

to resemble other known pituitary hormones of protein nature. Such passage between parabiotic animals of gonadotropic, adrenotropic and growth hormones has been previously described^{7,8,9} and was also observed in the present experiments. The steroid hormone estrogen, on the contrary, failed to be transferred to the partner.⁸ If the pituitary mammotropic substance has a lipoid character

as is reported by Lewis and Turner¹⁰ it is perhaps surprising that its behavior in parabiosis seems to link it more with the protein hormones than with the chemically more similar estrogen.

Since the pituitary gland of one rat was serving both members of a pair the amount of mammotropic factor which crossed to the hypophysectomized partner must have been small; yet this quantity was sufficient to alter the mammary response to estrogen from an unqualified negative to a definite positive. Furthermore the fact that the greatest mammary growth occurred where a female was furnishing the mammotropic hormone to the hypophysectomized parabiont suggests a sex difference in production and release of this hormone.

⁷ Møller-Christensen, E., *Acta Path. et Microbiol. Scand.*, 1933, **10**, 296.

⁸ Van Dyke, H. B., *The Physiology and Pharmacology of the Pituitary Body*, University of Chicago Press, 1936, 1939.

⁹ Greep, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 214.

¹⁰ Lewis, A. A., and Turner, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 435.

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Cell Count, Rate of Flow, and Protein Content of Cervical Lymph in the Rat.*

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Previous studies^{1,2} have shown that it is possible to collect thoracic duct lymph from the anaesthetized rat in amounts sufficient to carry out cell counts and chemical analyses. It was noted in these studies that there was an evident flow of lymph from the cervical lymphatic trunk in the preparations employed. Since spontaneous flow of lymph from the periphery (head and neck or extremities) of quiescent animals is unusual,³ it is thought noteworthy that there is, in the anaesthetized

rat, a steady flow of lymph from the main lymphatic trunks draining the head and neck. The present report describes a method of cannulation of the cervical lymphatic trunk, and presents data on the rate of flow, white cell count, and protein content of the lymph obtained. This data is compared with further measurements of the same type for thoracic duct lymph.

Technic of collection of cervical lymph. Adult female rats of the Long-Evans strain, unfasted, weighing between 250-300 g, were anaesthetized by intraperitoneal injection of a 1% solution of sodium pentobarbital. A midline incision was carried through skin and subcutaneous tissue from the symphysis menti to the sternal angle. The incision was carried sufficiently deep to expose the sternohyoid muscles. All superficial neck tissues were retracted laterally. The manubrium sterni was split lengthwise in the midline, using bone

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¹ Reinhardt, W. O., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 123.

² Reinhardt, W. O., and Li, C. H., *Science*, 1945, **101**, 360.

³ Drinker, C. K., and Yoffey, J. M., *Lymphatics, Lymph and Lymphoid Tissue*. Harvard University Press, Cambridge, Mass., 1941.

TABLE I.

Animal No.	Duration of lymph collection		Wt of lymph (mg)	Lymph flow/hr (mg)	WBC/emm	Protein* (g %)
	hr	min				
41	0	48	38.5	48.1		
42	2	0	96.0	48.0	4,800	
43	0	35	13.5	23.2	9,050	
44	6	20	196.3	31.0	2,400	4.12
45	5	50	300.1	51.4	4,250	2.46
46	4	10	98.9	23.7		3.31
47	7	0	177.9	25.4	5,200	3.44
49	6	5	204.8	34.0	1,525	3.44
50	1	45	112.2	64.2	15,050	2.87
51	9	55	418.9	42.3	8,500	2.56
52	8	0	218.5	27.2	2,375	3.00
53	8	40	375.3	42.3	17,500	2.44
Avg				38.5	7,065	3.07

* The total protein ($N \times 6.25$) represents g/100 g of lymph (not corrected for N P N).

forceps. The two halves of the manubrium were retracted laterally by means of hemostats. The attachments of the sternohyoid muscle to the manubrium were dissected away bluntly, exposing the carotid arteries, vagus nerve and internal jugular veins on each side of the neck. Lateral to and in the same layers of deep cervical fascia as the structures of the carotid sheath, course the cervical lymphatic ducts from the head and neck to empty into the venous system; on the left, via the common lymph sac by union with the thoracic duct, and on the right, to empty into the venous system at the junction of internal jugular and subclavian veins. The lymph vessels were isolated (under the binocular microscope) by blunt dissection at the site of their drainage into the venous system and were ligated by single silk ligatures to prevent reflux of fluid. Ligation of the lymph trunks produced immediate distention allowing one to choose for cannulation an area in the vessel wall free from the rather extensive plexus of vasa vasorum. The point of a 27 G hypodermic needle was thrust through the vessel wall, it being possible to distinguish the layers of the vessel wall grossly. When the needle passed through the intimal layer, a free flow of lymph ensued. A fine glass cannula with tip of the same size as the needle was then threaded into the vessel and left *in situ*. It was not necessary to tie the vessel on the cannula; the muscular wall of the lymphatic sufficed to maintain contact with the cannula. Crystal-clear lymph flowed readily into the cannula and, indeed, against

a pressure of 1-2 cm of water, since the end of the cannula rested on the thoracic cage. It was possible to cannulate both cervical lymph trunks in the same animal. Lymph was collected over a variable number of hours, expelled into weighing bottles, weighed, thoroughly mixed for white blood cell sampling (duplicate), and analyzed for total nitrogen content by a micro-Kjeldahl method.

Results. Table I presents the pertinent data gathered on cervical lymph in the anaesthetized rat.

The data on cervical lymph may be compared in Table II with similar data for thoracic duct lymph, obtained under identical conditions in another series of rats of the same age and weight.

It will be noted that the absolute lymphocyte count of cervical duct lymph is about $\frac{1}{3}$ that of the thoracic duct, and that the rate of flow is about $\frac{1}{10}$ as great.

It is evident that the motionless anaesthetized rat exhibits a free flow of cervical duct lymph. This is in distinction to other commonly used laboratory mammals.³ For this reason, the rat may be useful in the study of absorption of various particulate materials, protein solutions, or other foreign material, from organs or tissues of the head and neck, without the necessity of resorting to artificial methods of producing passive motion or massage to promote lymph flow.

Preliminary electrophoretic analyses of blood plasma and cervical lymph (pooled specimen) indicate that there is no essential

TABLE II.

	Thoracic duct lymph		Cervical duct lymph	
White cells/cmm	21,250 $\pm$ 1,580*	(37)†	7,065 $\pm$ 1,725	(10)
Lymph flow/hr	0.39 $\pm$ 0.03 cc	(27)	38.5 $\pm$ 3.75 mg	(12)
Protein conc. (whole lymph)	4.18 $\pm$ 0.11 (g %)	(19)‡	3.07 $\pm$ 0.18 (g/100 g)§	(9)

* Mean $\pm$ S.E.

† Figures in parentheses indicate number of animals.

‡ Copper sulfate gravity method. (Avg specific gravity of 19 specimens was 1.0187 $\pm$ 0.00014)

§ Micro-Kjeldahl. ($N \times 6.25$) (not corrected for N P N)

difference in the albumin-globulin ratio, but that there is a definitely higher content of gamma globulin in the cervical lymph as compared with blood plasma.

Summary. The quiescent anaesthetized rat exhibits a free flow of lymph from the cervical lymphatic trunks. A method of collecting

cervical lymph in the rat is described. The average rate of flow is approximately 40 mg/hr, the lymphocyte count averages 7,065/cmm, and the average protein content is 3.07 g %. These figures are compared with similar data for thoracic duct lymph.

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Comparison of the Toxicity of Antiseptics for Embryonic Tissue and Bacteria.

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(Introduced by A. C. de Graff.)

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This report describes a procedure for the determination of the toxicity index of antiseptics which appears to have certain theoretical and practical advantages over those previously presented.

The evaluation of antiseptics has long been a difficult problem, largely because of the many types of chemical compounds which have been found useful in this field. To date, no entirely successful procedure has been devised for comparing these drugs except when tests are made of closely related compounds. The use of a toxicity index (the ratio of the dilution of an antiseptic which will produce some readily determined toxic effect on tissue to that which will kill bacteria tested) permits interclass comparisons to some extent. The reliability of the results can be enhanced further by inclusion of a suitable reference standard to control shifts in tissue or bacterial susceptibility. In addition it is generally recognized that since the toxicity of an antiseptic is frequently

modified by the presence of organic matter, the tissue or bacteria should be implanted in the medium before contact with the antiseptic is permitted. In a recent paper Salle and co-workers¹⁰ have described a method which takes into account all of the foregoing considerations. They suspended chick heart fragments in serum and embryonic extract, added antiseptic, and then, after 10 min exposure, cultured the tissue in Carrel flasks. In a corresponding series of experiments, bacteria were exposed for 10 min followed by subculture in beef broth.

Salle's work and that of other investigators¹⁻¹⁰ in this field has been based on either

¹ Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

² *ibid.*, *J. Am. Med. Assn.*, 1916, **67**, 1300.

³ Lambert, R. A. and Meyer, J. R., *Proc. Soc. Exp. Biol. and Med.*, 1926, **23**, 429.

⁴ German, W. J., *Arch. Surg.*, 1929, **18**, 1920.

⁵ Buchsbaum, R., and Bloom, W., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 1060.