

tion, recordings were taken of potentials evoked in the cochlear nucleus by tones and clicks. Under both conditions each position of the cat was photographed, and schematic drawings were prepared from these pictures.

Figure 1 presents data obtained from cat 15 which are representative of our experimental results. Recordings taken under anesthesia are shown in the top half of the figure. Three different positions of the cat are represented in row *A*. Row *B* shows the responses of the microphone and the right cochlear nucleus to tone pulses in each of these positions. Row *C* shows the corresponding responses to clicks. The same format is used in the bottom half of the figure to present results from the unanesthetized animal.

It is evident in the figure that small changes in the cat's position produce marked modifications in the responses of microphone and cochlear nucleus to tone pulses. It is equally apparent that the responses of the cochlear nucleus to tones follow variations in the envelope and amplitude of the sound pulse. The inverse correlation between envelope-complexity and peak-to-peak amplitude of the sound pulses is preserved in the responses of the cochlear nucleus. For example, the cat was moved  $\frac{1}{2}$  inch between *A1* and *A2*, which resulted in a shift from double to single responses of both the microphone and the cochlear nucleus, along with an increase of peak-to-peak amplitude in both.

When click stimuli are used (Fig. 1, rows *C* and *F*) there is less change in microphone and CN responses as position is varied. In this acoustically deadened box decrements of over 50 percent in tone-evoked CN responses were observed, whereas the maximum decrement with clicks was 37 percent (cat 17). In a training box with the usual glass window and plywood walls, decrements as large as 50 percent have also been observed in click-evoked CN responses. These effects occur regardless of whether the tympanic membrane and ossicular chain are intact or destroyed. They are observed equally well from electrode placements yielding large or small CN potentials.

A number of recent reports (3) have described amplitude changes in evoked CN potentials during habituation, shifts in attention, and learning. Central neural influences have been cited as factors mediating these changes. Our results demonstrate

another factor, namely, the powerful effects on evoked CN potentials produced by small differences in position of the animal within the acoustic field. These effects on evoked auditory potentials pose methodological and theoretical problems for the investigation of auditory electrophysiology and behavior. Specifically, habituation, shifts in attention, and learning are associated with characteristic changes in position of the animal. The acoustic effects of these position changes have to be isolated from central influences on CN potentials. The possibility that particular changes in CN potentials may reflect acoustic or central neural influences, or both, complicates the interpretation of such phenomena.

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### An Apparently New Lethal Virus Disease of Infant Mice

**Abstract.** A hitherto unreported disease of infant mice is described. The agent reproduces upon passage, is filterable, is confined mainly to the gastrointestinal tract in infant and adult mice, causes death in infants and inapparent infection in adults, and is sensitive to mild heat, ether, and sodium deoxycholate. Unique and characteristic morphologic changes occur in the epithelial cells of the intestine in infected infant mice. These consist in the development of multinucleated giant cells designated "balloon cells."

A disease accompanied by diarrhea and death was noted in infants of a colony of C57BL mice in 1959. The disease was at first attributed to flagellates that were found in the intestinal

contents; but when these were lost upon passage, and when bacteria-free filtrates of intestines with contents were found to be infectious, it seemed likely that a virus disease was being dealt with. Because the agent kills infant mice with regularity, because the original material has to date been diluted at least  $10^{-25}$  by passage of intestinal filtrates, and because the agent is confined, primarily, to the intestinal tract, it has been tentatively designated "lethal intestinal virus of infant mice" (LIVIM). No description suggestive of the disease in mice has been found in the literature.

As noted above, diarrhea may occur in LIVIM infection, and mice manifesting the disease in mild form may simulate animals suffering with epizootic diarrhea of infant mice (EDIM) (1). The similarity ends there, however; for, unlike the EDIM-infected mouse, the LIVIM-infected mouse does not nurse, loses weight rapidly, becomes lethargic, and dies after a short period of cyanosis.

Upon sacrifice and autopsy of sick and dying animals, it is seen that gross pathology is limited to the digestive tract. The stomach is shrunken and devoid of milk. Bile-tinged material may be present throughout the intestinal lumen; and portions of the small intestine, distended by a large volume of gas, are so thin-walled that they may rupture during life.

Microscopically, infected mice show remarkable and unique changes. Numerous swollen multinucleated cells are noted throughout the intestinal tract but are most frequent in the small intestine. These cells appear to be modified epithelial cells. Phosphotungstic acid staining indicates that the nuclei are not separated by cell membranes. Of further interest is the fact that the nuclei have never been observed in any stage of mitosis. For want of a better term, to distinguish them from other multinucleated giant cells, and because of their appearance (Figs. 1 and 2), the cells are referred to as "balloon cells." Early in the course of the disease, many balloon cells display a distinct cytoplasmic basophilia. Later, more of them manifest cytoplasmic eosinophilia with a few large inclusions that are also eosinophilic. Inclusions are not seen in nuclei of balloon cells nor in neighboring normal cells.

In the small intestine the villi decrease in size and number. The mechanism of the disappearance of the cen-

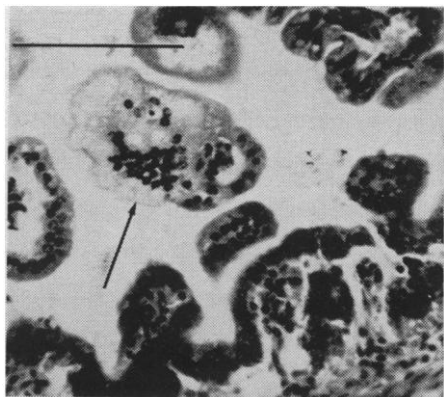


Fig. 1. Small intestine, infant mouse. Arrow indicates a multinucleated "balloon cell." Line at upper left represents 100  $\mu$ .

tral vessels and connective tissue of the villus is not understood at present. Inflammation is lacking throughout the tract; however, in long-lasting infections it may occur because of secondary bacterial invasion.

For study of the experimental disease CFW mice were used, and the methods of preparing intestinal suspensions and filtrates, of infecting animals *per os*, and of holding, handling, and breeding animals in filter cages were the same as those employed in studies of EDIM virus infection (2). Routes other than the oral resulted in sporadic "takes" and could not be used to obtain meaningful results.

Incubation period, severity of disease, and mortality rate vary with the dose of the agent and with the age at which it is fed. The shortest periods noted were 36 hours to onset of signs and 48 hours to death in infants less than 10 days old. Mice fed at 16 days

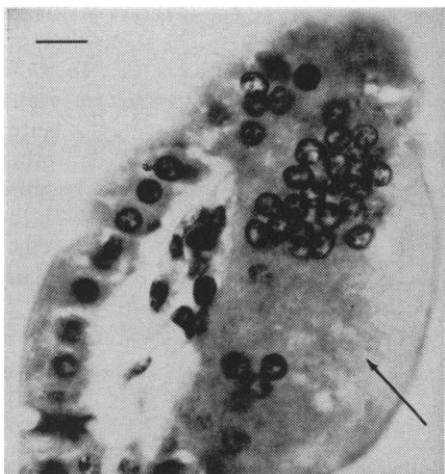


Fig. 2. Small intestine, infant mouse. "Balloon cell" (arrow) with normal appearing epithelium at left. Line at upper left represents 10  $\mu$ .

of age can react with death and gross pathology 4 to 6 days later. In these older animals, feces are pasty and soil the coat, but there is apparently no tendency toward rectal impaction as in EDIM virus infection (1). On autopsy the intestines appear chalky gray in color. Mice are susceptible until at least 1 year of age, but the infection after weaning and in adults is inapparent.

In mice younger than 10 days the new agent is found mainly in the intestinal tract. In two animals of 20 tested, however, it was also found in the liver in low titer. The titer attained in intestines and contents of infants has been found to be not greater than  $10^8$  ID<sub>50</sub>/g, whereas in EDIM virus infection it is not unusual to find a concentration of  $10^{10}$  ID<sub>50</sub>/g. In adult mice fed a single dose *per os* of  $5 \times 10^8$  ID<sub>50</sub>, the agent was shed constantly in feces for 15 days, beginning on the day after feeding.

Immunologic identification of the agent cannot at present be made with mouse serum; the same holds for EDIM virus (3). Hyperimmune rabbit sera, however, serve to differentiate it from EDIM virus. Whether the new agent is related to any other agent has not yet been ascertained.

Seitz filtrates of an intestinal suspension of LIVIM were passed through Millipore filters of 10-, 50-, 100-, 300-, and 450-m $\mu$  pore diameters. After this treatment, the agent passed without significant loss through the 300- and 450-m $\mu$  filters, but it could not be detected in filtrates passing through the others. The same results have been obtained with EDIM virus which, in electron micrographs, measures 65 to 75 m $\mu$  in diameter (4).

The new agent is susceptible to heating at 50°C for 30 minutes. Indeed, at least 99-percent infectivity may be destroyed when a filtrate is held at 4°C for 24 hours. Whether or not this is solely the function of temperature remains to be seen, for the virus is also sensitive both to ether and sodium deoxycholate. Incubation at 4°C for 24 hours with ether resulted in less than 1 percent survival of the agent when compared with an untreated control. Incubation with 1:1000 deoxycholate at 37°C with normal rabbit serum resulted in a reduction in titer of 2.3 log when compared with an untreated control.

Thus far the new agent has failed to multiply in L cells, mouse embryo, rhesus kidney, HeLa tissue cultures,

Ehrlich ascites tumor in vivo, and the chorioallantois of the chick embryo. Furthermore, agglutination of the following red cells in phosphate-buffered saline at pH 7.2 could not be demonstrated: sheep, mouse, guinea pig, rabbit, chicken, and human type O.

On the basis of its apparent size, its lack of growth on bacteriologic media, and its reproducibility by passage in mice, the agent described is assumed to be a virus. Further experimentation will be necessary to characterize it completely and to differentiate it from other known agents. It is described here as an apparently new disease of infant mice.

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#### Animals Used as Food by Late Archaic and Woodland Cultural Groups in New England

**Abstract.** Remains of vertebrates and invertebrates found at several New England Woodland sites confirmed results and conclusions of earlier investigators, and further indicated that amphibians and reptiles were not used much as food, if so used at all. Vertebrate remains found at Late Archaic sites indicate the presence of a largely contemporary fauna. Failure of Late Archaic people at sites studied to use mollusks in quantity as food may have been determined by cultural barriers, and not by lack of availability.

Since the middle of the last century investigators have been identifying remains of animals found in shell heaps and other sites of former human habitation in New England. Until two decades ago this investigation had involved only sites assignable to the Woodland cultural period. Animal remains at sites of an earlier vintage were not identified, even though some of these sites were found by amateurs.

The study reported here was undertaken to determine, among other things, animals used as food by successive cultural groups in New England. Ani-