Lymphocytic Choriomeningitis in a Hamster Colony Causes Infection of Hospital Personnel

Abstract. *A series of febrile illnesses, some severe, among hospital personnel was traced to an outbreak of lymphocytic choriomeningitis in a hospital research colony of Syrian hamsters. By rapid virus isolation and identification techniques, a definitive diagnosis was made 5 days after specimens were received. This outbreak emphasizes the dangers of working with experimental hamsters and tumor cell lines passaged in them.*

We report here a dangerous outbreak in hospital personnel of lymphocytic choriomeningitis (LCM) stemming from an LCM-infected research hamster colony. This virus is normally confined to wild mice (*1*, *2*), but it readily induces persistent infection (*3*) and is a well-known contaminant of colonies of laboratory mice (*4*, *5*) and hamsters (*6*, *7*). Human infection has almost invariably followed contact with infected mice (*8*) or hamsters (*6*) and may be inapparent, mild, or severe, with a few fatal cases (*2*, *9*). Serious laboratory-centered human outbreaks of LCM have been traced to hamsters carrying tumors contaminated by LCM virus (*6*, *7*), a not uncommon occurrence (*10*, *11*).

The present series of human cases began in 1971 and continued until April 1973. Most occurred in personnel visiting or using the basement of the radiotherapy department of a large hospital in upper New York State, particularly a room which housed about 200 Syrian hamsters and a photocopier. Clinically the cases were characterized mainly by sudden onset of fever (102° to 104°C), severe prostration headache, and myalgia. Convalescence was protracted, but there was little evidence of meningitis and there were no sequelae and no known associated deaths.

When LCM was suspected, serums from two patients, bled 9 and 16 days after onset, were immediately tested by indirect immunofluorescence for LCM antibody, with the use of LCM virus-infected mouse strain L cells as antigen. The titers of these serums were $< 1 : 4$ and $1 : 16$, respectively. On the day after the initial serological diagnosis, four more positive human serums were obtained, and three representative hamsters were received alive; tissue smears were conditionally immunofluorescent-positive when stained by the indirect technique with serum from animals immune to LCM. Isolation attempts were made on these tissues and on control tissues from normal mice, all tests being coded and tested "blind." Sentinel guinea pigs were used to check for airborne contamination. Suspensions (20 percent) of pooled spleen and kidney tissue were inoculated into groups of ten 12- to 14-g normal mice and mice immune to LCM, half of which were challenged on day 4 after inoculation with 100 μg of *Escherichia coli* endotoxin (Difco) by the intraperitoneal route; the nonimmune mice that had received hamster tissues by intracerebral inoculation sickened, and some died, within 24 hours of challenge. All mice immune to LCM inoculated intracerebrally survived this challenge, as did control normal mice. At this dosage, endotoxin was harmless to normal mice but lethal to mice with incipient clinical LCM (*12*) or mouse hepatitis virus (MHV); but MHV does not cause disease in man, nor does it cross-react by immunofluorescence with LCM virus. Thus all three hamsters were shown to have been infected with LCM virus.

A positive diagnosis of recent LCM infection in humans plus current LCM infection of the hamster colony was therefore made within 5 days of receipt of the first specimens. Further confirmation was obtained by day 12 after inoculation, by which time the unchallenged intracerebrally inoculated mice had died with typical signs of LCM infection and those inoculated in the footpad (*13*) had swollen feet. These last survived challenge on day 14 after inoculation with virulent LCM virus.

All mice inoculated with control tissues survived endotoxin challenge and had no footpad reactions. These mice and all sentinel guinea pigs remained well throughout and were fully susceptible to LCM challenge. Subsequently LCM virus was isolated by the above techniques from 10 or 11 more suspect hamsters, but from none of 4 mouse and 6 normal hamster controls. Further study of one isolate characterized it as a typical mouse-virulent neurotropic strain.

Additional serological tests of paired serums from patients and people with hamster contact were made by the complement fixation, indirect immunofluorescence, and LCM plaque-reduction methods. Good correlation was obtained between the three tests. A sample of 46 serums positive by plaque reduction included 40 which reacted by immunofluorescence; and 23 of the latter were also positive by complement fixation.

This outbreak of infectious disease was entirely preventable and should serve as a warning to research scientists to maintain adequate safeguards. Since this outbreak, other commercially distributed hamsters have been found to carry LCM virus and have caused human infections. The use of these animals and of tumor cell lines passaged in them must be regarded as dangerous procedures requiring special precautions. The location of a potential source of human infection within a patient care center compounds the danger, particularly when many of the patients can be expected to have impaired cellular immunity. It is well established that suppression of LCM infection is mainly T cell–mediated and that any cellular immune suppressant will impair the ability to fight this infection (*5*, *14*).

Contamination of an animal colony with LCM virus renders the colony useless for cancer research, since this virus exerts a well-documented sparing effect upon tumor-bearing hamsters, mice, and guinea pigs (*11*, *15*). Tumor tissue should be used for research purposes only if it is proven to be free of LCM virus. Large hamster colonies should be isolated from all humans except the animal care personnel, and it would be prudent to monitor such colonies continuously for LCM infection.

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Renal Lysosomes: Role in Biogenesis of Erythropoietin

Abstract. *The "light" mitochondrial pellet obtained from the kidneys of rats previously treated with Triton WR-1339 and rendered hypoxic was separated into subcellular component fractions by sucrose density gradient centrifugation in a zonal rotor. Selected fractions were pooled, disrupted by osmotic lysis and repeated freeze-thawing, and incubated in the presence and absence of normal rat serum. The incubation mixtures were assayed for erythropoiesis-stimulating activity (erythropoietin). High specific activity was identified only in fractions rich in lysosomes. Biochemical analysis of reference enzymes for the identification of lysosomes and mitochondria, supplemented by electron microscopic examination of the various separated fractions, supports the observed requirement for lysosomal constituents in the formation of erythropoietin by the kidney.*

In several studies (*1*, *2*) the biosynthesis of the hormone erythropoietin (Ep) has been related to the interaction between a renal erythropoietic factor, erythrogenin, presumably enzymatic in nature, and a substrate present in normal serum. The association of the renal erythropoietic factor with a particulate fraction of the rat kidney (*1*) has led to attempts to characterize the subcellular entity involved in erythrogenin formation or sequestration. Recovery of the factor from both nuclear (*3*) and "light" mitochondrial (*4*) fractions of homogenates of hypoxic kidney has been reported. Recently the lysosome has been implicated as the site of erythrogenin localization (*5*).

Male Long-Evans rats weighing 250 to 400 g received a single intraperitoneal injection of the nonionic detergent Triton WR-1339 (Rohm and Haas) at a dose of 85 mg per 100 g of body weight (*6*). After 77 hours, the rats were exposed to 0.45 atm of air for 19 hours in a hypobaric chamber and then immediately killed by cervical dislocation. Kidneys were rapidly removed, freed of perirenal fat and fascia, decapsulated, and finally homogenized in a Potter-Elvehjem homogenizer in ten volumes of 0.25*M* sucrose solution. These and all subsequent preparative steps were performed at 4°C. The initial homogenization sequence was accomplished with a Teflon pestle precisely milled so as to establish a mortar-pestle clearance of 0.51 mm. A final homogenization was achieved by substituting a wider pestle with a clearance of 0.14 mm. The homogenizer drive motor was operated at 720 rev/min.

The light mitochondrial pellet employed in these experiments was the material sedimenting between 6000*g* (10 minutes) and 21,000*g* (30 minutes) and was prepared in a rotor possessing a fixed angle of 34°. Pellets were resuspended in 0.25*M* sucrose solution and introduced into a Spinco Ti-14 zonal rotor which had been previously filled with a linear sucrose gradient extending from 36 to 52 percent. Centrifugation in the gradient was at 102,000*g* for 20 hours (about $12 \times 10^7 g$-min). Fractions were collected from the rotor in 65 10-ml portions. Sucrose density and protein determinations by the biuret method (*7*) were immediately conducted on alternate fractions; on the basis of these data and irregularly spaced cytochrome oxidase measurements (*8*) for the localization of the mitochondrial population within the gradient, fractions were pooled. Five such pooled fractions were selected for Ep bioassay in exhypoxic, polycythemic mice (*9*) following hypotonic disruption of the particles in distilled water and ten freeze-thaw sequences. The pooled fractions were incubated with either saline or normal rat serum (NRS) before assay. Values for 48-hour incorporation of ^{59}Fe into red blood cells (RBC) were converted to international reference preparation (IRP) units of Ep by reference to a standard dose-response curve prepared simultaneously with the assay of the fractions. Specific activities of the pooled fractions were expressed as net IRP units of Ep formed per milligram of protein administered to the assay mice.

Portions of pellets of the pooled fractions were prepared for electron microscopic examination. Portions of fractions from the zonal rotor taken prior to pooling were assayed for both cyto-

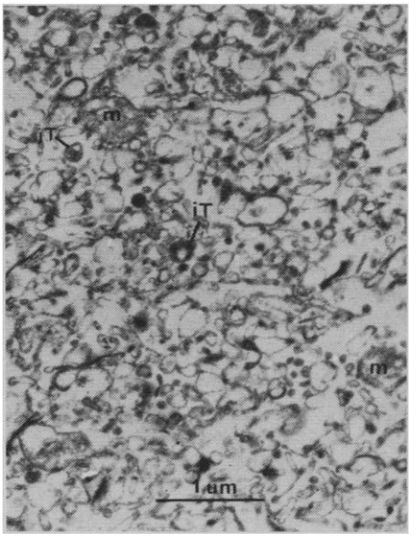

Fig. 1. Random electron micrograph through the midportion of the pellet of pooled fraction 18–22 from experiment B. The large, electron-lucid vesicles are Triton-treated lysosomes. While several mitochondrial profiles (*m*) are evident, the fraction is essentially free of other contaminants. Several lysosomes reveal dense matrices enclosing electron-lucid spaces. These are identified as lysosomes in which the Triton accumulation process is incomplete (*iT*). Fixation in 2.5 percent glutaraldehyde solution followed by 1 percent osmium tetroxide; sections stained with uranyl acetate.