serum seemed to warrant additional experimentation.

Materials and methods. Sera. Homologous hyperimmune monkey sera were employed as follows: the Petry strain (Leon type poliomyelitis) and the Brunhilde strain were received from Dr. Jonas Salk; Ilda and Stoddard strains (Brunhilde types), as well as MV and MEF1 strains (Lansing types) were received from Dr. Louis Gebhardt; and the Lansing strain from Dr. Robert Ward. Hyperimmune monkey and mouse sera prepared in this laboratory for the FA strain of murine encephalomyelitis virus were also employed, as were sera from monkeys hyperimmunized with the Texas-1 type of Coxsackie virus (Monkey 4197) using infant mouse brain as immunizing agent, and with the Easton-2 (Dalldorf's type 1) Coxsackie virus (Monkey 4766) using muscle-bone suspensions from infected infant mice as antigen. Specific hyperimmune Coxsackie virus mouse sera were prepared for all types of antigens used in the c.f. tests (see below)(2). Sera were obtained from 19 rhesus (*Macaca mulatta*) and 15 cynomolgus (*Macaca irus*) monkeys which had been in the laboratory for periods of time varying from 1 to 43 days. They had been uninoculated prior to bleeding. Ten of the rhesus monkeys were bled at 4- to 7-day

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