pounds in an attempt to relate some of the anabolic and catabolic aspects of steroid metabolism by strain U12-79.

Summary. Strain U12-79 of human uterine fibroblasts propagated *in vitro* for almost 5 years was grown in a medium containing acetate-2-C^{14}. C^{14}-cholesterol was isolated from both the cells and the medium.

1. Dougherty, T. F., Adrenal Cortex, Trans. 2nd Conf., Josiah Macy, Jr. Found., N. Y., 1955, p88.

2. ———, *Recent Progr. Hormone Res.*, 1952, v7, 307.

3. Berliner, D. L., Dougherty, T. F., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 3.

4. Berliner, D. L., Grosser, B. I., Dougherty, T. F., *Arch. Biochem. & Biophys.*, 1958, v77.

5. Sweat, M. L., Grosser, B. I., Berliner, D. L., Swim, H. E., Nabors, C. J., Jr., Dougherty, T. F., *Biochem. et Biophys. Acta*, 1958, v28, 591.

6. Swim, H. E., Parker, R. F., *Special Publications of N. Y. Acad. Sci.*, 1957, v5, 303.

7. ———, *J. Lab. Clin. Med.*,

8. ———, *Am. J. Hyg.*, 1957, v66, 235.

9. Earle, W. R., Nettleship, A., *J. Nat. Cancer Inst.*, 1943, v4, 213.

10. Zaffaroni, A., *Rec. Progr. Hormone Res.*, 1953, v8, 51.

11. Schwenk, E., Alexander, G. S., Fish, C. A., *Arch. Biochem. & Biophys.*, 1955, v58, 37.

Received June 17, 1958. P.S.E.B.M., 1958, v99.

Rate of Flow and Cell Count of Thoracic Duct Lymph in the Mouse. (24244)

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

This report describes a method for collection of thoracic duct lymph in anesthetized mice, and presents data on rate of flow, white cell count, and total lymphocyte output of lymph obtained. These data are compared with similar measurements of thoracic duct lymph in other animal forms.

Technic. Male mice (40 days old) of NIH-Webster strain, unfasted, 17-23 g B.W. were anesthetized by intraperitoneal injection of 6% sodium pentobarbital (7 mg/100 g B.W.). An incision was made through the abdominal wall, following the usual dorsal approach for left adrenalectomy. Manipulations and lymph collection were performed in a bloodless field under a dissecting microscope. The incised body wall was retracted to expose the abdominal aorta. Viscera within the field were carefully retracted, care being taken to avoid entering the peritoneal cavity. The aorta, cranial to the mesenteric lymph node (Aselli), was displaced gently away from posterior abdominal muscles caudal to the diaphragm. This procedure exposed the thoracic duct, contiguous to posterior aspect of the aorta. The dissection, carefully executed, resulted in formation of a depression or cup, bounded by the aorta on the left side and paravertebral muscles on the right. The superior border of this cup was demarcated by the aorta as it traversed the diaphragm; the inferior border of the cup was formed by juncture of the aorta, mesenteric lymph node, and paravertebral muscles. The floor of the cup was formed primarily by the left side of the thoracic duct.

The point of a 27 G hypodermic needle was used to make 2 slits in the exposed thoracic duct. The first opening was cephalic to a segmental artery crossing the duct. To insure complete drainage of the thoracic duct lymph into the structural cup, a second opening was made caudal to this segmental artery. After incising the wall of the duct, a free flow of clear or milky lymph drained into the anatomical cup or depression. The tip of a fine glass sequestrene treated cannula of small bore was then placed in the cup adjacent to the duct incisions. Lymph was drained through the cannula into a small glass vial under slight negative pressure. Lymph was collected for half hour periods, measurements being made at intervals during collection.

Results. Table I presents pertinent data

TABLE I. Thoracic Duct Lymph Flow and Lymphocyte Output in Anaesthetized Male Mice.

Exp.	No. of animals	Body wt, g	Collection time, min.	Lymph vol ml/hr	Lymph vol ml/hr/kg B.W.	Lymphocyte Content, cells/mm³ × 10³	Lymphocyte Output Cells/hr × 10⁶	Lymphocyte Output Cells/hr/kg B.W. × 10⁶
Controls	20	20 ± .5*	90	.10 ± .01	5.5 ± .7	11.7 ± 1.1	1.1 ± .2	57.0 ± 8.4
Diurnal variation								
A.M.	6	20 ± .6	90	.10 ± .02	4.9 ± 1.0	6.8 ± 1.2	.7 ± .1	34.6 ± 6.5
P.M.	5	19 ± .4	90	.11 ± .03	6.1 ± 1.3	18.6 ± 2.7	1.9 ± .3	104.6 ±15.6

* ± S.E.

determined on thoracic duct lymph from 20 anesthetized mice.

Lymph flow from the thoracic duct lymph varied in quantity from 0.03-0.24 ml/hr. Lymph flow remained fairly constant for each animal during collection, although the cell output had a tendency to rise during later sampling periods. The mean flow (ml/hr/kg B.W.) was greater than that reported for most other animals; the highest values obtained for lymph flow in the mouse (14 ml/hr/kg B.W.) were greater than recorded for other animals under similar conditions(1). There appeared to be no consistent relationship between lymph flow, lymphocyte output, and body weight. On the basis of average thoracic duct lymph flow of 0.1 ml/hr, it can be calculated that a volume of at least 2.4 ml of thoracic duct lymph passes into the venous blood in 24 hr period. This amounts to approximately one and one-half times the total blood volume(2). White cell counts for the thoracic duct lymph averaged 11,700 ± 1,100 and varied from 1,000-35,400 cells/mm³. The mean value for the thoracic duct lymphocyte output (cells/hr/kg B.W.) was similar to values obtained for the guinea pig(1).

Comparison of the values for lymph collections obtained from 9:30-10:30 a.m. with similar data obtained from 3:30-4:30 p.m. was used to determine extent of diurnal variation. A diurnal variation was apparent for average total lymphocyte output (cells/mm³, cells/hr, and cells/hr/kg B.W.) from the thoracic duct for mice in which lymph was collected for the morning hours, as compared to a similar collection period in the afternoon. The data showed a significantly higher value ($p<.01$) for mean cell count and output in the afternoon period.

Protein concentration as measured by the copper sulfate specific gravity method averaged 4.6 ± 0.3 g% for the thoracic duct lymph of 9 mice.

Summary. 1. A method is described for obtaining lymph from the abdominal thoracic duct of the anesthetized mouse. 2. Average rate of lymph flow was approximately 0.1 ml/hr, or 5.5 ml/hr/kg B.W. 3. The cell content averaged 11,700/mm³, and the total lymphocyte output averaged 1.10 x 10⁶/hr. 4. There was a significant diurnal variation in lymphocyte output which was greater in the afternoon than in the morning.

1. Yoffey, J. M., Courtice, F. C., *Lymphatics, Lymph, and Lymphoid Tissue,* Harvard Univ. Press, Cambridge, Mass., 1956.

2. Prosser, C. Ladd, *et al., Comparative Animal Physiology,* W. B. Saunders Co., Philadelphia, 1950.

Received June 17, 1958. P.S.E.B.M., 1958, v99.