Studies of Avian Sarcoma and Erythroblastosis (Strain 13). II. Virus Susceptibility to Ether and Chloroform.* (27658)

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(Introduced by D. S. Kronfeld)
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In 1935 Stubbs and Furth(1) described a leukosis of chickens (Strain 13, S 13) which was characterized by sarcomas, erythroblastosis, endotheliomas, or combinations of these tumors. Initially the transmitting cells were obtained from a chicken inoculated intravenously with dried blood from a fowl having Furth's Strain 1(1,2). Gottschalk(3) was able to transmit the disease characteristics of S 13 using filtered preparations. This implies that the transmissible agent was a virus. S 13 was distinguished from Strain 1 by the presence of sarcomas and the absence of myeloblastosis(4,1). To further characterize S 13 for the purpose of classifying it, we have studied its sensitivities to ether and chloroform.

The recent papers of Cooper(5), Andrewes *et al.*(6), and the often quoted communication of Andrewes and Horstmann(7) have established the value of determining the sensitivity or resistance of viruses to diethyl ether for classification purposes. Sensitivity to chloroform has also been suggested for a similar purpose(8). Although the ether and chloroform sensitivities of many viruses have been published, information concerning avian oncogenic viruses is limited to Rous sarcoma (RS) and Rubin's RIF virus(7,8,9,10). Both RS and RIF viruses are sensitive to ether; and RS virus is also inactivated by chloroform. Evidence to date indicates that, in general, viruses are either wholly resistant or highly susceptible to ether.

Materials and methods. Pool L-S S 13 virus was used in all experiments. The pool is a 5% breast muscle tumor extract clarified by centrifugation in a refrigerated Servall RC-2 high speed centrifuge. The first cycle was at 1300 $\times$ *g* for 30 min., the second cycle for the same time was at 2500 $\times$ *g*, and the final cycle was at 18,000 $\times$ *g* for 2 min. The clear supernatant was then passed through an 02 Selas filter. Test samples in thioglycollate medium and Sabourand's liquid medium (Difco) showed no evidence of growth after 7 days incubation at 35°C. The virus, stored at –65°C in narrow-mouth vials with rubber sleeve stoppers, was thawed in a 37°C water bath. After melting the vials were immediately placed in an ice-water bath.

Ether (freshly opened can of anhydrous reagent grade-Mallinckrodt) at 2°C was added with a needle and syringe through the rubber closure (one volume of ether to five volumes of virus suspension). The contents of the vial were then mixed by gentle shaking and allowed to stand overnight (16 to 17 hours) in the refrigerator at 2°C. Ether was removed from the virus-ether suspension by evaporation in a Petri dish; in our "sterile room" this took 30 min. Control virus was handled in a similar manner but without the addition of ether. The control and treated virus were then immediately titrated by wing web inoculation of chicks from the New Bolton Center strain of White Leghorns. These originated from Pennsylvania Farm Bureau Pure Line No. 1 and were random brother-sister mated for 9 years.

The experiment employing chloroform (analytical reagent-Mallinckrodt) was also performed at 2°C. Chloroform (one volume of chloroform to five volumes of virus suspension) was added to the virus extract in a small flask with a rubber stopper closure. The virus-chloroform mixture was agitated slowly on a magnetic stirrer for 10 min. and then centrifuged at 275 $\times$ *g* for 3 min. The virus phase was removed, immediately diluted, and inoculated into chicks as in the previous experiments. The chicks for this experiment were Pennsylvania Farm Bureau strain "60".

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Control virus was treated in the same way but without addition of reagent.

A palpable tumor in the wing web on two successive days was the criterion for considering a chick positive. The tumors, employing virus pool L-S, usually appear in 6 to 7 days. Nodules are firm, spherical to oval, and the skin is freely movable over their surface. On the cut surface they are homogenous, grey, smooth, and non-encapsulated. The histologic appearance is typical of S 13; a mass of spindle-shaped cells forming sheets and networks in various areas. The individual cells are elongated, have pale eosinophilic cytoplasm with an oval vesicular nucleus. The neoplastic cells are probably of endothelial origin(1). The birds on the ether experiment were examined every day from post-inoculation day five through post-inoculation day 14, and then three times during the next week, including day 21. Chicks used in the chloroform experiment were examined daily from post-inoculation day three through day 21.

Results. These experiments (see Table I) present evidence that sarcoma 13 virus is sensitive to both ether and chloroform. The first experiment, #2461, indicates that no virus survived ether treatment. An experiment was then designed to determine if a greater number of animals would indicate the presence of active virus when undiluted ether-treated material was inoculated. This experiment, #2561, proved that the virus had indeed been completely inactivated within the range of sensitivity of the experiment. Chloroform, experiment #262, also inactivated the virus completely.

Three days after the chicks were inoculated with the virus-chloroform mixture, swellings were detected in the wing webs of many chicks given the undiluted material which were *not* tumors. Chicks given the same amount of virus without chloroform treatment presented no evidence of swelling on post-inoculation day three. There was no indication of swelling in the diluent control group during the experiment. Before the final examination day the swelling regressed in all chicks inoculated with chloroform-virus mixture. Two affected chicks were sacrificed during the experiment for examination of the swellings. Microscopic examination of the wing webs revealed numerous focal accumulations of lymphocytes. In some instances these were adjacent to blood vessels; in others there were foci in the loose connective tissue. Fibroblasts in the web appeared to be slightly disorganized and swollen in a few areas, but there was no indication of neoplasia. The process appeared to be one of subacute inflammation.

Discussion. It has been demonstrated that S 13 virus is inactivated by ether and chloroform. These experiments help to substantiate the belief that S 13 virus is one of the leukosis complex group. Sensitivity of S 13 virus to these organic solvents appears to be identical to other members of the fowl leukosis complex viruses for which sensitivities have been established.

TABLE I. Effect of Ether and Chloroform on Sarcoma 13 Virus.

Exp. No.	Age of chicks (days)	Virus dilutions*	Virus†	Ether-treated virus	Chloroform-treated virus	Diluent controls
2461	5	$10^{-2.3}$	5/5	0/5		
		$10^{-3.3}$	5/5	0/5		
		$10^{-4.3}$	5/5	0/5		
		$10^{-5.3}$	2/5	0/5		
2561	3	$10^{-2.3}$	20/20	0/19		
262	4	$10^{-2.3}$	37/39		0/37	0/39
		$10^{-3.3}$	31/39		0/40	
		$10^{-4.3}$	17/39		0/39	
		$10^{-5.3}$	8/39		0/39	
		$10^{-6.3}$	3/39			

* Indicates virus introduced per chick.
† Number of chicks with tumors/Corrected number of chicks.

Final steps in defining the relationship of S 13 virus to other avian tumor viruses will include the establishment of serologic responses.

Summary. Ether and chloroform destroyed all detectable infectivity of S 13 virus as determined by wing web inoculation of chicks.

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Stimulation of Thoracic Duct Lymph Flow by Epinephrine and Norepinephrine.* (27659)

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Epinephrine has been observed to cause an increase in rate of lymph flow from the thoracic duct of dogs under pentobarbital anesthesia(1). We have previously reported that pentobarbital depresses the rate of lymph flow approximately 25%, and that although under this anesthesia the absolute amount of stimulation of lymph flow by epinephrine was diminished, the percent increase was of the same order of magnitude as in the unanesthetized preparation(2). This paper reports on the relative potencies of intravenous epinephrine and norepinephrine in stimulating thoracic duct lymph flow in conscious dogs.

Materials and methods. Dogs were prepared with chronic thoracic duct-venous shunts(2,3). The thoracic duct was cannulated in the neck with polyethylene tubing (I.D. 0.86 × O.D. 1.52 mm). This cannula was exteriorized and connected to another cannula which had been inserted into the precaval vein by way of a branch of the right external jugular vein. A stockinet bandage was placed around the neck to protect the external loop of tubing. No anticoagulants were administered. Duration of lymph flow in such shunt preparations has averaged 13 days. For the lymph collection periods, the dogs were loosely restrained and trained to lie on their left sides. To collect lymph the loop of tubing was separated and the lymph was drained into a graduated cylinder. The jugular segment of the opened shunt was available for infusing solutions. For the experimental periods the dogs were in the basal state—postcibal for at least 16 hours and rested in position on a padded table for one hour. A total of 12 paired experiments were performed on 4 dogs (13-18 kg) in order to compare the effects of epinephrine (L-Epinephrine Bitartrate—Winthrop) and norepinephrine (L-Arterinal Bitartrate—Winthrop) on rate of lymph flow. After a steady rate of flow had been established, 7.5 μg/kg of the pressor amine in 10 ml of isotonic saline were injected intravenously over a 10-minute period. During a given experimental period both epine-

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