Some Aspects of Lymph Production in the Rat[1] (34134)

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Several reports have shown the apparent superiority of NaCl solution over water as a lymphagogue; most of these studies have been carried out in *ad libitum* drinking animals (1). We have studied the effect of triglycerides, starch, casein, hormones, and Na^+/K^+ ratio on lymph flow. These studies were made possible by the development of a modified Bollman's technique for the cannulation of the abdominal thoracic duct in the rat (2), which includes the infusion of solutions into the duodenum at a constant rate to assure a uniform lymph flow rate. The substances listed above and found to be lymphagogic, with the exception of triglycerides, were only effective when the animals were infused with 0.85% NaCl solution rather than with water.

Materials and Methods. Female rats (300 ± 15 g) were purchased from Charles River Breeding Labs. (North Wilmington, Mass.) and were fed Purina chow. The surgical technique for the cannulation of the abdominal thoracic duct has been described in detail (2). In the present studies, cannulation of the thoracic duct was followed by cannulation of the avascular part of the stomach. After operation, the animal was put in a restraining cage; the catheter going into the stomach was connected to a compact infusion pump, model 975 (Harvard Apparatus Co., Inc., Dover, Mass.). A container covered with a wire gauze was placed underneath the animal to collect the excreta and to separate the feces from the urine. The latter was then measured during variable intervals corresponding to the periods of lymph collection.

Throughout these studies a constant infusion rate of 4 ml/h was used. The variables investigated were: concentration of NaCl, the effect of various ratios of 0.85% NaCl and 0.85% KCl, of 2% protein, of 1–20% carbohydrate, and of 2% triglycerides in either saline or water. In addition, subcutaneous injections of vasopressin (β-hypophamine), oxytocin (α-hypophamine) or deoxycorticosterone (Doca) were studied during the infusion of either 0.85% saline or water in these stomach fistula–thoracic duct fistula animals.

Results. As shown in Fig. 1, animal A was given saline at the beginning of the experiment and lymph flow of about 3 ml/hr was maintained for 23 hr. At the end of this period, when an emulsion containing 2% protein and 2% starch in isotonic saline was infused, a marked increase in lymph flow was observed for a period of 10 hr. In fact, lymph flow was greater than the rate of saline infusion (4 ml/hr). Upon return to infusion with isotonic saline, lymph flow decreased to about 3.6 ml/hr. Lymph production rose again to more than 4 ml/hr with the infusion of an emulsion containing 2% triglycerides and 2% starch in saline. Infusion with isotonic saline for the third time caused the lymph flow to return to the initial rate.

Much lower lymph flow was obtained when distilled water was infused in animal B (Fig. 1). Lymph flow with water infusion was only 1.5 ml/hr but rose to 3 ml/hr when 2% starch was infused in a water emulsion. This experiment was repeated in the same animal with similar results noted, Fig. 1.

To investigate the possible specific effect of

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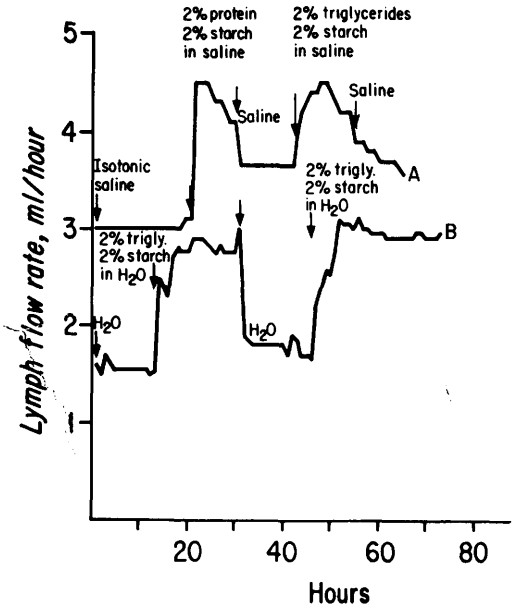

FIG. 1. The influence of protein (nonfat dry milk solids), starch (amylopectin special starch, Col-Flo 67, powdered; National Starch Inc., New Orleans) triglycerides (Wesson oil), saline, and water on lymph flow.

carbohydrates on lymph production, the studies depicted in Fig. 2 were performed. Animal A showed a lymph flow of about 3.3 ml/hr during the initial saline infusion which increased to more than 4 ml/hr with the infusion of 5% solutions of starch, or sucrose, or glucose in saline. Lymph production in the same animal was depressed by infusion of 5% glucose in water. In another animal (B in Fig. 2) a steadily decreasing lymph flow was

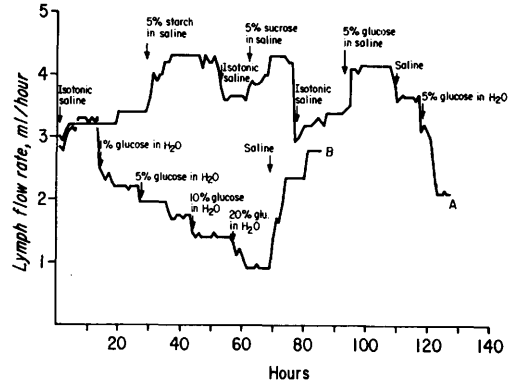

FIG. 2. The effect of carbohydrates in saline and in water on lymph flow.

observed upon infusion of glucose in concentrations increasing from 1 to 20% dissolved in water rather than saline. With the return to infusion with saline the lymph production increased again almost to the initial rate.

The infusion of solutions of NaCl, concentrations ranging from 0.2 to 2% at the same rate, showed that the maximum effect was attained with 0.85–1% saline. Similar findings have been reported by Kim and Bollman (3).

Owing to the requirements for the presence of saline in the infusate to demonstrate a lymphagogic effect of carbohydrate, it seemed necessary to explore the possibility that potassium might substitute for sodium. Animal A (Fig. 3) received solutions of sodium and

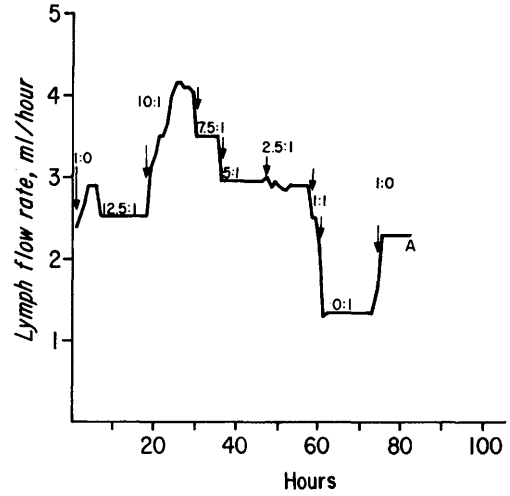

FIG. 3. The effect of Na^+/K^+ ratio on lymph flow. The curve represents the lymph flow response to 0.85% NaCl: KCl solutions whose Na^+/K^+ ratio is indicated.

potassium chloride (both 0.85%) differing in the concentration ratios NaCl/KCl. As shown, relatively small concentrations of KCl potentiate the effect of NaCl alone; thus, lymph flow of about 4.5 ml/hr was obtained when the sodium–potassium ratio in the infusate was 10:1; lymph flow decreased steadily as this ratio decreased. A minimal lymph flow rate was obtained when 0.85% KCl was infused alone. Lymph production returned to normal values, when NaCl (0.85%) was substituted for KCl.

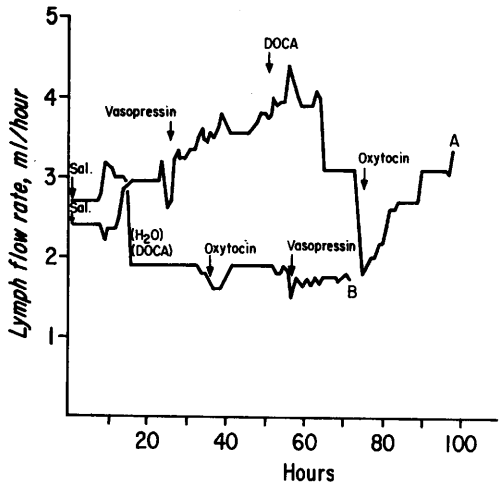

FIG. 4. The influence of saline on the lymph flow in response to vasopressin, oxytocin, or deoxycorticosterone, Doca.

Figure 4 shows the results of two experiments in which the effect of several hormones on lymph flow was tested. Animal A received saline throughout the experiment. After a 24-hr control period, during which lymph production ranged from 2.2 to 3.2 ml/hr, vasopressin[3] was administered subcutaneously. During the 24 hr following vasopression administration lymph flow ranged from 3.2 to 3.9 ml/hr. At this point deoxycorticosterone (Doca)[4] was injected subcutaneously and the lymph flow rate varied between 3.6 and 4.4 ml/hr for 14 hr, but dropping again to control values (3.1 ml/hr) during the next 10-hr period. Oxytocin[5] was then administered and lymph flow rose steadily from 1.8 to 3.1 ml/hr during the next 24 hr. In contrast, animal B, when given the same hormones during infusion with water rather than with saline, demonstrated no effect on lymph flow.

[3] Pitressin: 0.05 ml (250 mU) of a suspension of vasopressin tannate in peanut oil (Parke-Davis and Co., Detroit, Mich.). Each ml is equivalent to 5 pressor units.

[4] Doca: 0.02 ml (100 μg) of a solution of deoxycorticosterone acetate in cottonseed oil (The Upjohn Co., Kalamazoo, Mich.). Each ml contains 5 mg of Doca-acetate.

[5] Pitocin: 0.025 ml (250 mU) of a solution of oxytocin in sesame oil (Parke-Davis and Co.). Each ml is equivalent to 10 units.

The lymphagogic effect of Doca was investigated in experiments in which one rat (A in Fig. 5) was infused with saline and then injected with Doca and the saline infusion was continued. The infusion was then changed to water and the animal injected with Doca and the water infusion continued. A crossover type of experiment was performed on the other animal (B in Fig. 5). First it was infused with water, followed by treatment with Doca, and then infused with saline, followed by treatment with Doca. Both animals responded to Doca only when simultaneously infused with saline. All experiments reported here were repeated 2–3 times and similar results were obtained.

Urine formation by the kidney is apparently directly or indirectly influenced by factors that effect lymph flow in the fistulated animal. Under our experimental conditions in which lymph (comprised primarily of isotonic saline) was drained out of the animal's body owing to the fistula, the urine output remained constant as long as the infusion rate of saline was approximately equal to the lymph production rate. However, if the lymph production was less than the infusion rate of saline, saline was diuretic. Conversely, if the saline infusion rate was less than lymph production rate, the urine production

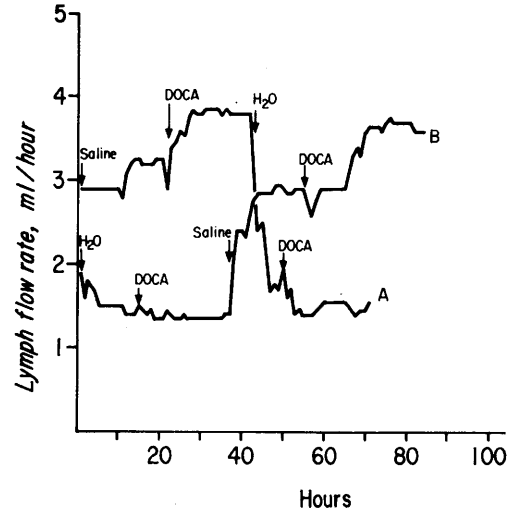

FIG. 5. The influence of saline on the lymph flow in response to deoxycorticosterone (Doca) administration.

was reduced. Glucose solutions in water (5%) were the most diuretic of all the solutions in terms of the volume of urine formed in relation to the volume of fluid infused. The least diuretic effects were obtained with the infusion of saline together with foodstuffs or with concomitant injection with hormones.

Discussion. In the present study, fluids of different composition were administered by a constant-rate infusion pump, thus combining infusion with analysis of lymph production and determining the influence of composition of infused fluids on lymph flow. Triglycerides, starch, glucose, protein, vasopressin, oxytocin, and Doca increased lymph flow when either mixed with saline and infused or given during saline infusion. Only triglyceride was lymphagogic in the absence of saline infusion.

It is evident that, in animals with a thoracic duct fistula, isotonic saline solution increased lymph flow but not urine production. Saline infusion in animals without a fistula produced diuresis, provided that kidney function was normal. In all cases where lymph flow was high, sometimes even higher than perfusion rate, a marked decrease in urine output was seen. Also, in instances where lymph flow was low and the composition of the infusate did not increase lymph flow, especially when water or glucose dissolved in water were infused, urine output increased markedly. This finding suggests the desirability of additional studies into the possible interrelationship between kidney function and lymph production.

There seems to be general agreement that the absorption of water from the intestine is greatly dependent, in direction and degree, on solute movement; energy is used during water absorption to satisfy the requirements of the sodium and chloride transport systems. Thus, the data obtained *in vivo* directly supports Crane's theory (4) on Na^+-dependent transport of water by the small intestine of the rat; lymph flow is closely related to specific rates of Na^+ infusion.

It is also evident that small amounts of K^+ potentiate the lymphogogic effect of Na^+, whereas large amounts of K^+ inhibit it. The existence of an optimal Na^+/K^+ ratio for stimulation of lymph flow is not surprising, owing to the fact that several systems exhibit an optimal Na^+/K^+ ratio for certain functions. For example, an optimal Na^+/K^+ ratio controls the secretion of aldosterone (5). The electrolyte pattern of thoracic lymph approximates extracellular fluid (6). Isotonic saline is the fluid of choice for expansion of the extracellular spaces; therefore, it seems reasonable that potassium does not substitute for sodium in stimulating lymph production.

In addition to the water and ions administered through infusion, large amounts of water and electrolytes are added to the intestine with the salivary, gastric, pancreatic, intestinal secretions, and bile in these thoracic duct–stomach fistula rats. Therefore, more thorough investigation of the mechanisms involved should include simultaneous study of the change of arterial and venous blood pressure, hydration status, electrolytes in serum and lymph, protein and glucose concentrations in lymph, as well as gastric emptying rate, intestinal motility, etc.

In our study the administration of emulsions of triglycerides in water produced an increase in lymph flow even though it was administered immediately following the administration of water alone. These findings agree with those of Shepherd and Simmonds (7) who investigated the absorption of coconut oil emulsions and observed that the maximum rate of transport into the lymph, and the flow of the latter, could be increased by the administration of water intraduodenally. Although mostly speculative, we should like to postulate that the mechanism by which triglycerides stimulate lymph flow may be as follows: the presence of triglycerides activates the duodenum to produce secretin, which in turn stimulates the flow of pancreatic juice, thus providing an increased concentration of sodium (and other ions) in the intestinal lumen. The electrolytes are absorbed, taking water with them, and thereby increasing the formation of lymph. Possibly, further clarification of this point could be obtained by the diversion of both biliary and pancreatic secretions from the duodenum by

fistula and then determining if triglycerides still stimulate lymph flow without saline. It is reasonable to postulate that this lymphagogic effect is mediated through the sodium ion.

Oxytocin (aside from its action on the uterus and the mammary gland) increases intestinal motility, but this would not explain its effect on lymph production because Simmonds (8) has demonstrated that, in dogs, lymph flow is not influenced by intestinal motility. Both, vasopressin and oxytocin influence the volume of the intestinal vascular bed and participate actively in the homeostatic adjustments of the cardiovascular system. These adjustments may include the absorption of water and electrolytes. Finally, Doca increases lymph flow only in the presence of NaCl solution and not in the presence of water, which is consistent with the hypothesis that its mechanism of action is mediated through electrolytes.

Summary. These *in vivo* studies were made possible by a modification of Bollman's technique for the cannulation of the abdominal thoracic duct of the rat. Thus, by combining a thoracic duct fistula with a stomach fistula, the influence of several substances on lymph flow was studied. Infusion with an isotonic solution of NaCl has a marked influence on lymph flow but it is not diuretic. Protein, carbohydrate, triglyceride, vasopressin, oxytocin, and Doca increased lymph flow only when accompanied by infusion with saline. Triglyceride emulsions had a pronounced lymphagogic effect even without infusion with saline. It is proposed that a unique mechanism is evoked by all substances tested that influence lymph flow; this mechanism involves electrolytes, and more specifically, the sodium ion. The findings reported here are in support of Crane's theory of Na^+-dependent transport of water by the small intestine.

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