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Received July 8, 1957. P.S.E.B.M., 1957, v96.

## Cataracts Following Mumps Virus in Early Chick Embryos.* (23437)

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Clinical reports dealing with the effect on the human fetus of German measles (rubella) in the mother, stressed the occurrence of cataracts along with heart damage, deafness, mental retardation and other defects. Other viruses have been suspected of being etiologically related to congenital defects and mumps virus has featured prominently among reports of such cases(1,2). Experimental work on the developing chick embryo in this laboratory has shown that infection with Newcastle disease and influenza A viruses resulted in specific damage to the developing lens, the extent depending on method of inoculation, stage of development of the embryo and virus titer of the inoculum(3-7). While these effects were definite and have been amply confirmed, these viral infections in the embryo were lethal within 48 hours. More recently, inoculation of the developing chick embryo with the Enders strain of mumps virus produced effects which were compatible with survival of many of the embryos for 3 to 15 days after inoculation and prominent in the results was the presence of cataracts.

*Materials and methods. Virus preparations.* The Enders strain of mumps virus, obtained from the American Type Culture Collection, was inoculated in $10^{-3}$ dilution into the allantoic cavities of 8-day chick embryos. The infectious allantoic fluids, harvested after incubation at 35°C for 7 days, served as stock virus suspension. In order to assure a high titered preparation, the fluid from each egg was harvested separately and tested for hemagglutinating activity at 1-100, 1-500 and 1-1000 dilutions. Much variation was observed, ranging from no hemagglutination to a titer of 1-1000. Fluids showing titers of 1-500 or more were pooled and dispensed in ampoules for storage at −70°C. For inoculating into early chick embryos the suspensions were diluted in buffered saline (pH 7.2) to the required infectivity. In some preparations 500 units of penicillin and 100 $\mu$g of streptomycin per ml were added to control bacterial growth. However, earlier mortality was noted when antibiotics were added to the mumps virus inoculum. In most instances, therefore, no antibiotic was added so as to eliminate the possibility that changes in the embryo were the result of a possible synergistic action of antibiotics and virus.

*Infectivity titrations.* Infectivity titers were determined by inoculating 10-fold dilutions of the virus suspensions into the allantoic cavities of respective groups of 8-day-old embryos. These were tested after 7 days for virus growth as indicated by hemagglutinability of the allantoic fluids. The $ID_{50}$ was calculated by the method of Karber(8). Young embryos to be tested for rise in virus titer were harvested whole, and ground in a Ten Broeck tissue grinder. A portion of tissue was then measured by volume and sufficient

* This investigation was supported by a research grant from the Microbiological Institute, Public Health Service.

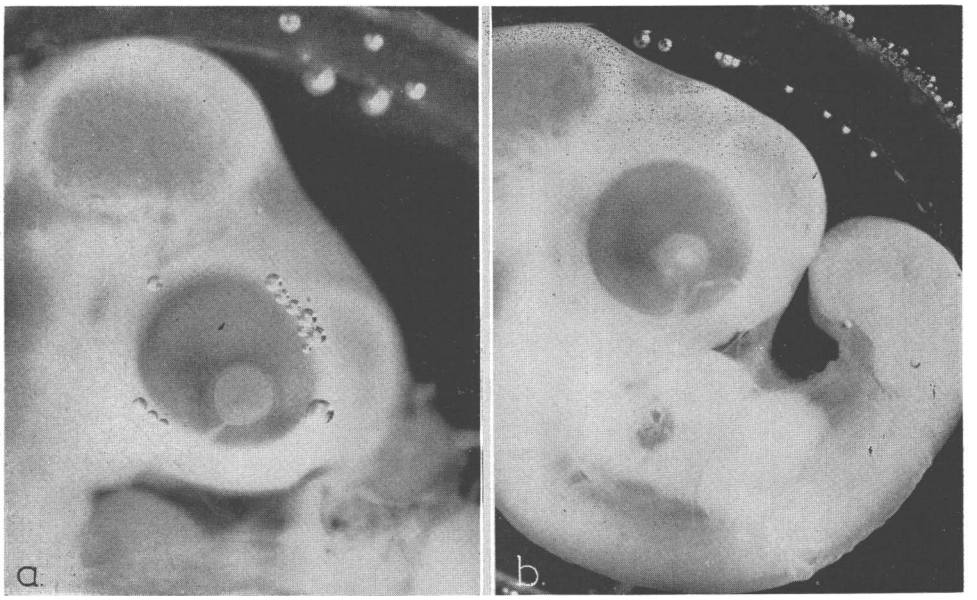


FIG. 1. a. Optic cup and lens of a control chick embryo of 5 days incubation. b. Lens opacity in a 5 day chick embryo inoculated 3 days previously with mumps virus.

saline added to make the initial 1-10 dilution.

*Inoculation of embryos.* Eggs incubated in a forced draft incubator at 98-99°F for approximately 48 hours were in stages of development from 10-14 according to Hamburger and Hamilton(9). Such embryos were prepared with openings and inoculated under the vitelline membrane and over the blastoderm (4) with 0.05 ml amounts of virus or control suspension. *Controls.* Embryos of the same incubation settings were inoculated with normal allantoic fluids in dilutions equal to those of the experimental groups. *Examination of embryos for abnormalities.* Embryos were examined daily under 24 power magnification by observation through the shell openings. Since development of the membranes obscured the view of the older embryos, in some experiments the embryos were harvested at appropriate intervals after inoculation for direct examination in the unfixed state.

*Experimental procedure and results.* Preliminary studies showed that embryos of 48 hours incubation which received $10^{5.7}$ to $10^{6.9}$ $ID_{50}$ of mumps virus, lived from 1-15 days after inoculation. Observation during the first 2 days revealed a few slight axis twists and some retardation of the amnion in the mumps infected group. By the third or fourth day, definite retardation of the amnion in the infected group was observed and permitted a view of many embryos which otherwise would be obscured by normal development of the membranes at this age. The developing crystalline lens of a significant number of these embryos showed areas of dense white opacity, centrally or excentrally located (Fig. 1). On daily observation, no regression was ever observed, the cataracts tending rather to increase in size. Experiments in which surviving embryos were harvested on the fifth day postinoculation, showed that the cataracts occurred either bilaterally or unilaterally, the unilateral ones involving chiefly the right lens. Other developmental deviations appearing about the same time included failure of the lower body axis to turn onto the left side resulting in mild abnormalities of axial torsion and in a "spraddling" of the lower limb buds on the yolk sac. Of 104 embryos receiving mumps virus, 60 lived 3 days or longer and in 46 embryos cataracts were observed grossly *in vivo*. Of 80 control embryos, 71 survived 3 days or longer and were harvested at intervals up to the fifteenth day after inoculation which was the longest time that a mumps-inoculated embryo survived. None showed cataract. In some of the longer surviving mumps-

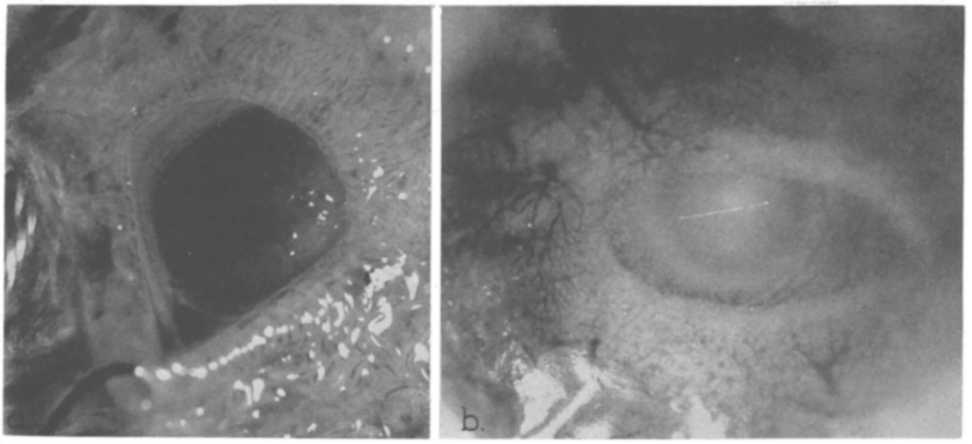

FIG. 2. a. Lens of a control chick embryo of 17 days incubation. b. Cataract in a 17 day chick embryo inoculated 15 days previously with mumps virus.

inoculated embryos there was evidence of other damage, such as irregularity of pigmentation of the choroid layer of the optic cup, hemorrhage into the lens and retarded development of the lids and skin around the eye, as well as a general retardation of feathering. The 2 longest surviving embryos died at 12 and 15 days after inoculation. Both embryos were very small, being approximately half the size of controls, and both showed cataract (Fig. 2).

Microscopic examination of lenses showing cataract at 3 days and 5 days after inoculation revealed extensive vacuolization and necrosis of the developing lens fibers (Fig. 3).

*Effect of virus titer of inoculum.* Embryos receiving $10^{3.8}$ $ID_{50}$ or less virus did not show the severe amniotic retardation or the axis abnormalities which were characteristic of embryos receiving the larger amounts of virus. Data presented in Table I show that the incidence of embryos with cataract decreases with dilution of the virus inoculum. Since the embryos were harvested for examination on the fifth day after inoculation the results of these studies are comparative.

TABLE I. Production of Cataracts by Mumps Virus in Chick Embryos of 48 Hours Incubation.

| Inoculum | Eggs inoc. | Embryos surviving 5 days post-inoc. | Surviving embryos showing cataract |
|---|---|---|---|
| | | | % |
| Mumps $10^{5.8}$ $ID_{50}$ | 78 | 33 | 28 (85) |
| " $10^{3.8}$ $ID_{50}$ | 45 | 31 | 10 (32) |
| " $10^{1.8}$ $ID_{50}$ | 45 | 37 | 2 ( 5) |
| Heated mumps* | 43 | 33 | 0 |

* Mumps suspension of titer $10^{5.9}$ was heated one hr at 60°C.

*Increase of virus titer in affected embryos.* Groups of embryos of 48 hours incubation, inoculated with $10^{5.8}$ $ID_{50}$ of virus, were harvested at 3 hours after inoculation and thereafter at daily intervals. Hemagglutination tests of the ground tissues showed an increase in titer from less than 1-40 at the 3 hour interval to a titer of 1-320 at the 2-day interval. Infectivity titrations on the ground tissues also showed an increase in virus titer up to the second day after inoculation, at which time a titer of $10^{6.7}$ $ID_{50}$ per 0.5 ml was obtained, a 16-fold increase over the titer obtained at the 3-hour interval. Thereafter the infective titer dropped to $10^{5.3}$ where it remained for the next 3 days. The decline in titer was not necessarily due to a decrease in virus production. Since the tissues of the embryo are growing rapidly during this period, the apparent decline in virus titer may have been due to a change in ratio between tissues not directly accessible to the virus, *i.e.*, mesodermal and entodermal tissues, and those directly exposed to the virus under inoculation procedures employed, *i.e.* ectoderm and organs differentiating from it at the time of inoculation. A change in susceptibility of various tissues as differentiation proceeds could also be a factor. It is clear, however, that multi-

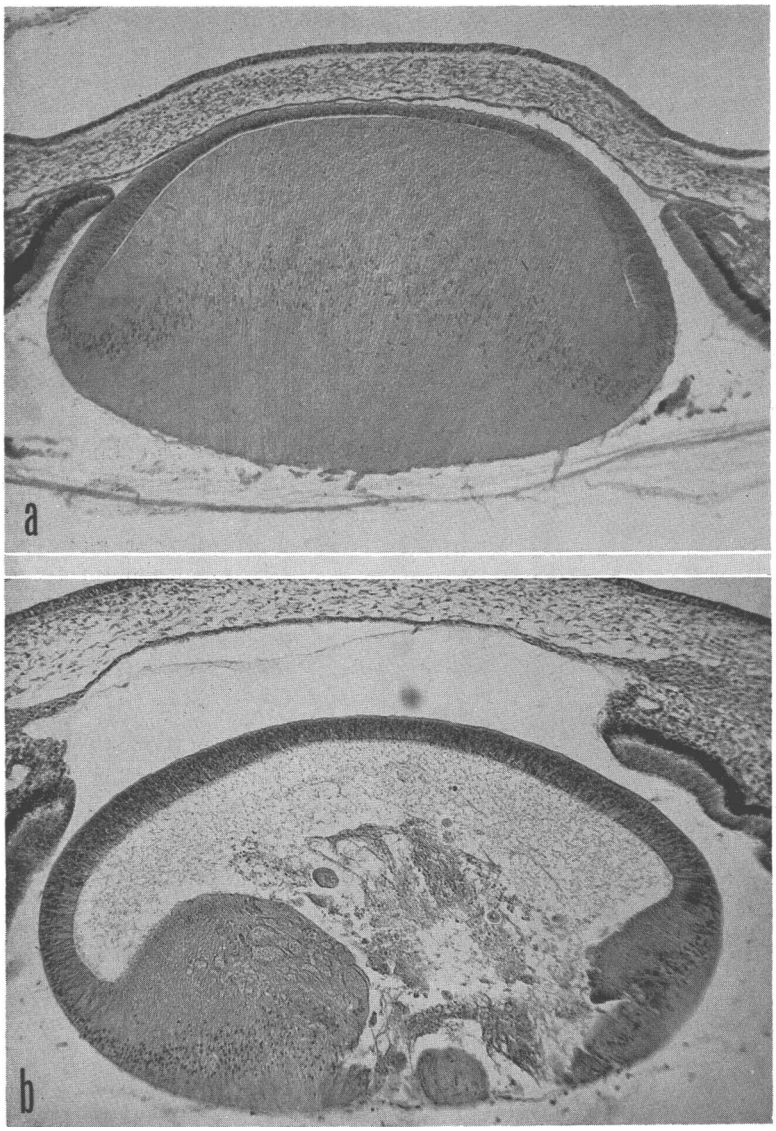


FIG. 3. a. Microscopic section of normal lens of a 7 day chick embryo. b. Microscopic section of a lens showing cataract in a 7 day embryo inoculated 5 days previously with mumps virus.

plication of the mumps virus occurs in chick embryos of this age even after inoculation of high titered virus inoculum. This is of interest since such a high titer of virus appears to be needed for optimal yield of defective embryos.

*Effect of heat inactivated virus.* An allantoic fluid suspension of mumps virus diluted in buffered saline to contain $10^{5.8}$ $ID_{50}$ per 0.05 ml, was divided into 2 portions, one remaining untreated while the other was heated 1 hour at 60°C. The preparations were then inoculated into respective groups of 48-hour embryos. Table I shows that heating destroyed the capacity of the virus to produce cataracts. No virus was recovered from the heated preparation when it was inoculated into the allantoic cavity of 8-day embryos.

*Discussion.* A variety of defects has been reported following virus infections of the early chick embryo, ranging from axis twists and collapsed encephalon following influenza A

(10,11), herpes and vaccinia(12) to specific defects such as absence or retardation of such organs as the lens, auditory vesicles or caudal neural tube following Newcastle disease and influenza A viruses(3-7). Earlier reports of the effects of mumps virus in early chick embryos(10) described no characteristic defects but reported a slight increase of various nonspecific changes of types commonly observed in the controls. The finding of cataracts and other defects in the present study may be accounted for by a difference in properties among strains of mumps virus, or may be due to variations in experimental technics employed.

It is of interest to observe that, in this laboratory, three viruses, all members of the myxovirus group, produced quite different effects when inoculated in high titer in young chick embryos under similar experimental conditions. Influenza A, within 24 hours, produced axis twists and collapsed encephalon as well as specific changes in development of the lens, auditory vesicles or neural tube. Newcastle disease, within 24 hours, produced only specific changes in the lens, auditory vesicles and neural tube. Mumps, however, inoculated in equally high titer produced no grossly observable defects within 24 hours. Only after 72 hours were mild axis twists and lens opacities observed. Although these different effects may reflect fundamental differences in cell-virus relationships, the slower growth of mumps virus, as observed in older embryos, may constitute another factor. A review of the results of virus-induced defects in chick embryos indicates that viruses producing the most severe defects also produce rapid death when inoculated into early embryos. On the other hand, the mumps virus, producing relatively mild defects, permits much longer survival of the embryos. This is in agreement with the impression that, in the human, the more virulent maternal infections tend to produce early abortion, whereas milder infections may permit survival of a congenitally defective infant.

The similarity of ocular changes produced by the mumps virus in the chick embryo and those observed with rubella infection in the human subject, suggests that further studies at the microscopic level be carried out in the chick embryo to ascertain whether the heart, brain and auditory systems are also damaged, as has been reported in the rubella syndrome.

*Summary.* Mumps virus, inoculated into embryonated chicken eggs of 48 hours incubation, produced a high percentage of cataracts in embryos surviving from 3 to 15 days after inoculation. Other defects observed included general growth inhibition, retardation in development of the amnion, mild axis twists, retardation of feathering, defects of the choroid layer of the optic cup and retarded development of the lids and skin around the eye. A decrease in the percentage of embryos showing defects was directly related to a decrease in the titer of the virus inoculated. Virus inoculated into the young embryos was shown to increase in titer over a period of 2 days after inoculation and was recoverable in high titer over a period of at least 5 days. Heat-inactivated mumps virus produced no cataracts or other defects.

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Received July 8, 1957. P.S.E.B.M., 1957, v96.