action through some other system. Regardless of the effect of serum potassium concentration on rate of secretion of aldosterone these experiments demonstrate that the characteristic kaliuresis accompanying potassium infusion is not dependent upon a potassium-sensitive receptor mechanism in the adrenal cortex.

Summary. 1) A technic has been described whereby the dog adrenal may be infused *in situ* without disturbing its arterial blood supply. 2) Utilizing this technic, infusion into left adrenal arteries of a solution containing high concentrations of potassium did not affect serum potassium concentration or urinary potassium excretion any differently than infusion of similar solution into a non-adrenal artery. It is concluded that the kaliuretic response to potassium infusions is not mediated by a direct action of potassium on the adrenal cortex.

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Electron and Fluorescence Microscopy of Mouse Hepatitis Virus.* (25981)

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Interest in comparative viral hepatitis stems from lack of methods for propagation and detection of the virus(es) of human hepatitis (1). Since identification of this agent depends on the hazardous exploitation of man, other host-specific viruses associated with hepatitis infections of dog(2), duck(3-5), and mouse (6-9) have been studied as possible prototypes. This communication describes procedures for visualization of the *Eperythrozoon*-free strain of mouse hepatitis virus (MHV 1)(10) by electron and by fluorescence microscopy.

Materials and methods. Newborn Swiss mice were infected *via* the peritoneal route with MHV 1(11). After 5 days, mice were sacrificed and infected livers were examined. Tissue imprints were prepared on coverslips, fixed without air drying in acid-alcohol, and stained with acridine orange fluorochrome at pH 3.8 and with fluorescein-tagged antibody by methods described for psittacosis infection of human cell lines(12). Antiserums were prepared in adult Swiss mice by repeated intraperitoneal inoculation of normal and of infective mouse-liver homogenates(13). Globulin fractions were conjugated with fluorescein isothiocyanate for use in the direct fluorescein-staining procedures(14). Paraffin sections were cleared in xylol, then stained with acridine orange and with fluorescein-tagged antibody. A Reichert Zetopan microscope and illumination system were used with appropriate ultraviolet light source and filters. Other paraffin sections and tissue imprints were stained by routine procedures with hematoxylin-eosin. Preparations of infected liver were fixed in Palade's(15) veronal acetate buffered osmium tetroxide, embedded in methacrylate, and sectioned for examination with an electron microscope (RCA EMU-3C).

Results. Tissue imprints and paraffin sec-

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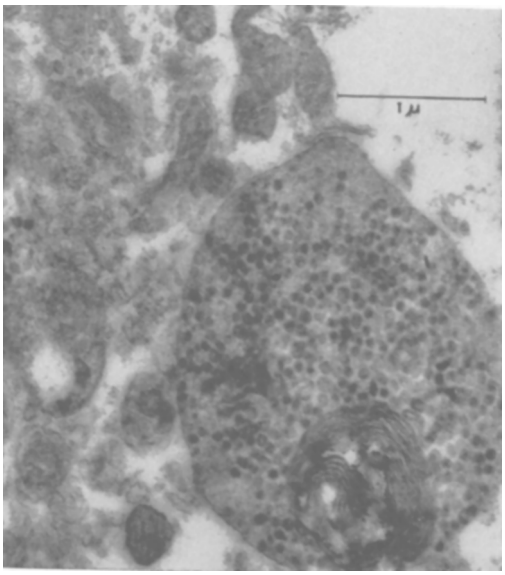

FIG. 1. Inclusion body in cytoplasm of hepatic cell infected with mouse hepatitis virus. Note limiting membrane and virus particles of varying densities. In some instances, dense-centered particles are surrounded by a single membrane. A portion of the nucleus is seen in upper-left corner.

tions which were stained with hematoxylin-eosin showed foci of necrosis in which pyknotic nuclei and cell fragments predominated. In comparison to the extensive liver damage, few cytoplasmic inclusions were observed. Although the dye binding nature of the inclusions could not be established in areas of cellular degeneration, eosinophilic bodies (1-5 μ) were present in otherwise normal appearing cells which surrounded necrotic areas. In parallel preparations stained with acridine orange, the cytoplasm of some hepatic cells contained abnormal accumulations of RNA-staining (red) material(16). Attempts to establish chemical identification of RNA-staining inclusions were made by treatment of infected tissue preparations with ribonuclease prior to staining with acridine orange. Ribonuclease removed RNA-staining (red) affinity of both cytoplasm and nucleoli and did not interfere with DNA-staining (yellow-green) affinity of the nucleoplasm for acridine orange. There was, however, some indication that ribonuclease did not change the RNA-staining property of a number of inclusions. With fluorescein-tagged antibody, positive-staining cytoplasmic inclusions were noted which corresponded to size, shape, and location of the RNA-staining inclusions. Observations by electron microscopy (Fig. 1) showed cytoplasmic inclusions (1-5 μ) which contained aggregates of virus particles (90 $\pm$ 20 mμ). Some inclusions were ruptured and elementary bodies were scattered throughout the cytoplasm. Other sections showed mature virus particles emerging from a viroplasm in which faint outlines of immature organisms could be seen.

Discussion. The cytological observations of Armstrong and Niven(16) on mouse livers infected with MHV 1 were confirmed. Thus, it was noted, also, that infected hepatic cells stained with acridine orange contained abnormal accumulations of material in the cytoplasm which fluoresced intensely red. Attempts to establish the chemical identity of these inclusions by treatment with ribonuclease prior to staining with acridine orange were not considered conclusive. Observations were hampered by 1) fragments of pyknotic nuclei, degenerated cells, and nuclear "blebs" which fluoresced both red and yellow-green; 2) the small size of the elementary body which precluded their detection unless larger aggregates were present; and 3) the possibility that if MHV 1 is an RNA virus, it would fluoresce the same color as the cytoplasm and hence be obscured. Although the nucleic acid composition of MHV 1 is still an open question, preparations stained with fluorescein-tagged antibody showed inclusions of similar size, shape, and location which were antigenic to homologous antiserum.

As determined by electron microscopy, intact cytoplasmic inclusions were surrounded by a limiting membrane(s). Virus particles within these inclusions had an average diameter of 90 $\pm$ 20 mμ (Fig. 1). This size range is comparable to that estimated by Gledhill *et al.*(17) who used filtration through membranes of graded porosity. Occasionally, the membrane around the inclusion was broken and single particles or small aggregates, some with limiting membranes, were noted throughout the cytoplasm. Presence of virus in similar liver samples was shown by infectivity tests in newborn mice.

Summary. Livers of mice infected with

mouse hepatitis virus (MHV 1) were examined by conventional staining procedures and by electron and fluorescence microscopy using acridine orange fluorochrome and fluorescein-tagged antibody. Preparations stained with acridine orange showed abnormal accumulations of RNA-staining material in cytoplasm of hepatic cells. Inclusion bodies of similar size, shape, and location were stained selectively with fluorescein-tagged antibody. Observations by electron microscopy showed virus particles in cytoplasm which measured 90 ± 20 mμ in diameter.

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Influence of Age at Time of Irradiation on Induction of Leukemia and Ovarian Tumors in RF Mice. (25982)

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Among physiological variables influencing susceptibility to radiation-induced leukemia in mice is age at time of irradiation(1,2). In man also, according to epidemiological surveys, susceptibility to leukemia induction may be relatively high during embryonic and fetal development(3), and the hematologic type of leukemia induced may vary with age at the time of irradiation(4). The present investigation was carried out to extend earlier observations on the relation between age at irradiation and leukemogenesis in mice of the RF strain.

Methods. Male and female RF mice of several ages from 3 days before birth to 180 days after birth were exposed to whole-body X radiation in doses of 100, 200, and 300 r (Table I). Radiation was administered with a 250-kvp General Electric Maxitron machine at 30 ma, with 0.1 mm of inherent and 3 mm of added Al filtration (hvl, 0.44 mm of Cu), TSD 93.7 cm, and a rate of 75-100 r/min. Suckling young were exposed together in intact litters, whereas other mice were exposed individually by the technic described previously(5). The mice were necropsied on death and tissues fixed in Zenker-formol for sectioning.

Results. Incidence of granulocytic leukemia was not significantly increased by irradiation *in utero* or during the first 9 days after birth but was increased by irradiation later in life, susceptibility to induction of the disease rising gradually to a maximum at 70 days of

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