The prolonged anticonvulsant effect of Dilantin shown by intravenous administration in mice, if true also in man, is certainly a very desirable property of the drug in management of status epilepticus or for prophylactic control of seizures in neurosurgery.

Summary. 1. Rate of onset and duration of anticonvulsant effect of intravenous Dilantin sodium against electrically-induced tonic-extensor seizures have been investigated in mice. At 15 mg/kg, the anti-convulsant effect of Dilantin reached a peak approximately 30 minutes after injection, and was maintained for 1½ hours; it then began to decline gradually, persisting more than 8 hours. 2. Dilantin, one minute after intravenous injection, was capable of abolishing the tonic-extensor seizures in mice at doses in proportion to the current strengths employed to produce convulsions.

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Canine Hepatitis Virus: Attempts to Find Relationship with Human Hepatitis.*† (24550)

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Two groups of investigators(2,8) described successful isolation and propagation of canine hepatitis virus in cell cultures of dog kidney. Since then the growth of this virus in cell cultures of kidney tissue from other animals has been reported(5,9). Fastier(6,7) described agglutination of fowl erythrocytes by this virus using infected tissue culture fluids as antigens. This agglutination was inhibited by immune sera from dogs naturally or experimentally infected with canine hepatitis virus. Although there is no epidemiologic evidence to suggest that the infectious agent of human hepatitis is the same as that of canine hepatitis, the possibility of some immunologic relationship between the two was felt to warrant exploratory investigation using recently developed methods.

Materials and methods. Virus. A strain of canine hepatitis virus, which had been passed 37 times in dog kidney tissue cultures, was kindly supplied by Dr. V. J. Cabasso, Lederle Medical Research Dept. *Cell cultures.* Kidneys from young puppies were trypsinized according to the method of Rappaport(16). Cultures were prepared by seeding each tube with about 300,000 cells contained in 1 ml growth medium. In the same way cultures were prepared from rhesus monkey and young pig kidneys. The growth medium consisted of 0.5% lactalbumin hydrolysate in Hanks' balanced salt solution (BSS)(13). For dog and pig kidney cell cultures this was supplemented with 10% calf serum; for rhesus kidney cell cultures with 2% calf serum. From continuous cell lines§ of human origin: Detroit-6(1);

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§ Original cell lines were kindly supplied by: Drs. I. W. McLean and W. A. Rightsel, Parke Davis and Co., Detroit, Mich., (Detroit-6 cells); Dr. Alice E. Moore, Sloan-Kettering Inst., N. Y., (Hep-2 cells); Dr. Jørgen Fogh, Virus Laboratory, Berkeley, Calif., (FL cells).

FL(10); and Hep-2(14), cultures were prepared by seeding each tube with approximately 100,000 cells contained in 1 ml of growth medium consisting of Eagle's medium (4) with 10% calf serum. Prior to inoculation of virus the Eagle's medium was replaced by 2 ml of medium 199(15). *Neutralization tests.* Serial dilutions of sera were made in medium 199. Each dilution was mixed with equal quantity of virus *i.e.* 0.2 ml of serum-virus mixture contained about 100 TCD_{50}. After one hour at 37°C, 0.2 ml of each mixture was inoculated into 2 or 3 tube cultures of dog kidney epithelium. Final readings were made at 5 to 7 days when control titration showed the desired amount of virus to be present. CF tests were carried out according to the method of Fulton and Dumbell(11) as modified by Svedmyr, Enders and Holloway (18). High titer tissue culture fluids were used as antigens. All sera were inactivated at 56°C for a half hour. Sera to be used as antigens in HA test were extracted with acetone and ether and reconstituted in saline according to method used by Havens for sera from human hepatitis patients(12). The HA reaction was carried out according to methods described by Clarke and Casals(3). Serial dilutions of antigens, either reconstituted serum samples or tissue culture suspensions of canine hepatitis virus were made in borate saline buffer (pH 9.0). To 0.5 ml of each dilution was added 0.5 ml of a 0.25% suspension of one-day-old-chick erythrocytes in various pH adjusting diluents consisting of phosphate buffers. These solutions were used to adjust the pH in final HA reaction to any desired pH between 6.0 and 7.6, when mixed with equal amounts of antigen diluted in borate saline.

Results. Canine hepatitis virus was routinely propagated in dog kidney monolayer cell cultures. Previously described(2,8) cytopathic effects (CPE) were observed. That this virus can be cultivated in porcine kidney cell cultures(5,9) was confirmed. Rhesus monkey kidney cultures, Detroit-6, FL, and Hep-2 cell cultures inoculated with dog kidney passage virus produced changes in each after 3-4 days. At various time intervals subcultures were made in cells of the same type. In no case, however, did CPE develop in series of 3-4 blind passages.

Two puppies were injected with canine hepatitis virus passed in dog kidney cell culture. Both survived the mild disease which resulted. Preinoculation serum samples contained no neutralizing, CF, or hemagglutination-inhibiting antibodies. Sera obtained 4 weeks after inoculation contained neutralizing antibodies in titer greater than $1 = 160$ and both inhibited 8 HA units at dilution of 1:40. No CF antibodies were demonstrable. The dogs were bled 5 times during first week after fever. We used these sera, after extraction with acetone and ether, as HA antigens in a reaction with one-day-old-chick erythrocytes. No HA was observed at pH values of 6, 7, and 7.6.

Neutralization experiments were carried out with various human sera against canine hepatitis virus. Paired sera from 7 (including 1 case of serum hepatitis) and single convalescent serum samples from 5 hepatitis patients were tested. As controls, serum samples were included from 4 normal persons. None of these sera gave neutralization in dilutions 1:5 and 1:10. In all tests sera from immune dogs were included as positive controls. These sera were also tested by CF for presence of antibodies against canine hepatitis virus. The antigen, consisting of undiluted culture fluid, had a titer of 4. As control an immune dog serum (titer 1:32) was used. All tests with human sera gave negative results at lowest dilution tested (1:8).

By using the above HA technic, maximal HA titers for canine hepatitis virus tissue culture antigens were at pH 6.0. At higher pH values tested, lower titers were obtained. This agglutination was not inhibited by normal human sera, 4 acute phase and 10 convalescent phase sera from human hepatitis patients. In each test, serum from an immune dog was included as control.

Discussion. Our purpose was to investigate the possibility of a serologic relationship between canine hepatitis virus and the infectious agent of human hepatitis. Such a relationship could not be demonstrated by the indirect method of testing acute and convalescent sera from human hepatitis patients

against canine hepatitis virus in complement fixation, neutralization or hemagglutination inhibiton tests. This indicates that there is no antigenic relationship between human and canine agents. Even though only one strain of canine hepatitis virus was included in this study, it is unlikely that the results might be altered by using other strains since by means of complement fixation and cross neutralization tests performed in dog kidney cell cultures Salenstedt(17) was unable to demonstrate any significant antigenic difference between 5 strains of canine hepatitis virus.

Fastier's findings(6) that canine hepatitis virus agglutinates chick erythrocytes and that this agglutination could be inhibited by specific immune serum were confirmed. In his system maximal titers were at pH 7.5 to 8.0. In the HA reaction employed by us, maximal titers occurred at pH 6.0. This may be accounted for by differences in the buffers used.

Havens(12) has described that acetone-ether extracted sera from human hepatitis patients may contain agglutinins for chick erythrocytes. In our study this method was applied to serial acute phase sera from 2 dogs inoculated with hepatitis. No hemagglutinins were demonstrated. It may be that this phenomenon does not occur in canine hepatitis, or that the disease in the 2 dogs was too mild.

By inoculaion of canine hepatitis virus into cultures of rhesus monkey kidney cells and cultures of various human cell lines characteristic cytopathic changes resembling those observed in dog kidney cell cultures, developed in the first passage, but not subsequently.

Summary. 1) Observations on growth and behavior of a strain of canine hepatitis virus in various tissue culture systems of human and animal origins have been described. 2) By testing acute and convalescent phase sera from human hepatitis patients against canine hepatitis virus in neutralization, complement fixation, and hemagglutination inhibitions tests, no antigenic relationship could be demonstrated between this virus and the infectious agent of human hepatitis.

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