the reverse is also possible and the isotopic experiments that have been reported do not distinguish between the two possible reaction pathways.

The experiment described below, with C^{14}-carboxyl-labeled glycine was performed to test this point. The results indicate that glycine is not directly reduced to ethanolamine and is so transformed only through serine as an intermediate.

Experimental. Three rats were injected intraperitoneally with 500,000 counts per min. (0.52 mg, 2.8 μc) each of C^{14}-carboxyl-labeled glycine. The animals were killed 4 hours after administration, the livers removed and pooled, and the phospholipids extracted, and then hydrolyzed by refluxing at the boiling point with 3.6% hydrochloric acid for 12 hours. The mixture was chilled, the fatty acids filtered off, and the filtrate concentrated *in vacuo* with repeated addition of water to remove the hydrochloric acid. The residue in about 75 ml of water was treated with neutral aqueous lead acetate, and then freed of excess lead with hydrogen sulfide. The filtrate was evaporated to dryness and extracted with small volumes of ethanol. The alcoholic solution was filtered, evaporated to dryness, and taken up in 1 ml of 1 M hydrochloric acid. The solution was mixed with an excess of calcium oxide and ethanolamine isolated by extraction with ether according to the method of Thierfelder and Schulze* (5). It was counted as the oxalate(6). None of the samples gave counts that were significantly above background.

To the phospholipid extract of the liver of another rat, to which the same dose of carboxyl-labeled glycine had been administered, carrier ethanolamine was added and the base was separated from serine by passage through a column of permutit(7) after acid hydrolysis of the phospholipids. The eluted ethanolamine was treated with periodate and the formaldehyde formed collected as the dimedon derivative. This too contained no activity.

Summary. Ethanolamine isolated following administration of C^{14}-carboxyl-labeled glycine contained no radioactivity. This indicates that glycine is not directly reduced to ethanolamine in the body and that this occurs through the intermediate formation of serine.

* Choline also was isolated and counted as the chloroplatinate. It contained no radioactivity.

5. Thierfelder and Schulze, *Z. physiol. Chem.*, 1915, v96, 296.

6. Keiser, B., *Ind. and Eng. Chem., Anal. Ed.*, 1940, v12, 284.

7. Artom, C., *J. Biol. Chem.*, 1945, v157, 585.

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A Small Hemagglutinating Component in Preparations of Newcastle Disease Virus.* (18306)

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The size of the virus of Newcastle disease of chicken (NDV) has been estimated to be between 80-120 mμ by filtration through gradocol membranes(1). This general order of size has been confirmed by electron microscopy(2,3), and ultracentrifugation(2,3). The sedimentation studies with virus obtained from infected allantoic fluids indicated that the hemagglutinating activity of NDV is

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1. Burnet, F. M., and Ferry, J. D., *Brit. J. Exp. Path.*, 1934, v15, 56.

2. Bang, F. B., *J. Exp. Med.*, 1948, v88, 241.

3. Cunha, R., Weil, M. L., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., *J. Immunol.*, 1947, v69, 55.

closely associated with the infectious unit(3). However, Burnet noted that upon Seitz filtration of allantoic fluid preparations the infectivity decreased to a far greater extent than the hemagglutinating capacity indicating a "partial dissociation of hemagglutinin and infective activity of NDV"(4).

Studies in this laboratory toward the development of complement fixing antigens for NDV led to observations which appear to substantiate the heterogeneity of NDV hemagglutinins. A comparison of infected allantoic fluids and suspensions of membranes revealed marked differences in the relative sedimentation in the high speed centrifuge of infectivity and hemagglutinins. Whereas in the allantoic fluids both properties sedimented at similar rates, a striking discrepancy became apparent when suspensions of membrane were employed, in that the infectivity was by far more sedimentable than the hemagglutinins. These experiments will be described in detail.

Methods and materials. Virus. The B strain of Newcastle disease virus, originally isolated by Beaudette, was kindly supplied by Dr. Bang. It had been maintained by passage through the allantoic cavity of 10-11-day-old chick embryos. Infected allantoic fluid was kept in glass-sealed ampules in the dry ice chest for stock virus. Suitably diluted seed (10^{-2} to 10^{-6}) was injected allantoically in 0.2 ml amounts. After further incubation of the embryos at 36 to 37°C for 24 to 72 hours the eggs were chilled and the allantoic fluids and membranes harvested aseptically. The allantoic membranes were washed thoroughly in sterile phosphate buffered saline solution of pH 7.2, drained on sterile filter paper, and weighed. A 20% suspension in sterile buffered saline solution was made by emulsifying the tissue for 5 minutes in an ice-cooled Waring blendor. After clarification by preliminary centrifugation of both allantoic fluids and membrane suspensions at 2,000 r.p.m. for 10 to 20 minutes the supernates were centrifuged simultaneously at 20,000 r.p.m. for 20 minutes; the upper two-thirds of the supernatant fluids were saved and the remainder discarded. The sediments were resuspended in buffered saline solution to the original volume. The preparations were found to be stable at 4°C for at least 10 days both as to infectivity and hemagglutinating property.

Infectivity titrations. The virus preparations were diluted in 10-fold steps in sterile brain-heart infusion broth to which 100 units of penicillin and 100 μg of streptomycin had been added per ml. Five 10- to 11-day-old chick embryos each were inoculated allantoically with 0.5 ml of one of the various dilutions. The allantoic fluids from individual eggs were collected separately after an incubation period of 48 hours at 36 to 37°C, the presence of virus was determined by their capacity to cause hemagglutination, and the 50% infectivity end point was calculated according to the method of Reed and Muench. The results are expressed in terms ID_{50}/ml. Since hemagglutinins may under certain conditions escape detection on account of rapid elution of the virus from red cells(5) a number of technics for their demonstration were tried and compared in individual tests. These methods were as follows: (a) to 0.4 ml of undiluted allantoic fluid were added 0.2 ml of a 1% suspension of thrice washed chicken red cells. The mixture was incubated at 4°C for 60 to 90 minutes and the test was read according to the pattern formed by the red cells in the bottom of the test tubes; (b) same as (a) except that the allantoic fluids were diluted 1:10; (c) each allantoic fluid was titrated according to the method described below; (d) one drop each of undiluted allantoic fluid and of a 10 to 20% suspension of chicken red cells were mixed on a glass slide and gently agitated by rotation. In positive samples agglutination became apparent within a few minutes; finally, (e) 0.5 ml of allantoic fluid was mixed with 0.1 ml of M/10 $NaIO_4$ and incubated at room temperature for 30 minutes, when 0.1 ml of a 40% solution of glucose was added in order to neutralize the periodate. After the addition of 0.2 ml of a 1% suspension of chicken red cells the test

4. Burnet, F. M., *Austral. J. Exp. Biol. and Med. Sc.*, 1945, v23, 178.

5. Burnet, F. M., *Austral. J. Exp. Biol. and Med. Sc.*, 1942, v20, 81.

was placed at 4°C until the red cells had settled, which on account of the larger total volume, required more time than in methods (a), (b) and (c). All 5 technics gave comparable results provided proper precautions were taken. For methods (a) and (b) the test had to be read as early as possible in order to avoid erroneous interpretation of results on account of elution of the virus; *i.e.,* with increasing delay in reading a "positive" pattern might gradually change to "negative." This did not occur in method (e) which, therefore, is preferred at present because of its independence of careful timing. Method (d), although highly reliable, was somewhat more cumbersome, and method (c) was too elaborate to be practical for the indicated purpose. The results of infectivity titrations presented in the text were determined by at least 2 of the technics and frequently 3.

Hemagglutinin titrations. To serial 2-fold dilutions of the various viral preparations in saline solution, using 0.4 ml volumes, 0.2 ml of a 1% suspension of chicken red cells was added. The test was incubated at 4°C (6) and read in terms of the patterns formed by the cells in the bottom of the tubes. The last initial dilution of the virus preparation giving definite evidence of agglutination was taken as the end point. In most instances the titrations were performed in duplicate and the mean computed. The results are expressed in terms of hemagglutinating (HA) units/ml.

Hemagglutination inhibition tests. Serial 2-fold dilutions of viral preparations were prepared in triplicate, using 0.2 ml volumes. To the first of these series was added 0.2 ml of saline solution; to the second 0.2 ml of 8-fold diluted serum of a patient convalescent from a laboratory infection with NDV(7); and to the third 0.2 ml of 8-fold diluted mumps convalescent serum. After incubation at room temperature for 30 minutes 0.2 ml of a 1% suspension of chicken red cells was added to each tube. The test was incubated at 4°C and read by the pattern formed. Thus, the reduction in hemagglutinin titer by a given dilution of serum was measured. The sera had been treated by $NaIO_4$ according to the method described above in order to minimize non-specific inhibitory activities(8). In addition slide tests were performed by mixing one drop each of undiluted virus preparation, undiluted serum, and 20% suspension of chicken red cells, and the presence or absence of agglutination recorded.

Experimental. Several centrifugation experiments with infected allantoic fluid and membrane preparations were conducted. Since the results were essentially in agreement, only 2 of the protocols will be discussed in greater detail. In the first of the experiments to be described 10-day-old chick embryos were infected with allantoic fluid containing NDV, diluted to 10^{-6}. The allantoic fluids and membranes from half of the living embryos were collected after an incubation period of 48 hours, from the survivors of the other half after 72 hours. In the second experiment a 10^{-2} and a 10^{-6} dilution of the same seed was used and the fluids and membranes were harvested after 48 hours. Emulsification of the tissues and high speed centrifugation were carried out as described in the section on methods and materials. The various preparations in each experiment were centrifuged simultaneously. The results of hemagglutination (HA) and infectivity titrations (ID_{50}) are recorded in Table I.

As can be seen in the table, the infective principle in *allantoic fluid* was largely sedimented by the centrifugal force employed, *i.e.,* only between 1 and 4.5% was left in the supernatant fluid. Sedimentation of hemagglutinins was of a similar order, although the percentage remaining in the supernate was somewhat larger, between 4.6 and 7.7%. This difference may be insignificant in the light of the relative crudeness of the tests employed. The corresponding results with the suspensions of *allantoic membrane,* however, revealed more striking differences. Sedimentation of the infective principle was somewhat greater than in the case of the allantoic fluids, in that only between 0.02 and 0.3% of this

6. Florman, A. L., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 458.

7. Keeney, A. H., and Hunter, M. C., *Arch. Ophthalm.,* 1950, in press.

8. Hirst, G. K., *J. Exp. Med.,* 1948, v87, 301.

TABLE I. Differences in Sedimentation of Infectivity and Hemagglutinating Capacity in Allantoic Fluids and Membrane Suspensions.

Exp.	Inoculum dilution	Incubation, hr	Material	Allantoic fluid					Allantoic membrane				
				ID_{50}/ml log	% of original	HA units/ml log	% of original	Ratio ID_{50}/HA log	ID_{50}/ml log	% of original	HA units/ml log	% of original	Ratio ID_{50}/HA log
1	10^{-6}	48	Original	10.13		3.51		6.6	9.03		3.46		5.6
			Supernate	8.30	1.5	2.38	7.7	5.9	5.97	.09	2.68	16.6	3.3
			Sediment	>9.80		3.16		6.6	8.33		3.05		5.3
	10^{-6}	72	Original	10.03		3.51		6.5	9.30		3.51		5.8
			Supernate	8.68	4.5	2.30	6.2	6.4	6.80	.3	2.90	25.0	3.9
			Sediment	>9.80		3.28		>6.5	>9.55		3.05		>6.5
2	10^{-2}	48	Original	9.76		3.41		6.4	8.80		3.65		5.2
			Supernate	7.80	1.1	2.20	6.2	5.6	5.68	.08	3.08	26.7	2.6
			Sediment	9.80		3.16		6.6	9.30		3.05		6.3
	10^{-6}	48	Original	9.93		3.41		6.5	8.93		3.51		5.4
			Supernate	7.93	1.0	2.08	4.6	5.9	5.13	.02	2.75	17.5	2.4
			Sediment	9.80		3.11		6.7	9.05		2.98		6.1

activity was retained in the supernate. Removal of the hemagglutinating capacity, on the other hand, was far less pronounced since between 17 and 27% of this property remained suspended. The redispersed sediments derived both from fluids and tissue suspensions indicated fairly complete recovery of the infective principle, whereas a slight loss of hemagglutinin was regularly apparent. Aggregation of particles in the sediment may possibly account for this deficit. These differences in relative sedimentation of the two activities became more evident by a comparison of the ID_{50}/HA ratios. Whereas these ratios remained fairly constant for all of the allantoic fluid preparations, *i.e.*, of the order of 10^6, they showed significant variations in the allantoic membrane preparations. The ratios in the resuspended sediments, likewise, were close to 10^6. On the other hand, those found in the supernate were of the order of 10^3 only. The ratios in the original membrane suspensions were somewhat lower than in the corresponding sediments or in the allantoic fluid materials, presumably because of the fact that they contained a mixture of high- and low-ratio materials.†

In further experiments infected allantoic fluids and membrane suspensions were subjected to 3 successive centrifugations at 20,000 r.p.m. for 20 minutes, followed by a fourth run at 30,000 r.p.m. for 1 hour; *i.e.*, the first supernate was used for the second centrifugation, the second for the third, and so forth. The results of ID_{50} and HA titrations in 2 such experiments are shown in Table II. As can be seen, the infectivity in the supernate of allantoic fluid in Experiment 1 had decreased by the third centrifugation to 0.003% and hemagglutination no longer was demonstrable. An increase in the centrifugal force (run No. 4) resulted in little additional sedimentation of infectivity. Again, strikingly different were the results obtained with the suspensions of membranes. After the third and fourth centrifugations only about 0.002% of the original infectivity was left in the supernate, but 5 and 1%, respectively, of the hemagglutinins remained suspended. The ID_{50}/HA ratios in the supernates decreased somewhat on successive centrifugation and approached a value of 10^2. Recentrifugation of the resuspended sediments of allantoic fluid or membrane gave similar results in that in the order of 95% of the hemagglutinins were found sedimentable in both instances (see Table IV).

These observations reveal a definite discrepancy between sedimentation of infectivity and hemagglutinins from allantoic fluid, on the one hand, and from allantoic membrane

† Similar experiments were conducted recently with the Victoria and California No. 11914 strains of NDV with essentially similar results.

TABLE II. Effect of Repeated Centrifugation Upon Infectivity and Hemagglutinating Capacity of Allantoic Fluid and Allantoic Membrane Suspension.

Exp.	Inoculum dilution	Incubation, hr	Material	Allantoic fluid					Allantoic membrane				
				ID_{50}/ml log	% of original	HA units/ml log	% of original	Ratio ID_{50}/HA log	ID_{50}/ml log	% of original	HA units/ml log	% of original	Ratio ID_{50}/HA log
1	10^{-6}	48	Original supernate	9.98		3.58		6.4	9.08		3.71		5.4
			1st	7.93	.9	2.20	4.2	5.7	5.68	.04	2.98	18.7	2.7
			2nd	6.30	.02	.70	.1	5.6	4.69	.004	2.68	9.3	2.0
			3rd	5.47	.003	<.07	<.1	—	4.30	.002	2.38	4.7	1.9
			4th	5.07	.001	<.70	<.1	—	4.30	.002	1.60	0.8	2.7
2	10^{-6}	48	Original supernate						8.47		3.41		5.1
			1st						4.93	.03	2.81	25.0	2.1
			2nd						4.56	.01	2.51	12.5	2.1
			3rd						3.68	.002	2.38	9.3	1.3

suspensions, on the other hand. The results seem to indicate that in the membranes, and possibly to a lesser extent also in allantoic fluids, 2 distinct hemagglutinating components may be found as a result of infection with NDV. One of these appears to be inseparable from, and therefore probably indentical with the infectious principle, the other seems to be of smaller size and probably noninfectious. This explanation has not been contradicted by a number of subsequent experiments which were devised to substantiate further this suggestion.

That both particles are products of NDV infection could be shown by hemagglutination inhibition tests. As can be seen in Table III the hemagglutinating capacity of both allantoic fluid and membrane preparations, as well as of the corresponding supernatant fluids, obtained after high speed centrifugation, were largely neutralized by a serum obtained from a patient convalescent from an infection with NDV whereas mumps convalescent serum failed to do so. In the slide test the specific serum completely prevented visible agglutination of the red cells by all virus materials tested. In the tube test the HA titers were reduced to a fraction of 1% in the cases of the more potent virus preparations and the tests became negative in the supernates showing lesser titers of hemagglutinins. These results clearly indicate a relationship between the non-sedimentable hemagglutinins and NDV.

TABLE III. Specificity of Hemagglutinins in Allantoic Fluid and Membrane Preparations.

Material	Serum	Slide test	Tube test HA units/ml
Allantoic fluid			
Original	Saline	+	1920
	Anti NDV	—	5
	" mumps	+	1920
Supernate	Saline	+	80
20,000 r.p.m.	Anti NDV	—	<2.5
	" mumps	+	40
Allantoic membrane			
Original	Saline	+	960
	Anti NDV	—	2.5
	" mumps	+	960
Supernate	Saline	+	120
20,000 r.p.m.	Anti-NDV	—	<2.5
	" mumps	+	120

It had been shown in the past that the medium in which the virus is contained may influence its shape; that is, when suspended in allantoic fluid or distilled water it assumes a spherical form, whereas in buffered saline solution it appears filamentous(9). In the light of this information attempts were made to interchange, as far as possible, the media of the two virus preparations prior to centrifugation studies. Consequently, in one set of experiments equal parts of normal allantoic fluid were mixed with suspensions of infected membranes, and, conversely, suspen-

9. Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 135.

TABLE IV. Effect of Medium on Sedimentation of Hemagglutinating Capacity in Allantoic Fluid and Allantoic Membrane Suspension.

Material	Medium added	Allantoic fluid HA units/ml Original	Supernate	Sediment	% of original in supernate	Allantoic membrane HA units/ml Original	Supernate	Sediment	% of original in supernate
Original	0	7680	240	1920	3.1	5120	960	1920	18.7
"	Saline sol.	1920	80	960	4.2	2560	320	480	12.5
"	Norm. mem. susp.	1920	80	1920	4.2	1920	320	960	16.6
"	Norm. fluid	1920	80	960	4.2	1920	480	960	25.0
Sediment 20,000 rpm	Buffered saline	2560	120	1920	4.7	—	—	—	
" "	Norm. mem. susp.	3840	120	3840	3.1	—	—	—	
" "	Buffered saline	2560	80	1920	3.1	1920	60	1280	3.1
" "	Distilled H_2O	3840	60	3840	1.5	1920	120	1920	6.2

sions of normal allantoic membranes were added to infected allantoic fluids. In a second series the sediments obtained by high speed centrifugation from aliquots of an infected allantoic fluid preparation were redispersed in a suspension of normal allantoic membrane, in distilled water, or in buffered saline solution. Similarly, sediments from infected membranes were suspended in distilled water or buffered saline solution. These various preparations were then subjected to centrifugation at 20,000 r.p.m. for 20 minutes and the various fractions tested for hemagglutinating activity. When the corresponding preparations were compared no significant differences became apparent in the sedimentation of hemagglutinins regardless of the medium in which they were suspended. These results are tabulated in Table IV. As far as has been ascertained, changes in the medium were without apparent influence upon sedimentation.

Furthermore, attempts were made to determine whether non-specific inhibitors of hemagglutination might have played a role in the experiments. Treatment of infected allantoic fluids and membrane suspensions with receptor destroying enzyme (RDE) as contained in culture filtrates of *V. cholerae*(10), or with $NaIO_4$(8), failed to change significantly the results of hemagglutination tests as compared to those obtained with untreated preparations. It was noted, however, that the hemagglutinin titers in membrane preparations after periodate treatment were on the whole lower but the percentage of hemagglutinins remaining in the supernate after high speed centrifugation was similar to that left suspended in untreated preparations.

Finally efforts were made to separate, if possible, the two types of hemagglutinating components by Seitz filtration. Infected allantoic fluids, as well as supernates and sediments of membrane suspensions obtained by high speed centrifugation were passed through Seitz E. K. filters of the Boerner type in 2 successive portions of 10 ml each. Whereas the allantoic fluids passed without great delay, difficulties were encountered with the membrane preparations and prolonged centrifugation was required to filter the amounts needed. Hemagglutination tests with these materials, as summarized in Table V, Section A, showed that no hemagglutination became measurable in the filtrates of the allantoic fluids and the high speed sediments of the membranes under the conditions of the experiments. On the other hand, filtration of the high speed supernates of the membrane suspensions clearly resulted in passage of hemagglutinins. These data would seem to be in agreement with the suggestion that two hemagglutinating units are formed in the in-

10. Burnet, F. M., McCrea, J. F., Stone, J. D., *Brit. J. Exp. Path.*, 1946, v27, 228.

TABLE V. Seitz Filtration of Allantoic Fluid and Membrane Suspensions.

Material	HA units/ml in			ID_{50}/ml in	
	Original	1st filtrate	2nd filtrate	Original log	1st filtrate log
A. Allantoic fluid (1) Original	1280	<5	<5		
" " (2) "	5120	<5	<5		
" " (3) "	2560	<5	<5		
Allantoic membrane (1) Supernate 20,000 rpm	480	120	320		
" " Sediment in buffered saline	320	<5	<5		
" " (2) Supernate	320	160	320		
Sediment in buffered saline	960	<5	<5		
B. Allantoic fluid (4) Original 20,000 rpm	2560	<5	<5		
" " Supernate	320	<5	<5		
" " Sediment in buffered saline	1280	<5	<5		
" " Sediment in NMS	1280	320	640		
Allantoic membrane (4) Original 20,000 rpm	7680	240	480		
" " Supernate	1280	960	1920	6.04	5.63
" " Sediment in buffered saline	2560	<5	<5	8.80	<2.30
" " Sediment in NMS	3840	120	320	8.68	6.82
C. Allantoic fluid (4) + Saline Solution Original	3840	<10	<10	9.13	2.52
" " + NMS Original	3840	80	960	9.52	7.97

fected chick embryo, one readily filtrable and found mainly in the membranes, the other largely retained by the Seitz filter and contained in the allantoic membranes as well as the fluids. However, since filtration is readily affected by various physical factors it was thought advisable to study the influence on Seitz filtration of the addition of supernatant fluid obtained from normal allantoic membranes by high speed centrifugation (NMS) to non-filtrable preparations of hemagglutinins. The method of filtration in these experiments was similar to that described in the previous experiments with the exception that smaller filters with a capacity of 2 ml were employed. As shown in Table V, section B, resuspension in NMS of hemagglutinins sedimented from allantoic fluids or emulsified membranes permitted their passage through the filter, whereas, when suspended in buffered saline solution, they failed to come through. Similarly, when NMS was added in equal volumes to infected allantoic fluids, hemagglutinins filtered through the Seitz pad quite readily (Table V, Section C). Thus, these experiments were unrevealing as far as differentiation between hemagglutinins was concerned. The infectivity titrations, where done, showed that the infective principle as well as the hemagglutinins passed through the filter in the presence of NMS.

Discussion. The hemagglutinins of various viruses have been found to be closely associated with the respective infectious principles, except for those observed in the pox group of agents(11). However, recently some evidence has been presented which indicates that early in the infectious cycle of influenza virus in the chick embryo a hemagglutinin appears which seems to be non-infectious as yet and which is considered an "immature" or "developmental stage" in virus production (12). Furthermore, injections of influenza virus into tissues, which, as a rule, do not support propagation of the agent to the extent of liberation of fully infectious virus, nevertheless may lead to the development of "incomplete" forms, *i.e.*, a non-infectious, hemagglutinating component(13). Upon

11. Burnet, F. M., and Stone, J., *Austral. J. Exp. Biol. and Med. Sc.*, 1946, v24, 1

12. Henle, W., and Henle, G., *J. Exp. Med.*, 1949, v90, 23.

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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13. Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 541.

14. Friedewald, W. F., and Pickels, E. G., *J. Exp. Med.*, 1944, v79, 301.

15. Gard, S., and von Magnus, P., *Ark. Kemi, Miner. och Geol.*, 1947, v24b, No. 8.

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