Fusion of Mouse Egg Cells Induced by Newcastle Disease Virus (36102)

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Ever since Müller (1) discovered multinucleate cells in 1838, there has been much speculation about their origin, function, and significance. Multinucleate cells have been observed in normal osseous and placental tissues, as well as in tumors and virus-induced lesions (2). In recent years, *in vitro* studies have been employed utilizing viruses for fusion of cells of the same or different species for studying multinucleation, or polykaryocytosis (3, 4). Some viruses can readily cause fusion and cytotoxic effect on certain types of cells, while other viruses are less effective (5). The present paper reports on the fusion of dissociated mouse blastomeres, or egg cells, which were cultured in the presence of live Newcastle disease virus (NDV).

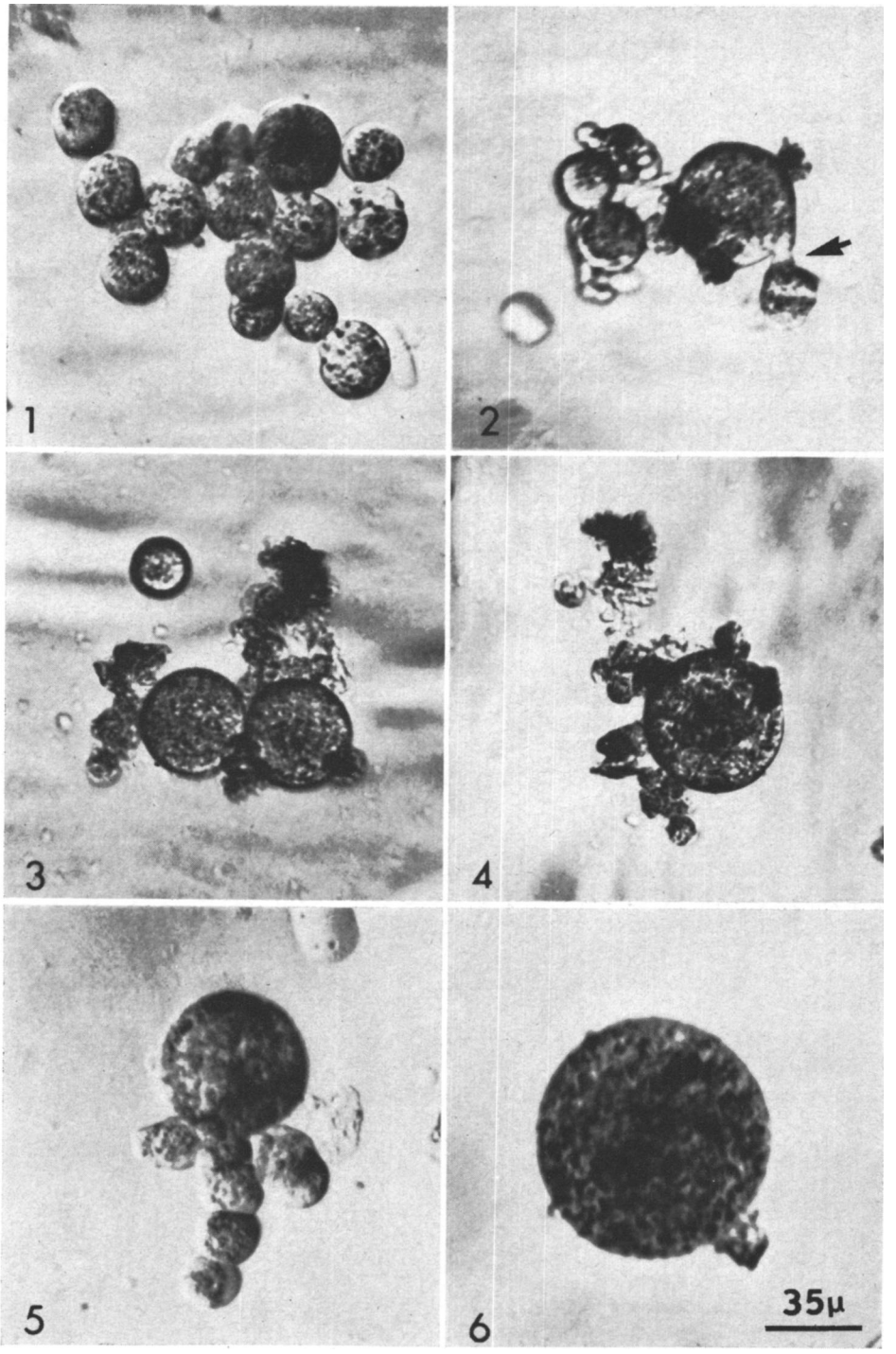
Materials and Methods. Female mice of black agouti (C3H/HeJ) or Bagg albino (BALB/c) strains were induced to ovulate and mate by injection of pregnant mare's serum and human chorionic gonadotropin (6). The fertilized eggs at 8–16 cell stages were flushed from the genital tract of these females at about 60 hrs after expected ovulation and mating. The zona pellucida of the eggs was ruptured and their blastomeres were dissociated by sucking the eggs up and down in a fine pipette in the trypsinated citrate–chloride–albumin (CCA) solution (7). The freed, spherical blastomeres were washed in three changes of CCA solution without trypsin and then cultured in Brinster's sterile balanced salt solution (8) for fusion in the presence of NDV. Usually 10–15 dissociated blastomeres obtained from the embryos were put into an oil-covered microdrop of about 0.2 ml of the balanced salt solution (see Fig. 1) containing approximately 10^4, 10^3, or 10^2 plaque forming units (PFU) of NDV of the L-Kan 1948 strain, which was prepared according to a described method (9). The preparation was gassed with 5% CO_2 in air for 30 min and incubated at 36–37° for 24 or 48 hr. When the blastomeres fused they formed giant cells, which were pipetted out of the microdrop culture, deposited and air-dried on a slide, fixed with the aceto-alcohol, and then stained by the Giemsa solution.

Results. Table I shows that NDV can induce fusion of dissociated egg cells of the mouse in the droplet culture. Blastomeres cultured in Brinster's medium without NDV, formed aggregates (7), but fusion of the cells never occurred. After culturing in viral concentrations ranging from 10^4 to 10^2 PFU/0.2 ml, fusion of about 60% of the blastomere preparations appeared within 24

TABLE I. Induction of the Fusion of Dissociated Mouse Blastomeres in Culture Preparations by Live Newcastle Disease Virus.

Virus conc (PFU/0.2 ml)	Total no. of preparations	Fusion at: 24 hr		48 hr	
		No. of preparations	%	No. of preparations	%
10^4	25	15	60	20	80
10^3	85	48	56	65	76
10^2	62	40	64	49	79
0	36	0[a]	0	0[a]	0

[a] Only aggregations of the blastomeres were observed.



FIGS. 1–6. Formation of giant cells in oil-covered microdrop culture of dissociated mouse egg cells with NDV: (1) A group of 15 dissociated blastomeres just placed into the culture medium containing 10^1 PFU of the virus. (2) A giant cell, formed after 48 hr culture in presence of 10^4 PFU of the virus, was composed of approximately 8 blastomeres; one blastomere is in process of

fusion by intercellular bridging (arrow). (3) Two giant cells formed in a preparation being cultured for 24 hr; each giant cell was composed of about 5 blastomeres; the giant cells are in contact. (4) Same preparation as (3) after 48 hr culture; two giant cells fused again and formed a larger cell. (5) A giant cell formed of 8–10 blastomeres; some blastomeres attached to the giant cell and 4 of them lined up in tandem for fusion. (6) A giant cell formed of more or less 15 blastomeres in a microdrop containing 10^3 PFU of the virus within 24 hr of cultivation.

hr, regardless of the concentration of NDV. As the culturing continued for 48 hr, about 20% additional fusion occurred. Failure of remaining blastomeres to fuse appeared to be the result of scattering or early necrosis.

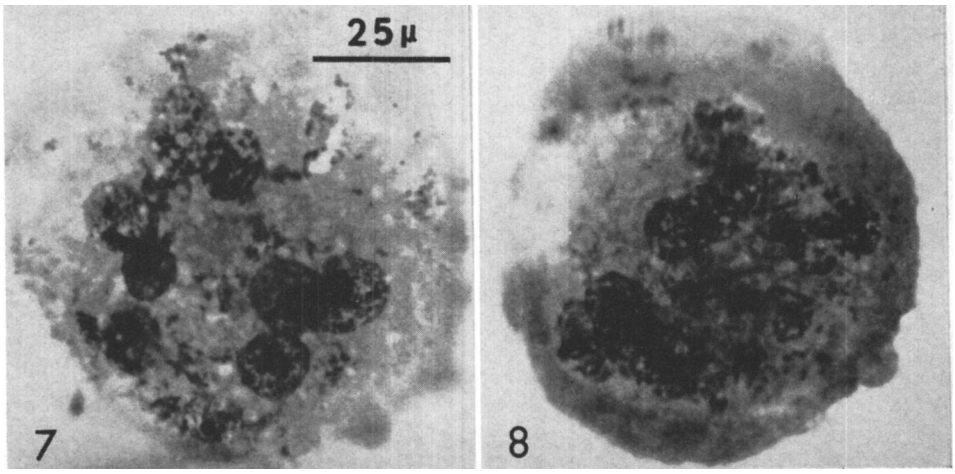
Some of the events which take place during fusion caused by NDV were examined further. The dissociated blastomeres aggregated in the droplet culture with their cell surface in contact, and fusion and formation of round giant cells occurred. Some blastomeres fused additively by surface contact or by intercellular bridging (Fig. 2). Two giant cells, after forming, appeared to join and fuse again (Fig. 3 and 4). Blastomeres, in rare cases, lined up in tandem to fuse into a giant cell (Fig. 5). As many as 15 blastomeres were observed to fuse into a giant cell in one preparation (Fig. 6).

Although deformed or necrotic blastomeres did not fuse, they may attach onto the surface of a giant cell (Fig. 3 and 4). Fused blastomeres appeared to have their cell membrane partially, if not entirely, incorporated into the new membrane of a fused giant cell. However, some membranous materials of a fused blastomere may be left out and adhered to the giant cells (Figs. 2 and 6).

In the stained preparation of fused blastomeres, the nuclei of a giant cell appeared to be gradually clumping in the course of time. As seen in the 24 hr culture preparations, the nuclei of the giant cell were scattered in an irregular fashion (Fig. 7); after 48 hr cultivation, all the nuclei contained in the cells were closely located (Fig. 8). Nuclear fusion could not be ascertained in the preparations.

The cytoplasm of the stained polykaryocytes appeared to be vacuolated or easily broken (Figs. 7 and 8). However, destruction of the giant cells due to the effect of NDV was not observed during manipulation.

Discussion. Adsorption and disruption of the cell membrane by viruses are known to



FIGS. 7–8. Multinucleation of giant cells with NDV. (7) A multinucleate giant cell obtained in a droplet culture of 15 blastomeres in presence of 10^4 PFU of the virus for 24 hr; at least 10 nuclei were seen clearly scattered in this cell. (8) A giant cell derived from a culture of 15 blastomeres with 10^2 PFU of the virus for 48 hr; all the nuclei were observed to be clumping or closely located within the cell; Giemsa stain preparation.

be the early steps for the process of cell fusion. After fusing, the infected membrane of fused cells has to repair its disruption sites, which may differ from cell types or viruses employed. NDV has been employed to induce fusion of various cells, but it has never been employed to fuse mammalian egg cells in the past. As we have observed in the fusion of mouse blastomeres by NDV in culture, some membranous materials have detached from the fused cells, NDV must have disrupted the membrane of the egg cells to a certain extent. However, the disrupted membrane must have been repaired for the formation of polykaryocytes.

Early embryonic cells of the mouse have also been induced to fuse by the popular technique of Sendai virus; the fused cells lysed in a short time (10). In our present study, NDV fused dissociated mouse blastomeres into multinucleated giant cells; the giant cells did not lyse for at least 48 hr. Therefore the effect of Sendai virus on the fusion of dissociated mouse egg cells does not appear the same as that of NDV, thus resulting in the difference in cellular disruption.

Few experiments have already begun employing the viruses for fusion of mammalian eggs for the study of embryological development. Some investigators (10, 11) were suspending the somatic cells and denuded mouse eggs for fusion in the presence of Sendai virus; the fused eggs usually became multinucleated. Using NDV (12), we have attempted to micropipette a single somatic cell, such as a rat lymphocyte or mouse erythroblast, into the perivitelline space for fusion within a zona-protected egg of the mouse; the NDV-treated cell first adhered to the vitellus and, in 3 hr of culturing, its nucleus fused into the periphery of the ooplasm. Such application of the virus method for cell fusion within the perivitelline cavity may lead to development of means for transfer of a nucleus for cloning of intact mammalian eggs. NDV appears to be a suitable virus for use in such studies.

Summary. Dissociated mouse blastomeres cultured in a microdrop of Brinster's medium containing live Newcastle disease virus ranging from 10^2 to 10^4 plaque forming units fused to form multinucleate giant cells. Regardless of the concentration of the virus employed, about 60% of the blastomere preparations fused in 24 hr and showed an additional fusion of 20% in 48 hr. The blastomeres fused additively by surface contact or by intercellular bridging. Membranous materials of the fused blastomeres appeared to be partially or entirely incorporated into the new membrane of a giant cell. A giant cell could be composed of up to 15 blastomeres. After 24 hr of culturing, the nuclei of a giant cell were usually seen in scattered fashion; and after 48 hr all the nuclei clumped within the cell. Under the conditions employed, neither mononucleate cells of giant size nor lysis of the polykaryocytes during manipulation was observed.

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