

Thoracic Duct Lymph Flow Changes Secondary to Alterations in Serum Calcium Levels: A Proposed Mechanism of Action

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Summary

The mechanism behind the dose-related increase in thoracic duct lymph flow (TDLF) produced by an injection of calcium gluconate was investigated in parathyroidectomized dogs divided into groups composed of three animals each. After skeletal muscle paralysis was induced by succinylcholine chloride, calcium produced a highly significant rise in TDLF ($P < 0.001$). This rules out skeletal muscle contractility as a factor in the response to calcium. Blocking the parasympathetic system with atropine also failed to inhibit the TDLF response to calcium.

Levaterenol augmented both TDLF and aortic blood pressure (ABP) while the fall in ABP produced by Isoproterenol was accompanied by a rise in TDLF. Sympathetic inhibition attempted by a combination of adrenalectomy, reserpine, phenoxybenzamine hydrochloride and propranolol significantly diminished TDLF after calcium ($P < 0.001$). An infusion of angiotensin completely abolished TDLF, probably by precapillary vasoconstriction. While angiotensin was still being infused, calcium significantly increased TDLF ($P < 0.001$). Papaverine was the only drug able to completely block the action of calcium on TDLF. These experiments suggest that lymph propulsion changes after calcium may be due to the action of this ion on the lymphatic channel wall.

We have reported that the intravenous injection of calcium gluconate is followed by a dose-related, highly significant increase in Thoracic Duct Lymph Flow (TDLF) (3). During those experiments, Aortic Blood Pressure (ABP) increased synchronously with the increase in TDLF. This reproducible effect of calcium on both TDLF and ABP provided us with means to analyze several factors involved in the physiology of lymph flow.

Lymph flow can be varied by changes in the capillary filtration rate, skeletal muscle activity,

and the contractility of smooth muscle intrinsic or extrinsic to the lymphatic channel wall. This series of experiments was performed in order to clarify the role that some of these mechanisms have in lymph propulsion, utilizing as a model the above mentioned response to a rise in serum Ca. One of our problems was to establish the significance of the associated rise in ABP, because it could, by itself, alter the capillary circulation, an event of significant importance in lymph production.

During these experiments, we observed that the action of calcium on TDLF and ABP took place in spite of a significant degree of skeletal muscle paralysis and sympathetic as well as parasympathetic inhibition. It also occurred in the presence of arterial hypertension produced by an angiotensin infusion. The rise in TDLF and ABP after calcium was maximally inhibited by papaverine, suggesting that the action of Ca is secondary to the contraction of the smooth muscle present in the lymphatic vessel wall.

Methods

Adult mongrel dogs of both sexes were utilized throughout the experiments. They were fed a regular chow and were allowed to drink tap water ad lib. Animals were parathyroidectomized through a transverse neck incision in order to allow us to raise the serum calcium without reaching hypercalcemic levels. The degree of parathyroid ablation was assessed by the subsequent fall in serum calcium. Postoperatively, milk was added ad lib to their diet to minimize tetany and muscle weakness which often leads to pneumonia.

Three days after the parathyroidectomy and after fasting for 12 hours, dogs were anesthetized with increasing doses of intravenous pentobarbital until spontaneous respiration was suppressed. They were then intubated with a cuffed endotracheal tube which was connected to an automatic pressure triggered respirator (Byrd). This apparatus was set at a respiratory rate of 45 respirations per minute in order to mimic the panting type of rhythm common to the canine species. A maximum inspiratory pressure of ten centimeters of water was delivered per cycle. Additional doses of pentobarbital were utilized thereafter to maintain this status for the entire duration of the experiment.

Through a right groin incision, the right femoral artery and vein were cannulated with polyethylene catheters which were advanced to the inferior vena cava and abdominal aorta, respectively. Pressures were amplified with transducers and recorded. The thoracic duct was exposed on the left side of the neck at its junction with the subclavian vein, and ligated. A polyethylene catheter of approximately the same diameter as the thoracic duct was inserted and the lymph was allowed to drain by gravity into tubes placed in a fraction collector. The distance between the thoracic duct and the end of the tube was maintained constant in every experiment to standardize the negative pressure created by the column of lymph. The tubes in the fraction collector were advanced every five minutes. One tenth of a milliliter of a solution containing 200 units of sodium heparin in 50 ml of normal saline was added to each tube to prevent clotting. Lymph was reinjected intravenously after its volume had been measured and an aliquot was removed for calcium determination. Lymph flow was expressed as ml of lymph per kg of body weight per minute. Lymph and venous blood serum calcium were measured by atomic absorption spectrophotometry at regular intervals (10).

After a control period of 15 minutes during which lymphatic flow (LF) and aortic blood pressure (ABP) were found to be stable, the drugs were injected in the highest concentra-

tions possible to minimize blood volume changes. This produced changes in TDLF which lasted a few minutes; TDLF then returned to a new stable baseline. When this level of TDLF was reached, calcium was added. Changes in ABP and TDLF were recorded at intervals for an additional 60 minutes. Changes in TDLF produced by calcium were referred to the control TDLF baseline and not to the TDLF change produced by the drugs. If the TDLF baseline was not found to be steady, after the onset of the experiment, or after the injection of the tested drugs, the experiment was discontinued.

The drugs utilized were injected in the following concentrations: Ca, in a bolus dose of 10% Ca gluconate, delivering 125 mm of Ca kg/B/W; Atropine sulfate, 0.2 mg/kg/B/W in a single intravenous dose; L tubocurarine chloride, 0.53 mg/kg/B/W divided into three intravenous doses at 10-minute intervals to minimize hypotension; Succinylcholine chloride, 0.2 mg/kg/B/W per minute dissolved in 10 ml of normal saline, delivered intravenously. Another group of 3 parathyroidectomized dogs, after a steady control TDLF baseline was obtained, received two separate doses of 4.5 micrograms of Levoarterenol i.v. Later, when the TDLF returned to control values, 20 micrograms of Isoproterenol were added to the infusion. The following doses were administered to the animals receiving the drug mixture: Phenoxybenzamine chloride, 4 mg/kg/B/W in a single intravenous dose; Propranolol, 1 mg/kg/B/W in a single intravenous dose; Reserpine, three doses of 2.5 mg/kg/B/W delivered intramuscularly 24 hours apart. This last dose was given one hour before the onset of the experiment. In this group, animals were adrenalectomized via a laparotomy two hours prior to the injection of calcium. After adrenalectomy, they received i.v. phenoxybenzamine chloride, i.v. propranolol, and the last dose of i.m. reserpine (the doses referred to above).

Angiotensin was delivered dissolved in 10 ml of normal saline at a concentration of 20 mg/minute to maintain a mean ABP above 155 mm of mercury.

Papaverine was injected in a single intravenous dose of 25 mg/kg/B/W 15 minutes prior to the Ca injection.

Every drug or group of drugs was tested on a group of 3 dogs who were exposed to one protocol only.

In a group of 5 control parathyroidectomized dogs, normal saline solution was injected intravenously in volumes equal to those calculated for calcium gluconate.

Animals did not receive any other fluids except the pharmacological agent tested.

The results are expressed as the mean of 3 values \pm standard deviation (S.D.) and analyzed using a paired t test for difference in means.

Results

The amount of milk consumed after parathyroidectomy varied from animal to animal and did not substantially alter the control serum calcium levels which fell, as expected, after parathyroidectomy. Milk apparently prevented the expected deterioration.

Lee White clotting times and partial thromboplastin times were found to be normal in the serum of these animals during and after the experiments. The intravenous injection of normal saline in volumes equal to those calculated for calcium gluconate did not alter the TDLF or ASP.

The summary of our results is depicted in Table 1. Parathyroidectomy lowered the serum Ca from a mean control of 9.8 ± 0.4 mg% to a mean of $6.6 \text{ mg}\% \pm 0.9 \text{ mg}\%$ and lymph Ca from 7.8 ± 1.2 to $5.1 \pm 0.9 \text{ mg}\%$. The control lymph flow after parathyroidectomy was $0.025 \text{ ml} \pm 0.003 \text{ ml/kg/B/W}$ per minute. In the control group receiving only Ca gluconate, the injection of Ca raised this flow to $0.065 \pm 0.003 \text{ ml/kg/B/W}$ per minute ($P < 0.001$).

Curare, if injected in one dose, produced in several animals severe systolic and diastolic hypotension which was then followed by cardiac arrest. It was injected thereafter in divided doses which minimized the hypotension. Curare lowered the TDLF from 0.025 ± 0.003

to $0.016 \pm 0.002 \text{ ml/kg/B/W}$ per minute. When Ca was injected after Curare, TDLF increased from 0.025 ± 0.003 to $0.050 \pm 0.002 \text{ ml}$ per minute ($P < 0.001$), while the ABP remained stable (Figure 1). Succinylcholine chloride reduced both TDLF and ABP. In animals totally paralyzed by this drug, Ca was able to increase TDLF from 0.030 ± 0.001 to $0.130 \pm 0.001 \text{ ml/kg/B/W}$ per minute ($P < 0.001$), while ABP rose from 125 ± 7 to $135 \pm 4 \text{ mg}$ ($P < 0.005$).

Atropine increased TDLF and ABP from the control values. In this group, Ca further altered both parameters from a control of 0.016 ± 0.001 to $0.125 \pm 0.002 \text{ ml/kg/B/W}$ per minute

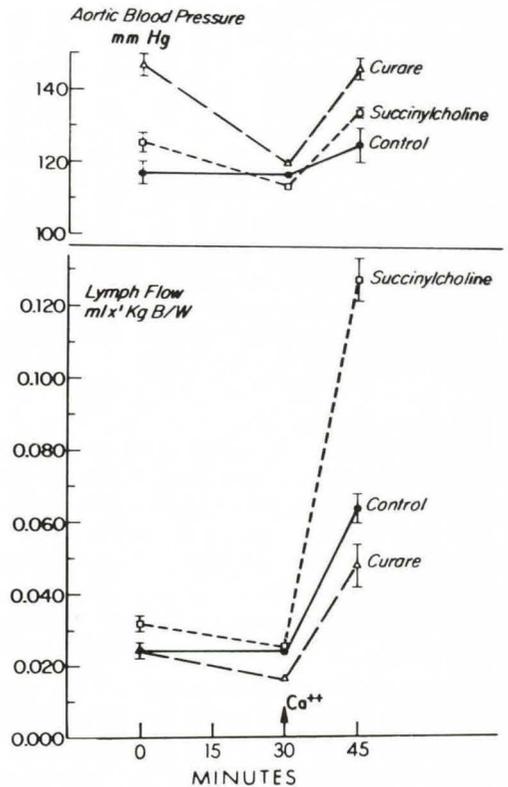


Fig. 1 Diagrammatic representation of variations in aortic blood pressure and thoracic duct lymph flow produced by L-tubocurarine chloride and succinylcholine chloride before and after the injection of calcium gluconate. Values represent the mean of 3 experiments \pm S.D. The control group received calcium gluconate only

Table 1 Changes in serum calcium, lymph calcium, lymph flow and aortic blood pressure in parathyroidectomized animals after the injection of various drugs and calcium gluconate. Values represent the mean of 3 experiments \pm S.D.

	Control Serum Calcium mg%	Serum Calcium After Calcium mg%	Control Lymph Calcium mg%	Lymph Calcium After Calcium mg%	Control Lymph Flow ml/kg B/W per minute	Lymph Flow After Drugs ml/kg B/W per minute	Lymph Flow After Drugs Calcium ml/kg B/W per minute	Control Arterial Blood Pressure mm Hg	Arterial Blood Pressure After Drugs mm Hg
Calcium Gluconate	6.6 \pm .8	10.5 \pm .8	5.1 \pm .9	7.0 \pm .8	0.025 \pm .003	0.065 \pm .003**	0.050 \pm 0.002**	117 \pm 8	126 \pm 4*
L-Tubocurarine Chloride	7.6 \pm .8	12.0 \pm .7	6.0 \pm .8	7.4 \pm .8	0.025 \pm .003	0.016 \pm .002	0.050 \pm 0.002**	147 \pm 10	120 \pm 8*
Succinyl-Choline Chloride	6.3 \pm .8	10.1 \pm .6	6.3 \pm .7	8.0 \pm .8	0.030 \pm .002	0.025 \pm .001	0.130 \pm 0.001**	125 \pm 7	116 \pm 6*
Atropine	7.2 \pm .9	10.2 \pm .8	5.5 \pm .8	7.0 \pm .9	0.016 \pm 0.001	0.025 \pm .002	0.125 \pm 0.002**	98 \pm 4	102 \pm 6*
Phenoxybenzamine Hydrochloride	7.0 \pm .8	10.0 \pm .7	6.0 \pm .9	8.1 \pm .9	0.030 \pm .003	0.025 \pm .002	0.075 \pm .002**	116 \pm 6	92 \pm 8*
Propranolol	7.1 \pm .9	10.5 \pm .8	7.2 \pm .7	8.2 \pm .8	0.040 \pm 0.002	0.030 \pm 0.001	0.090 \pm 0.003**	130 \pm 8	120 \pm 6*
Mixture	7.0 \pm .8	10.4 \pm .8	6.8 \pm .8	7.8 \pm .8	0.025 \pm 0.003	0.025 \pm 0.002	0.058 \pm 0.004**	126 \pm 4	110 \pm 2*
Angiotensine	7.2 \pm .8	10.5 \pm .8	5.7 \pm .9	7.1 \pm .8	0.025 \pm 0.003	0.001 \pm 0.002	0.092 \pm 0.002**	100 \pm 4	155 \pm 9*
Papaverine	7.3 \pm .9	10.2 \pm .9	6.2 \pm .8	8.1 \pm .6	0.019 \pm 0.001	0.023 \pm .002	0.024 \pm .001*	95 \pm 5	78 \pm 4*

*P < 0.10

**P < 0.001

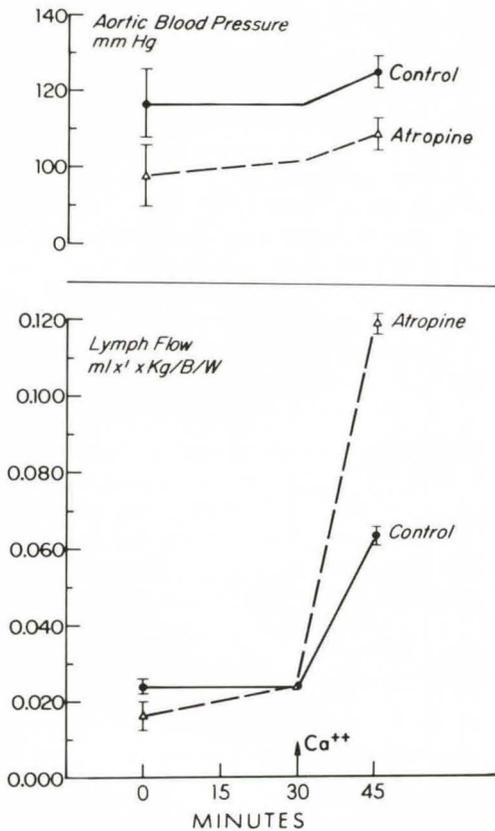


Fig. 2 Diagrammatic representation of variations produced by atropine in aortic blood pressure and lymph flow before and after the injection of calcium. Points represent the mean of 3 experiments \pm S.D. The control group received calcium gluconate only

($P < 0.001$) and ABP from 98 ± 4 to 110 ± 6 mm Hg ($P < 0.05$), (Figure 2).

The injection of Levoarterenol produced a rise in ABP and in TDLF. Isoproterenol produced a fall in ABP accompanied by a significant rise in TDLF. Phenoxybenzamine produced a fall in TDLF and ABP. Calcium augmented TDLF from 0.030 ± 0.003 to 0.075 ± 0.002 ml/kg/B/W per minute ($P < 0.001$). Its effect on ABP was non-significant ($P < 0.05$). A very similar response was elicited by propranolol, which lowered TDLF and ABP. After propranolol, Ca increased TDLF from 0.040 ± 0.002 to 0.090 ± 0.003 ($P < 0.001$). ABP did

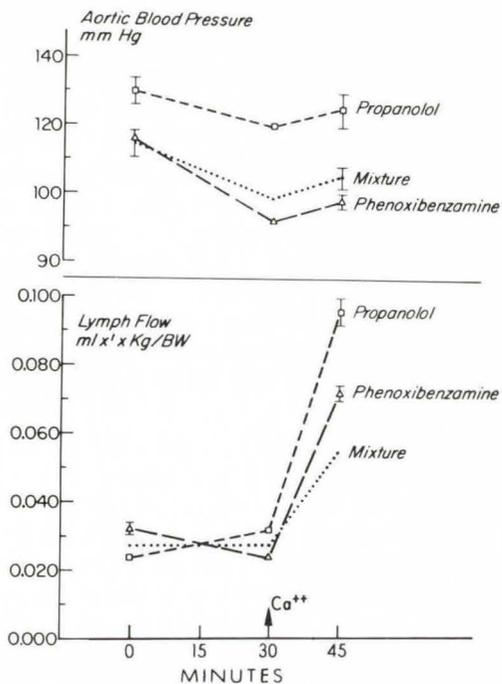


Fig. 3 Diagrammatic representation of changes produced by propranolol, phenoxybenzamine hydrochloride, and the combination of adrenalectomy, reserpization, propranolol and phenoxybenzamine before and after the injection of calcium. Points represent the mean of 3 experiments \pm S.D. Control values can be compared from previous figures

not change significantly ($P < 0.05$). In the group of adrenalectomized dogs, phenoxybenzamine, Propranolol and Reserpine did not change control TDLF values, although ABP was lowered minimally. After Ca, TDLF increased from 0.025 ± 0.003 to 0.058 ml/kg/B/W per minute ($P < 0.001$), while ABP remained virtually unchanged (Figure 3).

The angiotensin infusion was adjusted to maintain ABP at 155 ± 9 mm Hg. This was followed by a complete cessation of TDLF. Ca added while angiotensin was being infused rapidly produced a high TDLF and further augmented the ABP (Figure 4).

In intact as well as in parathyroidectomized dogs, papaverine produced non-significant changes in TDLF and ABP (Figure 5).

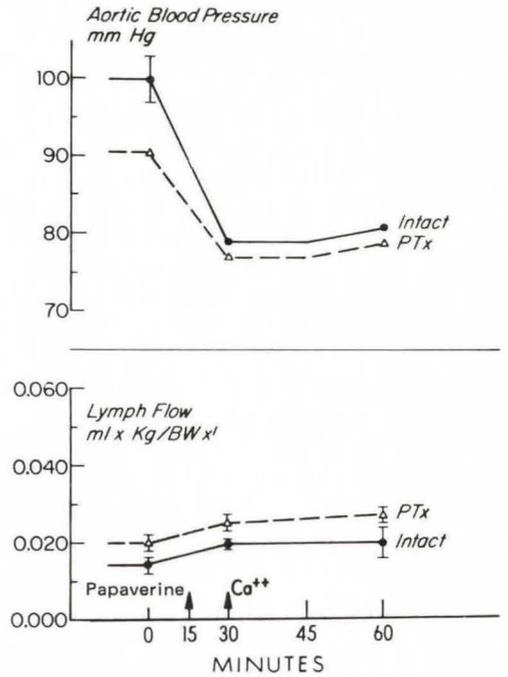
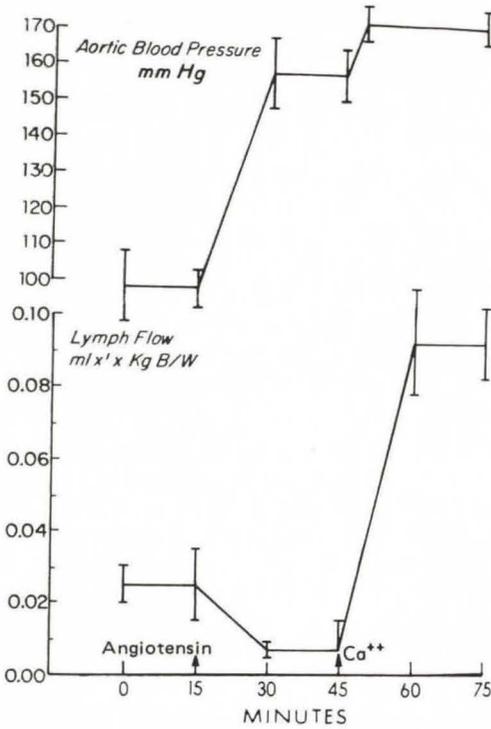


Fig. 4 Diagrammatic representation of changes produced by an infusion of angiotensin in aortic blood pressure and lymphatic flow before and after the injection of calcium. Points represent the mean of 3 experiments \pm S.D. Control values can be compared from previous figures

Fig. 5 Diagrammatic representation of variations produced by the injection of papaverine in aortic blood pressure and lymph flow before and after the injection of calcium in intact and PTX dogs. Points represent the mean of 3 experiments \pm S.D. Control values can be compared from previous figures

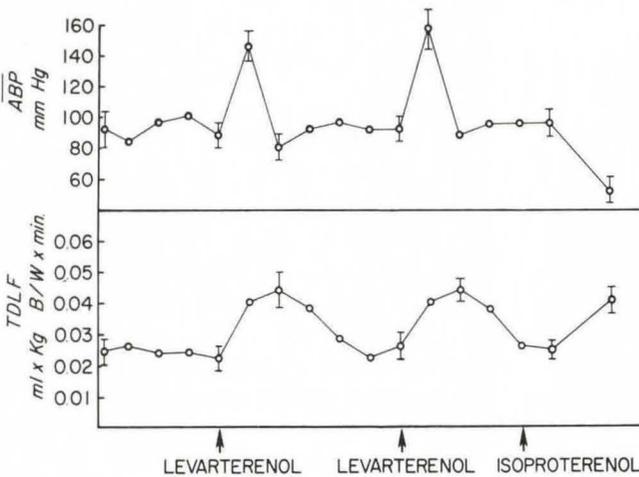


Fig. 6 Diagrammatic representation of the effect of Levarterenol and isoproterenol on TDLF and ABP in a group of 3 previously parathyroid-ectomized dogs. Points represent the mean of 3 experiments \pm S.D.

Discussion

In spite of extensive studies performed on the mechanisms of lymph transport, it is still a matter of speculation whether lymph is propelled by skeletal muscle activity or by the active contraction of the lymphatic wall or by both (6, *Hall*, 1969). Clarification of these areas should help us to understand the role that TDLF plays in the replenishment of the vascular compartment during septic and hemorrhagic shock, as it has been reported that, under those conditions, TDLF becomes an important source of volume, electrolytes and protein (2).

In our experimental design, it was not possible to establish the exact time relationship between the rise in ABP and that of TDLF secondary to the injection of Ca. We measured ABP at its immediate source, the aortic lumen, but we do not know if the lymph flow recorded at the TDLF level reflects in time the onset of changes occurring at the end-capillary lymphatic. The inertia of the system was, therefore, an unknown factor.

In order to reduce the possibilities that our Ca injections would produce hypercalcemia, the animals were parathyroidectomized, thus keeping the serum Ca levels within the normocalcemic range after the Ca injection. The effect of Ca on TDLF is almost identical in intact and parathyroidectomized dogs (3). In some dogs, serum Ca reached 12 mg% after the Ca was injected. This is considered the upper limit in the dog (7).

All drugs tested, including calcium, have an effect on TDLF of no more than 15 minutes. Thus, after the baseline had returned to control values, we injected calcium. Thereafter, the effect of Ca on TDLF and ABP was compared with the original TDLF and ABP baseline as recorded before any drug was tested.

Changes in serum Ca can alter the contractibility of the skeletal muscle system (8). These contractions are known to change TDLF by acting as peripheral pumps, compressing vessels in which the flow of lymph is governed by a competent unidirectional valve system (1). In our experiments, skeletal muscle paralysis produced by curare or succinylcholine

chloride failed to prevent the TDLF and ABP increases secondary to the injection of Ca. This ruled out the possibility that Ca may increase TDLF by augmenting the contractibility of the skeletal muscle system.

Atropinization produced significant increases in TDLF, accompanied by a non-significant rise in ABP. In this group of animals, Ca produced a vigorous TDLF response and a modest increase in ABP, suggesting that atropine, by blocking the parasympathetic system, may allow the sympathetic mechanism to freely react to Ca. This group of dogs started with a lower control TDLF.

Calcium can elicit a catecholamine discharge from the adrenal glands, augmenting ABP (4). If we postulate that such rise in ABP is not followed by a restriction in capillary blood flow, then an increasing capillary filtration should be expected, augmenting TDLF. However, if this is followed by an increase in precapillary resistance, capillary filtration will decline, as will perhaps, lymph flow. In our experiments, a rise in ABP produced by levoarterenol was accompanied by augmentation in TDLF. In contrast, the arterial hypotension which followed the injection of isoproterenol was accompanied by a rise in TDLF. In an attempt to inhibit the action of calcium on the sympathetic system, we utilized phenoxybenzamine and propranolol, both post ganglionic alpha and beta blockers. These drugs reduced the TDLF response to calcium. The action of Ca on TDLF and ABP was further inhibited by the combination of Phenoxybenzamine, Propranolol, Reserphine and adrenalectomy.

We observed that, after atropinization, Ca produced a marked increase in TDLF. This suggested that such parasympathetic inhibition may have allowed for a more unrestricted sympathetic response. This is supported by the observation that, in adrenalectomized animals receiving Alpha and Beta blocking, the action of Ca on TDLF and ABP was diminished. Later, we attempted to separate the rise in ABP from that of TDLF by maximally augmenting ABP with the angiotensin infusion. This was accompanied by a stoppage of TDLF. We interpreted this to be secondary to precapillary vasoconstriction, lowering the capil-

lary blood pressure and significantly reducing capillary filtration. While angiotensin was still being infused, Ca was able to override its effects, further raising ABP and producing a marked TDLF increase. So we have to postulate that, either Ca had a lymphoconstricting action, or that angiotensin may cause lymphatic spasm which was relieved by Ca. In order to investigate if Ca acted by contracting the smooth muscle present in the lymphatic vessel wall (6), it became necessary to use an agent which could block the action of Ca on such muscle. *Tomiyama et al.* (9) demonstrated that papaverine inhibits the action of Ca on KCl depolarized tenia coli muscles of the guinea pig and that papaverine also repressed ^{45}Ca uptake by this preparation. They concluded that papaverine may impair the availability of Ca to the contractile system (9).

In our hands, papaverine was able to prevent a rise in TDLF after Ca injection. The fall in ABP produced by papaverine alone was accompanied by a modest increase in TDLF, possibly the effect of an augmented blood capillary perfusion secondary to the vasodilating effect of papaverine.

These experiments suggest that Ca increases TDLF by the combination of several actions. The most significant may be the contraction of the muscle present in the lymphatic channel wall. Associated with this, there seems to exist some degree of sympathetic system po-

tentiation which may produce the rise in TDLF by an increase in the pressure at the arterial side of the capillary bed.

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