Thoracic Duct Lymph in Unanesthetized Mouse. Method of Collection, Rate of Flow and Cell Content.* (24992)

MARVIN M. SHREWSBURY (Introduced by W. O. Reinhardt)
Dept. of Biology, San Jose State College, San Jose, Calif.

A method has been described for short term collection of thoracic duct lymph from the anesthetized mouse(1). The following report is concerned with the possible difference between lymph flow and lymphocyte output in unanesthetized as contrasted with anesthetized state. In addition, thoracic duct lymph flow and lymphocyte output collected from abdominal or cervical regions of anesthetized mice are compared.

Technic. Male mice of NIH-Webster strain, having mean body weight of 24 g ± 1, maintained on Purina Laboratory Chow, were used. Operative procedures were carried out under sodium pentobarbital-induced anesthesia (7 mg/100 g B.W., injected intraperitoneally). With this dose level, the animals recovered consciousness and became active within approximately one hour. The abdominal thoracic duct was cannulated caudal to diaphragm with polyethylene tubing (internal diameter 0.011 in.), utilizing with minor variations a method employed for rats(2). For instance, a small thin-edged spatula was used to separate thoracic duct from aorta. A curved atraumatic needle(6-0), the thread of which was looped, was used to pull a second silk thread (50 gauge) around the thoracic duct to fasten the cannula in place; a second restraining tie was placed around the cannula in the duct and fixed to body wall to prevent dislodgment. After operation a thin glass rod was attached in the sagittal plane to the skin on dorsum of animal by means of wound clips. This rod partially restrained the mouse from forward, backward, or lateral movements. The mouse was placed by means of glass rod on upper and outer circumference of an activity wheel (Fig. 1). This arrangement permitted voluntary rest, or freedom to exercise, as well as access to fluids and food. After recovery from the anesthetized state, animals were active, ate food, and drank water during period of lymph collection, and appeared healthy at end of collection period. Lymph was collected at room temperature into a glass vial which contained a small quantity of sequestrene (ethylene diamine tetraacetate) to prevent coagulation. Lymph flow and lymphocyte output were measured for successive 6-hour samples over 24 hours from each of 17 mice. A few animals were permitted to drain lymph for 30 hrs; one animal continued lymph flow for 48 hrs. Duplicate white blood cell counts were carried out on each 6-hour sample of lymph. Occasional clotting of lymph, usually in the first 6 hrs., and at the distal end of cannula, was relieved by slight negative pressure at cannula tip. Comparative studies

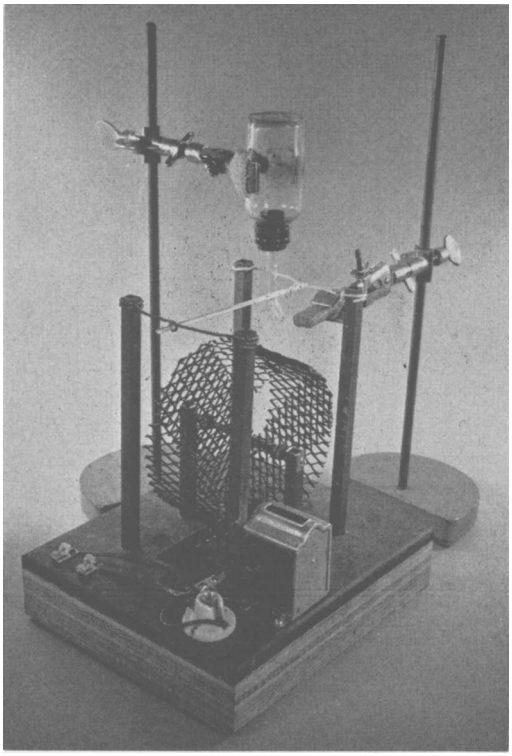

FIG. 1. Activity wheel for collection of mouse thoracic duct lymph. Water bottle, food, and adjustable restraining glass rod are shown.

* Assisted by grants from Santa Clara Heart Assn. and Nat. Inst. Health, U.S.P.H.S.

TABLE I. Effect of Anesthesia and of Site of Collection on Thoracic Duct Lymph Flow and Cell Content in Male Mice, in 2 Experiments.

Treatment	No. of animals	Collection time, hr	Lymph vol, ml/hr	Lymphocyte Content cells/mm³ (× 10³)	Lymphocyte Output cells/hr (× 10⁶)
Abdominal thoracic duct					
Unanesthetized	17	6	.06 ± .01*	30.8 ± 2.1	1.5 ± .2
Anesthetized†	10	3	.07 ± "	27.5 ± 4.4	1.4 ± .1
Anesthetized†					
Abdominal thoracic duct	7	1	.09 ± .01	20.0 ± 2.7	1.6 ± .2
Cervical " "	7	1	.08 ± "	21.0 ± 4.2	1.6 ± .3

* Mean ± stand. error. † Intraper. inj. of solution of sodium pentobarbital.

were made on 10 anesthetized mice by collecting abdominal thoracic duct lymph for 3 hours. Seven pairs of male mice were used in comparative studies of thoracic duct lymph flow and cell output in anesthetized animals, lymph being collected from the abdominal thoracic duct in one of each pair and from the neck in the other, using a technic for the latter as described for the rat(3). Because of previously demonstrated diurnal variation in the mouse(1), lymph collections were carried out at similar times for each pair of animals. Significance of difference of means was calculated, using Fisher's values for t, p values of $<.01$ being considered of probable significance.

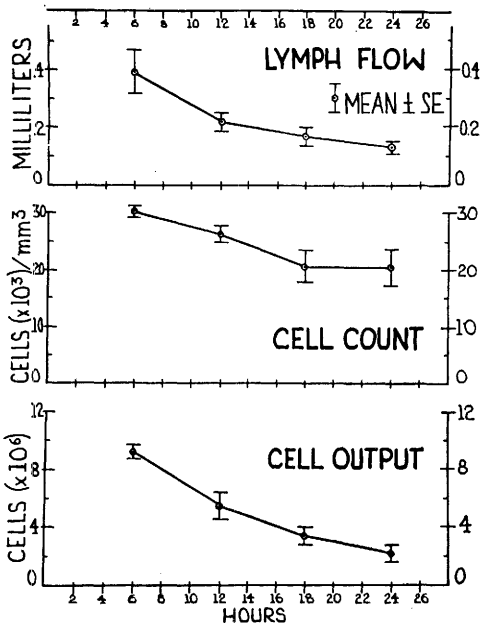

FIG. 2. Changes in lymph flow, lymphocyte count and total lymphocyte output, at 6 hr intervals, in mice with continuously draining abdominal thoracic duct lymph.

Results. Fig. 2 presents values, for each of 4 consecutive 6-hour periods, for lymph flow, lymphocyte content, and total lymphocyte output, for 17 male mice. Lymph flow decreased sharply after first 6 hours, then declined slowly over remaining collection period. Lymphocyte count decreased about one-third in the first 18-hour period, then leveled off. Total lymphocyte output decreased at every 6-hour interval. Comparison of first and second 6-hour samples revealed a significant decrease in lymphocyte output. The fall in cell output was further extended in animals maintained for 30 hours and in the animal allowed to drain lymph for 48 hours. In the latter animals, however, lymph volumes did not vary from those obtained at end of first 24 hours collection. These findings confirm the continuous fall in thoracic duct cell output reported for unanesthetized rats(4,5,6).

No consistent relationship was noted between lymph flow and lymphocyte output in either unanesthetized or anesthetized animals (Table I). Data derived from lymph studies in the unanesthetized state are therefore deemed to be representative of values obtained in anesthetized mice only for a limited period after recovery from the anesthetized state. Similar results have been recorded for rats(7).

Technics of collection of thoracic duct lymph from abdominal and cervical regions were assessed. Both technics are of equal difficulty and for short-term studies either technic is adequate. However, for longer drainage of lymph from anesthetized forms, the

abdominal approach is preferred. The cervical approach was generally accompanied by respiratory distress after more than one hour of lymph collection.

Table I also presents a comparison of data obtained from studies made on paired mice in which thoracic duct lymph was collected from either the neck or abdomen at same time of day. Mean values for lymph flow, lymphocyte content, and total lymphocyte output did not differ. Although mean values were similar for abdominal and for cervical lymphocyte output for paired animals, individual cell outputs collected simultaneously in paired animals on same day were closer to each other than in collections made at similar times but on different days.

On the basis of the mean total thoracic duct lymphocyte output of 1.5 cells/hr x 10^6 for anesthetized or unanesthetized mice, it can be calculated that at least 35 million cells enter the blood from this duct in a 24-hour period. This is 3.3 times the total calculated number of circulating lymphocytes.

Summary. 1. The mouse is a suitable animal for collection of thoracic duct lymph in the partially-restrained unanesthetized state. 2. Initial values for lymph flow and lymphocyte output did not vary significantly in the unanesthetized as compared to the anesthetized mouse. 3. A continuous decrease in lymph flow and lymphocyte output from the thoracic duct occurred over a 24-hour period in the unanesthetized mouse. 4. Values for thoracic duct lymph flow and lymphocyte output did not vary significantly, whether collected from the neck or abdomen.

1. Shrewsbury, M. M., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 53.

2. Bollman, J. L., Cain, J. C., Grindlay, J. H., *J. Lab. and Clin. Med.,* 1948, v33, 1349.

3. Reinhardt, W. O., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 123.

4. Mann, J. B., Higgins, G. M., *Blood,* 1950, v5, 177.

5. Shrewsbury, M. M., Reinhardt, W. O., *Am. J. Physiol.,* 1952, v168, 366.

6. Gowans, J. L., *Brit. J. Exp. Path.,* 1957, v38, 67.

7. Hungerford, G. F., Reinhardt, W. O., *Am. J. Physiol.,* 1950, v160, 9.

Received May 11, 1959. P.S.E.B.M., 1959, v101.

Cytopathology of Feline Viral Rhinotracheitis Virus in Tissue Cultures of Feline Renal Cells. (24993)

ROBERT A. CRANDELL AND EDWARD W. DESPEAUX (Introduced by F. D. Maurer)
Armed Forces Inst. of Pathology, Washington, D. C.

In the original report(1) on isolation of a feline virus associated with intranuclear inclusion bodies the agent was designated as C-27 virus. We now propose the name "feline viral rhinotracheitis" for the disease entity induced by this agent. The virus in this and future communications will be referred to as feline viral rhinotracheitis (FVR) virus. In this paper we describe the sequence of morphological changes with formation of multinucleated giant cells or syncytia in cell cultures infected with FVR virus, and report our results from the use of different methods of fixation for demonstration of inclusions.

Materials and methods. The original FVR virus obtained from the throat of a cat exhibiting clinical signs of upper respiratory infection was isolated in cultures of feline renal cells. The virus used was from 5th and 6th tissue culture passages. Primary cultures of feline renal cells were prepared on 11 x 22 mm coverslips in Leighton tubes. Tissue culture methods employed were those of Madin *et al.* (2). The cells were grown in nutrient medium consisting of 0.5% lactalbumin hydrolysate in modified Hank's salt solution containing 0.01 M Tris (Hydroxymethyl aminomethane), adjusted to pH of 7.4, and 10% lamb serum. The maintenance fluid used at time of inoculation was lactalbumin hydrolysate