Immunological Relationship Between Measles and Distemper Viruses.* (23442)

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The following experiments are being reported because of previous studies pointing to a definite pathological and immunological relationship between canine distemper and a human respiratory disease(1,2). Pinkerton, *et al.*(3) suggested a possible biological relationship between measles and distemper principally on the basis of pathological data. Both measles and distemper viruses produce characteristic cytoplasmic and intranuclear inclusion bodies in epithelial tissues, and also have the property of causing multinucleated giant cells(4). As yet, however, no evidence has been submitted of an immunological relationship between these two agents. The finding that measles or rubeola virus (Edmonston strain)(5) could be established in HeLa cell cultures(6,4) as well as KB cells(7) and Hep-2 cells(6), suggested a method of exploring the problem. The experiments reported here indicate that measles propagated in HeLa cell cultures could be neutralized by specific canine distemper (Carre's virus) antiserum obtained from immunized ferrets, and that distemper virus infection in animals could be neutralized by measles antiserum. These immunological cross-reactions suggest that common antigenic components are shared by the viruses of measles and distemper.

Materials and method. Methods of study. Three principal methods were employed to demonstrate the immunological relationship between measles and distemper viruses: 1. In tissue culture, measles virus was neutralized by specific distemper antiserum. The antiserum was prepared in ferrets by inoculation with either egg-adapted or mouse-adapted distemper virus. 2. In ferret protection studies, ferrets were immunized with measles virus and subsequently challenged with virulent distemper virus. 3. Mouse-adapted distemper virus was neutralized by human measles convalescent sera and by sera from ferrets that were immunized with measles virus.

Viruses. The Edmonston strain of the measles virus was successfully propagated and maintained in HeLa cell cultures. Cytopathic changes consisted of characteristic multinucleated giant cells with cytoplasmic and intranuclear inclusion bodies (Fig. 1). The identity of the virus propagated in HeLa cells was established by neutralization and complement-fixation tests (Table I). The neutralization test was carried out by mixing 200 TCD_{50} of the viral tissue culture supernatant with dilutions of acute and convalescent serum from patients with typical measles. These mixtures were incubated at 37°C for ½ hour and at 4°C for an additional ½ hour before inoculation of HeLa cultures. Supernatant fluids from infected cultures were also employed as antigens for complement-fixation tests. These tests indicate that significant increases in neutralizing and complement-fixing antibodies occurred in the serum of the patients with clinically diagnosed measles.

The Lederle strain of avianized distemper virus(8) was maintained by successive serial passages on the chorioallantoic (CA) membrane of 8-9-day-old chick embryo. A 10% CA membrane suspension of 1 ml amount was injected intraperitoneally into ferrets, which subsequently survived a challenge of 100 MLD virulent ferret distemper virus.

The mouse-adapted distemper virus(9,10, 11) was developed by successive serial passage in brain of suckling mice with egg-adapted distemper virus. The LD_{50} of this virus after 45 passages in suckling mice is about $10^{-3.5}$ with an incubation period of 4-5 days. Ferrets were inoculated intraperitoneally with 1 ml of 20% brain suspension from the 54th passage material. These ferrets subsequently survived a challenge of 100

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TABLE I. Measles Neutralization and Complement Fixation Tests with Human Sera.

Sample	Serum	Serum dilution — Neutralization titer	Serum dilution — CF titer
1	Acute	<1/10	<1/ 16
	Convalescent	1/80	1/128
2	Acute	<1/10	<1/ 16
	Convalescent	1/40	1/ 64

MLD virulent ferret distemper virus.

Neutralization of measles virus. The HeLa cell cultures were grown and maintained in medium containing 60% yeast extract medium(12), 20% Scherer's maintenance solution(13), 10% calf serum and 10% brain heart infusion broth. The medium was changed every 3-4 days. In the neutralization studies measles virus representing 200 TCD_{50} was mixed with equal amounts of diluted test sera. Mixtures were incubated at 37°C for ½ hour and placed in the refrigerator for an additional ½ hour. All test sera were inactivated at 56°C for ½ hour prior to use. A standard inoculum of 0.2 ml was added to the HeLa cell culture tubes and observed daily for 21 days. Tests were also carried out by mixing diluted serum with serial virus dilutions and incubating similarly before inoculation of HeLa cell cultures.

Ferret protection studies. In 3 separate experiments ferrets were immunized with measles virus and subsequently challenged with virulent distemper virus. In the first experiment, 8 ferrets were given 2 intramuscular injections of HeLa passage measles virus 2 weeks apart and challenged 2 weeks later with approximately 100 MLD virulent ferret distemper virus. In the second experiment, 5 ferrets were given 4 intramuscular injections of measles virus at approximately 3 week intervals. These animals were challenged 3 weeks after the final injection with approximately 50 MLD virulent ferret distemper virus. In the third experiment, 7 ferrets were inoculated intramuscularly with 1 ml of a mixture of measles virus and adjuvant(14) prepared as follows: 1 part arlacel A, 9 parts bayol F and 10 parts HeLa virus suspension. Approximately 1 month and 2 months later, the animals were again inoculated intramuscularly with 1 ml of measles virus without adjuvant. These animals were subsequently challenged with 100 MLD virulent ferret distemper virus. In each experiment, control ferrets included animals receiving no previous treatment and also animals receiving normal tissue culture fluid instead of measles virus suspensions.

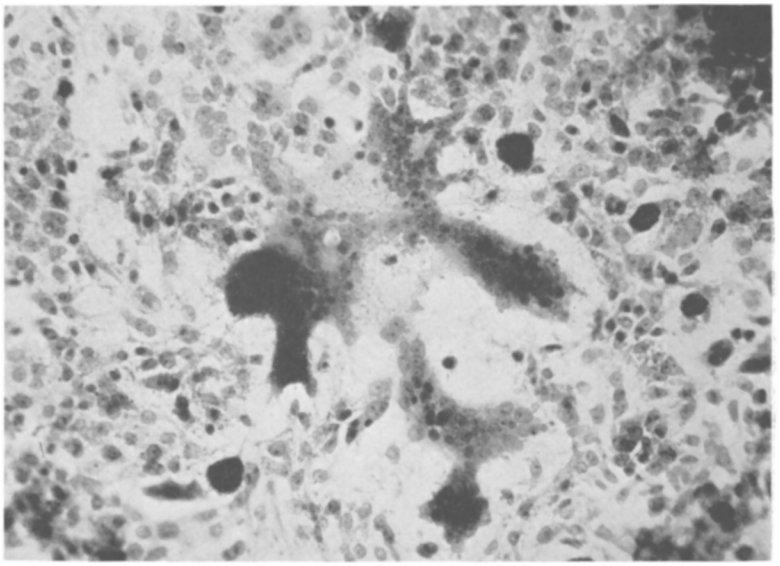

FIG. 1. Photomicrograph of HeLa cell tissue culture showing typical cytopathology with multinucleated giant cells produced by measles virus. Photographed by Timothy Dodge.

TABLE II. Effect of Addition of Fresh or Heated Normal Guinea Pig Serum on Neutralizing Capacity.

Antiserum samples	Neutralizing antiserum diluted with BSS*	Inactivated N.G.P. serum	Fresh N.G.P. serum
1	1/ 2	1/ 2	1/ 16
2	1/16	1/16	1/ 64
3	1/32	—	1/128

* BSS—Hank's Balanced Salt Solution.

Neutralization of distemper virus. Equal amounts of the mouse-adapted distemper virus and serum were incubated at 37°C for ½ hour and 4°C for additional ½ hour before injecting 0.01 ml intracerebrally into suckling mice. Neutralization was determined by survival of the animals. Tests were also carried out by mixing serum with serial virus dilutions and incubating similarly before inoculation. All test sera were inactivated at 56°C for ½ hour before being employed for neutralization studies.

Results. The neutralization data show that sera from ferrets immunized with mouse-adapted and egg-adapted distemper virus inhibited cytopathogenesis due to measles virus. Final serum titers of ½, ¼, ⅛ and rarely greater than 1/16 were recorded. These sera were drawn 4 weeks following the severe distemper challenge. In no case did preimmunization or normal serum show any inhibition of measles virus. A representative neutralization test which was carried out by mixing 1/5 dilution of ferret serum with serial virus dilutions shows that 2.5 logs of measles virus were neutralized by the serum from a ferret immunized with mouse-adapted distemper virus.

When complement as present in fresh normal guinea serum was added to the ferret sera, the enhancement of neutralizing properties was demonstrated; when normal guinea pig serum was inactivated by heating for ½ hour at 56°C no enhancement was evident (Table II).

Animal studies employing ferrets have been partially successful in confirming the findings in tissue culture. In the following 3 experiments ferrets were immunized with measles virus and subsequently challenged with virulent ferret distemper virus. Evidence of protection was revealed by prolonged incubation periods and modified illness with survivals. These results are summarized in Fig. 2. Among the 20 experimental animals, 8 survived and 12 died, whereas only 1 animal survived the challenge among 17 controls. Serum from the 1 surviving control ferret which received only distemper virus showed a rise in measles antibody from 0 to ⅛.

Representative results of neutralization of mouse-adapted distemper virus by specific measles antiserum prepared in ferrets are presented in Table III. Employing 300 LD_{50} of mouse-adapted distemper virus and undiluted measles antiserum obtained from ferrets in the preceding experiments, neutralization studies were carried out by inoculation of the mixture intracerebrally into 1- and 2-day-old mice. These results indicate that specific measles antiserum completely protected the mice against the lethal effects of the virus, whereas, preimmunization serum and control ferret serum had no inhibitory effect. Dilution studies revealed complete protection at ¼. Neutralization of the virus was also evident when mouse-adapted and egg-adapted distemper antisera were employed in the study. Similarly, when neutralization tests were carried out by mixing undiluted ferret sera with serial virus dilutions, 3.5 logs of distemper virus were neutralized by the serum from a ferret immunized with HeLa passage measles virus.

Table IV illustrates the fact that mouse-adapted distemper virus is neutralized by serum antibodies developing during the course of clinically diagnosed measles. Employing 100 LD_{50} of mouse-adapted distemper virus and sera obtained from acute and convales-

TABLE III. Neutralization of Mouse Adapted Distemper Virus with Ferret Measles Antiserum.

Serum samples	Suckling mice, # dead/# inj.
Normal ferret (pre-immunization)	8/8
Ferret immunized with measles	0/7
Normal ferret (pre-immunization)	4/4
Ferret immunized with measles	0/9
Ferret immunized with:	
Control culture	7/7
Avian-distemper	0/6
Mouse-distemper	0/7

TABLE IV. Neutralization of Mouse-Adapted Distemper Virus by Human Convalescent Measles Sera.

Sample	Serum	Neutralizing serum dilution*
1	Acute	<1/ 4
	Convalescent	1/64
2	Acute	<1/ 4
	Convalescent	1/43

* Expressed as dilution of serum which protected 50% of suckling mice from death.

cent stages from 2 patients with measles, neutralization tests were carried out in suckling mice. These results indicate that significant increases in distemper neutralizing substances occurred in the serum of both patients. These two patients also had significant increases in measles antibody in complement-fixation and neutralization tests.

Discussion. It would appear that measles and distemper are as yet 2 distinct entities caused by well recognized respiratory viruses in their respective hosts, man and dog. Although both of these diseases occur in epidemics with similar incubation periods and are characterized by similar symptom patterns including a rash, there exist many unsolved problems with respect to the antigenic properties of the agents. The measles virus is readily adaptable to many different tissue culture preparations whereas the distemper virus, although adapted to certain animals, mice and the chick embryo, has been found difficult to produce cytopathogenesis in tissue culture. The pathologic similarities have been emphasized in previous reports(3,4), including the striking formation of multinucleated giant cells, and typical cytoplasmic and intranuclear inclusion bodies produced by both viruses in epithelial tissues.

Cross neutralization studies reported in this paper have demonstrated the development of neutralizing factors in the serum of animals and man by both measles and distemper virus.

It has been demonstrated(1,2) that human serum was capable of neutralizing distemper infection in chick embryos and in ferrets, and in this paper human serum from patients recovering from measles has been shown to protect mice completely from death caused by distemper virus. The acute or early specimen

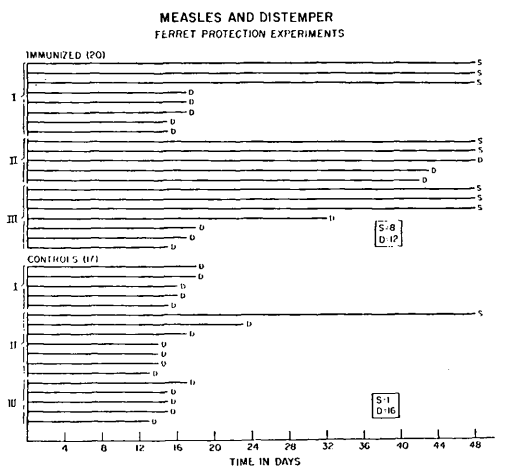

FIG. 2. Summary of 3 experiments showing results of challenge with distemper virus of ferrets immunized with measles virus; duration of illness (D) and survivals (S) is charted for comparison with the controls.

from these same patients has failed to protect the mice from dying. Recently both Karzon(15) and Carlström(10) demonstrated distemper antibodies in human beings. In both studies, the serum dilutions employed were 1/5. It is possible that cross-neutralization between measles and distemper might be responsible for the data presented.

Summary. In tissue culture studies, the Edmonston strain of measles virus was neutralized by distemper antiserum prepared in ferrets with the egg-adapted and mouse-adapted strains of distemper virus. All normal ferret sera failed to show any neutralization of measles virus. In animal studies, ferrets immunized with measles virus and subsequently challenged with virulent distemper virus showed some evidence of protection as revealed by prolonged incubation periods, modified illnesses and survivals. The mouse-adapted distemper virus was completely neutralized by measles antiserum prepared in ferrets, whereas, normal serum failed to show any neutralization. Mouse-adapted distemper virus was also neutralized by human measles convalescent sera. These results suggest that common antigenic components are shared by the viruses of measles and distemper.

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1. Adams, J. M., *Pediatrics,* 1953, v11, 15.
2. Imagawa, D. T., Yoshimori, M., Wright, S. W., and Adams, J. M., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 2.
3. Pinkerton, H., Smiley, W. L., and Anderson, W. A. D., *Am. J. Path.,* 1945, v21, 1.
4. Adams, J. M., Imagawa, D. T., Yoshimori, M., Huntington, R. W., *Pediatrics,* 1956, v18, 888.
5. Enders, J. F., and Peebles, T. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 277.
6. Black, F. L., Reissig, M., and Melnick, J. L., *ibid.,* 1956, v93, 107.
7. Dekking, F., and McCarthy, K., *ibid.,* 1956, v93, 1.
8. Cabasso, J. V., and Cox, H. R., *ibid.,* 1949, v71, 246.
9. Morse, H. G., Chow, T. L., and Bradly, C. A., *ibid.,* 1953, v45, 180.
10. Carlström, G., *Acta paediat.,* 1956, v45, 180.
11. Yoshimori, M., Master of Science Thesis, unpublished, U.C.L.A., 1957.
12. Robertson, H. E., Brunner, K. T., and Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 119.
13. Scherer, W. F., *Am. J. Path.,* 1953, v29, 113.
14. Salk, J. E., and Laurent, A. M., *J. Exp. Med.,* 1952, v95, 429.
15. Karzon, D. T., *Pediatrics,* 1955, v16, 809.

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Atherosclerotic Response of "Aggressive" and "Passive" Groups of Chickens To a Cholesterol Enriched Diet.* (23443)

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The possible effect of various emotional factors upon development of hypercholesteremia and atherosclerosis in laboratory animals also given a cholesterol enriched diet rarely has been studied. It was thought advisable to attempt such a study in respect to one emotional factor, namely, degree of aggressiveness as observed in young male chickens. This species was considered for such a study because of the well known pecking order (which might be considered a form of aggression) often observed in a flock of chickens(1,2) and susceptibility of chickens to dietary induced atherosclerosis(3).

Methods. In order to study male chickens ostensibly exhibiting either "aggressiveness" or "passiveness", a total flock of approximately 2500 male chickens (Avg. age: 6 weeks) was observed carefully by one of us (C.A.) for a number of hours both before, during and after feeding periods. A preliminary selection of 100 most "aggressive" chickens and 100 most "passive" chickens then was made. The characteristics of aggression were considered to be (1) insistence and speed of obtaining proffered feed, (2) over-all general physical activity and (3) agitation and belligerent resistance when handled. Passive characteristics were considered to be the converse of the preceding traits.

Following this preliminary selection, the 2 groups were housed in respective coops and were observed daily for 2 weeks during which time, the 30 most "aggressive" and 30 most "passive" chickens were selected from each. After this selection, the chickens were tagged with leg bands. Each group then was housed in 2 coops (approximately 5 feet square). The "aggressive" chickens were removed from their coops every 2 weeks, intermingled and then replaced in the coops. This was done in order to prevent a few from gaining group ascendancy. The "passive" chickens in the other 2 coops were treated similarly.

The animals were fed twice daily with a standard poultry mash (Albers Pullet Grower) enriched with cottonseed oil (5%) and cholesterol (2%). Routinely, the "passive" chickens were fed first and time allowed for feeding was one hour. At the end of this

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