Sensitivity of Strain R Avian Erythroblastosis Virus to Ether and Chloroform.* (30647)

ALFRED M. WALLBANK AND E. L. STUBBS (Introduced by D. S. Kronfeld)
New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pa.

Viruses of the avian sarcoma-leukosis complex are an intricate group that are very difficult to classify, except on the basis of pathologic effects. Therefore, any chemical or physical characterization of this complicated virus group is useful.

The value of ether sensitivity as a tool for classification of viruses was demonstrated by Andrewes and Horstmann(1). More recent work by Cooper(2) and Andrewes(3,4) supports the usefulness of ether sensitivity determinations. Feldman and Wang(5) suggested chloroform sensitivity determinations to provide similar information. After studying a number of viruses, Hamparian *et al* (6) indicated that chloroform gave more clear cut and reproducable results than did ether. Wilner's excellent monograph(7) lists sensitivity or resistance to chloroform or ether as one of the 4 basic criteria for virus classification.

Data published on Rous sarcoma virus (RSV)(1,5,8), the California strain of avian lymphomatosis (Rubin's RIF virus)(9), and the Stubbs-Furth strain of sarcoma and erythroblastosis (Strain 13) virus (S13V) (10) indicate all of these viruses were sensitive to ether. In addition, RSV and S13V were also inactivated by chloroform. The present experiments test the sensitivity of another avian oncogenic virus to ether and chloroform.

Materials and methods. Strain R erythroblastosis virus used for these studies was produced by intravenous inoculation of 50- and 58-day-old chickens. Birds presenting evidence of erythroblastosis(11), as determined by blood smear examination, were exsanguinated when they appeared to be moribund. Plasma obtained from these bleedings was passed through a Celite 512 mat in a Buchner funnel and then an 02 Selas filter. This plasma pool of Strain R virus was dispensed into serum vials and designated Pool RA. A sterility test in AC medium (Difco) was negative at 7 days.

The virus was tested by adding diethyl ether to Strain R Pool RA plasma (virus and ether cooled in cracked ice). Ether was added to the narrow mouth serum vial, sealed with a rubber sleeve stopper, using a needle and syringe. One volume of ether was added to 5 volumes of virus. The contents of the vial were mixed by gentle agitation and refrigerated 16½ hours at 2°C. Ether was removed from the virus-ether suspension by allowing the mixture to stand in an open Petri dish for 45 minutes. Control virus was handled in the same manner, without addition of ether. The tubes of control and treated virus were placed in cracked ice, dilutions of each preparation were made, and immediately titrated by intravenous inoculation of 59-day-old Strain 1 Pennsylvania Farm Bureau single-comb White Leghorns.

Chloroform was added to the virus (plasma) in the same proportion as in the ether experiment. The virus-chloroform suspension, in a small rubber stoppered flask, was mixed on a magnetic stirrer for 10 minutes in a refrigerator at 2°C. The mixture was centrifuged at 275 × *g* for 5 minutes, the virus phase then removed, dilutions made, and the chickens (51 days old) were inoculated. The control virus was subjected to the same conditions, excluding the addition of chloroform.

Inoculated chickens were observed for 21 days, using death as the recorded end point (11).

Results. The data (Table I) indicate that Strain R virus is sensitive to both ether (Exp. 2364) and chloroform (Exp. 2464). In Experiment 2364, 74% of —1.0 dilution virus controls were positive, as opposed to 4% positive for the ether-treated virus. Of the —2.0 dilution, virus controls were 76% positive *vs* 2% for the treated virus. There were

* This investigation was fully supported by research grant CA-04610-06 from Nat. Cancer Inst., N.I.H., Bethesda, Md.

TABLE I. Effect of Ether and Chloroform on Strain R Erythroblastosis Virus.

Exp No.	Age of chicks (days)	Virus dilutions*	Virus controls†	Virus controls (%)	Ether treated virus	Ether treated virus (%)	Chloroform treated virus	Chloroform treated virus (%)
2364	59	−1.0	37/50	74	2/49	4		
		−2.0	39/51	76	1/48	2		
		−3.0	27/49	55	0/49	0		
		−4.0	23/49	47	0/48	0		
		−5.0	8/51	16				
2464	51	−1.0	28/45	62			0/41	0
		−2.0	29/43	67			0/43	0
		−3.0	25/44	57			0/41	0
		−4.0	18/44	41				
		−5.0	7/43	16				

* Amt of virus introduced per chick.
† No. of chicks with tumors/corrected No. of chicks.

no positive chicks in the —3.0 dilution ether-treated virus, but 55% positive virus control chicks. Chloroform, as results of Experiment 2464 demonstrated, completely inactivated the virus.

Discussion. Strain R virus was shown to be sensitive to ether and chloroform, as has been the case with all other avian oncogenic viruses tested to date. An intriguing possibility that underscored the significance of this study was that, if one of the viruses of this group were ether and/or chloroform resistant, a convenient and specific method would be available for separating it from other avian tumor viruses. By this differentiation one could more easily prepare and study a pure strain of virus. Unfortunately, this did not prove to be the case. The mechanism of virus inactivation was elucidated by Bonar *et al*(12,13), studying BAI Strain A avian myeloblastosis virus. Ether and chloroform dissolved the lipids or phospholipids, altering the primary structure of the viral outer membrane. Thus altered, the outer membrane ruptures, releasing the nucleoid, and finally the inner membrane disintegrates.

Summary. Strain R erythroblastosis virus was sensitive to ether and chloroform, as determined by chick inoculation of treated and untreated virus. In this respect, Strain R resembles other members of the avian sarcoma-leukosis virus complex for which sensitivities have been established, *i.e.*, Rous sarcoma virus, the California strain of lymphomatosis (Rubin's RIF virus), and the Stubbs-Furth strain of avian sarcoma-erythroblastosis (Strain 13) virus.

Appreciation is expressed to Mrs. Ruth E. Matter and Mrs. Eleanor Steel for excellent technical assistance. For dependable care of the chickens, we acknowledge the assistance of Mr. Horace Heald.

1. Andrewes, C. H., Horstmann, D. V. M., J. Gen. Microbiol., 1949, v3, 290.
2. Cooper, P. D., Nature, 1961, v190, 302.
3. Andrewes, C. H., Burnet, F. M., Enders, F. F., Gard, S., Hirst, G. K., Kaplan, M. M., Zhdanov, V. M., Virology, 1961, v15, 52.
4. Andrewes, C. H., Viruses of Vertebrates, Williams & Wilkins Co., Baltimore, 1964, p2.
5. Feldman, H. A., Wang, S. S., Proc. Soc. Exp. Biol. and Med., 1961, v106, 736.
6. Hamparian, V. V., Hilleman, M. R., Ketler, A., ibid., 1963, v112, 1040.
7. Wilner, B. I., A Classification of the Major Groups of Human and Other Animal Viruses, Burgess Publishing Co., Minneapolis, 1965, p1, 3rd Ed.
8. Drayton, H. A., Brit. J. Cancer, 1961, v15, 348.
9. Friesen, B., Rubin, H., Virology, 1961, v15, 387.
10. Wallbank, A. M., Sperling, F. G., Stubbs, E. L., Hubben, K., Proc. Soc. Exp. Biol. and Med., 1962, v110, 809.
11. Eckert, E. A., Beard, D., Beard, J. W., J. Nat. Cancer Inst., 1956, v16, 1099.
12. Bonar, R. A., Heine, U., Beard, D., Beard, J. W., ibid., 1963, v30, 949.
13. Bonar, R. A., Heine, U., Beard, J. W., Nat. Cancer Inst. Monograph, 1964, v17, 589.

Received September 20, 1965. P.S.E.B.M., 1965, v120.