excess N^{15} was injected intravenously into mice. 2. The N^{15} was rapidly removed from the blood stream and after 10 minutes was found principally in the skin and carcass. The remainder of the N^{15} was accounted for in the gastrointestinal tract, liver, and urogenital tract. 3. Thirty to 180 minutes after injection the predominant portion of the N^{15} still appeared in the skin, carcass, gastrointestinal tract and with progressing time in the excreta. 4. On the average the N^{15} in the carcass showed a decrease after one hour, while the gastrointestinal tract showed an increase during the first hour and a decrease after 3 hours.

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Rate of Flow and Cell Count of Rat Thoracic Duct Lymph.*

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In the course of studies on the action of adrenocorticotrophic hormone on the leucocyte count in the rat,[1] it was thought advisable to study directly the effect of hormone administration on the lymphocyte cell content of thoracic duct lymph in this animal form. Review of the literature did not, however, reveal any description of the technic of cannulation and collection of lymph in the rat. The present report describes a method of cannulation of the thoracic duct in the rat, and observations on the white cell count, and rate of flow of lymph in the normal, adult female rat.

Technic. Rats of the Long-Evans strain, of weight 200-250 g, unfasted, were employed. Preliminary studies indicated the difficulty of accurate identification of the lymphatic trunks in the neck so that it was necessary to identify the vessels by intraperitoneal injection of ½ cc of 1% trypan blue, half an hour before cannulation was attempted. This amount of dye was sufficient to stain all the main lymphatic trunks and to demonstrate the site of entrance of the lymph stream into the junction of internal jugular and subclavian veins with the left superior vena cava (in the rat). When familiarity with the lymphatic vessel arrangement was attained, further use of the dye was obviated.

The animals were anesthetized by intraperitoneal injection of 50-60 mg/kg B.W. of sodium pentobarbital in 1% solution. Further amounts were injected as necessary to maintain the anesthetic state over long periods of time.

Two types of cannulæ were employed. Capillary tubes were used for drop-wise collection of the lymph. In other cases, 3 to 5 mm glass tubing was drawn out into a capillary tube at one end for the collection of larger samples of lymph. The capillary tubings were drawn to the size, externally, of No. 25 G-No. 27 G hypodermic needles, which were employed to make openings in the lymph vessels previous to insertion of the cannula. All manipulations and cannulation were carried out under the binocular dissecting microscope. Cannulæ were heparinized to prevent clotting. A midline incision was carried through skin and subcutaneous tissue of the neck, through the deep fascia, separating the submandibular salivary glands, dissecting to the ventral aspect of the sternohyoid muscles. Using a small pair of bone forceps, the manubrium sterni was split in the midline as far as the sternal angle. The two halves of the manubrium were retracted laterally by means of clamps and held apart exposing the length of

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1 Reinhardt, W. O., Aron, H., and Li, C. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 19.

the sternal attachment of the sternohyoid muscles. The left sternohyoid muscle was bluntly dissected away from its attachments to the manubrium, exposing the site of juncture of the left superior vena cava and the internal jugular vein. Lying in close dorsal approximation to the internal jugular vein is the small lymph sac formed by the thoracic duct, its jugulo-subclavian tributaries, and the site of union with the venous system. The main cervical lymph trunk was identified and followed down to the point at which the cervical trunk joined the thoracic duct. A No. 27 G hypodermic needle was thrust through the wall of the cervical lymph vessel, and the cannula was inserted into the vessel and threaded down the thoracic duct. Lymph rushed into the cannula immediately and could be collected by any desired method, either drop-wise for lymph cell counts or in larger quantities for chemical analysis.

In some experiments, cannulæ were inserted directly into the cervical lymph trunk and threaded into the thoracic duct. It is undoubtedly possible to cannulate and collect lymph from any of the tributaries of the thoracic duct or lymph sac. It may be noted that the living lymph vessels are very thin-walled and practically transparent, but are distensible and much larger in size than would be indicated by embalmed animal dissections.

Results. Under the binocular microscope, clear lymph of the cervical and subclavian lymph trunks may be seen mixing with the usually milky and opaque thoracic duct lymph prior to entrance into the venous system. The rate of flow of thoracic duct lymph varied from 0.13-0.7 cc per hour, averaging 0.45 cc in 10 animals. In one animal, 0.7 cc were collected in an hour; in another, 2.0 cc in 6 hours.

On the basis of a thoracic duct lymph flow of 0.5 cc/hr, it can be calculated that a volume of at least 12.0 cc of thoracic duct lymph passes into the venous blood stream/24 hours. This amounts to an estimated 75% of the total blood volume (6.5% of total B.W.) in rats of the size employed.

White cell counts were carried out on the thoracic duct lymph of 17 rats. The total white cell count averaged 19,050, and varied from 6,650-46,250 cells per cu mm. Supravital studies and Wright-stained differential cell counts disclosed that the white cells were almost uniformly small and medium sized lymphocytes.

The above values correspond closely to those for other animal forms studied.[2]

It should be remarked that the amount of lymph available on cannulation of the rat thoracic duct is more than sufficient to carry out most morphological or chemical studies.

Summary. 1. A technic is described for obtaining lymph from the thoracic duct of the rat. 2. The average rate of flow of thoracic duct lymph in the adult, female, unfasted rat, anesthetized with sodium pentobarbital was approximately 0.45 cc/hour (range: 0.13-0.7 in 10 rats). 3. The white cell count of the thoracic duct lymph in 17 rats varied from 6,650-46,250 cells/cu mm and averaged 19,050 cells/cu mm. 4. The rat is a useful and convenient animal to employ in studies on physiology of the lymph.

2 Drinker, C. K., and Yoffey, J. M., *Lymphatics, Lymph and Lymphoid Tissue,* Harvard University Press, Cambridge Mass., 1941.