oculated with normal blood or in every culture treated with JM blood. The former hypothesis is supported circumstantially by (a) the perfect correlation between CPE and MD infectivity of inoculated DEF cultures and (b) the cell association of both the MD agent and the factor responsible for the induction of CPE. This evidence strongly suggests that the CPE is the direct result of infection of DEF with the MD agent.

The induction of CPE by MD agent in DEF cultures might be utilized for the *in vitro* assay of the agent. However, the inconsistent induction of infection in cultures with JM blood would appear to limit the usefulness of this system at present. Greater consistency was obtained in recent trials when cultures were inoculated with 1.0 ml of blood. However, the dose dependency of this response as well as many other important parameters have yet to be determined. Further studies are in progress to determine the potential usefulness of this system for the *in vitro* assay of the MD agent.

Of potentially greater importance, however, were investigations in which infected tissue cultures were employed in an attempt to elucidate the fundamental nature of the MD agent. Preliminary studies by Nazerian *et al.* (5) have established through electron microscopy the presence of a herpesvirus in JM inoculated DEF cultures with CPE and have circumstantially implicated this virus in the etiology of MD.

Summary. A focal cytopathic effect (CPE) was observed in duck embryo fibroblast (DEF) cultures 11–25 days postinoculation with blood from the JM strain of Marek's disease (MD). All cell suspensions from such cultures reproduced MD when inoculated into chicks, while all of the morphologically normal DEF cultures were noninfectious. The CPE and infectivity were maintained in DEF cultures for 182 days. Both the induction of CPE in cell cultures and MD in chickens required intact cells in the inoculum. These data indicate that the JM strain of MD was successfully propagated in DEF cultures and produced a characteristic CPE.

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Studies on the Etiology of Marek's Disease. II. Finding of a Herpesvirus in Cell Culture (32650)

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The etiological agent of Marek's disease (MD) is easily transmitted from infected to susceptible chickens by direct or indirect contact (1, 2). Although the agent apparently passed a 0.3 μ filter in one trial (3), this observation could not be confirmed (Biggs and Payne (4); H.G. Purchase, personal communication; R.L. Witter, unpublished data). Infectivity of cell suspensions varied directly with the number of viable cells, thereby suggesting a direct association of the agent with the cell (4). However, chromosome studies in-

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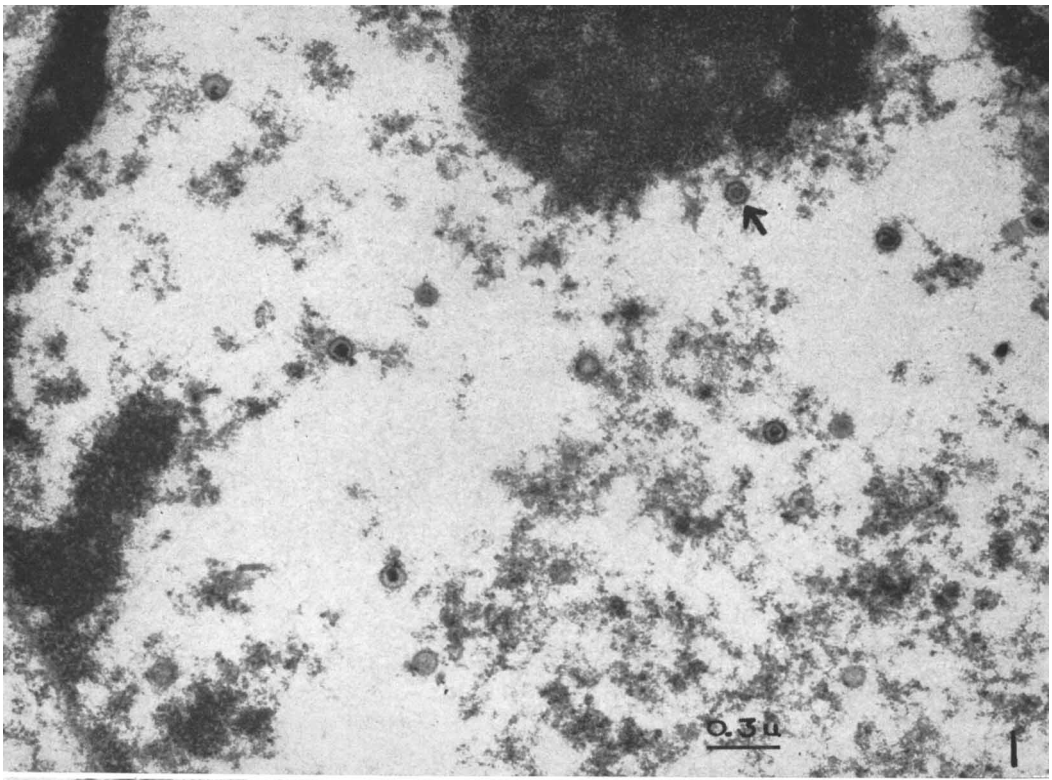
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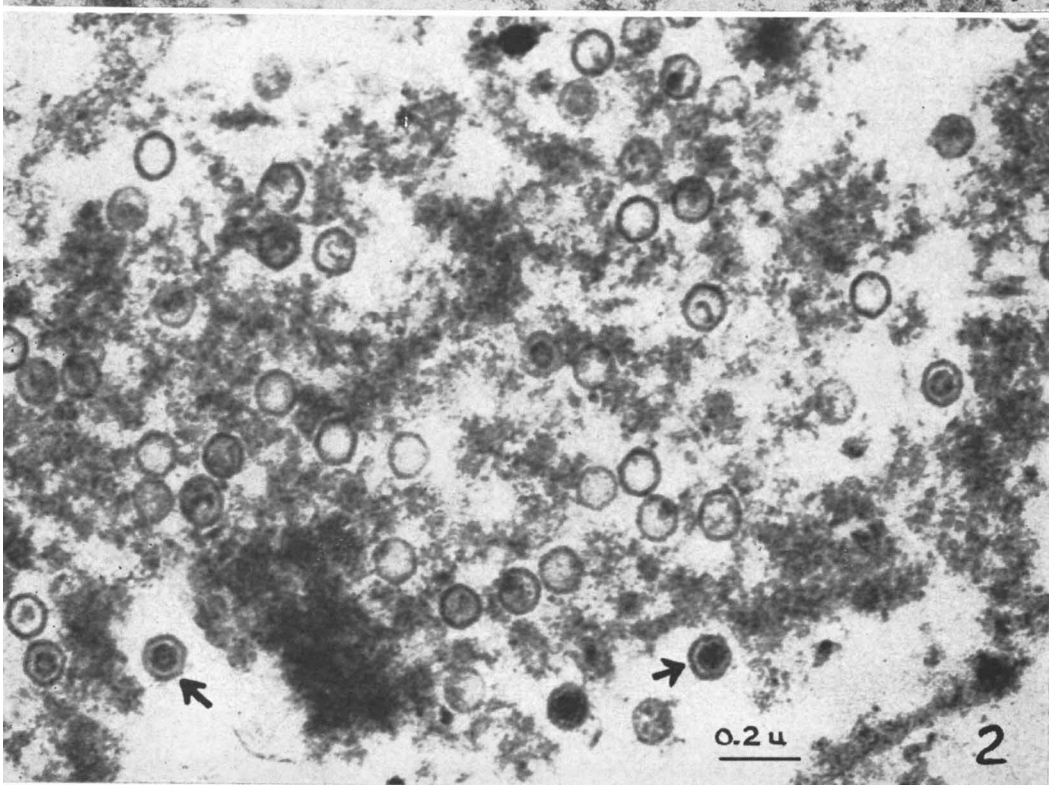
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FIG. 1. Electron micrograph of part of a cell from MD-infected DEF culture. Viral particles similar to naked herpesviruses (arrow) are seen in the nucleus. ×30,000.

FIG. 2. Naked herpes-like virus particles in a degenerating nucleus. Notice the hexagonal profile of the capsid (arrows). ×57,000.

dicate that experimental transmission with cellular inoculums is accomplished without transplantation (5). Reports on the morphological features of the agent are lacking. Therefore, pending its isolation and characterization, the fundamental nature of the MD agent must be considered unknown.

This report presents evidence for the involvement of a cell associated virus or its genome in the etiology of MD. This virus was recovered as a distinctive herpes-like particle in duck embryo fibroblast (DEF) cultures seeded with infectious blood. We have just learned of detection of similar particles in chick kidney cell cultures seeded with MD tumor suspension (6).

Materials and Methods. Source of the virus. Whole blood from birds inoculated with JM strain (3) of MD showing clinical signs of the disease was used as the source of virus. Inoculation of DEF monolayers with infectious blood and *in vivo* studies for determination of MD response to infected cultures are being reported simultaneously (7).

Passage of the virus in cell culture. The procedures and medium used were those already described (7). In addition, in one experiment undiluted spent medium from cultures with CPE, the same undiluted medium which had been frozen and thawed once and passed through 450 mμ Millipore filters, were added to separate fresh DEF monolayers and incubated at 38°C for 24 hours in a 5% CO_2 atmosphere. The medium was then changed and monolayers were incubated further. Light and electron microscopy were used to detect CPE and presence of virus particles.

Electron microscopy. Monolayers of DEF cultures with CPE, inoculated cultures that did not develop CPE, plus uninoculated control DEF cultures were scraped off the plastic petri dishes and fixed in 1% OsO_4 and embedded in Epon 812 (8). Thin sections obtained by a Porter-Blum MT-1 ultramicrotome were stained with uranyl acetate (9) and lead citrate (10) and were studied with a Siemens 1A electron microscope using double condensor illumination at 80 kV acceleration. Micrographs were taken at 10,000 instrumental magnification.

Results. Virus particles were found in the nuclei of cells from cultures showing CPE (Fig. 1) and the degenerating nuclei of such cells (Fig. 2). These particles had a hexagonal capsid with a mean diameter of 100 mμ and a central nucleoid of approximately 65 mμ in diameter. Empty capsids were also present. Cells with virus particles were grouped in preparations studied with the electron microscope and appeared to correspond to the focal areas of CPE seen with the light microscope. Virus particles were also observed in the cytoplasm and in the extracellular materials (Fig. 3), but they had the same morphology of naked intranuclear particles and lacked the outer envelope. The intranuclear origin and morphological features of these particles suggested them to be a herpesvirus.

The presence of CPE in DEF cultures, virus particles as revealed by electron microscopy, and the reproduction of MD in chickens by infected cultures are shown in Table I. Five out of seven cultures treated with JM blood developed CPE and all of the five cultures contained virus particles. In addition, two cultures seeded with spent medium of cultures with CPE developed typical foci and showed viral synthesis. Such spent medium when frozen, thawed, and passed through 450 mμ Millipore filter failed to reproduce the CPE in fresh DEF cultures and virus particles were not found in these cultures. All cultures showing CPE have caused a high MD response in inoculated chicks.

Virus particles were not detected in either the two inoculated cultures that did not develop CPE, in six uninoculated control cultures, or the cultures treated with noninfectious control blood. These cultures did not produce MD when inoculated into susceptible birds.

Discussion. Results reported here provide evidence that a herpesvirus or its genome is involved in the etiology of MD. This hypothesis is supported by the following obser-

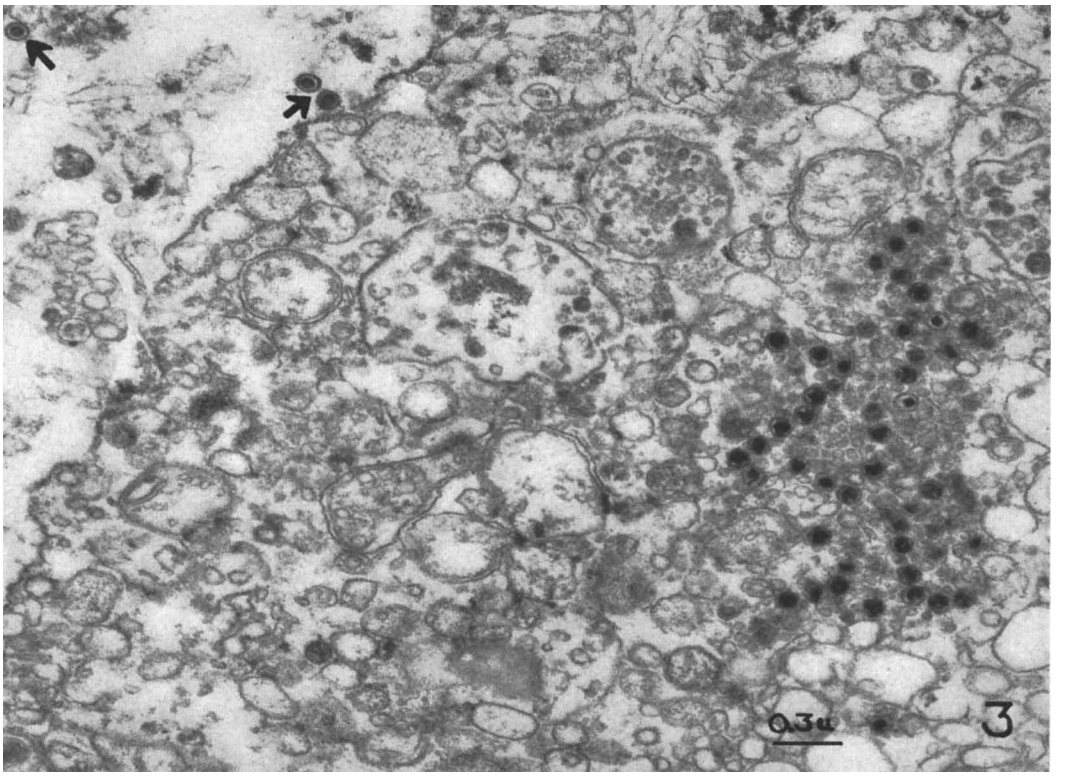

FIG. 3. Virus particles in the cytoplasm and in the extracellular materials (arrows) have the same morphology as the intranuclear virus particles (Fig. 1 and 2). ×30,000.

TABLE I. Relation between CPE, Infectivity, and Presence of Virus Particles in Duck Embryo Fibroblast Cell Cultures Inoculated with JM Blood.

Culture no.	Inoculum[a]	CPE	MD response in birds[b]	Viral synthesis
2–6	0.1 ml JM blood	+	+	+
4–1	2 ml spent media from culture 2–6	+	+	+
5–1	0.1 ml JM blood	—	—	—
5–2	0.1 ml JM blood	—	—	—
6–1	1 ml JM blood	+	+	+
6–2	1 ml JM blood	+	+	+
7–1	1 ml JM blood	+	+	+
7–2	1 ml JM blood	+	+	+
7–3	10 ml spent media from culture 6–1	+	+	+
9–1	10 ml spent media from culture 6–1, frozen, thawed and filtered	—	ND[c]	—
8–1	1 ml control blood	—	ND[c]	—
Controls (6)[d]	None	—	—	—

[a] Added to DEF monolayers in 100 × 20-mm Falcon plastic petri dishes. Inoculum for each culture came from different donor bird.

[b] More complete data on infectivity of these cultures are being reported simultaneously (8).

[c] Not determined.

[d] Some of these cultures were used as control for more than one inoculated cultures.

vations: (*a*) absence of CPE and viral synthesis in untreated DEF control cultures and cultures treated with normal blood, (*b*) the presence of infectivity only in cultures showing CPE and its absence in other cultures, (*c*) absence of any morphologically complete virus particle, and (*d*) the cell associated property of this virus which has also been noted in the experimental transmission of MD. Under most *in vivo* and *in vitro* conditions the virus appears to be hidden since extensive electron microscopy studies (Nazerian, unpublished data) of tumor cells and other tissues from MD infected chickens have not elucidated the nature of the agent or its morphological features.

Cytopathic effect of this virus in cell culture and its potentiality to produce MD in birds are both closely associated with infected cells. It is highly probable that unsuccessful attempts in passing the virus and its CPE using cell-free inoculum is due to the absence of complete virus in these infected cultures. Morphological evidences also indicate that the virus particles lack the outer envelope which has been shown to be essential for infectivity of herpes group of viruses (11). The virus found in the present experiments has many characteristics of the second group of herpesviruses exemplified by herpes zoster in which viral infectivity is closely associated with infected cells and the percentage of fully infectious particles with the other membrane is extremely low (12). The candidate virus of MD herein described probably contains DNA and is distinctly different from the leukosis-sarcoma group of viruses (13) which contain RNA and are similar to the myxoviruses.

While these observations explain the experimental transmission of the disease, their significance in the natural transmission of MD through the air (2) is as yet unknown. If these observations are in fact manifestations of the causative agent of MD, then a new concept becomes introduced in the field of avian viral oncology. These findings and nuclear projections in gonad tumor cells from MD infected birds (Nazerian, unpublished data) similar to the findings in Burkitt lymphoma (14) and malignant lymphoma (15) present a significant discovery and a possible model which can facilitate to a greater extent further research in neoplasia.

Summary. Duck embryo fibroblast (DEF) monolayer cultures seeded with whole blood from birds inoculated with the JM strain of Marek's disease (MD) and which showed a cytopathic effect (CPE), also contained intranuclear herpes-like virus particles. Complete virus particles with envelope essential for infectivity of herpesviruses were not found in either the infected cells or the extracellular materials of cultures containing such cells. All cultures showing CPE contained virus particles, and when inoculated into susceptible chicks caused MD. Cytopathic effect and viral synthesis could not be induced in fresh DEF cultures with spent media from infected cultures passed through 450 mμ Millipore filters. The perfect correlation obtained between CPE, presence of virus particles, and reproduction of MD by these cells suggests a cause and effect relationship and circumstantially implicates this virus in the etiology of MD.

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Cathepsin Activity of Liver and Muscle Fractions of Adrenalectomized Rats* (32651)

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The release of acid hydrolases from lysosomes has been associated with several physiological and pathological states. De Duve *et al.* (1) postulated that if lysosomal enzymes were to function in the normal economy of cell catabolism or renewal, mechanisms must exist whereby the release of these potentially injurious enzymes could be facilitated or retarded. *In vitro* and *in vivo* experiments have indicated that various physiological compounds may stabilize or labilize the lysosomal membrane.

Under several experimental conditions cortisone and cortisol have been shown to stabilize the lysosomal membrane. De Duve *et al.* (1) observed that these corticoids stabilized liver lysosomes in incubation at acid pH. Weissmann and Thomas (2) found that cortisone administered *in vivo* protects rabbits against the cartilage lesions of hypervitaminosis A; and lysosomes isolated from the tissues of animals treated with cortisone appear to be more resistant to incubation with labilizers.

If one of the effects of corticoids is to stabilize lysosomal membranes, it appeared of interest to study the results of a drastic reduction of corticoids in the organism, and thus our experiments were designed to measure lysosomal enzyme activity in liver and muscle tissues of adrenalectomized rats. In that adrenalectomized animals have a reduced food intake, other experiments were designed to study the influence of starvation on lysosomal enzyme activity.

Materials and Methods. Weanling male Long-Evans rats were maintained on Purina Fox Chow until they attained a body weight of 60–65 gm, at which time they were placed on experiment. A semipurified 20% casein diet (3) was fed *ad libitum* to all adrenalectomized rats and their intact controls. Other groups were pair-fed to the adrenalectomized rats.

Adrenal glands were removed under ether anesthesia through bilateral incisions in the lumbar region. Care was taken to remove the glands encapsulated with as much adherent fat as possible. In one series of experiments untreated adrenalectomized animals and their controls were sacrificed 5 days after operation. In another series the adrenalectomized animals were given 1% saline to drink, and sacrificed with their controls 10 days after operation. In a final series, intact animals were pair-fed 25% of the food intake of their intact controls.

Animals were sacrificed by decapitation. Liver and gastrocnemius muscles were rapidly excised and chilled in a sucrose solution. After rinsing the tissues in additional sucrose solution, the tissues were cut into small pieces with scissors, and prepared into a 10% (w/v) homogenate with 0.25 *M* sucrose containing 1 m*M* EDTA. The homogenization was done in chilled glass, conical, motor driven homogenizers with the pestle rotating about 1000

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