Virus Resistance in Rabbit Kidney Cell Cultures Contaminated by a Protozoan Resembling *Encephalitozoon cuniculi*[1] (37209)

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(Introduced by M. Ho)

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In the course of preparing cultures of rabbit kidney cells it was observed that certain lots were resistant to vesicular stomatitis virus. The resistant cultures were found to be contaminated by a protozoan parasite tentatively identified as *Encephalitozoon cuniculi* (Microsporida). In this report we describe the appearance of this phenomenon and show that the virus resistance is probably due to interferon.

Materials and Methods. Cell cultures. Preparation of cultures of kidney cells from weanling (300–400 g) albino rabbits (1) and 11 day old chick embryos (2) has been described.

Viruses. Vesicular stomatitis virus (VSV, Indiana strain) and eastern equine encephalitis virus (EEE) were grown in monolayer cultures of chick embryo fibroblasts and stored at −70°.

Staining. Monolayers on coverslips or on the surface of 5 cm plastic culture dishes were fixed in methanol, stained with Gram's stain or Giemsa's stain (1:20 in buffered saline, pH 7.2) and air dried. Coverslips were mounted, cells down, on slides prior to examination. Plastic culture dishes were examined directly.

Interferon assay. The semimicromethod described by Armstrong, (1) was used. The results are expressed as the dilution of test material giving 50% inhibition of cytopathic effects (CPE) due to VSV.

Results. Rabbit kidney cell cultures were prepared weekly from the kidneys of weanling rabbits for use in assays of rabbit interferon. The interferon assay involved infecting cultures in microwells (6 mm diam) with about 400 plaque forming units (PFU) of VSV (1). From time to time it was observed that cultures were resistant to this dose of virus which normally causes extensive CPE within 18 to 24 hr. Such cultures were found to be resistant to 4×10^4 PFU of VSV and up to 10^6 PFU of EEE. Microscopic examination of such cultures in the living state revealed large cytoplasmic vacuoles filled with granular material. Gram's stain showed that the granular material consisted of small gram + bacillary to coccobacillary bodies (Figs. 1 and 2). With Giemsa's stain the longer forms stained blue with a barely discernible red nucleus centrally located. The short form took Giemsa's stain poorly (Fig. 3). In cells containing a large, well-filled vacuole, the long forms, presumably proliferative forms, were arranged peripherally while the short forms, thought to be spores, occupied the central area of the vacuole. The vacuoles or pseudocysts had no well-developed wall. Some cells were also seen to contain as few as 3–4 proliferative forms without a well-defined vacuole. All intermediate states between these extremes were also observed. Comparison of this organism with published photomicrographs of *Encephalitozoon cuniculi* (Microsporida, Nosematidae) (3), the positive Gram's stain and the absence of a cyst wall served to tentatively identify the parasite as *E. cuniculi* and to distinguish it from *Toxoplasma gondii*. Further evidence that this organism was not *T. gondii* was evidenced by its failure to show specific staining in the fluorescent antibody test using high titered antiserum to *T. gondii*.

Parasitized cell cultures were dispersed with trypsin and transferred to new culture

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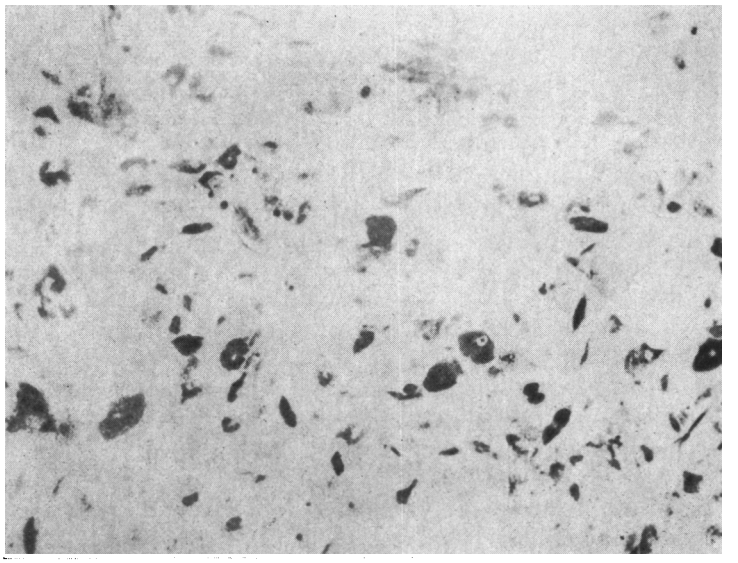

FIG. 1. Parasitized rabbit kidney cell culture. Gram's stain. ×48. The dark patches are cytoplasmic pseudocysts packed with spores. The cells are almost unstained.

vessels. The daughter cultures were also parasitized and resistant to VSV. Passage was continued at approximately weekly intervals but after the fifth passage it was necessary to add an equal number of cells from an unparasitized culture in order to maintain cell numbers at an adequate level. At the 14th passage level the cells remained resistant to VSV and EEE and parasites were observed. These results suggest that the parasite was multiplying and spreading from cell to cell under these conditions. Attempts to serially transmit the antiviral factor to rabbit kidney cells using the fluid portion of the cultures were unsuccessful.

Medium from each of the first five passages of parasitized cultures was tested for antiviral activity in rabbit kidney and chick embryo

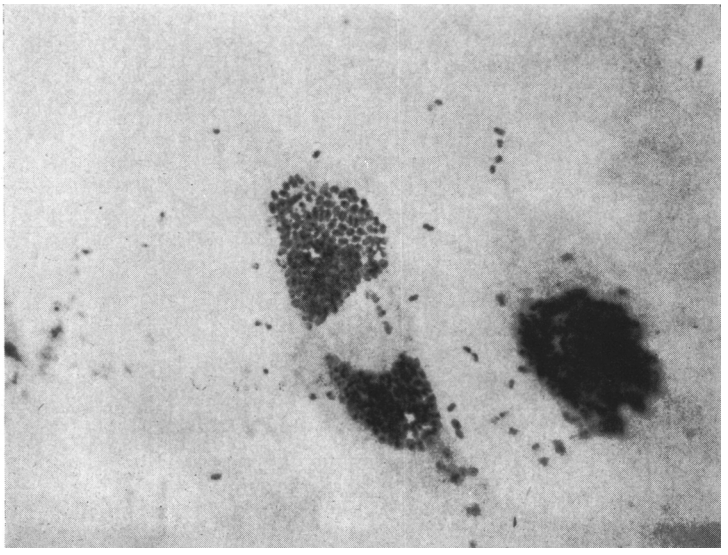

FIG. 2. A single cell from the culture shown in Fig. 1. ×800.

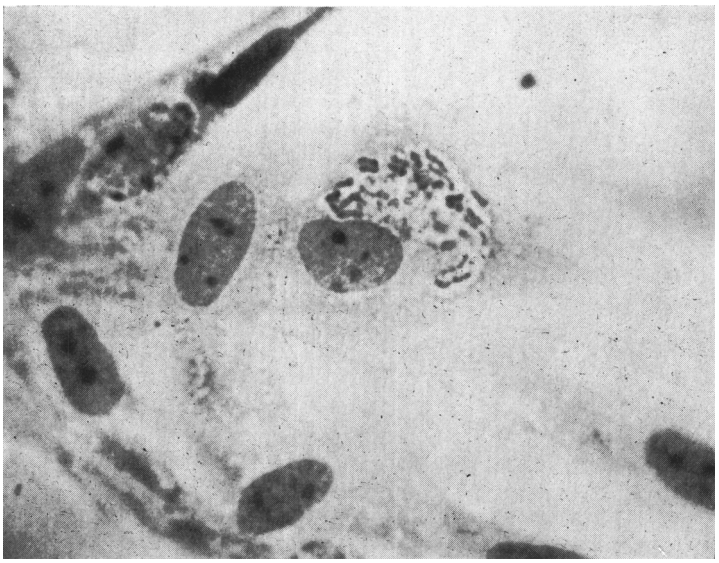

FIG. 3. Parasitized rabbit kidney cells. Giemsa's stain. ×800. Elongated proliferative forms and shorter spores can be seen.

cells. No antiviral activity was detected in chick embryo cells at a 1:2 dilution of the medium. In rabbit cells, inhibition of CPE due to VSV was seen at all five passage levels at dilutions from 1:28 to 1:64. These materials were sterile on bacteriological media, did not form plaques on chick embryo cells and the antiviral activity was resistant to heating for 1 hr at 56°.

Healthy rabbits from the parasitized colony were examined for the presence of the parasite. Peripheral blood smears and impression smears of brain, liver, kidney and spleen were stained with Giemsa's stain. The parasite was seen only in the kidney. It was not common.

The prevalence of the parasite, as measured by the presence of parasites in kidney cell cultures was variable. Frequently no cultures prepared from a batch of six rabbits were positive but frequencies as high as 4 out of 6 rabbits have been observed in a colony known to have the parasite. Another colony of rabbits has not shown a single positive out of several hundred used.

Discussion. The organism described here morphologically resembles *E. cuniculi* but this identification must remain provisional until comparison can be made with an authentic culture or antiserum. The discovery and taxonomic history of *E. cuniculi* have been described by Sprague and Vernick (4) and Shadduck (5) isolated and cultivated *E. cuniculi* in monolayer cultures of rabbit choroid plexus cells. Shadduck and Pakes (3) have also pointed out how widespread this parasite is and how it has frequently interfered with the interpretation of experimental data. Our experience is based on the use of rabbit kidney cell cultures for the routine assay of interferon. Parasitized cultures are quite useless for this purpose since the cells are resistant to virus challenge and produce an interferon-like inhibitor, which, however, has not yet been fully characterized. Other protozoans such as *T. gondii* (6, 7), *Plasmodium berghei* (8) and *Trypanosoma cruzii* (9) have been reported to induce interferon.

Summary. Cultures of rabbit kidney cells were sometimes found to be contaminated with an intracellular protozoan parasite resembling *Encephalitozoon cuniculi.* Such cultures were resistant to vesicular stomatitis and eastern equine encephalitis viruses owing to production of an inhibitor which resembles interferon.

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