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EXPERIMENTAL INFECTION OF RABBITS WITH THE VIRUS OF INFECTIOUS BOVINE RHINOTRACHEITIS

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Summary.—Adult and newborn rabbits were infected with the LA strain of infectious bovine rhinotracheitis virus. Adult rabbits developed only mild, self-limiting conjunctivitis, but had microscopic inflammatory foci in the liver and adrenal glands. Newborn rabbits developed a severe, sometimes fatal, generalized infection with focal and diffuse necrosis of the liver and adrenal glands.

THE HERPES VIRUS associated with infectious bovine rhinotracheitis (IBR) is an important cause of infection of the respiratory, genital and nervous systems of cattle (Saxegaard, 1970). The virus grows well in tissue cultures of foetal bovine kidney (Timoney and O'Connor, 1971) and in organ cultures of nasal, tracheal and vaginal epithelium (Shroyer and Easterday, 1968) but, although experimental infection of pigs has been reported (Nelson, Mare and Glock, 1972), natural infection appears to be confined to cattle and no suitable experimental animal model for the study of this virus infection exists at the present time.

Several herpes viruses produce lesions when injected experimentally into rabbits and it seemed likely that in this respect IBR virus might behave like other members of the group. This paper describes experiments in which this possibility was tested.

MATERIALS AND METHODS

Virus stock.—The IBR virus used in these experiments was supplied by Dr W. C Lawrence of the Laboratory of Microbiology, School of Veterinary Medicine, University of Pennsylvania. It was derived from the LA strain of the American Type Culture Collection. It had been passaged at least 47 times in foetal bovine kidney cell cultures and 3 times in the Madin–Darby bovine kidney cell line. The titre was $1{\cdot}5 \times 10^8$ plaque-forming units/ml.

Experimental animals.—Adult rabbits weighing approximately 2 kg were used. They were of a stock laboratory type obtained from Huntingdon Farms, Pa. Non-pregnant and pregnant females were obtained. The latter were observed carefully at the end of pregnancy so that newborn rabbits were available for infection in the first few hours of life.

Infection.—Adult female rabbits were infected with 0·5–3·0 ml of virus suspension by the following routes, either singly or in combination: i.v., i.p., intra-conjunctival (conj). Intra-peritoneal and intra-cerebral routes were used for infection of newborn rabbits (between 1 and 10 days of age). Control animals were injected with the same volumes of uninfected minimum essential nutrient medium + 10% lamb serum.

Infected animals were observed carefully at short intervals. Moribund rabbits were killed by cervical dislocation. Complete post-mortem examinations were carried out and tissues fixed in Bouin's solution. Tissues were embedded in a paraffin/polymer mixture (Paraplast ®, Arthur H. Thomas, Philadelphia), 5-μm sections were cut and stained with haematoxylin and eosin (HE).

Some of the moribund newborn rabbits were anaesthetized by i.p. injection of pentobarbitone sodium. The abdomen was opened and 1-mm cubes cut from the surfaces of the liver and adrenals and fixed in a mixture of glutaraldehyde and paraldehyde (Karnovsky, 1965). After post-osmication the blocks were dehydrated and embedded in Araldite. Thick sections (1 μm) were stained with hot 1% toluidine blue in 1% borax for selection of areas suitable for electron microscopy. Thin sections were cut

with glass knives, stained with uranyl acetate and lead citrate, and were examined on uncoated copper grids in a Philips EM 201 electron microscope.

RESULTS

Infection of adult rabbits

The results are summarized in Table I. Only infection of the conjunctiva produced any untoward clinical effects. Between 6 and 11 days after infection there was hyperaemia and oedema of the conjunctiva, scleral congestion and swelling of the lids. The eyes of control rabbits remained normal. One rabbit died 2 days after infection; the cause of death was not determined. The remaining rabbits were killed at intervals between 6 and 29 days after infection. Histological examination revealed conjunctival and corneal inflammatory hyperaemia and oedema, with infiltration of connective tissues by neutrophilic leucocytes. In one rabbit killed 6 days after infection some of the corneal epithelial cells contained Type A intranuclear inclusions (Fig. 1). Gross lesions of the viscera were found only in the rabbit killed on Day 6. It had multiple punctate white foci throughout the substance of the liver. Histologically these foci contained fibrin, polymorphonuclear leucocytes and macrophages. The adrenal cortex of some rabbits contained multiple foci of coagulative necrosis; these were recognized only on histological examination. The foci contained a central mass of cell debris. At the junction with viable cortex damaged cells had fragmenting nuclei. Adjacent sinusoids contained thrombi of platelets and fibrin and there was a light scattering of polymorphonuclear leucocytes and macrophages around the necrotic foci. There was no evidence of infection in the concepta of injected does.

Infection of newborn rabbits

The results are summarized in Table II. Fifteen rabbits were infected and 7 of these died between 1 and 6 days after infection. Gross changes were seen only in rabbits examined up to 3 days after

TABLE I.—*Infection of Adult Rabbits with IBR Virus*

Rabbit	Routes of infection	Clinical signs of conjunctivitis (duration)	Killed (K) or died (D) after infection (days)	Lesions (see text)		
				Eye	Adrenal	Liver
1	i.v.; i.p.	—	D2	—	—	—
2	i.v.; i.p.; conj.	+ (Day 6)	K6	+	+	+
3	i.v.; i.p.; conj.	+ (Days 8–10)	K17	—	+	—
4	i.v.; i.p.; conj.	+ (Days 7–11)	K20	+	—	—
5	i.v.; i.p.; conj.	+ (Day 7)	K22	—	—	—
6	i.v.; i.p.; conj.	+ (Day 7)	K24	—	—	—
7	i.v.; i.p.; conj.	+ (Day 6)	K29	—	—	—
8	—	—	K10	—	—	—

TABLE II.—*Infection of Newborn Rabbits with IBR Virus*

Experiment	Age at infection (days)	Routes of infection	Killed (K) or died (D) after infection (days)	Focal coagulative necrosis in	
				Adrenal*	Liver
1	<2	i.p.	D(2); D(2); K(2)	0/3	0/3
	<2	i.p.	K(2); K(2); K(2)	0/3	0/3
2	2	i.p.	D(1); K(2)	0/2	2/2
3	3	i.p.	D(2); D(3)	2/2	2/2
4	7	i.p.	K(1); D(6); D(6)	2/3	2/3
5	10	i.p.	K(12); K(12)	0/2	0/2
	10	None	K(12)	0/1	0/1

* Number affected/number examined.

infection. There was slight fibrinous peritoneal exudation and the liver contained pale punctate foci of necrosis, up to 2 mm in diameter (Fig. 2). Histological lesions were found in the liver and adrenals up to 6 days after infection, and there was evidence of virus infection of cells in the wound at the injection site.

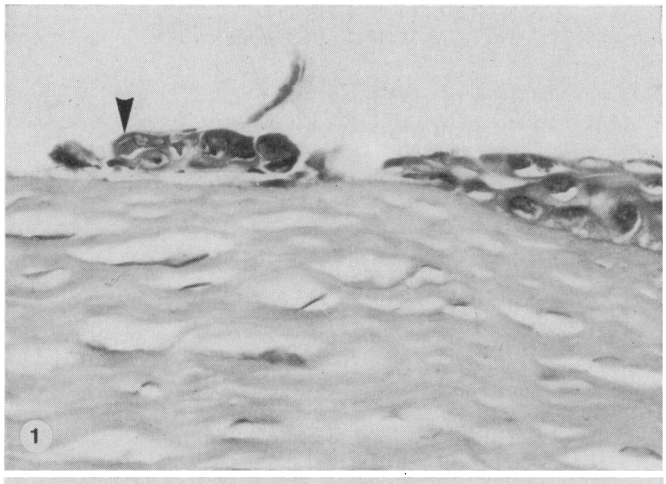
The earliest changes in liver consisted of focal or more diffuse degeneration of parenchymal cells (Fig. 3), which was manifested by cytoplasmic shrinkage and increased eosinophilia. Cells affected in this way frequently had margination of chromatin inside the nuclear membrane around a faint central intranuclear inclusion. More severe parenchymatous degeneration was represented by distinct focal and diffuse necrosis, with pyknosis, karyorrhexis and fragmentation of the cytoplasm. Parenchymal cells at the junction between the affected and normal tissue often contained eosinophilic Type A intranuclear inclusions (Fig. 4). The more advanced lesions contained cellular debris and polymorphonuclear leucocytes. There was acute fibrinous serositis of the hepatic capsule and adjacent portal connective tissues. Adrenal lesions were found in rabbits which died between 2 and 6 days after injection (Table II). The lesions consisted of microscopic foci of coagulative necrosis randomly scattered throughout the cortex. The lesions were essentially similar to those found in adult rabbits. Examination of the adrenals later than 6 days after infection revealed no focal necrosis, but randomly scattered foci of lymphocytes and foamy cells were frequently encountered throughout the cortex (Fig. 5).

Ultrastructural observations on infected livers

At low magnification the infected foci were immediately conspicuous because of the severe degenerative changes in the hepatocytes. Nuclei of these cells were enlarged. There was margination and clumping of nuclear chromatin around the nuclear membrane, leaving a large electron-lucent central zone in which only few chromatin granules remained (Figs. 6, 7). Small numbers of particles with a typical herpes morphology were present in the nuclei of these cells (Fig. 8). Many of the affected cells had, in addition, elaborate folding of the nuclear membrane. Cytoplasmic changes included swelling of mitochondria (Figs. 9, 10) and increased electron-lucency of the cytoplasm due to vesicular swelling (Fig. 10). Mature virus particles were found within cytoplasmic vesicles, and within extracellular spaces (Figs. 9, 10). Crystalline intranuclear arrays of virions, such as those seen with some strains of herpes simplex virus (Rabin *et al.*, 1968), were not seen. In sinusoids adjacent to infected parenchymal cell foci there were numerous thrombi consisting of fibrin and cell debris.

DISCUSSION

These observations indicate that histological lesions may occur in rabbits in the course of infection with IBR virus. No attempt was made to re-isolate the virus from the rabbits, but it is reasonable to assume that the lesions which developed were a direct result of infection with this virus since no similar lesions were found in uninoculated rabbits and ultrastructural examination of the lesions revealed numerous herpesvirus particles. In adult rabbits the only clinical effect was the presence of a mild keratoconjunctivitis. The ocular lesion in rabbits infected with this unadapted strain of virus was mild and appeared to resolve within 3 weeks. Conjunctivitis is one of the recognised lesions associated with IBR virus infection in cattle (Dawson *et al.*, 1962; Mohanty and Lillie, 1970). The latter group commented that clinical keratitis was not a feature of the ocular infection in cattle, but they did not examine the eyes histologically. The rabbit may be particularly suitable for experimental studies on the role of herpes viruses in ocular disease for herpes simplex virus produces lesions similar to those reported here when



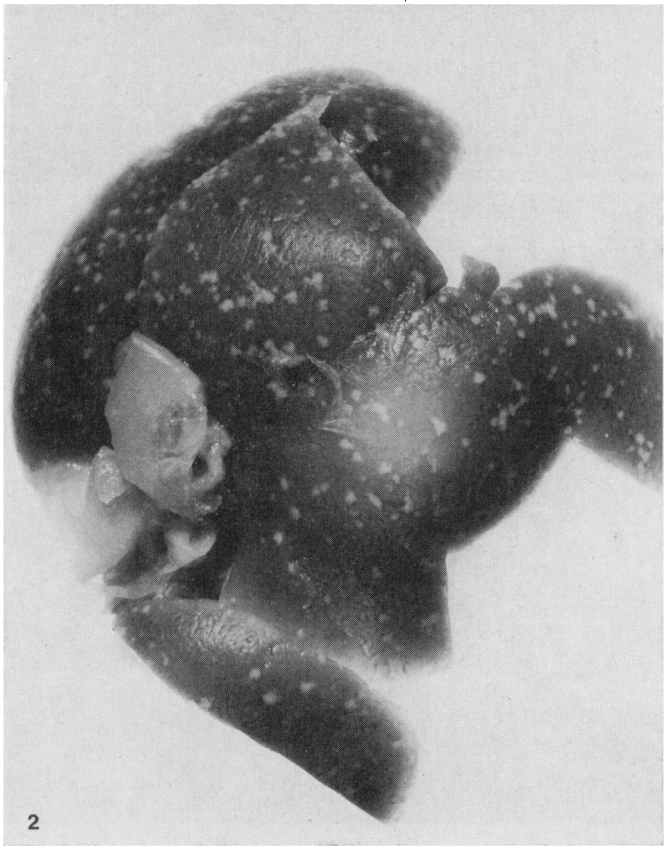
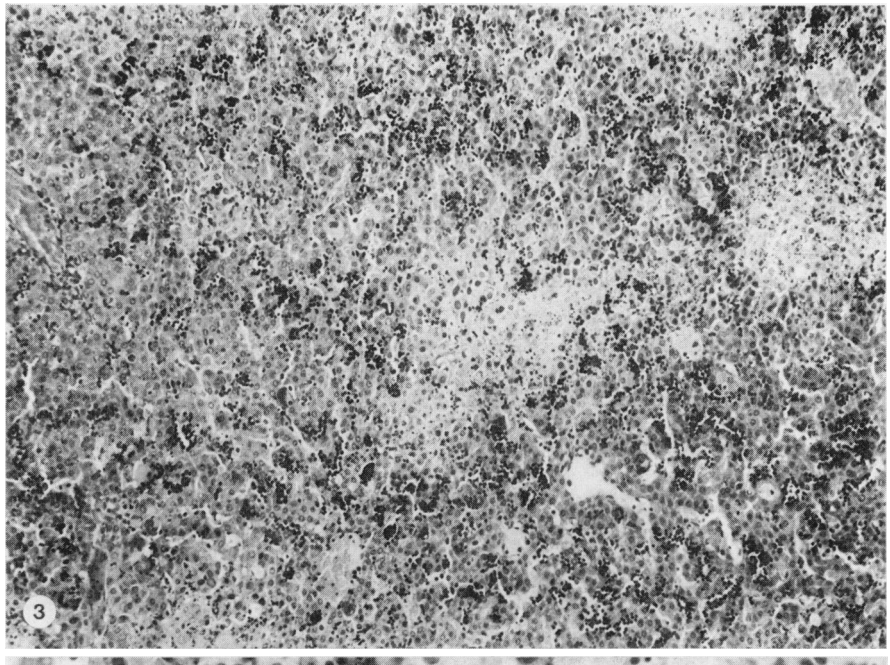


FIG. 1.—Cornea 6 days after IBR virus infection, showing epithelial ulceration and intranuclear inclusion (arrow). H. and E. ×375.

FIG. 2.—Liver from a young rabbit showing focal necrotizing hepatitis 5 days after IBR virus infection. ×1·5.



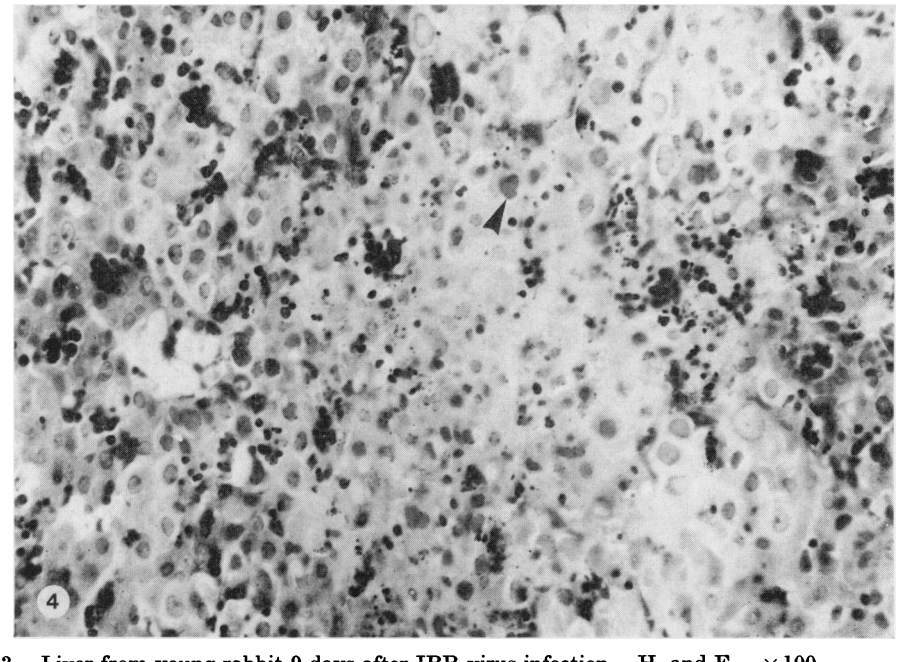


FIG. 3.—Liver from young rabbit 9 days after IBR virus infection. H. and E. ×100.
FIG. 4.—Liver from young rabbit 2 days after IBR virus infection. Parenchymal cell nuclei are enlarged and have beaded nuclear membrane. There is dispersion of nuclear chromatin, and intranuclear inclusion bodies are present in many cells (arrow). H. and E. ×240.

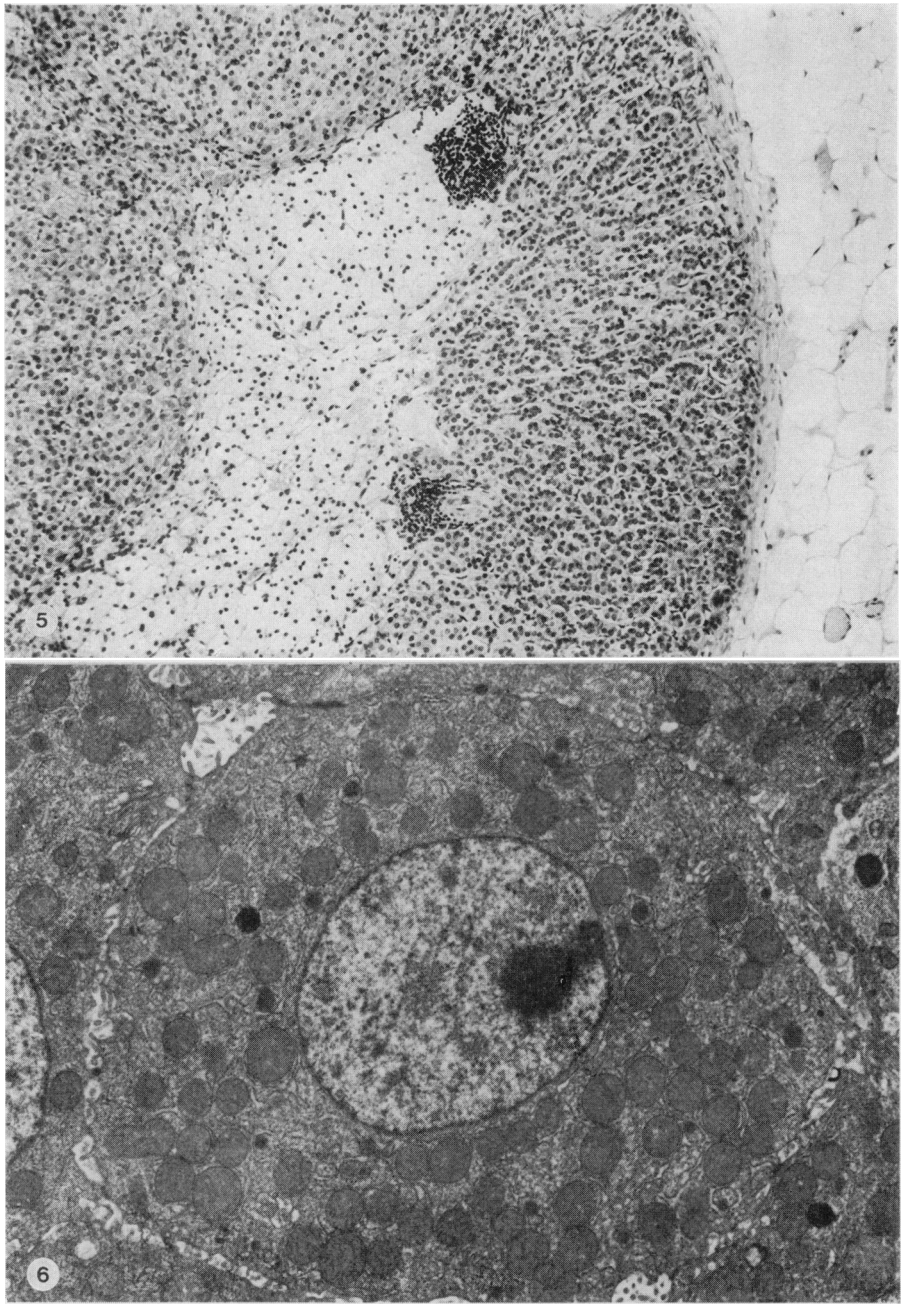
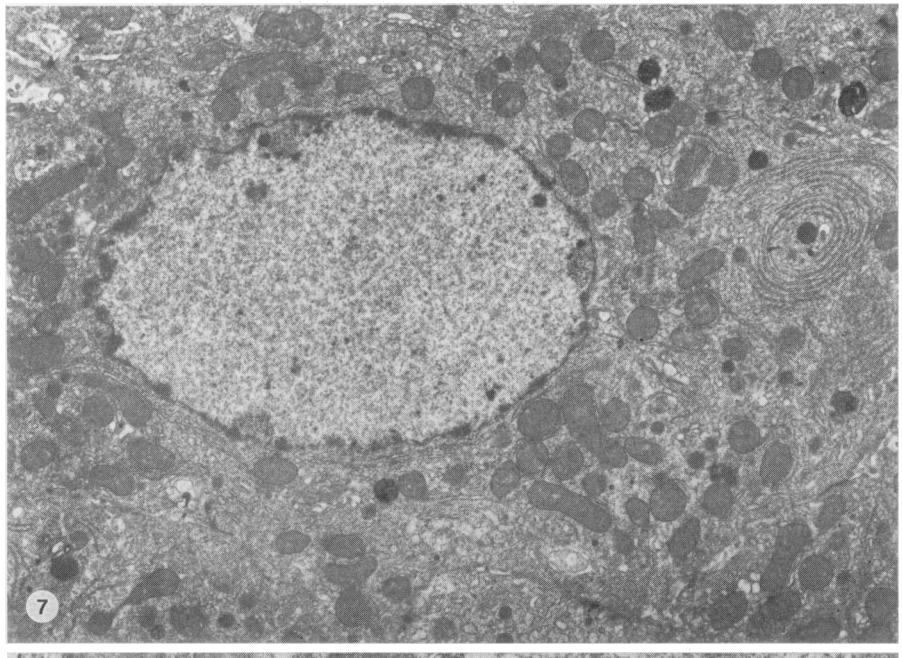


FIG. 5.—Adrenal from a rabbit 35 days after IBR virus infection, showing focal lymphocyte accumulation and " foamy cell " appearance of cortex. H. and E. ×100.

FIG. 6.—Liver. Electron micrograph (EM) of a parenchymal cell showing normal nucleus with dispersed nuclear chromatin and nucleolus. ×4800.



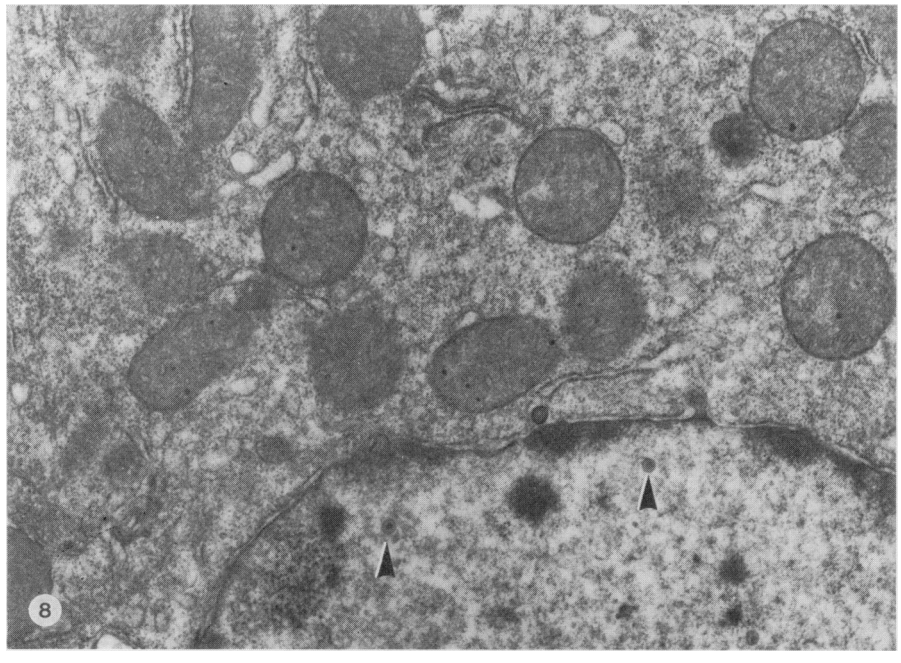


FIG. 7.—Liver EM. The nucleus is enlarged and has a crenated membrane. The nuclear chromatin is widely dispersed and clumped on the nuclear membrane. ×4800.

FIG. 8.—Liver EM. Herpes virus particles (arrows) are present in the outer part of the nucleoplasm. ×24,000.

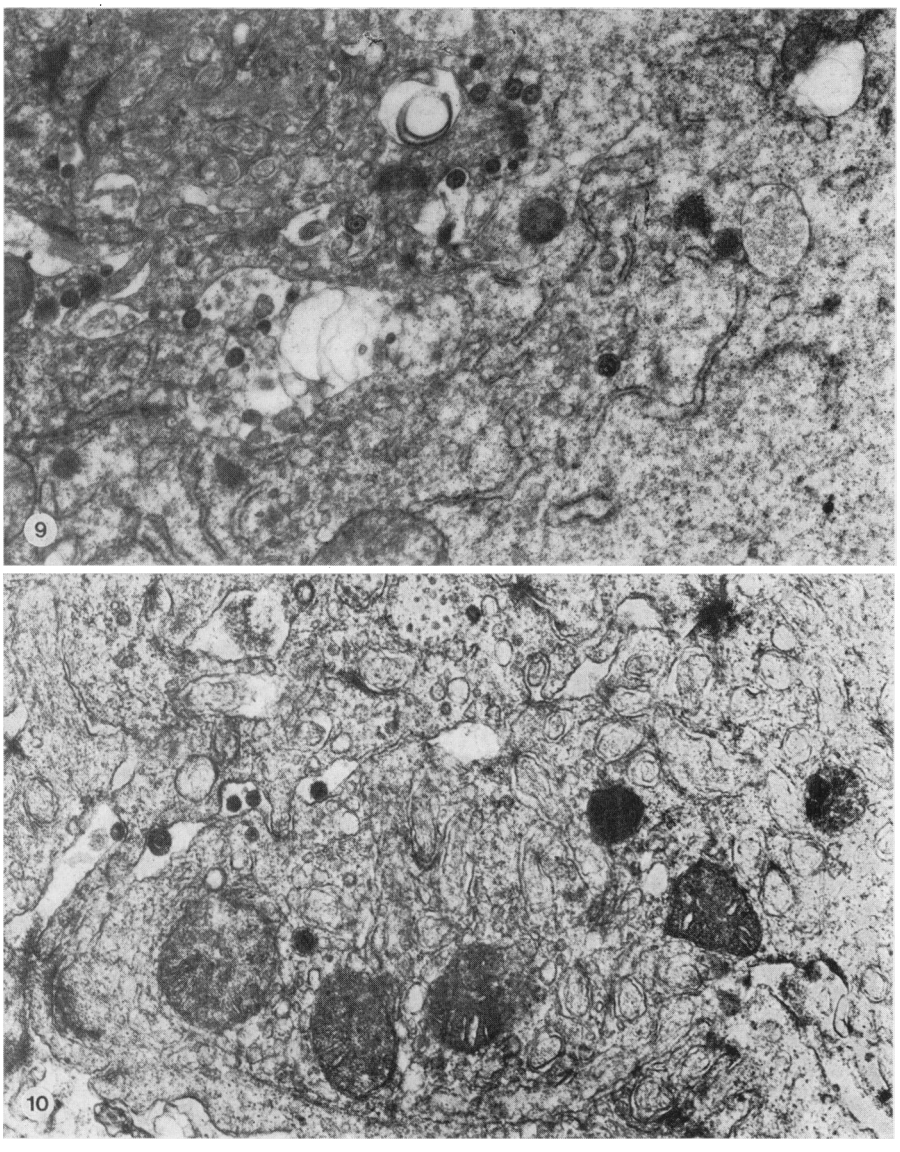


FIG. 9.—Liver EM. Herpes virus particles are in the cytoplasm of a parenchymal cell and within expanded vesicles. × 16,800.

FIG. 10.—Liver EM, showing herpes virus particles in intercellular space between parenchymal cells. × 24,000.

introduced into the eye (Oh, 1970; Oh *et al.*, 1972). The infection in baby rabbits was much more severe and extensive than in adults. In general the lesions in this experimental infection were those of a multifocal necrotizing process, which was most severe in liver and adrenals. The lesions are similar histologically to those described in aborted bovine foetuses from dams with IBR infection (Kennedy and Richards, 1964; Molello *et al.*, 1966). There are, however, two points of difference in that IBR inclusion bodies are relatively easy to find in rabbits, but the

lesions are less extensive than those seen in aborted calves. The results of attempts to infect rabbits at 10 days of age suggest that the apparent susceptibility of the neonate to IBR virus may be short-lived (Table II). The ultrastructural features of the hepatic and adrenal lesions are similar to those described in cultures of bovine kidney cells infected with IBR virus (Jasty and Chang, 1972; Jasty *et al.*, 1972). At the intact organ level the ultrastructural lesions also resemble those of herpes simplex hepatitis in mice (Rabin *et al.*, 1968) and equine rhinopneumonitis virus infection of hamster liver (Kapp, 1972). The results of the present work suggest that the baby rabbit may be very useful as an experimental host for studies on the pathogenesis of IBR infection.

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