analytical centrifugation of influenza virus preparations, obtained under various conditions, 2 components were noted, both resulting from the infection, one identical with the infectious principle, the other smaller but apparently endowed with hemagglutinating activity(14,15). This material has been considered a "precursor" of fully active virus (15). Neither the "immature" or "incomplete" forms nor the "precursor" have been isolated as yet for more extensive analysis of their physical and biological properties.

The data presented above, which confirm and extend earlier observations by Burnet(4), may be considered in the light of the problems raised by the studies on influenza virus. The available information appears to indicate the presence in chick embryos infected with NDV of 2 hemagglutinating components, one associated with the infectious principle, the other distinctly smaller and according to all indications non-infectious. As in the influenza problem, the question arises whether this smaller component represents a breakdown product of the seed virus or of the new generations of the agent, or whether they constitute a developmental stage in virus reproduction. The fact that this component is found mainly in the infected tissue and not in the allantoic fluid into which the new generations are liberated, seems to favor the latter hypothesis.

Summary. Disproportionate sedimentation in the high speed centrifuge of hemagglutinins and infective principle of Newcastle disease virus in suspensions of infected allantoic membranes has been reported. This suggests the presence in such preparations of two hemagglutinating particles of different sizes; one of these appears to be identical with the infectious principle, the other, smaller one, according to all indications is non-infectious. The specificity of this smaller hemagglutinin has been established by hemagglutination-inhibition tests. The medium in which the virus is suspended apparently does not effect the sedimentation of hemagglutinating capacity. Experiments employing Seitz filtration have been unrevealing.

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Studies of Newcastle Disease Virus (NDV) Propagated in the Cave Bat (*Myotus lucifugus*). (18307)

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The egg-adapted Newcastle disease virus (NDV) strains: California, Colorado, Delaware, Kentucky, Minnesota, and Connecticut, used for these studies were obtained in 1946 from the Bureau of Animal Industry, Department of Agriculture, Washington, D. C. These strains were isolated by the Bureau from infected chicken tissue from the corresponding states before the introduction of commercial live virus vaccines for Newcastle disease.

The cultures had been carried in 10-day embryonated chicken eggs through 6 serial passages. In our laboratory they were carried 6 additional passages in 10-day embryonated White Leghorn eggs. Embryo passage of the California strain was continued through the 24th passage. Allanto-amniotic fluid from several eggs dead of each strain was harvested and pooled separately for each strain. All strains of the virus were confirmed to be NDV by neutralization tests conducted in 10-day embryonated chicken eggs. The tests were performed according to the procedure recommended by the Bureau of Animal Industry(1). Newcastle-immune chicken serum neutralized the virus in all cases while normal

chicken serum had no effect. Five-hundredths ml of pooled, infected allanto-amniotic fluid from embryonated egg passages of all strains was inoculated in the right frontal lobe of each of 4 cave bats. Within 4 to 6 days the bats from all strains showed typical Newcastle virus symptoms: irritability, malaise, rhythmical contraction of muscles starting in the limbs and spreading over the entire body, paralysis of the hind legs, prostration, and death. There was no evidence of excessive salivation from pharyngeal paralysis which is characteristic in other animals infected with the hamster-adapted NDV California strain 11,914(2). When nervous symptoms developed in each bat, the brain was removed aseptically, ground with alundum, and diluted to a 10% suspension with physiological saline. The brain suspension from each strain was inoculated into the allantoic sac of 10-day embryonated eggs. All embryos died within 72 hours. Pooled allanto-amniotic fluids from dead eggs of each strain was prepared. Neutralization tests were done on these infected pools against NDV antiserum and normal chicken serum using embryonated chicken eggs as the test host. The results of these tests proved the virus in all strains to be that of Newcastle disease.

The California strain of egg-adapted NDV was carried through 10 passages in the cave bat as shown in Table I. Brain material for passage was chosen from bats sacrificed when nervous symptoms developed. Amounts of 0.05 ml of 10% brain suspension from the preceding passage were injected intracerebrally throughout the 10 passages. These passages are being continued at the present time. Brain material from the 2nd intracerebral bat passage was injected into 10-day embryonated eggs, and upon death of all embryos in 72 hours the allanto-amniotic fluid was harvested, concentrated, and examined under the Emu electron microscope. The virus particles showed morphological characteristics similar to those previously described by the authors(3).

TABLE I. Bat Passage of a Strain of Egg-Adapted Newcastle Disease Virus (California Strain No. 11,914).

Passage No.	No. bats inoculated	No. bats paralyzed, moribund, or dead	Min. to max. incubation period (days)
1	4	3	4-6
2	6	6	4-6
3	8	5	3-5
4	4	3	2-4
5	6	6	2-5
6	8	6	3-4
7	6	4	3-4
8	6	4	2-4
9	6	6	2
10	10	10	3-4

TABLE II. Results of Neutralization Test of Bat-Adapted NDV (10th Passage).

Material inoculated	Virus dilutions 10^{-1}	10^{-2}	10^{-3}
Virus bearing brain material	4/4	2/4	2/4
Same + normal chicken serum	3/4	3/4	2/4
Same + Newcastle-immune chicken serum	0/4	0/4	0/4

Numerator of fraction denotes the number of deaths.

Denominator of fraction denotes the number of bats inj.

A virus neutralization test was conducted using brain suspension of the 10th intracerebral bat passage with specific Newcastle-immune and normal chicken sera and with bats as the test host. Specific immune sera completely neutralized the virus while normal chicken sera did not. The virus titered $10^{-2.5}$ according to the Reed-Muench method of calculation(4). Details are given in Table II.

Summary. The cave bat, *Myotus lucifugus,* is susceptible by intracerebral inoculation to the 6-egg-adapted NDV strains: California, Colorado, Connecticut, Delaware, Kentucky, and Minnesota, used in this study. The California strain was carried 10 passages in the bat by intracerebral inoculation. Serial passage in bats was not attempted with any of the other 5 strains.

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