A Comparison of Canine Distemper and Measles Viruses.* (26696)

CARLTON R. PALM AND FRANCIS L. BLACK
Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Conn.

A relationship between canine distemper (CDV) and measles (MV) viruses has been suggested on the basis of similar histopathology(1,2) and antigenic cross reactions(3, 4,5). Since evidence of a relationship has to date been primarily biological and the serological findings have been subject to controversy(6), it seemed pertinent to compare physical properties of these viruses. The filterability, and sensitivity to irradiation, heat and ether of CDV and MV have been studied.

Materials and methods. Virus stocks. Canine distemper virus kindly supplied by Dr. G. Rockborn(7) was grown in dog kidney tissue culture with a medium composed of Earle's salt solution plus 0.5% lactalbumin hydrolysate and 2% calf serum. Stock virus titers up to 10^4 per 0.1 ml were obtained. The Edmonston strain of measles virus was grown in Hep-2 cultures with Eagle's medium plus 10% calf serum; titers of 10^5 per 0.1 ml were obtained. *Titrations.* CDV titrations were carried out in tubes at half log intervals and checked for cytopathogenic effect at 7-14 days. The measles titrations were assayed in African monkey (*Erythrocebus patas* or *Cercopithecus aethiops*) kidney cultures by the plaque method. *Gamma irradiation.* Both viruses were subjected to gamma radiation from the Cobalt-60 source of the Yale University Dept. of Biophysics. This unit delivered 270,000 roentgens per hour. *UV irradiation.* The viruses were exposed to UV radiation from a Westinghouse G 30T8 Sterilamp which gave 1590 ergs/sec cm^2 as measured by a SM-600 meter (Westinghouse). *Gradocol membranes* were obtained from the Wright-Fleming Institute, London.

Results. The identity of the virus stocks was confirmed by titration against human sera collected before and after measles, and against sera from dogs before and after distemper. None of the "pre" specimens neutralized either virus. Post CDV specimens neutralized the CDV only, while post-measles specimens neutralized MV to high titer and CDV at low titer. Typical antibody titers are given in Table I.

TABLE I. Neutralization of Virus Strains.

	Canine sera		Human sera	
	Pre-CD	3 yr post-CD	Pre-measles	2 mo post-measles
CDV	<2	45	<2	6
MV	<2	<2	<2	128

Filtration experiments were carried out to obtain an estimate of the size of the CDV. The results are given in Fig. 1. On some occasions infectious virus passed membranes with a rated average pore diameter (APD) as low as 180 mμ, but never smaller. In several tests it was held back by membranes with APD values as high as 250 mμ, but it passed every membrane with larger diameters. On the basis of a ratio of 0.64 between virus and pore diameter(8) these data would indicate a size for CDV between 115 and 160 mμ, a range similar to that previously reported for measles virus(9).

Inactivation curves of CDV and MV by gamma radiation are presented in Fig. 2.† The slopes of the curves for the 2 viruses were indistinguishable in both wet and frozen preparations. The difference between the 2 viruses is always less than a log unit and hence within the range of error to be expected from the endpoint method. Unfrozen (0°C) preparations of both viruses showed a more rapid rate of inactivation than frozen (-70°C) specimens. The diameter of the effective targets of CDV and MV based on gamma sensitivity in the wet preparations was 42 mμ. When calculated on the basis

* This investigation was supported by a grant from Nat. Inst. Health, U.S.P.H.S.

† The data for measles inactivation by gamma rays at 0°C and by UV were obtained by Mr. Paul Ackerman, Yale Univ. School of Med.

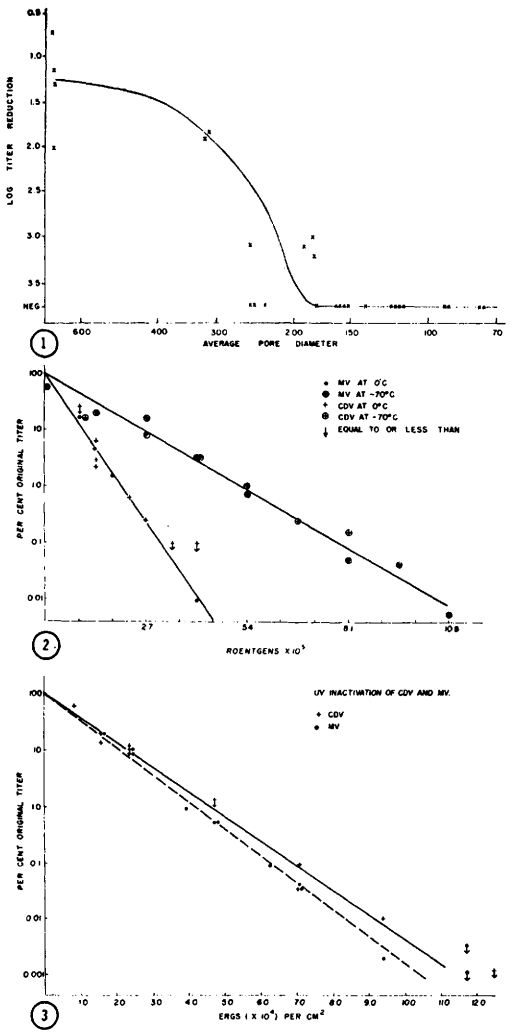

FIG. 1. Filtration of canine distemper virus through collodion membranes. Relations between avg pore diameter and reduction of titer in filtrates.

FIG. 2. Inactivation of canine distemper and measles viruses by irradiation from a cobalt-60 source at two temperatures.

FIG. 3. Inactivation of canine distemper and measles viruses by irradiation with ultraviolet light at 22°C.

of the frozen preparations, a figure of 35 mμ was obtained for the diameters of both viruses.

Data on sensitivity of the 2 viruses to UV are shown in Fig. 3. This non-ionizing form of radiation gave similar rates of inactivation for CDV and MV. Both curves were exponential over a 5 log range and titer of both viruses was reduced about 1.0 log unit per 22,000 ergs/cm^2.

Tests for thermal sensitivity were carried out with undiluted virus from tissue culture fluids. Preparations of each virus were sealed in ampules and immersed in water baths held at 37°C and 56°C. Ampules were removed at several intervals and titrated. Titer of both viruses fell 1.2 to 1.4 log units in 1 hour at 37°C and both were reduced more than 3.7 log units in 8 hours at 37°C or ½ hour at 56°C. Thus, as has been previously reported with MV(10), CDV was relatively labile at elevated temperatures.

Both CDV and MV were sensitive to ether. Preparations of both viruses shaken with an equal volume of ether and allowed to stand at 4°C overnight were completely inactivated, whereas controls without ether retained full titer.

Discussion. Biological and serological properties have frequently been used to group various viruses simply because they have been the properties most readily investigated. However, physical and chemical criteria may reflect more fundamental characteristics of viruses and hence offer some advantages. Since there has been controversy regarding the serological relationship between CDV and MV, the paucity of data from other lines of investigation has been particularly noticeable. The present study has shown CDV and MV to be similar with respect to size, and sensitivity to irradiation, to heat, and to ether; hence, our observations support the hypothesis that these viruses are members of a common group.

The filtration data put both viruses in the same general size range as herpes virus, varicella, salivary gland and rabies viruses. MV and CDV differ from herpes virus in their greater sensitivity to UV and ionizing radiation(11) and from rabies in their sensitivity to ether. Physical and chemical data on salivary gland and varicella viruses are insufficient to provide a basis for comparison.

The rate of inactivation by gamma radiation found in these studies at 0°C was only slightly less than that reported to occur with measles in serum free media at 25°C by Musser and Underwood(12). However, the

viruses were considerably more resistant to gamma rays when in frozen media. This latter effect was in contrast to results obtained with poliovirus(9) but similar to vaccinia results obtained by J. F. McCrea Freezing presumably suppresses indirect effects of irradiation either through restricting mobility of radicals formed in the medium, or by reducing transfer of charges from non-essential to essential portions of the virus particle. Regardless of the mechanism, the effect of freezing provides another point of similarity between the viruses under consideration.

Summary. Comparisons of certain physical properties of canine distemper (CDV) and measles (MV) viruses have yielded data compatible with the hypothesis that they belong to the same group. Ultrafiltration of CDV indicated a particle size between 115 and 160 mμ, a value similar to that previously reported for measles. Both viruses were inactivated by ultraviolet light at a rate of 4.5 log units per erg/cm^2 × 10^5. Irradiation with 10^5 roentgens of gamma particles reduced the titer 0.4 log unit at −70° and 1.0 log unit at 0°C for both CDV and MV.

1. Pinkerton, H., Smiley, W. L., Anderson, W. A. D., *Am. J. Path.*, 1945, v21, 1.
2. Adams, J. M., Imagawa, D. T., Yoshimori, M., Huntington, R. W., *Pediatrics*, 1956, v18, 888.
3. Carlstrom, G., *Lancet*, 1957, v273, 344.
4. Adams, J. M., Imagawa, D. T., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 240.
5. Warren, J., Nadel, M. K., Slater, E., Millian, S. J., *Am. J. Vet. Res.*, 1960, v21, 111.
6. Cabasso, V. J., Avampata, J. E., Kiser, K. H., Stebbins, M. R., *Bact. Proc.*, 1960, v60, 106.
7. Rockborn, G., *Arch. ges. Virusforsch.*, 1959, v8, 485.
8. Black, F. L. *Virology*, 1958, v5, 391.
9. Benyesh, M., Pollard, E. C., Opton, E. M., Black, F. L., Bellamy, W. D., Melnick, J. L., *ibid.*, 1958, v5, 256.
10. Black, F. L., *ibid.*, 1959, v7, 184.
11. Powell, W. F., *ibid.*, 1959, v9, 1.
12. Musser, S. J., Underwood, G. E., *J. Immunol.*, 1960, v85, 292.

Received May 8, 1961. P.S.E.B.M., 1961, v107.

Disappearance Rate of Normal Bactericidins in Irradiated Mice.* (26697)

LOTTIE KORNFELD** AND C. PHILLIP MILLER
Argonne Cancer Research Hospital† and Departments of Medicine and Microbiology, University of Chicago, Chicago, Ill.

In an earlier publication(1) we reported that normal serum bactericidal activity for a strain of *Escherichia coli* was lost within 9-12 hours after total body exposure of CF-1 mice to a midlethal dose of X-rays and remained absent for about 10 days. The disappearance of bactericidins could not be associated with development of an inhibitor demonstrable *in vitro* or with *in vivo* absorption by enteric microorganisms through the damaged intestinal mucosa.

The rapidity of the loss of bactericidins indicated that their production was promptly interrupted by irradiation and that they must have a very short biological half-life. Recent findings suggested that a block in the synthesis of bactericidins develops after X-ray exposure(2). It is the purpose of this communication to show that normal bactericidins for *E. coli* disappear very quickly after passive transfer in irradiated mice.

Materials and methods. CF-1 mice were exposed to 500 or 700 r total body X-radiation‡ and were used on the following day when no serum bactericidal activity was demonstrable. They were injected intravenously *via* a tail vein with pooled mouse sera of known bactericidin titer and exsangui-

* This work was supported in part under U. S. Atomic Energy Commission Contract.

** Address after Oct. 1 Scripps Clinic and Research Foundation, La Jolla, California.

† Operated by University of Chicago for U. S. Atomic Energy Commission.

‡ The authors wish to thank Mr. James Bland for irradiating the animals.