PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **147**, 158–161 (1974)

Lymphatic Flow Alterations Secondary to Changes in Total Serum Calcium Levels[1] (38303)

MAXIMO DEYSINE, MILAN MADER, ELISEO ROSARIO, AND CHARLOTTE MANDELL
(Sponsored by J. V. Klavins)

Department of Surgery, Long Island Jewish-Hillside Medical Center, New Hyde Park, New York 11040, and Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York

In spite of the numerous investigations directed to study lymph propulsion, wide discrepancy exists about the mechanisms of action of drugs altering thoracic duct lymph flow (1–3). During our previous studies on the transport of calcium by the lymph we observed that between the onset of a hypocalcemic challenge produced by ethylenediaminotetracetate infusion and the return of serum calcium to normal levels, thoracic duct lymph flow (TDLF) was markedly increased (4). Further experiments determined that the rise in serum calcium that followed hypocalcemia was responsible for the increase in TDLF. The purpose of this investigation was that of analyzing and quantifying such a response.

Methods and Materials. Experiments were performed in adult mongrel dogs of both sexes divided in groups of five animals each. They were fed a chow containing all essential nutrients in their adequate proportions and tap water *ad lib*. One group of these animals was parathyroidectomized three days prior to the experiment. The success of the ablation was assessed by the fall in total serum calcium. Whole milk was administered *ad lib* postoperatively to the parathyroidectomized animals to prevent tetany. The amount of milk consumed varied from animal-to-animal but it was helpful in slowing down the general deterioration observed in parathyroidectomized dogs.

On the morning of the experiments, dogs were anesthetized with intravenous pentobarbital in successive doses to the point of apnea. This level of anesthesia was maintained throughout the experiment by additional pentobarbital doses in order to avoid changes in respiratory rate, depth and rhythm, which can alter TDLF. Animals were intubated with a cuffed endotracheal tube connected to a positive pressure ventilator (Bird). Respiratory rate was regulated to produce a panting-type of respiration (45/min at 15 cm of water of maximum inspiratory pressure). The right femoral artery and vein were dissected at the groin and catheterized with a polyethylene catheter advanced to the abdominal aorta and the inferior vena cava levels, respectively. Pressures were recorded using transducers and a multichannel apparatus.

The thoracic duct was exposed over the left side of the neck, ligated at its entrance into the jugular vein and catheterized with a polyethylene catheter. Lymph was allowed to flow by gravity into a fraction collector. Lymph flow was expressed as ml of lymph/kg body wt/min. After a period of stabilization during which blood pressure and lymph flow were found to be constant, a bolus dose of a 10% solution of calcium gluconate in water was injected and calculated to provide 0.5 mg of ionic calcium/kg body wt. The same amount of calcium was reinjected when lymphatic flow and aortic blood pressure (ABP) returned to a new steady baseline. In two additional groups of intact and parathyroidectomized animals, a solution of 0.9% sodium chloride in water was injected in volumes equal to those calculated for calcium gluconate, according to their weight. In these animals sham operations were performed during which the parathyroid glands were exposed but not removed. Serum and lymph calcium were measured using atomic absorption spectrophotometry.

Results. The injection of 0.9% sodium chloride in water in intact or parathyroidectomized dogs did not alter TDLF or ABP.

[1] This work was supported by a grant from The John A. Hartford Foundation, Inc.

Figure 1 depicts our results in intact dogs. In this group of animals serum calcium rose from 9.5 ± 0.2 mg/100 to 12.3 ± 0.1 mg/100 (± SD). This was accompanied by a rise in lymph calcium from 6.2 ± 0.4 mg/100 to 9.3 ± 0.2 mg/100 (± SD). TDLF augmented from a baseline level of 0.018 ml/kg body wt/min to a peak of 0.053 ml/kg body wt/min. At this time serum calcium was 12.3 mg/100. Thereafter as serum calcium reached further hypercalcemic levels TDLF showed a tendency to decrease. Mean control ABP in this group of animals was 122 mm of mercury. This pressure increased an average of 4.3 mm Hg immediately after each injection of calcium. Inferior vena cava pressures did not change in any of the groups throughout the experiments.

In the parathyroidectomized group the injection of calcium raised the total serum calcium from a mean control level of 7.1 ± 0.5 mg/100 to a level of 10.7 ± 0.5 mg/100. This was followed by a rise in TDLF from 0.019 to 0.065 ml of lymph/kg body wt/min. Lymph calcium was elevated from 5.7 ± 1 to 9.5 ± 0.5 mg/100 (±SD). Mean control ABP in these animals was 120 mm of mercury which was raised an average of 12 mm Hg after each calcium injection.

Discussion. These experiments demonstrate that increases in serum calcium levels are accompanied by an augmentation of TDLF and ABP in intact and parathyroidectomized dogs.

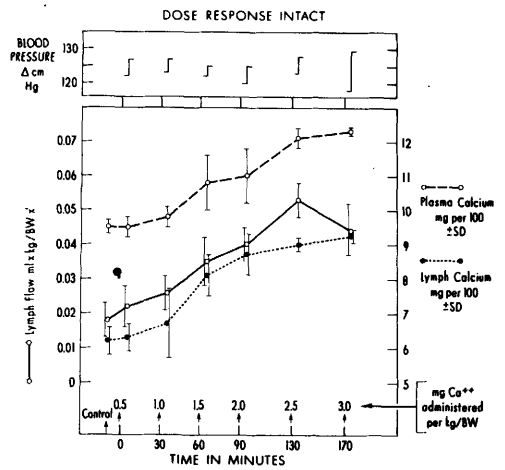

FIG. 1. Changes in aortic blood pressure, thoracic duct lymph flow, serum and lymph calcium in a group of five intact dogs receiving intravenous calcium in increments of 0.5 mg of Ca^{2+}/kg body wt. Points represent the mean of five experiments ± standard deviation.

Although lymph production is a relatively well understood phenomenon, the passage of this fluid into the end capillary lymphatic is still a subject of debate. Accordingly, valve trapping of lymph, low interstitial fluid pressure and direct passage through the capillary walls have all been utilized as possible explanations (6). Once into the end capillary lymphatic, lymph is probably propelled by a combination of extrinsic (skeletal muscle contractions and changes in pressure of body cavities) or intrinsic forces (activity of the smooth muscle present in the lymphatic vessel wall). The relative value of each of these factors is still a matter of controversy. However, clarification of these points is an overdue necessity because lymph return to the venous circulation has been considered an important factor in the survival from septic and hemorrhagic shock (7). Furthermore, the fact that a dose related response can be elicited by a calcium injection provides a useful tool to study the pharmacodynamics of agents altering lymphatic flow.

In our experimental design, several factors could have been responsible for the rise in TDLF. The most obvious one, an increase in ABP, could perhaps be the most significant and could have increased lymph flow by raising the head of arterial pressure entering the capillary beds; thus, augmenting the filtration rate in that area.

Calcium has been demonstrated to be an important mediator in the response of the adrenal medulla to acetycholine (8) and to produce a vigorous release of catecholamines from the adrenals. Fujii (9) demonstrated that lymph flow increases, parallel elevations in blood pressure produced by vasopressors. He also observed after the injection of a vasopressor, lymph flow remained higher and longer than the arterial blood pressure. Corroborating his work, Baisset (1) showed that sympatheticomimetic drugs increase TDLF. It is therefore possible that in our experiments calcium may have produced a primary increase in ABP which would have increased TDLF by augmenting the filtration rate across the capillary bed.

It has been postulated that TDLF may be propelled by three types of skeletal muscle pumps; peripheral, abdominal or thoracic (10). Our observed rise in TDLF could have also been the result of changes in skeletal muscle activity because calcium has been demonstrated to alter

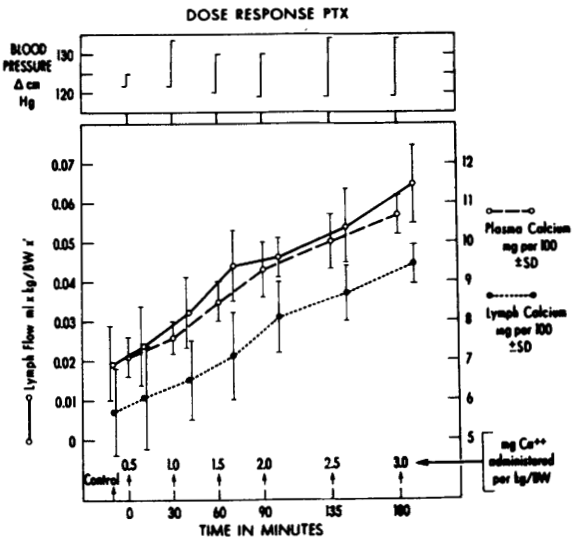

FIG. 2. Changes in aortic blood pressure, thoracic duct lymph flow, serum and lymph calcium in a group of five parathyroidectomized dogs receiving intravenous calcium in increments of 0.5 mg of Ca^{2+}/kg body wt. Points represent the mean of five experiments ± standard deviation.

the contractibility of the skeletal muscle system (11). Such contractions are known to alter lymph flow by externally compressing a vessel in which the flow of lymph is governed by a unidirectional valve system. In spite of the heavy doses of nembutal utilized, some of our parathyroidectomized dogs exhibited fibrillatory muscle activity in their tongues demonstrating the ability of the skeletal muscle system to contract. In an unpublished group of experiments, tubocurarine chloride and succinylcholine chloride were unable to prevent the action of calcium on both TDLF and ABP.

Finally, calcium may have acted directly on the smooth muscle present in the lymphatic and arterial wall, causing them to contract, and thus producing parallel increases in ABP and TDLF. Some investigators have insisted that the contraction of the lymphatic channel is the most important factor in lymph propulsion (2). Corroborating these theories Hall demonstrated that lymph flow occurs against gravity in animals that, like the bat, rest upside down (5). Contractions of the regional lymphatics of the human abdomen have been observed and described by Kinmouth (12). These contractions have not been documented yet. Attempts to physically record them in isolated perfused lymphatic channels either by kimography or by time-lapse photography have met with failure in our hands.

We did not attempt in these experiments to establish a correlation between the onset of the ABP elevation and the rise in TDLF. We measured ABP at its immediate source, the aortic lumen but it is our impression that TDLF is a final common pathway from multiple lymph sources and we do not know if the lymph flow recorded at thoracic duct levels reflect the exactly true onset of the phenomenon as it occurs in the end capillary lymphatic. Thus, the possibility of a time delay factor has hampered our ability to correlate both ABP and TDLF. In other series of unpublished experiments we have attempted but failed to block the rise in ABP utilizing alpha and beta sympathetic blockers alone or in combination with reserpine and adrenalectomy. In this group, calcium still produced a rise in TDLF and ABP.

TDLF changes were similar in both intact and parathyroidectomized dogs. It is therefore possible that the action of calcium is not mediated by a response of the parathyroid glands. In the parathyroidectomized group, calcium produced a greater response in ABP, a phenomenon we cannot explain.

Conclusions. These experiments demonstrate that rises in serum calcium produce in intact and parathyroidectomized dogs an augmentation of lymph flow from an average of 0.018 ml/kg body wt/min to 0.06 ml/kg body wt/min. A rise in ABP, more marked in the parathyroidectomized groups, accompanied the TDLF augmentation.

The associated rise in ABP may be sufficient to produce this effect by increasing the precapillary pressure. Other possible reasons for this effect could be an increase in skeletal muscle activity, or the contraction of the smooth muscle present in the lymphatic vessel wall.

1. Baisset, P. A., Cotonat, J., and Montastruc, P., Therapie **XXV,** 517 (1970).

2. Hall, J. G., Morris, B., and Woolley, G., J. Physiol. **180,** 336 (1965).

3. Lewis, G. P., and Winsey, N. J. P., Br. J. Pharmacol. **50,** 446 (1970).

4. Deysine, M., Koch, M., and Wilder, J. R., Surg. Gynecol. Obstet. **130,** 509 (1970).

5. Hall, J. G., Nature **220,** 910 (1968).

6. Hall, J. G., New Eng. J. Med. **281,** 720 (1969).

7. Cope, O., and Litwin, S. B., Ann. Surg. **156,** 655 (1962).

8. Douglas, W. W., and Rubin, R. P., J. Physiol. **159,** 40 (1961).

9. Fujii, J., and Wernze, H., Nature **216,** 956 (1966).

10. Calnan, J. S., Pflug, J. J., Reis, N. D., and Taylor, L. M., Brit. J. Plastic Surg. **23,** 305 (1970).

11. Shanes, A. M., J. Cell. Comp. Physiol. **57,** 193 (1961).

Received February 27, 1974. P.S.E.B.M., 1974, Vol. 147.