Electron Microscopy of Equine Abortion Virus. (24171)

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That strain of equine abortion virus (EAV) which has been adapted to the Syrian golden hamster provides, together with this animal, an excellent biological system for a variety of studies of virus-host relationships. One of the many advantages of the system is the extreme rapidity with which the virus acts to produce a fatal disease in hamsters, invariably culminating in death of animals in 18 to 21 hours after inoculation. Thus, the time factor involved during the disease precludes many undesirable side effects, such as anorexia. Systematic and reproducible studies, both cytological and biochemical, may readily be made of the sequential changes which occur. The pathological picture, both gross and cellular, is unmistakable. These changes have been described elsewhere(1,2), but it is of interest to note here that substantially all the hepatic cells of infected animals contain well-defined intranuclear inclusions. Certain phases of investigations employing EAV have been hampered by the fact that the morphology of the virus, as well as the number and intracellular orientation of the elementary bodies, has been unknown. It has been difficult to interpret the observed sequential biochemical changes in the host cells(3). The question has been whether these changes are due principally to the obligate biochemical response of the host cells to the presence of the virus, or to the simple physical presence of large numbers of virus particles. The present study was undertaken with several purposes in view: to elucidate the morphology of EAV; to gain some knowledge of the number and site of the elementary bodies parasitizing the host cells; and to attempt electron microscopic visualization of the characteristic intranuclear inclusion bodies seen by light microscopy.

Materials and methods. Virus. Material from the 186th passage of EAV in hamsters comprised the inoculum. The virus and inoculation of animals has been previously described(2). *Electron microscopy.* Small portions of liver removed from ether-anesthetized animals were cut into small blocks and fixed by immersion for 2 hours in chilled 1% osmium tetroxide buffered by veronal acetate approximately to pH 7.4. Following dehydration in graded alcohols, the blocks were embedded in a mixture of 25% methyl-75% butyl methacrylate catalyzed with benzoyl peroxide, sectioned on a Porter-Blum (Servall) microtome and examined with an RCA Model EMU-2B electron microscope.

Results. The virus. Structures which were presumably virus particles or stages in development thereof were seen in several forms. These forms may be categorized as: (1) poorly defined "nucleus," with no outer membrane, as shown in (a) of Fig. 1; (2) double membrane with apparently no "nucleus," as exemplified by (b) in Fig. 1; (3) well-defined "nucleus," surrounded by a single membrane, as seen in (c) of Fig. 1; (4) dense "nucleus" surrounded by a double membrane, shown in (d) of Fig. 1 and 2. It should be noted that some of these apparently different forms may be due, in actuality, to the plane of sectioning and/or the level of focus. The structures were ovoid, and those possessing a double membrane (or halo) extended approximately 146 mμ in diameter across the long axis to the limits of the outer membrane. The inner membranes measured approximately 90 mμ across the long axis, and the dense central bodies, or "nuclei," averaged about 40 mμ in diameter. Compression by the microtome knife probably was responsible for the ovoid shape of the particles. The basis for considering the observed structures

* The authors express their gratitude to Sir MacFarlane Burnet for his interest and helpful suggestions and criticisms advanced during the course of this work.

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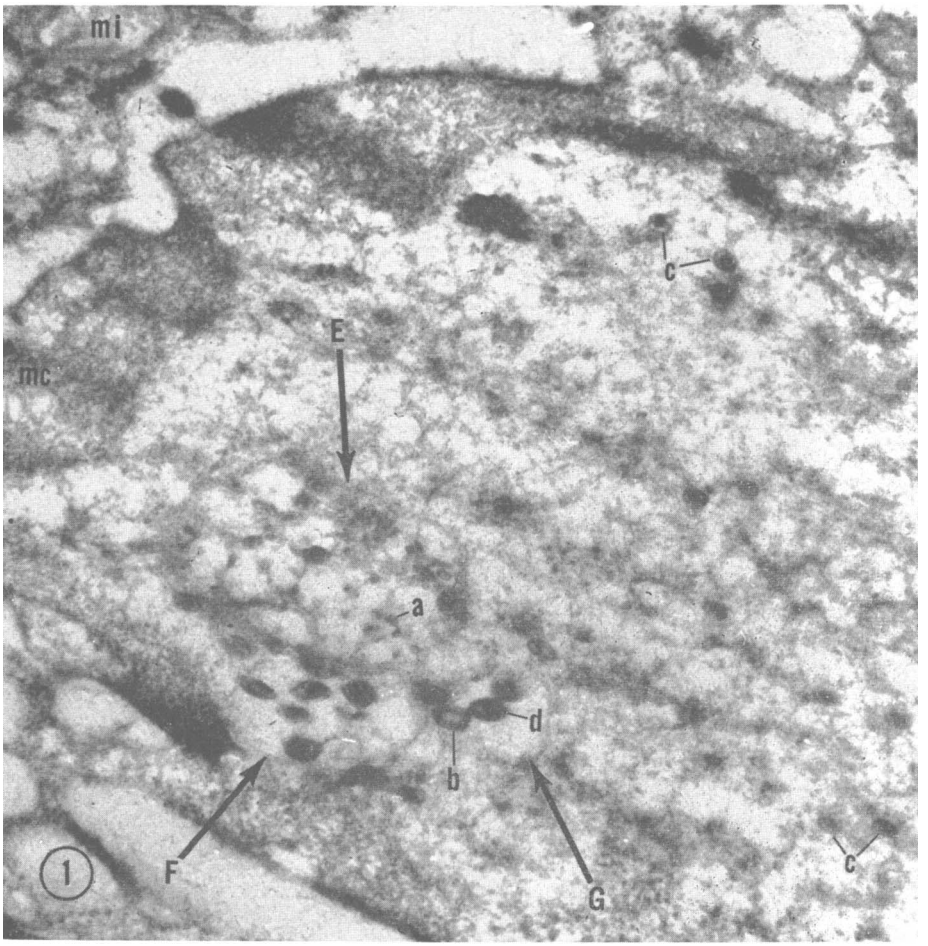

FIG. 1. Hepatic parenchymal cell of hamster inoculated with EAV. Various forms of intranuclear virus particles are shown, (a), (b), (c) and (d), as well as 3 viral "nests," (E), (F) and (G). Note margination of chromatin (mc). Mitochondrion (mi). Osmium fixation. 42,000 ×.

to be virus particles is predicated principally upon the fact that they are notably absent in control sections (Fig. 3) and are present in all examined sections of infected tissues.

Orientation of virus. A striking feature, readily apparent in Fig. 1, is that the elementary bodies are present in large numbers in the nuclei of the host cells, but were noticed only occasionally in the cytoplasm. Three distinct, although contiguous, "nests" may be discerned in the lower left quadrant of the nucleus in Fig. 1. One of these (e) seems to be composed of small central bodies enmeshed in a rather coarse reticulum; the other 2 ("f" and "g") contain structures which are considered to be more or less mature virus particles grouped together in vacuolated areas.

Cellular pathology. Inclusion bodies. No intranuclear inclusion bodies either of amorphous or crystalline structure were recognizable in our electron micrographs. *Margination of chromatin.* The represented figures of infected cells clearly show margination of chromatin around the periphery of the nuclei. The arrangement, thickness, and density of the marginated chromatin closely parallels that observed by light microscopy. *Nuclear membranes.* The nuclear membranes appeared distorted and were discontinuous in several areas. A comparison with a normal control (Fig. 3) serves to emphasize this cytopathogenic effect. *Cytoplasm.* Although

the infected cells apparently are undergoing general disorganization and degeneration, it is of interest to note that mitochondria, prominent in the control section (Fig. 3), appear less conspicuous in the cytoplasm of infected cells. Large, well-defined vacuoles are present in the cytoplasm of the infected cells.

Discussion. As more and more types of animal viruses are studied *in situ* with host cells by electron microscope a common structural pattern has become apparent. The virus particles are generally typified by a dense central body surrounded by one or two rings. Examples which may be cited include herpes

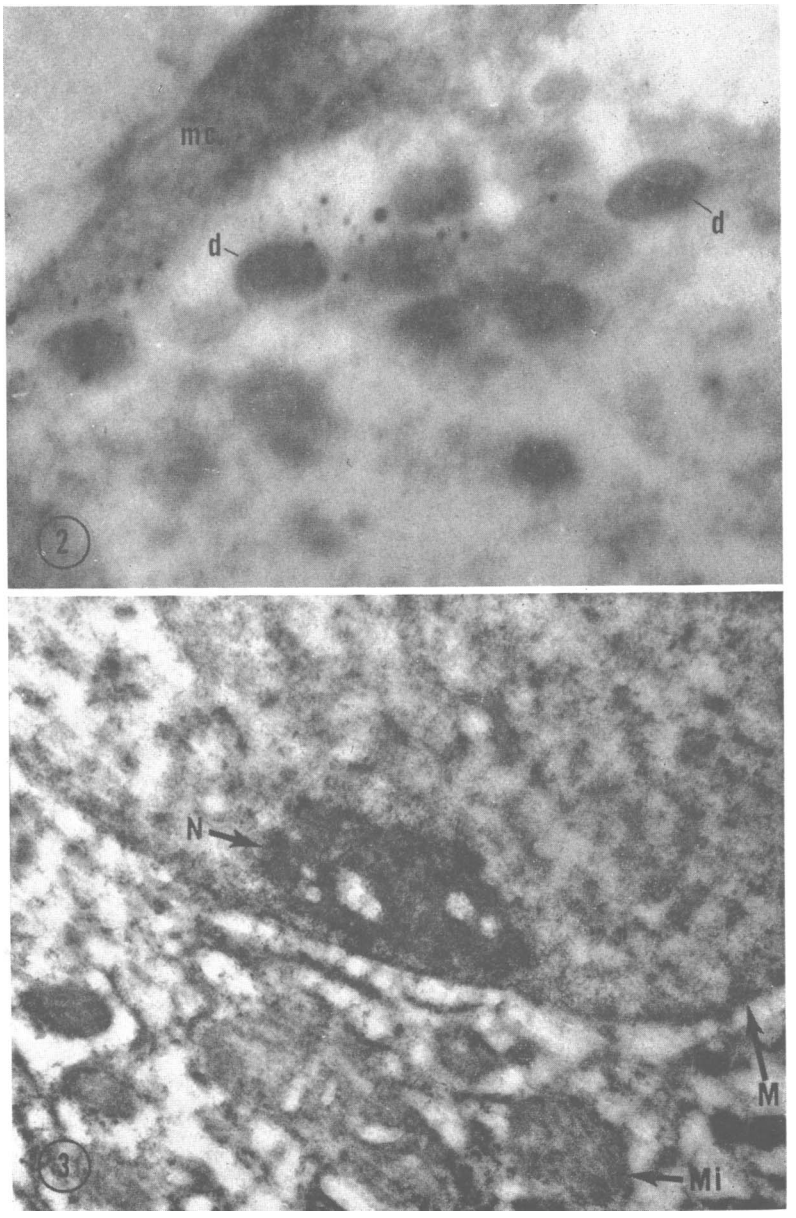

FIG. 2. Hepatic parenchymal cell of inoculated hamster showing double-ringed form of intranuclear elementary body, (d). Marginated chromatin (mc). Osmium fixation. 89,000 ×.

FIG. 3. Hepatic parenchymal cell of normal control hamster. Nuclear membrane (M), nucleolus (N), mitochondrion (Mi). Osmium fixation. 23,000 ×.

simplex reported by Morgan, *et al.*(4), influenza virus also observed by Morgan and co-workers(5), the salivary gland viruses reported by Luse and Smith(6), and the virus-like structures seen by deHarven and Friend in mouse leukemia material(7), and by Fawcett in frog adenocarcinoma(8). The EAV herein reported conforms very closely to the pattern. These examples of correlation in structure of different types of animal viruses serve to accent the importance of the questions raised by Morgan(9) concerning the relationship between structure and function.

The subject of intranuclear inclusion bodies associated with viral lesions has been of sustained interest to virologists for many years. Until recently no incontrovertible evidence for the existence of viral inclusion bodies in infected cells had been demonstrated by electron microscopy. It may be, at least in some instances, that the substances comprising the inclusion bodies which are readily seen by light microscope are too similar in electron density to the surrounding nuclear material to be visualized by the electron microscope. However, conclusive electron microscopic evidence of intranuclear inclusion bodies has been presented recently by Fawcett, *op. cit.*, and by Luse and Smith, *op. cit.* It is of considerable interest that margination of chromatin was prominent in the renal tumor cells studied by Fawcett, but was not a feature of the infected salivary gland cells employed by Luse and Smith. In contradistinction the EAV material, although revealing no intranuclear inclusion bodies did show conspicuous margination of chromatin. Whether these contrasting characteristics are features of the different viruses or of the different host cells constitutes a problem which might be explored.

Summary. 1) Young Syrian golden hamsters were inoculated with equine abortion virus (EAV). Hepatic cells from these animals, in late stages of infection, were studied with the electron microscope, and compared with similar material from uninfected (control) animals. 2) Various forms in which virus particles were seen, are described, and their similarity to other viruses is discussed. 3) Virus particles were observed predominantly in the nuclei. 4) "Nests" of virus particles were seen, but these were not considered to be equivalent to the intranuclear inclusion bodies characteristically seen by light microscopy. No structures identifiable as inclusion bodies were seen. 5) Other cytopathogenic effects are described.

1. Doll, E. R., Bryans, J. T., McCollum, W. H., Crow, E. W., *Cornell Vet.*, 1956, v46, 68.
2. Randall, C. C., Bracken, E. C., *Am. J. Path.*, 1957, v33, 709.
3. Bracken, E. C., Randall, C. C., *ibid.*, 1957, v33, 1009.
4. Morgan, C., Ellison, S. A., Rose, H. M., Moore, D. H., *J. Exp. Med.*, 1954, v100, 195.
5. Morgan, C., Rose, H. M., Moore, D. H., *ibid.*, 1956, v104, 171.
6. Luse, S. A., Smith, M. G., *ibid.*, 1958, v107, 623.
7. deHarven, E., Friend, C., *J. Biophysic. Biochem. Cytol.*, 1958, v4, 151.
8. Fawcett, D. W., *ibid.*, 1956, v2, 725.
9. Morgan, C., Rose, H. M., Moore, D. H., *Bull. N. Y. Acad. Med.*, 1958, v34, 85.

Received June 2, 1958. P.S.E.B.M., 1958, v98.

Maintenance of Gonads of Frog Larvae on Synthetic Media.* (24172)

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Recent studies on gonads of adult and larval amphibians have shown that these organs can be grown and maintained *in vitro* on media composed of chick embryo extract and cock plasma, and on chick embryo extract and agar(1). To determine what factors influence differentiation of the amphibian gonad,

* Aided by grant from U.S.P.H.S.