

administration of IEC-QH with the fluorescent qualitative technic described.

(1) The gastric secretion of the group in whom the quininium cations appear in the urine during the first 2 hours after the administration of the indicator compound will have free hydrochloric acid. (2) The gastric secretion of the group in whom the quininium cation does not appear in the urine until the third hour after the administration of the indicator compound will have no free hydrochloric acid. (3) The group in whom the quininium cation is not excreted in the first hour but appears in the second hour will require quantitative photofluorometric examination of the urine extracts to determine the presence or absence of free gastric hydrochloric acid.

The use of this cation exchange indicator compound should find an important place in both clinical diagnosis and in preventive

medicine.

Summary. The preparation of a cation exchange indicator compound called quininium resin indicator compound has been described. The rationale and method of its use have been stated.

In vitro tests have demonstrated that the quininium cation present in this compound will be displaced by the hydrogen ions of dilute hydrochloric acid solutions and of the gastric juice.

In vivo experiments have shown that, by administering this cation exchange resin indicator compound orally and noting the time of appearance of the quininium cation in the urine, the presence or absence of free hydrochloric acid in the gastric juice can be determined without subjecting the individual to intubation.

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Transmission and Multiplication of Newcastle Disease Virus (NDV) in Brains of Suckling Mice. (17860)

LAWRENCE KILHAM

From the Microbiological Institute, National Institutes of Health, Bethesda, Md.

Adult mice have generally been found resistant to Newcastle disease. Brandly *et al.* (1) concluded that NDV failed to multiply on intracerebral inoculation of weanling mice. Recently Reagan *et al.* (2) report transmission of Newcastle disease to adult mice, using virus from the 203rd hamster passage. The results of present investigations indicate that 3-day-old mice are almost all susceptible to at least 2 strains of NDV and that this infection is readily transmissible to successively older mice.

Materials and methods. The California strain of NDV (No. 11,914) was obtained from Dr. Erwin Jungherr of Storrs, Conn.

1. Brandly, C. A., Moses, H. E., Jungherr, E. L., and Jones, E. E., *Am. J. Vet. Res.*, 1946, v7, 289.

2. Reagan, R. L., Lillie, M. G., and Brueckner, A. L., *Proc. Soc. Exp. Biol. and Med.*, 1949, v70, 5.

Dr. Alfred S. Evans kindly supplied an Australian strain of NDV. Suspensions of these viruses were prepared in amniotic and allantoic (AA) fluids as described elsewhere (3). The Newcastle disease immune hen serum used as a standard was kindly supplied by Dr. Clarence H. Thompson, Jr. of the Bureau of Animal Industry, U.S. Department of Agriculture. Hen erythrocytes were employed in antihemagglutination tests as elsewhere described (3).

Mouse passages. The Australian and California strains were passed in essentially the same manner in separate series of Swiss albino mice. Virus suspensions were diluted in buffered saline (pH 7.6) and inoculated intracerebrally in amounts of 0.03 ml. Infected brain tissue was ground without

3. Kilham, L., Jungherr, E., and Luginbuhl, R. E., *J. Immunol.*, 1949, v63, 37.

TABLE I.
Hemagglutination Titrations and Antihemagglutination Tests in Which AA Fluids from Embryonated Eggs, Inoculated with Mouse Brain Suspensions Were Used.

Strain of NDV	Mouse brain passage used for egg inoculation	AA fluids obtained from eggs inoculated with infected mouse brain	
		Hemagglutinin titers	Antihemagglutinin titers of standard serum
California	4th	1: 128	1: 8192
"	8th	1:1024	1:32768
Australian	8th	1: 512	1: 2048
"	10th	1: 128	1: 1024

abrasive for dilution and transfer. On the initial passage, 3-day-old mice received infected AA fluids diluted 10^{-1} . All mice inoculated became ill in 4 to 6 days. Weaned mice, 4 weeks old, failed to become ill when similarly inoculated. In subsequent passages, infected brains of the suckling mice were transmitted in dilutions of 10^{-2} or 10^{-3} , inoculated mice becoming ill in 2 to 3 days. The age of mice at time of inoculation was successively increased. Twelve- to 14-day-old mice were used for the 10th brain passages. Manifestations of Newcastle disease in afflicted mice were similar for both strains of NDV. Younger mice had humped backs and used their hind legs stiffly. As the disease progressed, especially in older mice, neurological manifestations were striking. Manifestations varied, some mice turning repeatedly in one direction while others had paralyses or uncontrolled movements of various limbs. Some mice had convulsions. Death occurred 2 to 3 days after the appearance of illness. Suspensions of brain tissue from each passage, as well as all egg fluids, were cultured for bacterial contaminants in thioglycollate and in meat infusion broths. Contaminants appeared on 2 occasions, but were eliminated by the addition of penicillin and streptomycin.

Passage of infected mouse brain suspensions in embryonated eggs. Two per cent suspensions of infected mouse brains along with penicillin and streptomycin were inoculated into allantoic sacs of 10-11-day-old eggs. The 4th passage brain suspensions of the Cal. NDV and 8th passage suspensions of the Aust. NDV killed all of the embryonated eggs inoculated with each within 44

hours. As presented in Table I the AA fluids when harvested had hemagglutinin titers of 1:128 for the Cal. NDV and of 1:512 for the Aust. NDV. Eighth mouse brain passage of the Cal. and 10th passage of the Aust. NDV were inoculated into a later set of eggs. The AA fluids of these eggs had HA titers of 1:1024 for the Cal. NDV and of 1:128 for the Australian. All of the infected AA fluids, when diluted in amounts containing 8 hemagglutinating units, gave substantial titers with the standard NDV-immune serum in anti-hemagglutination tests (Table I). These tests served to indicate that the agents passed were actually Newcastle disease viruses.

Infection of chicks with mouse brain suspension. Mouse brain suspensions from the 8th passage of Cal. NDV and 10th passage of Aust. NDV were inoculated intracerebrally into 2-week-old chicks in amounts of 0.05 ml. All chicks became ill and moribund within 2 to 4 days. They appeared hunched, with drooping wings, and had difficulty standing. Brains of moribund chicks were harvested and inoculated into 10-day-old embryonated eggs. Antihemagglutination tests against the standard serum indicated that the AA fluids of these eggs contained NDV.

Discussion. Australian and California strains of NDV have been transmitted through 10 passages in the brains of suckling mice. As both agents increased in virulence with passage, there apparently was an active multiplication of virus. The agents transmitted showed many features characteristic of NDV. They multiplied rapidly in embryonated eggs, killing them in less than 2 days and producing hemagglutinins in the AA fluids. These fluids yielded high titers

when run in antihemagglutination tests with a standard NDV-immune serum from the Bureau of Animal Industry. Inoculated mice developed encephalitis suggestive of Newcastle disease in fowls. Viruses from infected mouse brains, produced Newcastle disease when inoculated intracerebrally into 2-week-old chicks. The agents transmitted were resistant to freezing and thawing, penicillin, and streptomycin. The ease with which 2 different strains of NDV are transmissible in the brains of suckling mice may have value in investigations of the immunology and

other aspects of Newcastle disease. Further passages, now in progress, suggest that weanling and older mice are susceptible to NDV following preliminary passages already described.

Summary. Suckling mice of the Swiss albino strain are susceptible to Newcastle disease of fowls following intracerebral inoculation. This has been demonstrated by 10 consecutive passages of the California and of an Australian strain of NDV.

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Unique Physico-Chemical Properties of Japanese B Encephalitis Virus Hemagglutinin.* (17861)

ALBERT B. SABIN AND EDWARD L. BUESCHER†

From The Children's Hospital Research Foundation, Department of Pediatrics, University of Cincinnati College of Medicine.

The discovery of the phenomenon of hemagglutination with the influenza viruses(1,2) has provided an important new tool for the study of certain basic properties of a number of viruses(3,4). No such hemagglutinins, however, could be demonstrated for the neuronotropic viruses affecting the human nervous system. Recently, the phenomenon has been found to occur with the virus variously known as the "Columbia S.K.," "M.M.," encephalomyocarditis (EMC), or Mengo encephalomyelitis(5-7) and with the GDVII strain of mouse encephalomyelitis(8). During a visit to the University of Leiden

(Holland) in September, 1949, Prof. J. D. Verlinde informed one of us (A.B.S.) that he obtained the same results with the Nakayama strain of Japanese B encephalitis as with the "S.K.-M.M." virus, and that by hemagglutination inhibition tests it was indeed not possible to distinguish these viruses. Olitsky and Yager(6), however, shortly thereafter in reporting their own studies with the "S.K.-M.M." virus indicated that they obtained only negative results with the Japanese B encephalitis virus and a number of other viruses affecting the human nervous system. In an attempt to clarify this situation it was discovered that the virus of Japanese B encephalitis was indeed associated with a specific hemagglutinin. Its properties, however, were found to be so different from all others previously described that it became evident that Verlinde and DeBaan(7) were dealing not with the Japanese B encephalitis virus, originally obtained from this laboratory, but with

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† Captain, M.C., U.S.A., on active duty.

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2. McClelland, L., and Hare, R., *Canad. Pub. Health J.*, 1941, v32, 530.

3. Hirst, G. K., *J. Exp. Med.*, 1950, v91, 161.

4. Burnet, F. M., *Lancet*, 1948, v1, 7.

5. Hallauer, C., 4th Internat. Cong. Microbiology, July 1947, Copenhagen, 1949, p. 257.

6. Olitsky, P. K., and Yager, R. H., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 719.

7. Verlinde, J. D., and DeBaan, P., *Ann. Inst. Pasteur*, 1949, v77, 632.

8. Lahelle, O., and Horsfall, F. L., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 713.