Serum Neutralization of Distemper Virus in Chick Embryos.* (21266)

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Virus-neutralization tests in embryonated hen's eggs have been employed by several investigators to provide evidence for the presence of distemper neutralizing antibodies (1-5). The successful cultivation of canine distemper virus in chick embryos(1,2,6) made available a new and valuable tool in distemper research and provided a cheap and adequate means of determining the presence of distemper antibody in serum.

The purpose of this paper is to present data showing that a definite correlation exists between the results of virus-neutralization tests in chick embryos and ferret protection tests, and that some samples of human serum and gamma globulin have distemper neutralizing substances which are as abundant as those known to exist in immune ferret serum.

Materials and methods. The Lederle strain of egg-adapted distemper virus(1) was employed in this study. The virus was maintained by successive serial passages on the chorioallantoic (C-A) membrane of embryonated hen's eggs. When inoculated on the C-A membranes of 8-9 day old embryos, the virus causes a marked thickening of the membrane with numerous macroscopic light-gray lesions. These lesions tend to coalesce and form large inflammatory and necrotic areas. Photomicrographs of infected and normal membranes are shown in Fig. 1. When no virus is present or when virus is neutralized by

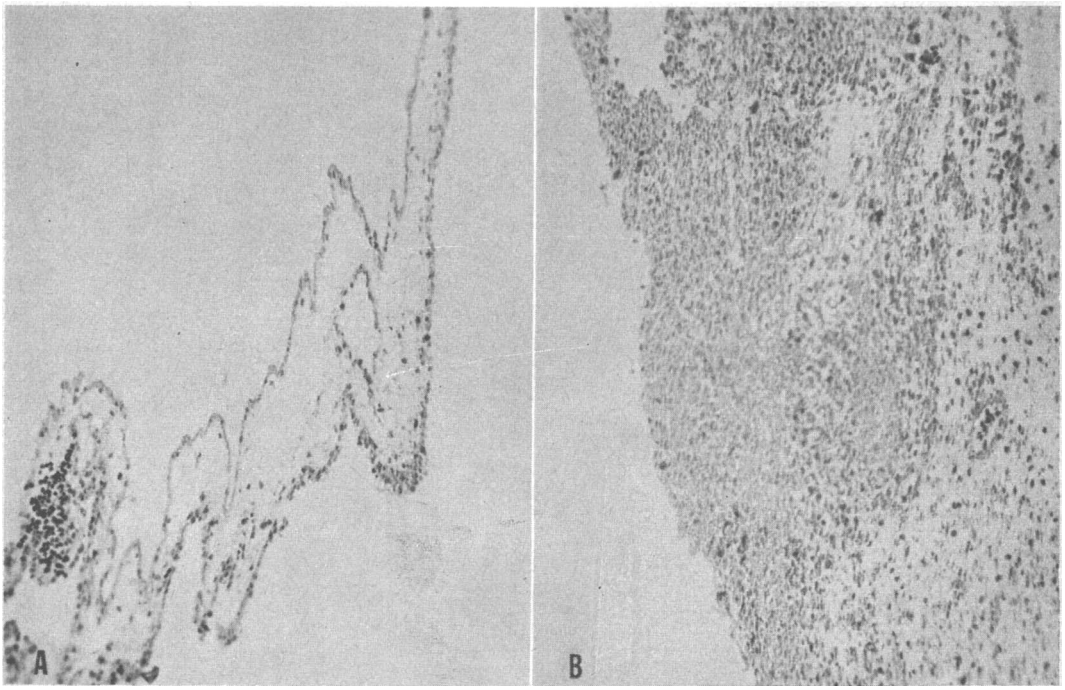

FIG. 1. Photomicrographs: A. Normal C-A membrane (×240). B. Inflammatory and necrotic membrane, 7 days after inoculation with egg-adapted distemper virus (×240).

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TABLE I. Results of Quantitative Serum-Virus Neutralization Tests in Eggs, Employing 20 ID_{50} Egg-Adapted Distemper Virus and Samples of Ferret Serum.

Ferret No.	Serum dilutions 1:40	1:80	1:160	1:320	1:640	Results of challenge with 100 MLD ferret virus
N*74	2/4†	5/5	5/5			Died
N 76	—	4/5	5/5			"
N 90	3/3	4/4	4/4			"
N 91	4/5	4/5	4/5			"
I 72			0/4	0/4	—	Survived
I 85			0/5	0/5	2/4	"
I 86			0/4	0/4	0/5	"
I 88			0/5	0/4	1/5	"

* N = Normal; I = Immune.
† Numerator indicates No. of eggs showing lesions. Denominator indicates total No. of eggs.

specific antiserum, the C-A membrane remains typically thin and transparent with no apparent lesion.

Immune sera were collected from ferrets which had been immunized with the Lederle egg-adapted strain and subsequently challenged with virulent ferret-adapted distemper virus. The hyperimmune canine distemper antiserum, and the virulent ferret distemper virus used for challenge exposure of ferrets, were obtained from Lederle Laboratories through the courtesy of Drs. Herald Cox and Victor Cabasso.

Two methods were employed in the virus-neutralization tests: a) serum-dilution method and b) virus-dilution method. In the serum-dilution method, the serum was diluted in serial 2-fold dilutions. Equal volumes of diluted serum and 20 ID_{50} of egg-adapted distemper virus were mixed, incubated at 37°C for 2 hours and placed in a refrigerator for an additional one-half hour. The serum-virus mixture was inoculated in a dose of 0.3 ml per egg; groups of 5-6 eggs per dilution were employed. The C-A membranes were observed following the incubation of the eggs for 6-7 days.

In the virus-dilution method, the virus was serially diluted in 2-fold dilutions. Equal amounts of diluted virus and 1:10 dilution of serum were mixed, incubated and inoculated on the C-A membranes. All samples of serum were inactivated at 56°C for one-half hour before being used in the neutralization test.

Results. For evaluation of the neutralizing capacities, sera from immune ferrets were compared with sera from ferrets with a history of no known exposure to distemper virus. The results from a typical experiment are shown in Table I. It can be seen that there were no detectable antibodies in 1:40 dilutions of the sera from normal ferrets. That these animals had no protective antibody can be further shown by the results of challenge exposure with approximately 100 MLD of ferret virus. All the ferrets showed typical symptoms of distemper and were dead by the 16th day.

On the other hand, immune ferret sera showed demonstrable antibody when diluted to 1:640. All these animals survived the challenge exposure of 100 MLD of ferret virus.

Table II shows the results from one typical quantitative neutralization experiment employing 20 ID_{50} of egg-adapted distemper

TABLE II. Results of Quantitative Serum-Virus Neutralization Tests in Eggs, Employing 20 ID_{50} Egg-Adapted Distemper Virus and Various Samples of Serum.

Serum samples	Serum dilutions 1:80	1:160	1:320	1:640	1:1280	1:2560	PD_{50} of serum†
Canine hyperimmune	0/5*	0/5	0/5	0/4	2/5	5/5	1:1436
Human gamma globulin	0/6	0/4	0/6	4/6	5/5		1:537
Immune ferret	0/5	0/5	0/5	2/4			1:640
Normal ferret	5/5	5/5	5/5				<1:20

* Numerator indicates No. of eggs showing lesions. Denominator indicates total No. of eggs.
† Expressed as dilution of serum which protected 50% of embryos from infection.

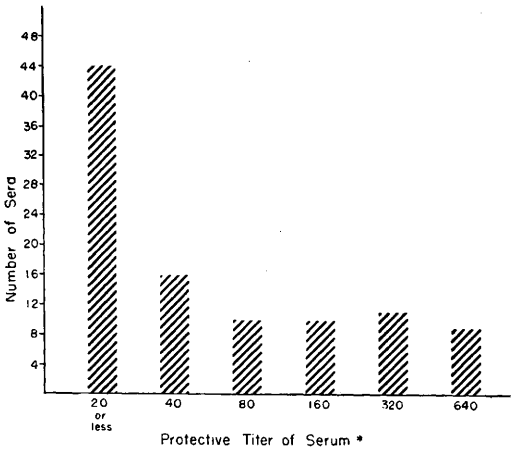

FIG. 2. Results of neutralization tests in eggs, employing egg-adapted distemper virus and 100 samples of human serum.

* Expressed as reciprocal of dilution of serum which protected 50% of embryos from infection.

virus and various samples of serum. The resulting titers were expressed as the dilution of serum calculated to protect 50% of the embryos from infection (PD_{50}). The results indicate that human gamma globulin† has a protective titer equal to that found in immune ferret serum.

A few samples of serum from adult human beings have also shown protective titers (1:640) as high as those known to exist in immune ferret serum. The data on the first 100 samples of human serum which were tested for protective titers are presented in Fig. 2. Forty-four per cent of the sera showed some protective substance at 1:20 dilution or less. Protective titers of 1:320 dilution or greater were found in 20% of the tested samples and 9% of the sera showed titers at 1:640 dilution. Tests on samples of serum from 12 normal premature infants have shown some protective substances at 1:20 dilution of serum but not at 1:40 dilution.‡

The results of neutralization tests employing the virus-dilution technic are shown in Tables III and IV. The control materials represent sera from respective ferrets before the animals were immunized. The titer of the virus is expressed as the dilution calculated to infect 50% of the embryos. The resulting neutralization index was determined by obtaining the difference between the log titer of the virus found with the immune serum and that obtained with the control or normal serum. The results in Tables III and IV show that the neutralization indexes of the immune sera fell between 10-16. If the average for log ID_{50} titer of virus with normal serum is taken as 3.0, the neutralization index of the human gamma series will be as great or greater than that shown with the immune ferret serum. These results would again indicate that human gamma globulin has a protective titer equal to that found in specific immune sera.

Discussion. The retention of the antigenicity of the canine distemper virus even after continued passage in embryonated hen's eggs (1,2,6) has led to its use as a tool for neutralization studies. The data presented in this report indicate that the results of neutralization tests employing the egg-adapted distemper virus show a definite correlation with ferret protection tests. Animals with high titers of distemper antibodies as shown by the neutralization studies will survive the challenge exposure of virulent virus. Moreover, animals without demonstrable neutralizing antibody succumb to the challenge exposure. Therefore, the neutralization test is believed to offer a suitable method for the estimation of distemper antibody levels in serum.

The nature of the endpoint employed for the neutralization test is the presence or absence of gross macroscopic lesions on the C-A membrane. Since a constant lesion is produced by a fixed dose of virus, it is probably preferable to employ the serum-dilution technic. However, the present study also indicates that the virus-dilution technic may be employed with satisfactory results. The levels of neutralization indexes obtained with the immune sera in the present study might be explained by the relatively low level of infective titer of the avianized distemper virus in embryonated eggs. The virus was prepared in 2-fold dilutions rather than in $\log_{10}$ increments.

The presence in high titer of distemper an-

† E. R. Squibb and Sons, N. Y.

‡ Dr. Robert Chinnock and Dr. Phyllis Wright kindly furnished these samples of serum.

TABLE III. A Representative Example of a Neutralization Test Employing the Virus Dilution Technic.

Serum samples	Virus dilutions 1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	ID_{50} titer log of virus dilution	Neutralization index
N*F89	—	4/4†	4/4	3/5	3/5	0/5	0/5	2.8	
I F89	2/4	2/5	0/4	0/4	0/4	0/5	—	1.8	10

* N = Normal; I = Immune.
† Numerator indicates No. of eggs showing lesions. Denominator indicates total No. of eggs.

tibody in human gamma globulin and some samples of human serum was also demonstrated. The significance of these findings can not be determined at the present time. A possible direct relationship between canine distemper and a respiratory disease of humans has been suggested by Adams(4).

Summary. Virus-neutralization tests were carried out in embryonated hen's eggs employing the egg-adapted distemper virus and various samples of serum. Ferrets whose sera contained no demonstrable antibody at 1:40 dilution all succumbed to 100 MLD of ferret distemper virus, whereas ferrets showing antibody titer to 1:640 dilution of serum all survived the same challenge dose. The data presented demonstrate that the results of serum-neutralization tests in chick embryos have a definite correlation with ferret protection tests. Human gamma globulin and some samples of human serum have neutralizing substances in titers as high as those known to exist in immune ferret serum. On the other hand, samples of serum from normal premature infants did not show any neutralizing substances in 1:40 dilution of the serum.

TABLE IV. Results of Serum-Virus Neutralization Tests in Eggs, Employing the Virus Dilution Technic.

Serum samples	Log ID_{50} titer of virus: Control serum	Immune serum	Neutralization index
F85	3.2	2.1	13
F86	3.0	1.9	13
F89	2.8	1.8	10
F88	3.0	1.8	16
Human gamma globulin		1.4	
Canine hyperimmune serum		.7	

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Effect of Dicumarol on Localized Shwartzman Reaction.* (21267)

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There have been numerous theories as to the pathogenesis of the Shwartzman reaction. One theory is that it is caused by excessive concentration of toxin in the prepared site after the intravenous or provocative injection due to a localizing effect of the inflammatory reaction. This would appear to be contradicted by the fact that local injection of large amounts of toxin into the skin does not pro-

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