and a dihydrostreptomycin-polymixin-pectin-adsorptive clay mixture were ineffectual despite their relatively low solubility. The characteristic low-body fat of the lactose-fed rat was not affected by chlortetracycline administration.

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Lymphocyte Output and Lymph Flow of Thoracic and Right Lymphatic Ducts of Anesthetized Rats. (24401)

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The present experiments were undertaken to ascertain rate of flow and cellular content of lymph simultaneously collected from right and thoracic lymph ducts in male rats.

Methods. Preliminary observations of lymphatic channels draining into right jugulo-subclavian angle of the rat (after injection of trypan blue) indicated that quantitative collection of right duct lymph was feasible using a procedure previously described by Reinhardt(1) for thoracic duct cannulation. Ten unfasted male Long-Evans rats about 100 days old were anesthetized by IP injection of sodium pentobarbital (7 mg/100 g body weight). The neck region was shaved and midline incision made from symphysis menti to sternal angle to expose sternohyoid muscles. Manubrium sterni was split lengthwise in the midline and the halves of the manubrium along with superficial neck muscles were retracted laterally, care being taken not to occlude or damage underlying vessels. Sternal attachments of the sternohyoid muscles were bluntly dissected away, exposing carotid arteries, vagi, and the jugulo-subclavian venous angles. Animals were tracheotomized and cervical lymphatic ducts ligated. Under dissecting microscope, thoracic and right lymphatic ducts were identified at entrance into jugulo-subclavian angles. The lymph ducts were nicked with a sharp needle and an L-shaped Pyrex capillary cannula was introduced into each duct. Lymph was collected in small vials. Coagulation of lymph was prevented by occasional application of powdered heparin to tips of cannulae. Compared to difficulties usually encountered in cannulating the right lymphatic duct in dogs, cats, and rabbits, this method is simple and successful, in most instances, in the rat. Lymph flowing through each duct was collected for various intervals (1 to 4 hours) and volume measured with graduated tuberculin syringe or micropipette. Total white cell counts were made in duplicate; all cells observed in the counting chambers were considered to be lymphocytes.

Results. Table I gives results obtained for each animal. Rate of lymph flow from thoracic duct is about 6 times as great as for right lymphatic duct. Average rates of flow, in terms of body weight, are 1.8 and 0.3 ml/hr/kg for thoracic and right lymphatic ducts respectively. Whereas lymphocyte content of thoracic duct lymph is 25% higher than that of right duct lymph, total lymphocyte output of the thoracic duct is more than 7 times that of the right duct. Total lymphocyte output, calculated/unit body weight, is 42.9 and 5.7

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TABLE I. Lymphocyte Content and Volume of Lymph Flow in 10 Male Anesthetized Rats.

Body wt (g)	Lymphocyte count: Thoracic duct (cells/mm³) × 10³	Lymphocyte count: Right lymphatic duct (cells/mm³) × 10³	Vol of lymph: Thoracic duct (ml/hr)	Vol of lymph: Right lymphatic duct (ml/hr)	Lymphocyte output: Thoracic duct (cells/hr) × 10⁶	Lymphocyte output: Right duct (cells/hr) × 10⁶
334	18.7	14.9	.70	.11	13.1	1.6
296	24.8	13.0	.36	.07	8.9	.9
294	34.2	23.7	.28	.07	9.6	1.7
300	16.1	15.7	.55	.14	8.9	2.2
322	28.2	14.7	.45	.06	12.7	.9
302	25.2	16.4	.65	.10	16.4	1.6
304	19.6	15.0	.58	.10	11.1	1.5
298	21.1	19.5	.62	.10	13.1	1.9
300	29.7	22.9	.57	.12	16.9	2.7
280	22.6	16.8	.84	.13	19.0	2.2
303 ± 4.7*	24.0 ± 1.7	17.3 ± 1.1	.56 ± .08	.10 ± .01	13.0 ± .9	1.7 ± .2

* Mean ± stand. error.

million cells/hr/kg, respectively, for the thoracic and right lymphatic ducts. It is of interest to note that lymph collected from the right lymphatic duct was always clear; lymph collected from the thoracic duct was invariably milky. This may indicate that intercommunications between thoracic and right lymphatic ducts which have been observed in other species do not occur to any appreciable extent in the rat. This viewpoint was further substantiated by observation of radiograms made after IP or intra-thoracic injection of colloidal thorium dioxide. In radiograms, such intercommunications were not seen, whereas main lymph channels were clearly visualized.

Discussion. Employing these data, it is possible to calculate the Daily Replacement Factor (DRF) for blood lymphocytes. If the total number of lymphocytes circulating in the animal, and total number of lymphocytes emptied into blood by the lymphatic system each day is known, it is possible to calculate the number of times the blood lymphocytes are replaced by the lymphocytes discharged/day into the blood stream. Such DRF values have generally been estimated utilizing only thoracic duct lymphocyte outputs and making assumptions about the lymphocyte outputs from other lymphatico-venous anastomoses. Assuming a 5% blood volume for rats of this age and weight, and blood lymphocyte counts of 7,000 cells/mm³, a total of 350 million lymphocytes would be present in the circulating blood volume per kg rat. The data obtained in these experiments indicate that 1,150 million and 137 million lymphocytes/day/kg respectively are discharged by the thoracic and right lymphatic ducts. Assuming further that the lymphocyte output of each subclavian duct is at least equal to if not greater than the output of the right lymphatic duct (an assumption which seems justified considering the findings of Hughes *et al.*(4) for the rabbit), a total of 274 million lymphocytes/day/kg would be discharged by both subclavian ducts. Data reported by Reinhardt(2) indicate that the cervical duct outputs would be about 60 million lymphocytes/day/kg. Total output of the thoracic, right, cervical, and subclavian ducts would be 1,620 million lymphocytes/day/kg. Thus, the lymphocytes which are released into the blood stream through these lymphatico-venous channels are sufficient to replace the 350 million cells of the circulating blood 4.6 times daily, indicating that the mean time (of the lymphocyte) in the circulating blood is about 5.2 hours. This value compares favorably with DRF values of 2.05(2) and 5.0-6.0(7) which have been reported for rats. Whaler and Widdicombe(4) have recently estimated a DRF of 24.0 in unanesthetized rats. These values are all undoubtedly minimal because of the assumptions involved in such calculations (5,6,8).

It is of interest to compare these results with data available for other species. It should be pointed out that any such comparison between different species and, in fact, be-

tween individuals of the same species, is hindered by the number of variables which may influence rate of lymph flow and lymphocyte content of lymph collected from any region of the body. The right duct lymph flow in 21 dogs(6) of an average weight of 21 kg was found to be 0.1 ml/hr/kg and in 10 cats (8) of an average weight of 3.4 kg it was 0.15 ml/hr/kg. The white cell content of the right duct lymph of the cat has been reported to be 2,310 cells/mm^3, whereas the thoracic duct lymph in the same animal contained 4,120 cells/mm^3. Right duct lymph flows of 0.46, 0.35, and 0.24 ml/hr in each of 3 rabbits with cell counts of 39,500, 39,800, and 43,600 cells/mm^3 have been reported(3).

Summary. 1. Lymph and lymphocyte outputs from thoracic and right lymphatic ducts of male rats have been measured. 2. Rate of flow and lymphocyte output of the thoracic duct was 1.8 ml/hr/kg and 42.9 million lymphocytes/hr/kg; right lymphatic duct values were 0.3 ml/hr/kg and 5.7 million lymphocytes/hr/kg. 3. Utilizing these data a Daily Replacement Factor for blood lymphocytes of 4.6 was obtained for the anesthetized rat. 4. These results have been compared with similar findings reported for other species.

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Co^{60}Vitamin B_{12} Binding Capacity of Normal Human Saliva.*† (24402)

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Microbiological or radioisotope-dialysis estimates of vit B_{12} binding capacity of normal human saliva have given comparable results (1,2,3). Preliminary to further investigation, this capacity was reevaluated using Co^{60}vit B_{12} and exhaustive dialysis and its relationship to age, sex and multiple factors in saliva was studied.

Methods. Co^{60}vit B_{12} having a specific activity of 0.823 mc/mg was used. Saliva stimulated by chewing gum was collected from 103 healthy adolescents and adults 1-4 hours after breakfast. Specimens were centrifuged to remove cells and debris. To 1 ml aliquots of clear supernatant in Visking bags varying amounts of Co^{60}vit B_{12} ranging from 1 to 100 mμg were added. After 2 hours incubation at room temperature, each bag was subjected to exhaustive dialysis in cold running tap water for 48 hours. Each sample was placed in a 4 ml vial then filled with concentrated sulfuric acid dissolving the bag, and allowing complete mixing within the vial. Standards were prepared in similar vials and included entire range of Co^{60}vit B_{12} added to the bags. Counting was done in a well-type scintillation counter. "Bound" vit B_{12} was measured in terms of residual radioactivity. Refractive index was determined with Bausch and Lomb Dipping Refractometer. Specific gravity was measured with an hydrometer. Optical density at 280 mμ was obtained with Beckman Recording Spectrophotometer D.K. Leukocyte counts were made on undiluted saliva with Neubauer hemocytometer. Studies for ABO blood group types and for secretion in saliva of ABO substances were per-

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