Voluntarily Ingested Sodium Chloride as a Lymphagogue in the Rat.*† (17494)

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In the course of studies on factors influencing the rate of lymph flow, experiments were carried out to determine the effect of fasting, feeding, and alterations in electrolyte intake on the formation of thoracic duct lymph in the unanaesthetized rat. The results of these experiments indicate that the ingestion of 1% NaCl solution by the fed animal causes an increase in rate of lymph flow which is out of proportion to that seen in control animals.

Methods and Materials. Male rats of the Long-Evans strain maintained under comparable environmental conditions were prepared for lymph collection by the method of Bollman, Cain and Grindlay.(1) This technique involved the insertion of a plastic cannula into the thoracic duct immediately caudal to the left crus of the diaphragm, at which point the cannula was tied in place, the delivery end being drawn out through the abdominal wall. In these experiments, a ventral midline abdominal operative approach was employed. The rat was then placed in a cage(2) which permitted *ad libitum* food and fluid intake, but which restrained activity to the point of allowing collection of lymph samples in the unanaesthetized state. The animals were anaesthetized for the preparation of the fistula by means of intraperitoneal administration of a 2% aqueous solution of sodium pentobarbital in the amount of 7 mg/100 g body weight. Following recovery from the anaesthetized state, the animals were placed on free oral intake of the regular laboratory diet (XIV) or were fasted as indicated. Each group of animals, as indicated in the tables, was given access to tap water or 1% NaCl for drinking purposes.

Results. The experimental results are presented in Tables I and II. Table I presents the results of experiments showing the rate of lymph flow in the fasting and fed animals with free access to water, as compared with the rate of lymph flow in the fasted and fed animal given 1% NaCl for fluid intake, and, finally, the effect of alternation for 24-hour periods of water and 1% NaCl in the fed animal. It will be noted that lymph flow is least in the group of animals fasted and given access to water. Animals fed, with access to water, or fasted with oral intake of 1% NaCl produced comparable amounts of lymph as expressed in ml of lymph per hour per 100 g body weight. On the other hand, rats given access to both food and 1% NaCl for drinking purposes markedly increase their lymph flow to an amount 5-6 times the volume of that of the fed animal on water. The difference in rate of lymph flow is seen most markedly in the last group of animals in this table in which water and 1% NaCl were alternated for 24-hour periods. The output of lymph during the period on saline solution is markedly higher than is the output during water ingestion.

The increase in lymph flow produced by oral intake of 1% NaCl increases with duration of the experiment. Table II presents the average daily lymph volumes in a group of rats maintained on water and 1% NaCl in the fed state. It will be noted that the lymph flow in the animals maintained on water is maximal on the second day and falls off gradually, whereas the flow of lymph in the animals maintained on NaCl increases daily to an amount which exceeds 50% of the body weight of the animal. In one in-

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† A portion of the experimental data was presented at the meeting of the American Association of Anatomists at Philadelphia in April, 1949.

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

2. Bollman, J. L., *J. Lab. Clin. Med.*, 1948, v33, 1348.

TABLE I.
Effect of Varying Food and Fluid Intake on Rate of Lymph Flow.

Diet	Fluid intake	No. of rats	Avg BW (g)	Time of collection, (hr)	Lymph flow (ml)	Rate of flow (ml/hr/100 g)
Fasting	Water	4	160* (110-250)	29* (18-47)	11.4* (7.3-18.6)	0.26* (0.12-0.38)
"	1% NaCl	6	250 (145-320)	25.5 (22-30)	29 (15-61)	0.48 (0.16-0.98)
Fed	Water	4	229 (212-240)	105 (93-118)	106 (64-163)	0.44 (0.29-0.62)
"	1% NaCl	3	280 (170-315)	124 (111-140)	727 (657-784)	2.45 (1.98-3.29)
"	1% NaCl	9	239 (183-386)	52 (40-72)	165 (137-302)	1.45 (0.88-2.2)
Fed†	Water	5	153 (130-168)	43 (20-72)	60 (13-123)	0.86 (0.39-1.21)
	1% NaCl			44 (27-72)	216 (57-457)	3.3 (1.5-4.7)

* Average value for the group. Figures in parentheses indicate ranges.
† The animals in this group were maintained alternately for 24 hr periods on 1% NaCl solution and water *ad lib.*

TABLE II.
Average Daily Output of Lymph in Fed Rats Maintained on Orally Ingested Water or 1% NaCl Solution.

Fluid intake	No. of rats	Avg BW (g)	Avg vol. (ml) of lymph per consecutive day				
			1	2	3	4	5
Water	4	229	9	31	27	21	18
1% NaCl	3	280	28	76	125	138	186

stance (not recorded in table) 302 cc of lymph was collected in 23 hours from a rat weighing 145 g.

Discussion. The experiments reported here indicate that the fluid intake of the rat rises remarkably in the presence of a lymph fistula, if the animal ingests added sodium chloride. The control rat with a thoracic duct fistula is apparently able to make up for the loss in nutrients and electrolytes by establishing a dietary and fluid intake which is satisfactory for the maintenance of a temporary equilibrium. If, however, sodium chloride is added to the diet, the requirement for fluid intake rises considerably. The abrupt increase in intake of saline drinking solution suggests that the animal is vigorously attempting to repair loss of fluids and electrolytes via the lymph fistula.

It is well known that the intravenous injection of large amounts of crystalloids will produce an increased lymph flow from the thoracic duct. The explanation for this effect(3) is that an hydremic plethora results, with consequent lowering of plasma colloidal osmotic pressure and increased capillary hydrostatic pressure. There is, then, a resultant loss of fluid to the tissue spaces, with consequent increase in lymph flow. This does not, however, explain the reason for the greatly increased *voluntary intake* of 1% NaCl solution and resultant lymph output as seen in the experiments cited here.

Summary. 1. A comparison is made of the

3. Evans, C. L., *Principles of Human Physiology,* 9th ed., p. 661, 1945, J. & A. Churchill, London.

rates of thoracic duct lymph flow in unanaesthetized male rats maintained under the experimental conditions of fasting and feeding. with free access to either water or 1% NaCl solution.

2. Thoracic duct lymph flow is at a low level in the fasted animal with free access to water. The rate of flow is increased by feeding or access to 1% NaCl solution. The rate of flow in the *fed* animal with free access to 1% NaCl is increased appreciably over that of the controls, and out of proportion to any hitherto employed method for *voluntarily* increasing lymph flow in the otherwise normal animal. In some instances, the lymph flow of the NaCl treated animal exceeded the body weight of the animal in a 24-hour period.

3. Alternation of periods of access to water and saline solution produced corresponding low and high rates of lymph flow in the fed animals.

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Specificity of Paper Partition Chromatography for Analysis of Free Amino Acids in Unhydrolyzed Urine.* (17495)

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The paper partition chromatographic technique devised by Consden, Gordon, and Martin(1) may be more specific for detecting free amino acids in urine than microbiological methods. The number of amino acids found by microbiological assay in unhydrolyzed urine excreted by normal human subjects is greater than the number detected by paper partition chromatography.

Paper partition chromatography has been used to investigate the amino acids excreted in the urine by normal subjects and selected cancer patients. Aliquots of a 24-hour urine collection or of pooled samples obtained over a 5 or 6 day period were standardized by Kjeldahl analysis to contain either 10 mg of total nitrogen or by the Van Slyke ninhydrin method(13,14) to contain 0.5 mg of α-amino nitrogen per milliliter. All samples were stored at 3°C under toluene. Aliquots requiring concentration were lyophilized and re-diluted to the desired volume. Other aliquots were hydrolyzed by boiling with 50% hydrochloric acid for 24 hours. After removal of the acid under reduced pressure, the hydrolysate was also standardized to contain 10 mg of total nitrogen per milliliter.

Standardization of the urine permits comparison of urine specimens from period to period and from patient to patient. The concentration selected yields chromatograms of satisfactory color intensity and insures detection of most of the amino acids present in the specimen. Concentration of normal urines to contain more than 40 mg of total nitrogen per milliliter prevents adequate separation since salts and other substances interfere. Removal of salt by common ion effect precipitation, impregnation of the filter paper and saturation of the solvents with salt has been unsatisfactory.

A two-dimensional chromatogram on Whatman No. 1 filter paper was prepared from each of the samples(1). One hundred microliters of the standardized solution were applied to the paper, and 25 microliters of 30% hydrogen peroxide were superimposed on the spot to effect quantitative oxidation of cystine and methionine to cysteic acid and

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1. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 1944, v38, 224.

13. Van Slyke, D. D., MacFayden, D. A., and Hamilton, P., *J. Biol. Chem.*, 1941, v141, 671.

14. Van Slyke, D. D., Dillon, R. T., MacFayden, D. A., and Hamilton, P., *J. Biol. Chem.*, 1941, v141, 627.