drine. It is of interest that Moe and Seevers[5] reported central impairment of sympathetic reflexes in dogs treated with plasmochin, a closely related 8-aminoquinoline.

Summary. 1. The administration of pentaquine in high dosage to patients with essential hypertension frequently produced a significant reduction in resting arterial blood pressure, usually accompanied by the development of postural hypotension. This depressor effect occurred abruptly after several days of therapy and receded gradually over a period of several days to several weeks following cessation of therapy.

2. Patients with malignant hypertension exhibited a similar response, but did not require as high dosage. With the fall in blood pressure there was some regression of neuroretinitis, headache and gross hematuria: but no significant improvement in renal function.

3. Pentaquine appeared to exert its effects primarily through a sympatholytic action.

4. Toxic reactions to the drug were too frequent and severe to consider its use practicable in the treatment of essential hypertension.

[5] Moe, G. K., and Seevers, M. H., *Fed. Proc.*, 1946, **5**, 193.

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Hemagglutination with Newcastle Disease Virus (NDV).

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Newcastle disease of fowl, an acute infection associated with severe intestinal, respiratory and nervous symptoms, was first recognized as a distinct entity by Kraneveld[1] in the Dutch East Indies and by Doyle[2] in Newcastle, England. Doyle gave the disease its present name and demonstrated that the causative agent is filterable.

Recently there has been increased interest in the virus which causes Newcastle disease, especially because of its capacity to cause hemagglutination.[3] In addition, although the western hemisphere was thought to be free of the disease, Beach[4] showed that there is an immunological similarity between classical Newcastle disease virus (NDV) and the virus of avian pneumoencephalitis, a disease which is of frequent occurrence in this country. The potential human pathogenicity of the virus for man was indicated in 1943 with the report of an accidental laboratory infection.[5] This resulted in a brief, mild illness associated with conjunctivitis and general symptoms. In 1946 2 similar laboratory infections[6] and a small epidemic of conjunctivitis among women who had been exposed to infected fowls were reported.[7]

Burnet[3] has raised the possibility of "an affinity between NDV and the influenza virus group" chiefly on the basis of hemagglutinating capacity, infectivity for the chick embryo and apparent particle size. However, no immunological relationship could be demonstrated, and recently Bang[8] and others[9] have shown by electron microscopy that NDV

* Aided by a grant from the Welt Fund of Mt. Sinai Hospital, New York.

[1] Kraneveld, F. C., *Nederl. Indisch. Blad. Diergeneesk*, 1926, **38**, 448.

[2] Doyle, T. M., *J. Comp. Path. and Therap.*, 1927, **40**, 144.

[3] Burnet, F. M., *Austral. J. Exp. Biol. and Med. Sci.*, 1942, **20**, 81.

[4] Beach, J. R., *Science*, 1944, **100**, 361.

[5] Burnet, F. M., *Med. J. Austral.*, 1943, **2**, 313.

[6] Anderson, S. G., *Med. J. Austral.*, 1946, **1**, 371.

[7] Yatom, J., *J. A. M. A.*, 1946, **132**, 169.

[8] Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 5.

[9] Cunha, R., Weil, M. L., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., *J. Immunol.*, 1947, **55**, 69.

TABLE I.
Successive Titrations of Newcastle Disease Virus at Room Temperature.*

No. of days chicken red cells were stored†	Dilution of virus‡												End point
	4	8	16	32	64	128	256	512	1024	2048	4096	8192	
1	1	2	2	2	2	2	2	2	2	0	0	0	1024
2	1	2	2	3	3	3	3	3	3	2	±	0	2048
3	1	1	2	1	1	2	3	2	2	2	1	0	2048
4	±	1	2	3	3	3	3	3	3	2	1	0	2048

* Room temperature = 22°C to 27°C.
† Red cells obtained from one chicken, and stored at 4°C, concentration = 0.75%.
‡ Reciprocal of final dilution of pool 3.
Results were read at 30 minutes.

TABLE II.
Successive Titrations of Newcastle Disease Virus at 4°C.

No. of days chicken red cells were stored*	Dilution of virus†											End point
	4	8	16	32	64	128	256	512	1024	2048	4096	
1	4	4	4	4	4	4	3	2	1	±	0	512
2	4	4	4	4	4	4	4	4	2	1	0	1024
3	4	4	4	4	4	4	3	2	1	0	0	512
4	4	4	4	4	4	4	4	3	2	±	0	1024

* Red cells, identical to Table I.
† Reciprocal of final dilution of pool 3.
Results were read at 60 minutes.

is strikingly different from the influenza viruses.

In a study of the phenomenon of hemagglutination, NDV was investigated. It was found that the methods which have been developed for influenza viruses were not satisfactory with NDV. When hemagglutination titrations were carried out at room temperature, there was much less marked agglutination with low virus dilutions than with higher ones. Even with more dilute chicken red cell suspensions and a shorter reaction time as recommended by Brandly *et al.*[10] this disturbing effect persisted and the reactions were difficult to read and to reproduce. It is the purpose of this communication to show how this effect can be circumvented by carrying out hemagglutination reactions with NDV in the cold and to offer an explanation for this peculiar property of the virus. In addition, some of the quantitative aspects of hemagglutination by NDV will be presented.

Virus. A strain of Newcastle disease virus (NDV) was obtained from Dr. G. K. Hirst who had received it from Dr. F. R. Beaudette. Further passages were carried out by inoculation into the allantoic fluid of 9-12-day-old chick embryos. After approximately 48 hours incubation at 37°C, the eggs were refrigerated at 4°C for 3-18 hours, after which the allantoic fluid was collected. Infected fluid was stored either at −70°C or at 4°C.

Sera. An authentic antiNDV serum was obtained from the Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C. Specific antisera were prepared in rabbits by slowly injecting into the ear vein 10 cc of allantoic fluid containing the virus. A second intravenous injection, identical to the first, was given after 6 weeks and serum was obtained 2 weeks later.

Saline. An 0.85% solution of sodium chloride containing 0.01M phosphate buffer at pH 7.2 was used throughout.

Chicken red cells. Blood was obtained from the wing vein of individual chickens in an excess of 2% sodium citrate. The cells were washed twice in the usual manner, and suspensions of desired concentration were prepared in saline from cells which had been packed by 10 minutes centrifugation at 1500 r.p.m.

[10] Brandly, C. A., Moses, H. E., Jungherr, E. L., and Jones, E. E., *Am. J. Vet. Research*, 1946, **7**, 289.

TABLE III.
Effect of Temperature on Titrations with Newcastle Disease and Influenza Viruses.

Temp.,°C	RBC 1.5%	Virus	Dilution of serum† 8	16	32	64	128	256	512	1024	2048	End point
22	chicken	NDV	0	±	1	1	2	3	3	1	0	512
4	”	”	4	4	4	3	3	2	±	0	0	256
22	”	PR8	4	4	4	4	4	4	4	2	1	1024
4	”	”	4	4	4	4	4	4	3	3	0	1024
22	”	Lee	4	4	4	4	4	4	3	1	±	512
4	”	”	4	4	4	4	4	4	3	±	0	512
22	human	NDV	1	2	2	3	3	3	2	0	0	512
4	”	”	2	1	±	0	0	0	0	0	0	8
22	”	PR8	4	4	3	3	3	2	2	1	0	512
4	”	”	4	4	3	3	2	2	2	0	0	512
22	”	Lee	4	4	3	3	3	3	1	0	0	256
4	”	”	4	4	3	3	2	0	0	0	0	128

* Reciprocal of final dilution.
Results were read at 60 minutes.

Test tubes. Pyrex tubes 75 by 10 mm were found to be satisfactory.

Titration of virus. As was stated above, when a titration of NDV is carried out by the hemagglutination technic at room temperature, an effect which simulates a serological prozone appears. This is evident from the results shown in Table I. However, if the reaction is carried out in cold (4°C), as originally suggested by Burnet,[3] this effect disappears and clear cut end-points are obtained, as can be seen from the results shown in Table II. Therefore, the following titration technic was employed: Serial 2-fold dilutions of infected allantoic fluid are made in cold saline. To each 0.4 cc of dilution an equal volume of chilled 1.5% chicken red cell suspension is added. The tubes are shaken and immediately placed in the refrigerator (4°C) for one hour. The patterns of sedimented cells are then noted while the tubes are still cold. Complete agglutination is considered to have occurred if a solid plaque of cells, which does not stream on tilting the tube, is seen. It is recorded as 4. Partial agglutination is represented by an even thin deposit of cells on the bowl of the tube, 3; a similar cell pattern with a small central mass of unagglutinated cells, 2; and by a larger central mass with only a few clumps of agglutinated cells along its edge, 1. The end-point is taken as the highest dilution in which a 2 reaction occurs.

When virus titrations are carried out in the cold, the end-point obtained with NDV is usually lower by 2 to 4 times than when the test is performed at room temperature. (Compare the results in Tables I and II). However, the pattern of hemagglutination and titration end-points obtained in the cold is sufficiently reproducible to permit a reasonably accurate estimate of the dilution which corresponds to one agglutinating unit (*i.e.*, the highest dilution giving a 2 reaction). This estimate is of importance in agglutination-inhibition tests for antibodies against the virus.

Care in the selection of a source of red cells for hemagglutination tests with NDV is important. Although human Group O erythrocytes are agglutinated by NDV, they give much poorer reactions in the cold than at room temperature. Results similar to those shown in Table III were obtained with red cells from each of 3 persons; the end-points were approximately 64 times lower at 4°C than at 22-27°C. Erythrocytes from occasional individual chickens are also markedly insensitive to agglutination by the virus in the cold. By selection, however, chickens can be obtained, the red cells from which give end-points not more than 2 times lower at 4°C than at room temperature. Erythrocytes from such chickens are employed as a routine. Chicken red cell suspensions are stored at 4°C and may be used for at least 4 days without appreciably changing the results of titrations, as is shown in Table II.

The weak agglutination seen with low dilu-

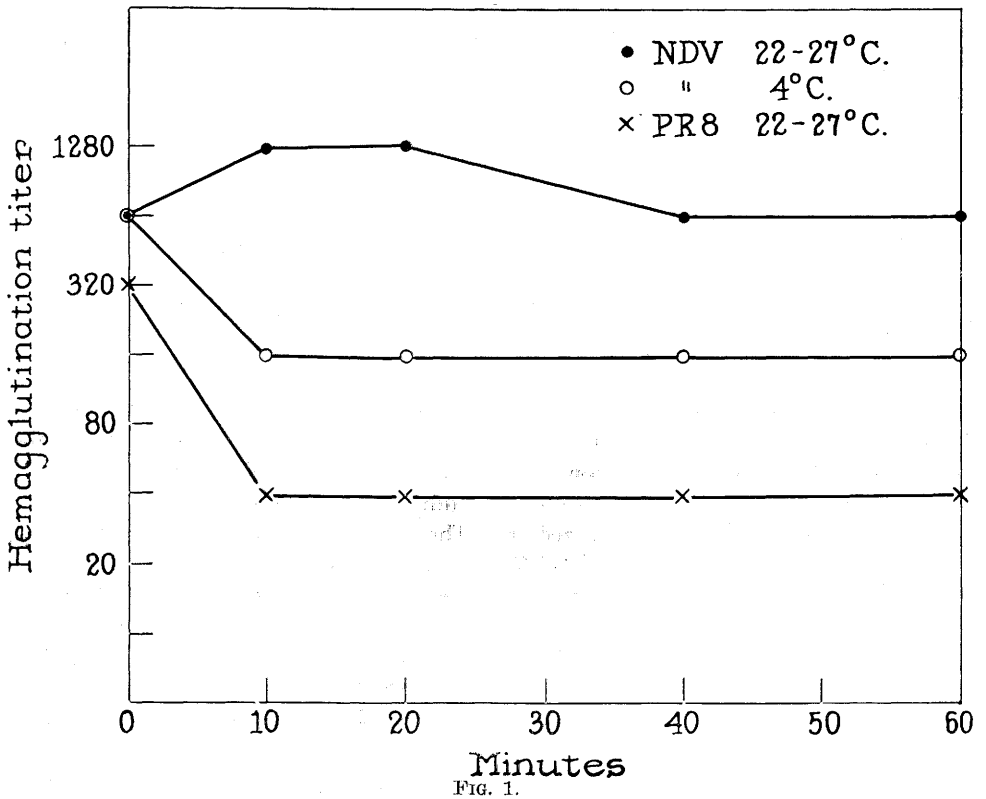

FIG. 1.

Adsorption of NDV and PR8 by chicken red cells. Hemagglutination titers of supernatant at various intervals after mixing red cells and virus.

tions of NDV at room temperature as compared to the complete agglutination observed at 4°C suggested that the difference might be dependent upon the rates of adsorption and elution of NDV from red cells at these temperatures. Experiments were carried out to determine this at 4°C and at room temperature. Equal volumes of 3% chicken red cell suspensions and NDV-infected allantoic fluid were mixed. At intervals aliquots were removed and immediately centrifuged. The supernates were tested for the presence of residual virus by the hemagglutination method. For comparison a similar experiment was performed with influenza A virus at room temperature. The results of these experiments are presented graphically in Fig. 1. Although no evidence was obtained that adsorption of NDV by red cells occurred at room temperature, it seems probable that it actually occurs but is masked because of very rapid elution of the virus from erythrocytes. Adsorption of NDV by red cells can be demonstrated when the mixture is held at 4°C and at that temperature elution of NDV from erythrocytes does not occur for at least 60 minutes. Hirst[11] has shown that temperature influences the rate of elution of influenza viruses from erythrocytes in a similar manner. It appears probable that the difference noted when NDV is titrated at room temperature and at 4°C is dependent on the elution of virus which occurs at room temperature[3] with the result that agglutination is masked in lower dilutions of virus. However, Cunha and his associates[9] have recently reported the presence in allantoic fluid of a substance which interferes with red cell agglutination by NDV.

Hemagglutination caused by NDV may be simply and readily differentiated from that produced by influenza viruses on the basis of the weak agglutination which is obtained with both chicken and human red cells at

[11] Hirst, G. K., *J. Exp. Med.*, 1942, **76**, 195.

TABLE IV.
Agglutination-inhibition Tests with NDV and Antiserum.

Preliminary incubation of serum-virus mixtures*	Rabbit serum	Dilution of serum†									End point
		20	40	80	160	320	640	1280	2560	5120	
None	Normal	3	2	3	4	4	4	4	4	4	
	Anti-NDV	±	0	±	0	1	4	4	4	4	1:480
1 hr at 4°C	Normal	4	4	4	4	4	4	4	4	4	
	Anti-NDV	0	0	0	0	0	2	4	4	4	1:640
1 " " 37°C	Normal	4	4	4	4	4	4	4	4	4	
	Anti-NDV	0	0	0	0	0	0	0	4	4	1:1920

* Virus sufficient to yield 8 agglutinating units in the final mixture was employed.
† Reciprocal of final serum dilution.
Results were read after 60 minutes at 4°C.

room temperature with low dilutions of NDV, as well as by the relative failure of human red cells to show agglutination with NDV in the cold. These differences are clearly evident in the experiments summarized in Table III in which parallel titrations of NDV, PR8 and Lee viruses were made with the same suspensions of chicken and human red cells. Allantoic fluid infected with NDV usually gives hemagglutination titers ranging from 1:320 to 1:512, whereas 50% infectivity end-points as determined in chick embryos are of the order of 10^{-10}. This indicates a difference between hemagglutination and virus titers with NDV which is 10 to 100 times greater than that usually obtained with influenza viruses.[12-14]

Titration of antibody. Because of the distinct advantages of performing hemagglutination tests with NDV in the cold, the usual method for measuring antibodies against the virus by means of agglutination-inhibition was modified as follows: The virus is titrated in the cold with the same chicken red cell suspension that is to be used in the agglutination-inhibition test. The final dilution of allantoic fluid which is equivalent to 32 agglutinating units is determined, prepared, and its actual titer checked by a second hemagglutination test. The sera to be tested are heated at 56°C for 30 minutes and diluted in 2-fold series. To each tube containing 0.2 cc of serum dilution, 0.2 cc of virus (diluted so as to equal 32 units) is added. The mixtures are shaken and incubated for 60 minutes in a water bath at 37°C. They are then cooled to 4°C and 0.4 cc of cold 1.5% chicken red cell suspension is added to each tube. After 60 minutes at 4°C the tests are read and the lowest serum dilution showing 2 plus, or greater, agglutination is taken as the end-point.

The results of parallel agglutination-inhibition tests with antiNDV serum are presented in Table IV. The conditions of preliminary incubation of the serum-virus mixtures were varied as indicated and the advantage of incubation at 37°C for one hour before the addition of red cells is clearly demonstrated. This modification was originally suggested by Burnet.[5]

To determine the reproducibility of serum dilution end-points (*i.e.* antibody titers) obtained by means of the agglutination-inhibition method described above, the following experiment was performed: Sera from each of 4 rabbits which had been immunized against NDV were tested separately against 3 different amounts of virus (*i.e.*, 32, 16 and 8 units, respectively). The results are shown graphically in Fig. 2. The end-points obtained were closely similar with each of the 4 immune sera and a constant amount of virus. Moreover, as the quantity of virus used was *increased*, the serum dilution end-point *decreased* proportionately. These results indicate that the reaction between NDV and specific antibody *in vitro* shows a quantita-

12 Taylor, A. R., Sharp, D. G., Beard, D., Beard, J. W., Dingle, J. H., and Feller, A. E., *J. Immunol.*, 1943, **47**, 261.

13 Sharp, D. G., Taylor, A. R., McLean, I. W., Jr., Beard, D., Beard, J. W., Feller, A. E., and Dingle, J. H., *J. Immunol.*, 1944, **48**, 129.

14 Friedewald, W. F., and Pickels, E. G., *J. Exp. Med.*, 1944, **79**, 301.

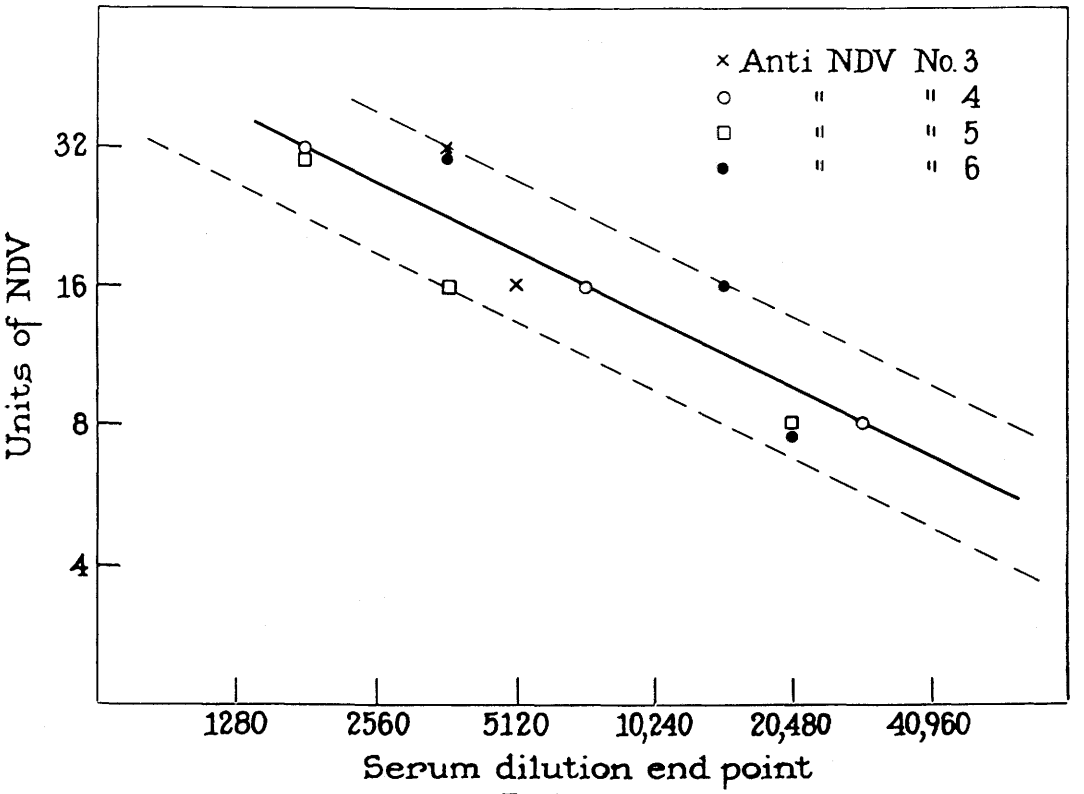

FIG. 2.

Distribution of serum dilution end points in agglutination-inhibition tests with varying amounts of NDV. Antisera from 4 rabbits immunized against NDV. Dotted lines represent the limits of twofold deviations (a single tube) from the solid line.

tive relationship analogous to that previously demonstrated with certain other viruses [*i.e.*, influenza and pneumonia virus of mice (PVM)] as well as with classical antigen-antibody reactions.

Summary. Hemagglutination by Newcastle disease virus (NDV) is markedly affected by temperature. At room temperature the reaction is difficult to read, especially with low dilutions of virus. At 4°C the reaction is easily read and titration endpoints are readily determined.

At 4°C NDV appears to be adsorbed more completely by and to elute less rapidly from chicken red cells than at room temperature.

Details of satisfactory methods for titration of virus and antibodies against it are presented. Both tests are carried out at 4°C.