lishing the existence of such a reflex in a crucial way. Had a material fall of right atrial pressure taken place in these experiments while arterial pressure was kept from rising, no one would have contested the conclusion that the Bainbridge reflex is responsible for the degree of slowing shown in Fig. 2 and 3. Having unwittingly devised an experiment in which neither right atrial nor arterial pressure altered significantly, it becomes apparent that these changes in heart rate must be attributed to still a different factor.

The possibility has been suggested[8,9] that differences in pulse pressure, aside from alterations in mean arterial pressure, may operate to cause reflex alteration in heart rate. However, this factor is also excluded in the curves of Fig. 2 and 3, in which the pulse pressure likewise remains unaltered before the heart rate slows. In short, the slight subsidiary changes in heart rate which are still found upon compression of an A-V fistula remain undetermined.

Summary. An attempt was made to determine whether reflexes other than those from arterial pressor receptors are concerned in modulation of the heart rate when an A-V fistula is closed or opened. In dogs under chloralose anesthesia, bilateral A-V shunts were established between femoral arteries and veins, one of which being so arranged that blood could be drained from the animal from the arterial side during closure of the shunt in an amount sufficient to stabilize arterial pressure at a fairly constant level.

In such experiments it was found that slight slowing of the heart rate still occurs on closing a shunt even when arterial pressure falls a little and the pulse pressure is unaffected. Since supplementary experiments of a similar type failed to reveal significant changes in right atrial pressure, changes in central venous pressure were also excluded. These experiments call attention to the difficulty of establishing the operation of the Bainbridge reflex in a crucial manner.

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8 Bronk, D. W., *Harvey Lec.*, 1934, **29**, 245.

9 McCrea, F. D., and Wiggers, C. J., *Am. J. Physiol.*, 1933, **103**, 417.

17080. A Newcastle Disease Virus (NDV) Hemolysin.

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The first observations of a virus hemolysin were those by Morgan, Enders, and Wagley[1] in regard to mumps virus. An hemolysin associated with Newcastle Disease Virus (NDV) has not hitherto been described, although its presence has been observed.[2] The methods employed in the present investigation were modifications of those described by Morgan *et al.*[1]

Dr. Erwin Jungherr kindly supplied the California strain of NDV (no. 11,914) used in all experiments. Infected allantoic and amniotic (AA) fluids were harvested together 36 hours after intra-allantoic inoculation of 10-day-old embryonated eggs with NDV. A single pool of fluids, stored in CO_2 ice, was used throughout. In hemagglutination (HA) tests, 0.5 cc of 0.25% hen erythrocytes was added to an equal amount of each virus dilution. Readings were taken after 1¼ hours at room temperature. Two per cent cells were used in hemolysis (HL) tests which were incubated for 2 hours at 37°C. Except for 1 test run at pH 6 (Table I), all dilutions were made in isotonic phosphate buffer pH 7.2.

Some properties of the NDV hemolysin are illustrated in Table I. Hemagglutination and hemolysis tests run on the original AA fluids served as controls. These were of especial

1 Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.*, 1948, **88**, 503.

2 Florman, A. L., personal communication.

TABLE I. Effects of Heat, pH, and Species Differences in Erythrocytes on Hemagglutination and Hemolysis by Newcastle Disease Virus (NDV).

	Test	Dilutions of NDV										Buffer cont.
		8	16	32	64	128	256	512	1024	2048	4096	8192	
Control pH 7.2	HA	4*	4	4	4	4	4	4	4	4	3	0	0
Titration	HL	4	4	4	3	2	1+	1	±	0	0	0	0
Effect of heat													
51°C 15 min.	HA	4	4	4	4	4	4	4	4	3	0		0
	HL	3	3	3	2+	2	1+	1	±	0	0		0
60°C 8 min.	HA	0	0	0	0	0	0	0	0	0	0		0
	HL	0	0	0	0	0	0	0	0	0	0		0
Effect of pH													
pH 6	HA	4	4	4	4	4	4	4	4	4	0		0
	HL	4	3	2	2—	1+	1	±	0	0	0		0
	(Brown)												
Erythrocytes different species													
Sheep	HA	4	4	4	4	4	4	4	0	0	0		0
	HL	4	4	3	2	1	0	0	0	0	0		0
Human "O"	HA	4	4	4	4	4	4	4	0	0	0		0
	HL	4	4	3	2	1	±	0	0	0	0		0

* Figures according to a scale ranging from 0 to 4, denote (HA) degree of hemagglutination and (HL) degree of hemolysis.

importance in reading the hemolysin (HL) tests, as the figures given were arrived at by direct comparison with the control tubes used as standards of the degree of hemolysis present. In a 1:8 dilution of the original AA fluid, 100% of the hen erythrocytes were hemolyzed, only cell stroma sedimenting to the bottom of the tube. Heating at 51°C for 15 minutes lowered the hemagglutinin and hemolysin titers only slightly, but both activities were destroyed after heating at 60°C for 8 minutes. The experiments carried out at pH 6 were not wholly satisfactory. For one thing, hen erythrocytes tend to agglutinate spontaneously in the absence of virus as the pH is lowered, a phenomenon regularly observed below pH 5. Secondly, hen hemoglobin is apparently altered at pH 6, as it becomes brown. Readings of the hemolysin were made by adding a small amount of HCl to the control tubes, to render the standards comparable. With these reservations in mind, it would appear the hemagglutination and hemolysis by NDV show some decrease in titer at pH 6. Sheep and human "O" cells gave lower titers in the hemagglutination and hemolysis tests than did hen erythrocytes. Normal AA fluids gave no hemolysis.

The NDV hemolysin can be absorbed and eluted from red blood cells as indicated in Table II. In these experiments 1 cc of NDV-infected AA fluids was added to 5 cc of a 10% suspension of hen erythrocytes and the mixture centrifuged, after incubation for one hour at 4°C. All of the hemolytic and the greater part of the hemagglutinating activity of NDV was removed from the supernatant by adsorption on the erythrocytes as shown under A of Table II. The sedimented erythrocytes plus adsorbed virus were then resuspended in buffer and incubated at 37°C for 2 hours. As indicated under B of Table II the erythrocytes recovered by subsequent centrifugation were refractory to the hemagglutinating and hemolytic activity of a fresh virus suspension. Tests on the supernatant fluid using fresh erythrocytes demonstrated that NDV had eluted, for the hemagglutination titer was even higher than in the original AA fluid. This elevation of hemagglutination titer has been explained by others[3] on a sup-

TABLE II.
Adsorption and Elution of Newcastle Disease Virus (NDV) Hemolysin and Hemagglutinin from Hen Erythrocytes.

	Test	Dilutions of NDV											Buffer cont.
		2	8	16	32	64	128	256	512	1024	2048	4096	8192	
Original AA fluid	HA		4*	4	4	4	4	4	4	4	4	3	0	0
	HL		4	4	3	2	1+	1	±	0	0	0	0	0
A. Incubation 4°C 1 hr														
Supernate	HA		4	4	3	0	0	0	0	0	0	0	0	0
	HL	0	0	0	0	0	0	0	0	0	0	0		0
B. Resuspended RBC from (A) incubation 37°C, 2 hr			0	0	0	0	0	0	0	0	0	0		0
Eluted RBC +	HA		0	0	0	0	0	0	0	0	0			0
Fresh NDV	HL													
Eluate and fresh RBC	HA		4	4	4	4	4	4	4	4	4	4	4	0
	HL not done as eluate too colored by previous hemolysis.													

* Figures according to a scale of 0 to 4, denote (HA) degree of hemagglutination and (HL) degree of hemolysis.

position that adsorption and elution serve to break up aggregates of virus and give greater dispersion. Hemolytic activity of the supernate could not be tested, since previous hemolysis resulting from incubation at 37°C rendered it unsuitable.

Inhibition of the NDV hemolysin by specific immune sera is demonstrated in Table III. In these tests the original AA fluids, titrated as in Table I, were used in a dilution of 1:32. Paired sera from a hen experiencing a natural attack of Newcastle disease and a pair of sera from a rabbit immunized with NDV, both gave a 64 fold rise of antibody titer in the inhibition of hemolysin test. Sera of 2 rabbits immunized against influenza virus, one with the PR 8 strain and one with LEE B, and a pair of acute and convalescent phase mumps sera were without specific inhibitory action.

To determine whether the infectivity of the NDV suspension was associated with the hemolytic activity, the AA fluids were treated with 0.2% formalin and incubated over night at 4°C. The hemagglutination and hemolysis tests showed no decrease in titer when formalin treated virus was used. An infectivity titration was carried out in embryonated eggs, using an aliquot of the formalin treated suspension. The formalin treatment lowered the LD_{50} end point of the NDV suspension from 10-9.45 to 10-1. Inoculation of controls indicated that 0.2 per cent formalin in broth was not lethal for embryonated eggs.

Discussion. Although it is impossible on the basis of present data to state whether the hemolysin associated with NDV is identical with the hemagglutinin or represents a separate factor the behavior of both is more or less parallel in the above experiments. This parallelism is noteworthy in regard to adsorption and elution from red blood cells, the response to heating and to lowering of the pH, as well as in reaction to erythrocytes of different animal species and the inhibitory effect of specific immune serum. Neither activity appears to reflect the infective potency of an NDV suspension as judged by the action of formalin. The NDV hemolysin exhibits points of similarity with the hemolysin associated with mumps virus. However,

TABLE III.
Inhibition of NDV Hemolysis by Specific Immune Serum.

Source of serum	Serum phase	Dilution of NDV 16	32	64	128	256	512	1024	2048	Serum cont.
Hen—natural	Acute	1*	2	3	3	3	3	3	3	0
Attack with NDV	Conval.	0	0	0	0	0	1	2	3	0
Rabbit—immunized	Pre-inoc.	1	2	3	3	3	3	3	3	0
with NDV	Immune	0	0	0	0	0	1	2	3	0
Rabbits immunized	PR8-immune	1	2	3	3	3	3	3	3	0
with influenza virus	LEE B- "	1	2	3	3	3	3	3	3	0
Mumps patient	Acute	1	2	3	3	3	3	3	3	0
	Conval.	1	2	3	3	3	3	3	3	0

* Figures denote degree of hemolysis as compared to control titration. Table II).

in other respects, the NDV hemolysin as demonstrated above differs from the description of the mumps virus hemolysin given by Morgan *et al.*[1] especially as regards the effect of heat and a lowering of the pH. Whether these differences are real or a reflection of the fact that the NDV hemolysin is more potent is not easy to determine. In our experience, it has been difficult to find a mumps virus hemolysin of sufficient potency for comparative tests.

The fact that hemolysins are associated with both NDV and mumps virus, but not with influenza virus,[1] is of interest in indicating a possible affinity of mumps and Newcastle viruses, as first suggested by Burnet[3,4] in his work on "receptor gradients." Recent demonstration of serologic relationships between NDV and mumps virus,[5] evidenced by the presence of neutralizing and antihemagglutinating antibodies in nearly half of patients convalescent from mumps, lends further support to this hypothesis.

Summary. (1) A hemolysin associated with the virus of Newcastle disease is described. (2) The activities of this hemolysin paralleled those of the NDV hemagglutinin in regard to adsorption and elution from hen erythrocytes, inhibition by specific immune serum, inactivation by moderate heating, and in its behavior with erythrocytes of different animal species. (3) A suggestion is made that similarities in behavior between the hemolysin associated with NDV and that associated with mumps virus are further indication of possible relationship between the 2 viral agents.

[3] Enders, J. F., Levens, J. H., and Robbins, F. C., personal communication.

[4] Burnet, F. M., *Australian J. Sci.*, 1945, **8**, 81.

[5] Jungherr, E., Luginbuhl, R. E., and Kilham, L., to be published.

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