Summary. The ability of a strain of Coxsackie virus, group B type 1, to propagate in cultures of mouse interscapular fat pad tissue and in fibroblasts derived from that tissue was investigated. The virus multiplied in primary cultures containing both explanted host cells and outgrowing fibroblasts, as well as in cultures of fibroblasts carried through 3 tissue culture passages. In each instance the growth of virus was accompanied by progressive cytopathogenic effects which continued until nearly all of the fibroblasts were destroyed.

1. Dalldorf, G., *Bull. N. Y. Acad. Med.*, 1950, v26, 329.

2. Pappenheimer, A. M., Daniels, J. B., Cheever, F. S., and Weller, T. H., *J. Exp. Med.*, 1950, v92, 169.

3. Godman, G. C., Bunting, H., and Melnick, J. L., *Am. J. Path.*, 1952, v28, 233.

4. Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, v81, 233.

5. Contreras, G., Barnett, V. H., and Melnick, J. L., *J. Immunol.*, 1952, v69, 395.

6. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

7. Shaw, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 718.

8. Stulberg, C. S., and Schapira, R., *J. Immunol.*, in press.

9. Scherer, W. F., and Syverton, J. T., *J. Exp. Med.*, 1952, v96, 389.

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Variables in Agglutination and Lysis of Human Red Cells by NDV. (19972)

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An hemolysin associated with the Newcastle disease virus particle has been described by Kilham(1) and also by Burnet and Lind(2). Both groups of workers found that chicken red cells were hemolyzed more readily than human red cells. This paper reports the hemolysis of human O-type red cells by the vaccine strain of NDV and outlines the variables affecting the agglutination and lysis of these cells. Freshly harvested virus did not agglutinate or lyse fresh human red cells but previously frozen virus did both. Furthermore, fresh human red cells which were not agglutinated by fresh virus were agglutinated after storage of the red cells at 4°C for 5-7 days.

Materials and methods. Virus. The Blacksburg or Hitchner(3) strain of NDV which is currently used as a commercial live vaccine for newly hatched chicks, was furnished by Dr. Herald Cox of Lederle Laboratories. This virus had several passages in chick embryos at our laboratory and is referred to here as the Vaccine Strain(4). In contrast to other strains, the vaccine virus causes a transient agglutination of chicken red blood cells and the reaction is inhibited by cold(4). Pools of virus were prepared by allantoic inoculation of 11-day-old embryos and the allantoic fluid was harvested after 2 days of incubation at 35°C. *Human O-type red blood cells.* In most of the experiments, the red cells were from one individual. Blood was obtained by intravenous puncture and put into a large volume of chilled 0.85% NaCl buffered at pH 7.2 with 1% 1/15 M phosphate, without any citrate or anticoagulant. The red cells were washed 3 times with buffered saline and a 1% cell suspension was used in the experiments. *Glassware.* Since the persistence of slight amounts of detergents may give irregular results in the hemagglutination tests(5), no lysol or detergents were used in our laboratory for sterilizing or cleansing glassware. All glassware was cleansed by overnight immersion in chromic acid cleaning solution. Following that, the pipettes and tubes were washed 7 times in running tap water and twice

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TABLE I. Effect of the Vaccine Strain NDV on Fresh Human O-Type Red Cells.

Virus dilution		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Untreated virus	H.A.*	0	0	0	0	0	0	0	0
	H.O.*	0	0	0	0	0	0	0	0
Virus frozen and thawed	H.A.	—	—	—	0	0	0	0	0
	H.O.	4†	4	3	1	1	0	0	0

* H.A. = Hemagglutination; H.O. = Hemolysis.
† Figure denotes degree of hemolysis on hemagglutination. 4 is max; 0 is neg.

TABLE II. Effect of Calcium Ion on Hemolysis and Hemagglutination.

Virus dilution		1:10	1:20	1:40	1:80	1:160	1:320	1:640
PO_4 buffered saline	H.A.	—	—	—	±	0	0	0
	H.O.	4	4	3	2	0	0	0
Ca " "	H.A.	2	3	4	4	3	2	0
	H.O.	0	0	0	0	0	0	0

in distilled water. The glassware was then dried and used. *Titrations.* All titrations were carried out in 0.85% NaCl buffered at pH 7.2 with 1% M/15 phosphate. Serial 2-fold dilutions starting with a 1/10 dilution were set up in 10 × 100 mm Wassermann tubes. Agglutination or hemolysis was graded as 0 to 4+ by direct examination. The end-point of the titration was considered to be that dilution which produced at least a 2+ reading.

Results. Effect of the virus on fresh human red cells. Freshly harvested virus or virus left at 4°C for 2 weeks did not agglutinate or hemolyze fresh human red cells. However, the same batch of virus after it had been frozen at —70°C in a CO_2 box did hemolyze the same red cells (Table I).† Hemolysis occurred at room temperature within 30 minutes after the virus and red cells were mixed. The treated virus (previously frozen) lysed the red cells so completely that it was impossible to determine whether any agglutination took place. Hemolysis was inhibited by calcium ion and in the presence of calcium the hemagglutination pattern appeared (Table II).

Effect of the virus on "aged" human red cells. Although fresh human cells were not agglutinated by fresh virus, they were agglutinated after being stored at 4°C for 5-7 days. However, the fresh virus did not hemolyse these "aged" red cells. Treated virus agglutinated and hemolyzed the "aged" red cells just as well as the fresh ones (Table III).

Inhibition of hemolysis by specific immune sera. The specific relation of the hemolysis to the virus was tested determining the degree of inhibition by convalescent immune chicken sera. Hemolysis was completely inhibited by immune sera obtained from convalescent chickens inoculated with NDV, either the B strain or vaccine strain. Normal chicken serum or serum from a chicken immunized to swine influenza or mumps had no inhibitory effect (Table IV). All tested sera were inactivated by heating at 56°C for 30 minutes.

Discussion. It seems likely that the hemolysis reported here actually is a property of the Newcastle infection since it was inhibited by specific sera and was a constant property of the virus. The remarkable conversion of a preparation which did not hemolyze cells to one which did merely by freezing and thawing may indicate that this procedure has broken up the virus particles. Indeed it has been previously demonstrated(6) that freezing and thawing of allantoic fluid containing NDV will cause a much larger portion of the virus to remain in the supernatant fluid after high speed centrifugation. However, the present experiments are not extensive enough to rule out other explanations.

† Drs. A. Granoff and W. Henle have recently informed us that a similar effect of freezing and thawing has been observed in their laboratories.

TABLE III. Effect of Vaccine NDV on Different Ages of Human Red Cells.

Virus dilution			1:10	1:20	1:40	1:80	1:160	1:320	1:640
Virus at 4°C for 7 days	2 day 0–cells	H.A.	0	0	0	0	0	0	0
		H.O.	0	0	0	0	0	0	0
	7 "	H.A.	0	1	1	3	2	0	0
		H.O.	0	0	0	0	0	0	0
Virus at −70°C for 7 days	2 "	H.A.	—	—	—	—	2	0	0
		H.O.	4	4	3	1	1	0	0
	7 "	H.A.	—	—	—	2	2	0	0
		H.O.	4	4	3	1	0	0	0

TABLE IV. Inhibition of Hemolysis by Immune Sera.*

Serum dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Normal chicken	2	2	2	2	2	2	2	2
Ch. homologous imm. ser.	0	0	0	0	0	±	2	2
Ch. heterologous imm. ser. (B strain NDV)	0	0	0	0	0	0	0	0
Ch. swine flu imm. ser.	2	2	2	2	2	2	2	2
Ch. mumps imm. ser.	2	2	2	2	2	2	2	2

* Performed against 8 hemolytic units.

The capacity of the vaccine strain of NDV to agglutinate aged human red cells is clear. However, the titers indicate that this system needs as great a concentration of virus particles to agglutinate these relatively resistant cells as it does to lyse them. Thus it was possible to set up titrations in which lysis of the cells was the only apparent reaction.

This differs from the effect of washed untreated virus on chicken red cells(7), in which agglutination is produced by a fraction of the number of particles necessary to produce hemolysis.

Summary. The vaccine (Blacksburg) strain of NDV harvested from allantoic fluid of chick embryos, after freezing at −70°C and subsequent thawing, agglutinated and hemolyzed human O-type red blood cells. The same virus, untreated, did not agglutinate or lyse fresh human red cells. However, if the red cells were stored at 4°C for 5-7 days, they were agglutinated but not hemolyzed by the untreated virus.

1. Kilham, L. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 63.

2. Burnet, F. M., and Lind, P. E., *Australian J. Exp. Biol. and Med. Sci.*, 1950, v28, 129.

3. Hitchner, S. B., and Johnson, E. P., *Vet. Med.*, 1948, v43, 525.

4. Bang, F. B., and Foard, M. A., *Am. J. Hyg.*, 1952, v55, 363.

5. Bang, F. B., *Bull. Johns Hopkins Hosp.*, 1949, v84, 497.

6. ———, *J. Exp. Med.*, 1948, v88, 251.

7. Bang, F. B., and Libert, R., *Am. J. Hyg.*, 1952, v55, 373.

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