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Observations on Erythrocytic Inclusion Bodies from Mice Infected with Rabies Virus. (29007)

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It has long been argued that there exists no demonstrable viremic phase in clinical rabies. Koprowski and Cox(1) cite numerous reports on both affirmative and negative attempts to isolate rabies virus from the blood of several species of rabid animals. None of our several attempts to induce rabies experimentally in new-born mice after injection of the blood of rabid animals has been successful. It seems logical, however, that at some time in the progression of rabies, virus would be present in the peripheral blood. Wong and Freund(2) found rabies virus in the blood shortly after intracerebral injection. Reports have shown rabies virus to be present in such non-nervous tissue sites as renal tubular epithelium, mammary gland, pancreas, brown fat and respiratory mucosal epithelium of infected animals(3-10). The thought that virulent virus could proceed to these sites solely by way of the nervous system seems tenuous at best. There is also mounting evidence of the transmission of rabies virus via aerosol from flying bats, presumably arising from inflight urination(11).

In studies with canine distemper virus it has been shown that intracytoplasmic inclusion bodies are present in conjunctival, respiratory and urinary bladder epithelial cells in addition to the cells of the central nervous system(12-15). Recent work has shown that neutrophils and monocytes from peripheral blood as well are found to contain distemper inclusions when specifically stained. Fluorescent antibody studies have proven their specificity(13,14,16,17).

This report concerns an investigation as to whether the peripheral blood of laboratory mice would yield similar structures during the course of rabies.

A pilot study was made by randomly sampling the peripheral blood of mice in various stages of clinical rabies induced with several sylvatic strains of virus. During this preliminary study, several of the stained smears showed erythrocytes with inclusion bodies strikingly similar to those seen in leukocytes and epithelial cells from dogs with clinical distemper. Following this initial discovery, a systematic approach was taken to find just when these bodies first appeared.

Materials and methods. Webster-Swiss mice 21 days old were divided into groups of 12 each with 6 mice per cage. Equal numbers of males and females were present but with the sexes separate. Group I was the control. Group II consisted of 12 mice inoculated with virulent rabies originally isolated from a rabid coyote (no. L488, mouse pool 4), and subsequently identified by serum neutralization, fluorescent antibody studies and Seller's staining for Negri bodies. They were inoculated intramuscularly in the right hind leg with 0.1 ml of a 10^{-1} dilution of mouse brain suspension with an intramuscular titer of $10^{-1.75}$(18). Following inoculation, one-half of each group (6 animals) was bled on alternate days by clipping the tail tip to obtain free flowing blood to prepare film pairs. The slides thus prepared were fixed while still wet in absolute alcohol anhydrous diethyl ether, equal parts, and allowed to remain

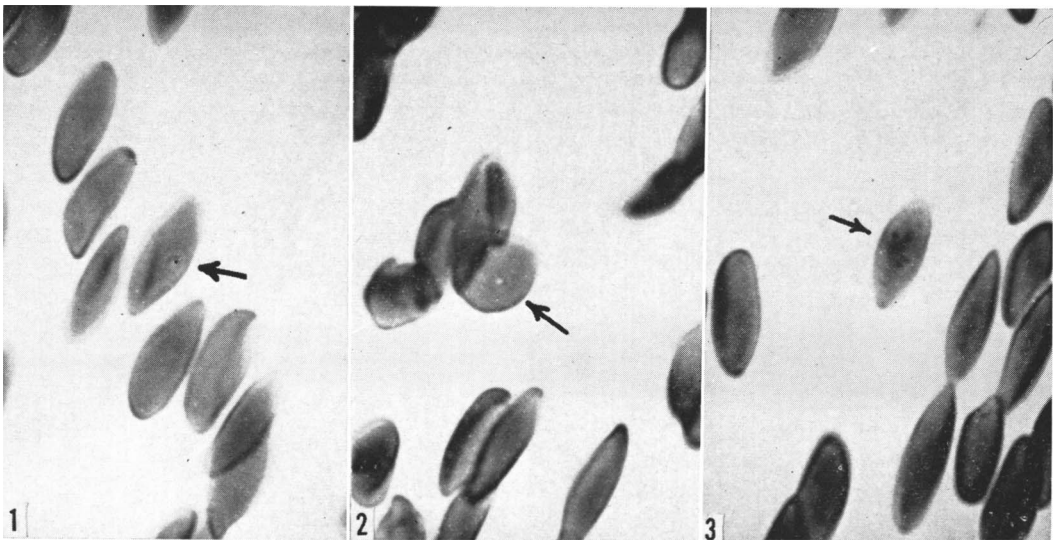

FIG. 1 and 2. Mouse erythrocytes containing single intracytoplasmic inclusions. Note distinct halo around periphery of body. Shorr's stain. Center of body carmine red, with darker red edge. The fusiform shape of the erythrocytes is due to wet fixation. 788×.

FIG. 3. Mouse erythrocyte containing multiple bodies, each with its own halo.

over night. The slides were then stained by Shorr's modified technique(12,19) and permanently mounted. Additional slide pairs were fixed in Bouin's fixative and stained by Kleineberger-Nobel's method(20) using a weak Giemsa stain for 24 hours. Photomicrographs of blood films considered significant were taken at 788× magnification.

Results. It was found that starting at the fifth postinoculation day, small, round, acidophilic inclusions were present in approximately 0.5-1.0% of the erythrocytes stained by Shorr's technique; those stained by K-N method were dark magenta. They were first noticed in reticulocytes which became more numerous due to the frequent bleeding. They are characterized as having a diameter of less than 2.0 μ and surrounded by a distinct clear halo (Fig. 1, 2, 3). The control mice at no time showed these bodies. Both groups were treated equally with respect to care and handling of the blood films. In mice injected with diluent only, these bodies could not be demonstrated. The slides were examined by a blind, coded method making foreknowledge of their origin impossible. The peak occurrence of the inclusions was at postinoculation day 8, with 11 of the 12 mice in Group II, evidence of bodies in their erythrocytes. The rabies-infected mice began to show early clinical rabies signs at postinoculation day 8, with most deaths occurring at postinoculation day 13.

Discussion. The exact nature of these inclusions is not clear. They may, in fact, be viral antigen or merely represent a host response to the virus. Fluorescent antibody studies have provided equivocal results. Apparently, there is some non-specific fluorescing substance present in the mouse blood. In any event, the negative control blood films were almost as fluorescent as those from rabid animals.

Summary. Rabies was induced in Webster-Swiss mice injected intramuscularly with 0.1 ml of a mouse brain suspension of rabies virus, the titer of which was $10^{-1.75}$. Peripheral blood films stained by Shorr's modified technique and Kleineberger-Nobel's method showed approximately 1.0% of the erythrocytes to contain discrete intracytoplasmic inclusions. The highest incidence of these bodies appeared at postinoculation day 8, with most deaths occurring at postinoculation day 13. The specificity of these inclusions has not been established. While these inclusions are related to the development of rabies in the host, it has not been demonstrated con-

clusively by either fluorescent antibody microscopy or mouse inoculation that these bodies are due to infection with rabies virus *per se*.

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Glomerulosclerosis in Mice with Myeloid Leukemia. (29008)

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Although aging RF mice spontaneously develop sclerotic changes in renal glomeruli (1,2) such lesions were also observed in young mice of this strain with passaged myeloid leukemia. This report describes these changes and their induction by leukemic filtrates, ultracentrifugates, and cells.

Methods. The mice used were derived from a non-inbred production stock of the RF/Up strain. The passaged myeloid leukemia originated in an irradiated RF male and had been serially transmitted through more than 25 passage generations in mice of the same strain (3,4). It was transmitted by intravenous inoculation of leukemic spleen cells, ultracentrifugates, ultrafiltrates, and plasma ultracentrifugates. The spleen cells were prepared for injection by mincing the spleen of a freshly killed donor in cold Tyrode's solution, to give a suspension of 100-150 $\times$ 10^6 nucleated cells/ml. Ultracentrifugates of the spleen cell suspension were prepared by centrifugation in the cold at 1800 $\times$ g for 20 minutes, followed by further centrifugation of the supernatant fluid at 30,000 $\times$ g for 1 hour. The ultracentrifugates were prepared by filtration of the 1800 $\times$ g supernatant fluid through a Selas 03 filter under negative pressure, with simultaneous filtration of added *Escherichia coli* to check the integrity of the filter. The plasma ultracentrifugates were prepared from heparinized whole blood by the same procedure as that used for preparation of the spleen cell ultracentrifugate.

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