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INTRINSIC LYMPH PROPULSION:
THE DYNAMIC RESPONSE TO HEMORRHAGIC AND ENDOTOXIN SHOCK
IN THE ANAESTHETIZED SHEEP

BY

ALLEN H HAYASHI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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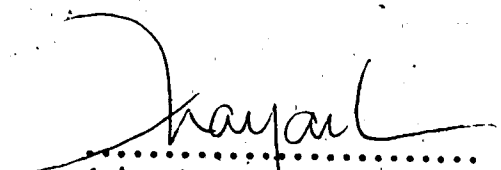

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ...INTRINSIC LYMPH PROPULSION: THE DYNAMIC RESPONSE TO HEMORRHAGIC AND ENDOTOXIN SHOCK IN THE ANAESTHETIZED SHEEP.....

submitted by ... ALLEN H HAYASHI .....

in partial fulfillment of the requirements for the degree of ... MASTER OF SCIENCE IN EXPERIMENTAL SURGERY .....

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(Supervisor)

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Date: 25 August 86

To my family.....

In appreciation for their strength, support, wisdom and most of all, eternal love.

AHH

ABSTRACT

Shock is a vast and complex subject, and remains a principal preoccupation of both the clinician and basic scientist. In both hemorrhagic and septic shock, it is fundamental that the effects on fluid dynamics be recognized for diagnosis and successful management. Traditionally, shock has been considered solely from a cardiovascular viewpoint such that our current concepts have completely ignored the lymphatic system and its role in shock. The return of protein and fluid to the bloodstream by the lymphatic system is a necessary and important component for the maintenance of homeostasis. In addition, it has not been appreciated that the lymphatics spontaneously contract to return significant volumes of lymph to the circulation daily. This implies a dynamic role for the lymphatic vessel, however, it has not been possible to assess the activities of the lymphatic pump during shock using current methods. Since the measurement of lymph flow represents only the end result of complex interactions between lymph formation and lymph propulsion, the major challenge has been in developing a technique which would discriminate between these two determinants.

We believe that the intrinsic contractile activity of the lymph vessel may play an important role in the regulation

of lymph return during shock. By adopting this new perspective we have:

(1) defined the changes in lymph flow that occur in mesenteric lymphatics during hemorrhagic and endotoxin shock in anaesthetized sheep

(2) developed an in vivo model system which permitted the study of intrinsic lymph propulsion

(3) determined the effects of hemorrhage and endotoxin on the lymph pump (lymphatic contractile activity)

With the removal of 25% of the sheep's blood volume, an increase in lymph flow over the next 6 hours was observed. Peak flows approached 365% of basal control rate at one and four hours after hemorrhage. Mean arterial pressure dropped initially to below 50% of control but was restored by the third posthemorrhage hour. In another series, endotoxin was used to simulate the conditions of septic shock. The intravenous injection of 3.3 mcg/kg of endotoxin led to the development of pulmonary edema and a 6.4 fold increase in mesenteric lymph flow 40 minutes after infusion.

In order to determine the effects of hemorrhage and endotoxin on lymphatic pumping, it was necessary to develop a new model system to distinguish the activities of the lymph pump from the microvascular events which affect lymph flow. A 10-15 cm segment of mesenteric lymphatic was catheterized at both proximal and distal ends and surgically "isolated" from all lymph input. Surgical dissection was minimized in

order to preserve the vessel's neurovascular supply. The vessel segment was supplied with fluid from a reservoir and was triggered to contract spontaneously by applying a transmural distending pressure. In the absence of a hydrostatic gradient, flow generated from this system could only occur if the lymph vessel contracted and propelled fluid. Utilizing this "double catheter" preparation, sheep bled 25% of blood volume demonstrated an increase in fluid propulsion. When compared with similar preparations in sheep not bled, flow rates were up to 6 times greater after hemorrhage. The blood loss stimulated an increase in lymphatic contractile frequency and force. In sheep injected with endotoxin (3.3 mcg/kg), a rapid fall in fluid output was observed. By 50 minutes, fluid propulsion averaged only 6.7% of basal control rate. A significant suppression of lymphatic contractile activity was concomittantly seen.

We conclude that lymph propulsion is regulated independent of changes in lymph formation during shock. Hemorrhage stimulates lymphatic contractile activity, while endotoxin is inhibitory. We speculate that the lymphatics may be an important factor in re-expanding plasma volume following hemorrhage, and may contribute to the genesis of interstitial edema associated with sepsis.

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INTRODUCTION

Shock is a major and complex clinical problem; an entity which commonly accompanies major trauma. The clinical picture is extremely variable often with controversy surrounding each level of diagnosis and management. But fundamentally, the basic underlying pathology relates to an acute reduction in tissue perfusion as a consequence of a diminished effective circulating fluid volume (ECFV). Of the many forms of shock, the two most challenging and commonly encountered in trauma and surgery are hemorrhagic and septic shock.

Hemorrhagic shock occurs following a major loss in blood volume and ultimately will lead to global cellular dysfunction unless compensatory mechanisms can be effectively recruited to restore hemodynamic stability. Acute compensatory mechanisms, characterized by peripheral vasoconstriction, tachycardia, and a decrease in venous capacitance, are mediated primarily by sympathetic nervous activity to ensure that vital perfusion to cerebral and coronary circulations are maintained (Chien, 1967; Bond and Johnson, 1985; Colantuoni et al, 1985; Zhao et al, 1985).

Restoration of intravascular volume and protein, while occurring over a more chronic period of time is also important for survival. Prior to medical intervention, this

is accomplished by the mobilization of endogenous stores of fluid and protein and transfer to the contracted vascular compartment (Lucas and Ledgerwood, 1983). It is in this regard that we believe the lymphatic system may play an important role.

With septic shock, the reduction of effective circulating fluid volume occurs through different mechanisms. Direct injury to the capillary beds occurs which leads to an increase in microvascular permeability to plasma proteins (Demling et al, 1984; Avila et al, 1985). This facilitates the extravasation of fluid and protein into the interstitium. It has been assumed that the flood of protein and fluid into the interstitium simply overwhelms normal lymphatic transport mechanisms which then becomes manifest clinically as progressive interstitial edema and intravascular volume depletion (Robin et al, 1972; Demling et al, 1984).

Despite the serious implications of hemorrhage and sepsis, considerable defects still exist in our understanding of the homeostatic mechanisms that serve to promote survival or that fail and contribute to an animal's demise. Traditionally shock has been investigated from a purely cardiovascular standpoint. Little, if any, attention has been directed toward the dynamic events which involve the interstitium and lymphatic system. It is our intention to explore shock from this new perspective.

Lymph is essentially derived from interstitial fluid and protein. The volume of interstitial fluid is considerable and accounts for approximately 25% of total body water or about 3 times normal plasma volume (Shires et al, 1964b; Courtice, 1971). Under normal circumstances, over a 24 hour period, lymph is returned back to the circulation in volumes comparable to the intravascular pool (Mayerson, 1963; Yoffey and Courtice, 1970). In man, between 1 and 3 litres of lymph is returned to the circulation by the Thoracic duct daily (Yoffey and Courtice, 1970).

The mass of interstitial protein is similarly large. Estimates have ranged between 1 and 3 times the amount normally held within the vascular space (Sellers et al, 1966; Courtice, 1971). The return of extravascular protein to the bloodstream remains essentially a function of the lymphatic system. Although some reports have claimed that interstitial protein may directly enter into the bloodstream (Jepson et al, 1953; Szabo et al, 1973; Perry, 1981), by far the bulk of literature maintains that the lymphatics provide the major route for interstitial protein removal (Starling, 1896; Drinker and Yoffey, 1941; Hollander et al, 1961; Courtice, 1971; Fernandez et al, 1983). In man, more than 100 grams of protein is transported back to the circulation per day (Yoffey and Courtice, 1970).

The return of protein to the vascular space is important primarily because its oncotic properties ensure the

maintenance of normal plasma volume. Usually a small amount of intravascular protein continuously leaks out through the capillary walls and back into the interstitium. Some have estimated that about 7% of intravascular albumin enters the interstitial space hourly (Lucas and Ledgerwood, 1983). More conservative estimates have calculated that 50% of the total circulating protein escapes into the interstitium daily (Mayerson, 1963). In any event, without effective lymphatic function, a substantial amount of extravasated protein could accumulate over the course of a day. Normal oncotic forces would become disrupted, and favour the escape of intravascular fluid into the interstitium, with resultant edema formation and circulatory collapse. This scenario fortunately does not normally occur but illustrates the importance of the lymphatic system for the maintenance of both interstitial and intravascular integrity.

Anatomic Considerations

Lymphatic vessels have been described as early as 1622 when Aselli discovered chyle filled lacteals within the mesentery of well fed dogs. Anatomically, the lymphatics represent an "open loop" system beginning in the periphery as small highly permeable capillaries and ending centrally as large muscular walled trunks. Certain "privileged" sites like the cornea, lens, and parts of the central nervous system lack lymphatic circulation (Adair and Guyton, 1985a; Olszewski, 1985). These tissues, however, usually have small interstitial clefts and channels which permit tissue fluid to drain into nearby lymphatic channels. Essentially then, all vascularized tissues of the body are subject to drainage by the lymphatic system.

Lymphatic Capillaries

Lymphatic capillaries are also known as "initial" or "terminal" lymphatics (Casely-Smith, 1970; Zweifach and Prather, 1975). They originate as blind ending endothelial lined structures within the interstitium. Lymphatic capillaries are anatomic counterparts to blood capillaries but have several important differences.

(1) They are wider, larger, and flattened with irregular shaped lumens and lack a definite basement membrane (Zweifach and Prather, 1975; Huth and Bernhardt, 1977; Olszewski, 1985).

(2) The endothelial lining is attenuated with intercellular junctions which easily separate to form open channels (Leak and Burke, 1968; Zweifach and Prather, 1975).

(3) Anchoring filaments have been described which support the lymphatic capillary within the interstitium by binding regions of the abluminal endothelial membrane to adjacent connective tissue elements (Leak and Burke, 1968; Huth and Bernhardt, 1977).

(4) The endothelial cells have been noted to overlap slightly. With the strategic placement of the anchoring filaments (3), it has been hypothesized that a "flap valve" effect is created where protein and fluid may easily enter the capillary, but meets with greater resistance when moving in the opposite direction (Leak and Burke, 1968; Adair and Guyton, 1985a,b).

All of the above characteristics render the lymphatic capillaries highly permeable, and promote the rapid removal of fluid, protein and cells from the interstitium.

Lymphatic Collecting Vessels

Lymphatic capillaries converge with others to form "collecting" lymphatic channels and can be distinguished from the former by the presence of valves and a more prominent vessel wall (Zweifach and Prather, 1975). Mislin (1967) has defined the basic functional unit of the lymphatic vessel as the "lymphangion", which represents that segment of vessel subtended by its adjacent valves. Collecting vessels have a

well developed basement membrane and the first appearance of smooth muscle cells, elastin and collagen within the vessel wall is observed. The small collecting vessels continue to converge to form large afferent (prenodal) lymphatic ducts which then pass through one or more lymph nodes and emerge from the nodal hila as efferent (post nodal) lymphatic ducts. Lymph is then returned to the innominate veins in the neck by the Thoracic duct or Right Lymphatic duct. These major lymphatic trunks are histologically similar in structure to large veins. Their walls characteristically have 3 distinct layers: tunica intima, tunica media, tunica adventia. The smooth muscle incorporated into the media of these large collecting ducts vary between 3-8 cell layers thick (Johnston, 1985). Ohhashi et al (1977) has defined 3 distinct muscle layers within the media: the internal longitudinal, intermediate circumferential, and external longitudinal. While the Thoracic duct has extensive smooth muscle development, smaller collecting vessels are also well endowed with muscle fibers (Smith, 1949). Functionally, smooth muscle is a necessary component for the generation of intrinsic lymphatic contractions.

Blood Supply

The collecting lymphatic vessels are supplied with a rich network of nutritive blood vessels (vasa vasorum) located within the connective tissue matrix of the adventitia (Yoffey and Courtice, 1970; Zweifach and Prather, 1975). The

vasa vasora have also been found to penetrate deep into the smooth muscle layers of the media (Ohhashi et al, 1977). The rich blood supply is presumed necessary to satisfy the high metabolic demands imposed by the smooth muscle cells (Yoffey and Courtice, 1970; Ohhashi et al, 1978).

Nervous Supply

Also lying within the adventitia are nervous plexuses of myelinated and nonmyelinated fibers. Nonmyelinated nerve fibers appear to penetrate into the media and are considered to be motor to the smooth muscle layer (Vajda, 1966; Ohhashi et al, 1982). The distribution of lymphatic innervation occurs only in vessels which contain smooth muscle cells and are therefore not found in terminal lymphatics (Carleton and Florey, 1927; Vajda, 1966). Histochemical studies have demonstrated lymphatic innervation to be of greatest density in regions adjacent to valve attachments (Ohhashi et al, 1982), however, others have found no such regional differences in nervous distribution (Alessandrini et al, 1981). Most investigators concur that lymphatic innervation is derived from autonomic origin. Both alpha and beta adrenergic receptors have been identified in bovine mesenteric lymphatics (Ohhashi et al, 1978), however, evidence claiming that differences exist in distribution of these receptors between valvular and intervalvular segments must be viewed with skeptism.

Permeability of Lymphatic Collecting Vessels

The difficulty which proteins (and other large molecules) have transgressing the lymphatic collecting vessels, directly contrasts with the ease in which they enter into the lymphatic capillaries. Mayerson et al (1962) found that while water and other small molecules were free to diffuse across all lymph vessels, free exchange decreased significantly when substances greater than a M.W. of 2300 were tested. Tight intercellular junctions and a continuous basement membrane of the intimal layer are held responsible for restricted passage of circulating proteins (Casely-Smith, 1969). The permeability characteristics of the lymphatic system permit the effective resorption of interstitial fluid and protein and the efficient transport of these products back to the general systemic circulation.

Dynamics of Lymph Flow

Lymph flow is critically dependent upon two principle factors, lymph formation (production) and lymph propulsion. These determinants of lymph flow are intimately associated, but appear to have different regulatory mechanisms.

Lymph Formation

Lymph formation is synonymous with the movement of fluid and protein from interstitium into the lymphatic capillaries, however, the dynamic events truly begin at the blood capillary-interstitial interface. Starling (1894; 1896) was the first to describe the forces involved in the determination of fluid filtration across the semipermeable capillary membrane. These forces are expressed in Starling's equation (table I) developed by Pappenheimer and SotoRivera (1948), and Kedem and Katchalsky (1958) and reviewed by Pappenheimer (1953) and Staub (1974).

The Starling equation represents the interaction of both colloid osmotic and hydrostatic forces of the interstitial and capillary spaces. The transcapillary hydrostatic pressure gradient (Pc-Pi) provides an outward driving force and is opposed by the colloid osmotic pressure gradient (IIc-IIi) which tends to drive fluid back into the capillary.

Table I

The Starling Equation

$$Jv = Kf\,[(Pc - Pi) - q\,(IIc - IIi)]$$

Jv = rate of fluid filtration between vascular and interstitial compartments

Kf = capillary filtration coefficient

Pc = hydrostatic pressure within the blood capillary

Pi = hydrostatic pressure (interstitial fluid pressure) within the interstitium

q = osmotic reflection coefficient, indicating the degree of permeability the capillary wall has to plasma proteins

IIc = colloid osmotic pressure within the blood capillary

IIi = colloid osmotic pressure within the adjacent interstitium

Normally a slight overall imbalance between hydrostatic and oncotic forces is present [(Pc-Pi) > (IIc-IIi)] favoring a positive filtration of fluid into the interstitium. Over the course of a day, 5000 litres of plasma circulate through the capillaries in man but yield just 1-3 litres of ultrafiltrate (Yoffey and Courtice, 1970). This relatively small but significant addition to the interstitial pool is

easily dealt with by the lymphatics. Alteration of any one of the parameters of the Starling equation however, will obviously have significant effect on transcapillary fluid filtration and therefore lymph formation.

It has been assumed for many years that the formation of lymph was a passive process whereby interstitial fluid moves down a hydrostatic pressure gradient into the terminal lymphatics. Indeed, many early studies appeared to support this hypothesis. McMaster (1947) found when inserting fine needles into various tissues in animals, that the "tissue pressures" were positive and averaged 1.9 cm H_2O. These measurements were slightly but consistently greater than pressures recorded within the terminal lymphatics (1.2 cm H_2O). The accuracy and validity of these measurements were questioned for two reasons. First, the needles used to measure these tissue pressures were several hundred times greater in width than the tissue (interstitial) spaces themselves. Second, it is commonly known that 99.9% of interstitial fluid exists in the "gel" state and not as free fluid. Both gel and cellular material could easily plug the lumen and make measurements inaccurate (Adair and Guyton, 1985a,b).

Interstitial Fluid Pressure

Recently it has been proposed that several different types of tissue pressures coexist. Guyton et al (1971) postulated that total tissue pressure was a function of

both solid tissue pressures and interstitial fluid pressure. His arguments however, led to the conclusion that only the interstitial fluid pressure was relevant in the determination of fluid movement through the interstitium and into the lymphatics.

Guyton (1963), in attempts to accurately measure interstitial fluid pressure, implanted hollow porous plastic spheres into various tissues of the body. The implanted capsules artificially created small "pockets" of free fluid in direct communication with the surrounding interstitium. Pressures measured within the capsule by direct puncture with a fine needle would therefore reflect interstitial fluid pressure. After surgical inflammation was allowed to resolve, capsular pressures were found in many tissues to be negative (subatmospheric). These surprising findings were later confirmed by others (Anas et al, 1968; Hopkinson et al, 1968; Gibson and Gaar, 1970; Taylor et al, 1970) and by those using other techniques (Scholander et al, 1968; Ladegaarde-Pedersen, 1970).

Changes in interstitial fluid pressure, as measured by the capsule technique, tend to reflect the dynamic events that happen within the interstitium. Guyton (1963) found that by elevating capillary hydrostatic pressure, capsular pressures would also increase, while increasing intravascular oncotic pressure with intravenous dextran caused capsular pressures to rapidly become more negative.

Interstitial Fluid Pressure and Lymph Flow

Taylor et al (1970) determined the relationship between lymph flow and interstitial fluid pressure using the capsule method. They found that as interstitial fluid pressure was made to rise from -6 mmHg to 0 mmHg, lymph flow increased 12 to 50 fold. After this point was reached, no further increase in flow occurred but instead edema ensued.

Mechanisms of Lymph Formation

The mechanisms by which interstitial fluid and protein enter the terminal lymphatics to become "lymph" are still largely unknown. In order for lymph formation to occur, it is necessary that an appropriate fluid pressure gradient becomes established. If one accepts the evidence that interstitial fluid pressure is subatmospheric, and that the average pressures measured within the terminal lymphatics are near atmospheric, then the passive process of lymph formation becomes an untenable hypothesis. Rather, an active process appears essential for the removal of interstitial fluid and protein and for the continued maintenance of negative interstitial fluid pressure (Guyton et al, 1971; Granger, 1979).

Many have proposed that the intermittent motion of tissues from movement or ambulation, provides increases in tissue pressure capable of "pushing" interstitial fluid into the lymphatic capillaries. Alternatively, others suggest that tissue motion intermittently compresses the terminal

lymphatics and the resultant recoil of these vessels draws interstitial fluid in by virtue of the negative pressures generated (Guyton et al, 1971; Adair and Guyton, 1985b).

The pumping action of the larger lymphatics (because of their spontaneous nature to contract) represents an obvious mechanism for drawing fluid into the lymphatic capillaries. Intrinsic contractions and unidirectional valves could establish pressure gradients leading to the movement of interstitial fluid into the terminal lymphatics and on into the collecting vessels (Taylor et al, 1973; Nicoll and Taylor, 1977). Nicoll and Hogan (1978) and Hogan (1981), using micropipette techniques on bat wings, were successful at simultaneously measuring both terminal lymphatic and adjacent interstitial pressures. Lymphatic pressures were found to be phasic and were less than interstitial fluid pressure for at least part of the time. So despite the fact that the average lymphatic pressure was greater than interstitial fluid pressure, the hydrostatic gradient necessary for lymphatic filling was present during 43% of each contractile cycle. Thus the pumping action of the lymphatics could, by their intrinsic contractions alone, generate enough suction to draw fluid in from the interstitium.

Other theories of lymph formation include the osmotic pull hypothesis (Casley-Smith, 1983), and the vesicular transport theory (O'Morchoe, 1980; Niiro and O'Morchoe,

1985). Both theories have not gained wide acceptance.

Lymph Propulsion

Only recently has serious attention been given to the mechanisms by which the lymphatic vessels may regulate lymph flow. Under resting conditions, lymph return is said to be "coupled" to lymph formation. The rate of lymph return must match the rate of lymph formation in order to maintain both intravascular and interstitial homeostasis (Hall et al, 1965; Guyton et al, 1971; Nicoll and Taylor, 1977). Conditions where lymph formation exceeds lymph propulsive capacity, result in edema.

Important for effective propulsion of lymph is the interposition of numerous valves along the course of each collecting vessel. These delicate appearing structures may contain between one to 5 leaflets each (Gnepp, 1976). They are deceptively sturdy and able to withstand intraluminal pressures as high as 68 mmHg without becoming incompetent (Ohhashi, 1980). Each valve serves to ensure unidirectional (centripetal) flow and to prevent the reflux of lymph back into the tissue spaces. Intrinsic and extrinsic mechanisms have both been cited as being important for lymph propulsion.

Intrinsic Mechanisms

It has become increasingly evident that intrinsic contractile properties of the lymphatics provide a major part of the propulsive force necessary for lymph transport (Hall

et al, 1965; Campbell and Heath, 1973; McHale and Roddie, 1976; Reddy and Staub, 1981).

Hewson, in 1774, first observed spontaneous rhythmical contractions of mesenteric lacteals in birds, dogs and horses. Since then, many have studied lymphatic contractile activity in a variety of species including guinea pigs, rats, mice, squirrels, cats, rabbits, dogs, cows, sheep, bats, and man (Florey, 1926, 1927; Smith, 1949; McHale and Roddie, 1976; Hall et al, 1965, Yoffey and Courtice, 1970; Nicoll and Taylor, 1977; Olszewski and Engeset, 1980; Armenio et al, 1981; Olszewski, 1981). Florey (1927) and Smith (1949) observed most lymphatic contractions to be propagated centripetally in a peristaltic pattern. Occasionally, in some of their preparations, lymphatic contractions showed little coordinated activity. These and many other studies have demonstrated that most animals have lymphatic vessels which possess intrinsic contractile activity.

Chronic catheterization of lymph vessels for prolonged monitoring of lymph flow and pressures had to await the advent of flexible plastic catheters. Before this development, glass pipettes were utilized to measure these lymphatic parameters under experimental conditions requiring constant supervision. Lymph clots and vessel perforations were frequent and frustrating complications.

Hall et al (1965) used plastic catheters to cannulate lymph vessels and was successful in measuring lymph pressures

and flow over prolonged periods of time. Lymphatic pressures were rhythmically pulsatile with a discernable amplitude and frequency. Lymph flow, similarly, was pulsatile and not significantly influenced by extrinsic forces. Campbell and Heath (1973) strengthened the relationship between intrinsic lymphatic contractility and pulsatile lymph flow by demonstrating a positive correlation between pulse pressure and flow rates. Using similar techniques, Nicoll and Taylor (1977) observed lymphatic contractions to directly result in centripetal flow in the bat's wing. In other studies, the onset of lymphatic contractions were found to coincide exactly with the initiation of pressure pulses measured from indwelling micropipettes (Zweifach and Prather, 1975; Nicoll and Hogan, 1978). In summarizing these data, it appears that lymph flow and pulsatile pressures correlate closely with the generation of intrinsic contractions by the lymphatics.

Intrinsic contractions have been studied in vitro by many investigators (Mawhinney and Roddie, 1973; McHale and Roddie, 1976; Johnston and Gordon, 1981). Short segments of bovine mesenteric lymphatics suspended in tissue baths perfused with physiologic solution (Krebs) showed rhythmical contractions to occur with measurable frequency and force (Mawhinney and Roddie, 1973).

The ability for lymphatics to contract appears to reside within the properties of the smooth muscle cells within the tunica media. Using the sucrose gap technique, Azuma et al

(1977) studied the electrical activity of the lymphatic smooth muscle. In rhythmically contracting lymphatic segments, resting membrane potential was estimated to be -32.7 +/- 4.2 mV. Each spontaneously generated action potential was characterized by an initial slow decay of the resting membrane potential, followed by sudden depolarization (spike) and finally a slow repolarization phase. An important observation made was that each action potential was always accompanied by a phasic contraction. In other words each lymphatic contraction was preceded and initiated by a single action potential (Azuma et al, 1977; Allen et al, 1983).

The role of calcium for intrinsic contractility has been studied. Deysine et al (1980) demonstrated that raising serum calcium levels in parathyroidectomized dogs led to a dose-related increase in Thoracic duct lymph flow. Their experiments suggested that the administration of calcium produced lymph propulsive changes as a result of a direct effect on the vessel wall.

The importance of calcium for action potential generation and smooth muscle contraction has been documented for many smooth muscle containing tissues of which the lymphatic vessel is no exception (Nicoll, 1975). The removal of external calcium from the tissue bath or the addition of D600 (a calcium antagonist) to a spontaneously contracting lymph vessel will quickly abolish all electrical and mechanical activity (McHale and Allen, 1983). Although the

actual mechanisms are still debated, it appears that a freely available source of extracellular calcium is important for membrane stability, normal pacemaking, generation and propagation of action potentials, and for excitation-contraction coupling of lymphatic smooth muscle (Nicoll and Taylor, 1977; Ohhashi, 1977; McHale et al, 1980; McHale and Allen, 1983).

The Regulation of Lymphatic Contractile Activity

Concept of Myogenesis

Lymphatic contractility appears to be myogenically controlled. The degree of contractile force that is expressed by the lymphatic depends on the amount of stretch (or vessel distension) that is applied. In cannulated lymphatics of conscious sheep, Hall et al (1965) demonstrated pulsatile lymphatic pressures rose dramatically in both frequency and force (from 25 mmHg to 60 mmHg) when the vessel was obstructed by clamping the outflow catheter. Contractile activity was also shown to increase after the vessel was made to distend by direct injection of fluid or by increasing lymph formation with rapid plasmapheresis (Campbell and Heath, 1973; Hargens and Zweifach, 1977).

Isolated lymphatic preparations in vitro (McHale and Roddie, 1976) and in vivo (Reddy and Staub, 1981; McHale and Thornbury, 1986) show a positive relationship between transmural distending pressure and the volume of fluid pumped by the lymph vessel. This bears a marked resemblance to the

Frank-Starling ventricular function curve of the heart. An increase in distending pressure was associated with an increase in contraction frequency, force and fluid propulsion. Overdistension however, led to a decrease in contractile effectiveness. Myogenic regulation is an effective mechanism whereby the lymphatics can adapt to variable rates of lymph input by appropriately adjusting its contractile activity.

Recent evidence suggests that arachidonate metabolites and in particular thromboxane, may be important endogenous mediators of myogenesis. Inhibitors of arachidonic acid (aspirin, indomethacin, B.W. 755C) and the leukotriene antagonist ,FPL 55712, have been shown not only to suppress spontaneous contractions but also the contractile activity induced by prostaglandin agonists and non-prostanoid compounds like noradrenalin, histamine and serotonin (Johnston and Feuer, 1983; Johnston and Gordon, 1981; Allen et al, 1984; Johnston, 1985). They are believed to act specifically on inhibiting the generation and propagation of action potentials (Allen et al, 1984).

Thromboxane has been found to be one of the most potent stimulators of lymphatic contractility. Its action may relate to its influence on transmembranous and microsomal calcium ion transfer (Heaslip and Rahwan, 1981; Kutsky et al, 1983). While it is uncertain whether substrates for thromboxane metabolism are derived from endogenous sources or

obtained systemically, the lymphatic vessel appears capable of converting them into stimulatory compounds to effect spontaneous contractile activity (Johnston, 1983). It is conceivable then, that the lymphatics might respond to a myogenic "stretch" stimulus by synthesizing active arachidonate compounds and thereby autoregulate contractile activity.

Other regulatory factors include neurogenic mechanisms (McHale, 1985) and numerous humoral mediators (Johnston, 1985). As noted previously bovine lymphatics possess alpha and beta adrenergic receptors. Noradrenalin stimulates contractile activity in lymphatic vessels in vitro, while phentolamine and beta agonists are inhibitory (Ohhashi and Roddie, 1981; McHale and Roddie, 1983). The spontaneous nature of lymphatic contractions however appears not to be directly under neurogenic control since tetrodotoxin (a nerve blocker), and alpha and beta adrenergic blockers do nothing to alter the vessels' inherent rhythmicity (McHale and Roddie, 1976; Azuma et al, 1977; McHale and Allen, 1983; Roddie et al, 1980). The role of adrenergic factors may be to modulate myogenic activity.

Numerous humoral mediators have also been found to affect contractile activity. These include bradykinin, serotonin and other classic mediators of inflammation which are found in abundance in lymph draining inflammatory sites (Ohhashi et al, 1978; Azuma et al, 1983; Johnston, et al, 1983).

Extrinsic Mechanisms

It has been a longstanding belief that lymph flow is the sole result of the dynamic interactions that occur within the microvascular beds. Many investigators have the conception that lymph return is a passive event in which the lymphatic vessels act merely as "passive" conduits for lymph transport. Strong proponents of this theory claim that extrinsic forces propel lymph by a "massaging" action on the lymph vessels, forcing lymph to move from one lymphangion to the next (Mayerson, 1963).

Skeletal Muscle Contractions

Episodic skeletal muscle contractions can intermittently raise local tissue pressures. This is thought to provide propulsion sufficient for lymph return from the extremities. Variable increases in intralymphatic pressures (Mayerson, 1963; Calnan et al, 1970; Olszewski, 1981) and lymph flow (White et al, 1933; Engeset et al, 1977; Olszewski et al, 1977) have been found in extremities after active muscle contraction and exercise. Much of the increased lymph flow, however, is likely secondary to increased lymph formation from augmented blood flow to these tissues (Olszewski et al, 1977; Coates et al, 1984; Johnston, 1985). The contribution of lymph from the extremities to Thoracic duct flow is less than 5% (Schad et al, 1978); it is therefore unlikely that muscular contractions are important in the overall regulation of lymph flow.

Vascular Pulsations

Lymph vessels are often found closely associated with blood vessels. Parson and McMaster (1938), in an interesting experiment, perfused rabbit ears to study the effects of arterial pulsations on lymph flow. When the perfusate was delivered under constant pressure, lymph flow was minimal, but when perfusion was made pulsatile, lymph flow was enhanced. Cressman and Blalock (1939) noted the position of the cisterna chyli to lie adherent along the posterior wall of the aorta. While it was clearly demonstrated that Thoracic duct and cisternal pulsations were transmitted from adjacent aortic pulses, forward flow of lymph could not be shown (Browse et al, 1974).

Respiratory Pump

The influence respiration has on the regulation of Thoracic duct lymph flow has been studied by many investigators (Hall et al, 1965; Campbell and Heath, 1973; Browse et al, 1974; Dumont, 1975; Woolverton et al, 1978; Schad et al, 1978; Drake et al, 1982; Staub, 1974). Some believe that the pumping action of respiration is the principal mechanism for Thoracic duct lymph return (Dumont, 1975). Lymphatic pressure fluctuations from respiratory motion were shown to vary according to rate and depth of respiration (Hall et al, 1965; Schad et al, 1978) and were superimposed upon intrinsic contractions (Hall et al, 1965; Campbell and Heath, 1973). Schad et al (1978) estimated the

"respiratory pump" could contribute up to 35% of Thoracic duct flow in anaesthetized dogs, but Campbell and Heath (1973) showed that negligible changes in lymph flow occurred after the "respiratory pump" was blocked by inducing apneusis. In conscious hypercapnic sheep, hyperventilation stimulated by hypoxia had no effect on lung lymph flow (Coat[illegible] et al, 1984).

The use of anaesthetized animals may be, in part, the reason for these conflicting reports. Since anaesthetic agents tend to depress intrinsic lymphatic contractility, this may give the "respiratory pump" proportionately more importance under conditons of anaesthesia (Staub, 1974). Although some evidence tends to suggest that respiration does contribute to lymph return, it appears to be a minor component in conscious states (Hall et al, 1965).

Summary

The Lymphatic circulation plays an important role in the regulation of both intravascular and interstitial fluid dynamics. Lymph flow is governed by factors which contribute to either lymph formation or its transport (propulsion). Lymph propulsion is achieved through extrinsic and intrinsic mechanisms, however intrinsic mechanisms are likely the most important components for lymph propulsion. The basic regulation of intrinsic lymphatic contractility is myogenic. Neural and other humoral factors may modulate contractile activity, however few in vivo studies have been reported to date.

Very little is known about lymphatic function during the pathophysiologic states of shock. One of the major stumbling blocks in assessing intrinsic propulsion (lymphatic pumping) in vivo has been in finding a suitable experimental model. The difficulty has been in separating the effects shock has on the pumping activity of the lymphatic vessels from its effects on lymph formation. Considering the dynamic shifts of fluid that occur during hemorrhage and sepsis, the measurement of lymph flow alone, will not discriminate between factors that affect lymph formation from those that significantly alter lymphatic contractile activity.

From this review there is good evidence to suggest that the lymphatics perform important homeostatic functions under physiologic conditions. The implication that the lymphatic

system may play an active role in the regulation of fluid dynamics during hemorrhagic and septic (endotoxin) shock is an attractive yet unproven hypothesis. Since no study to date has undertaken to examine this important issue, the objectives of this thesis were to:

(1) develop and test an in vivo model system which would permit the study of intrinsic lymph propulsion.

(2) utilize this new model system to study the effects of shock on lymphatic pumping.

In these studies, a conceptually simple technique was developed. The "double catheter" preparation, by eliminating the influences of lymph formation permitted the specific evaluation of lymphatic contractile activity. This technique was then applied to shock models to determine the effects of hemorrhage and sepsis on lymph propulsion.

MATERIALS AND METHODS

Animal Preparation

A total of 58 healthy sheep of either sex, weighing between 25 - 35 kg. were used in these experiments. All animals were deprived of food for 48 hours and both food and water were withheld 24 hours prior to surgery. Thirty-six sheep were allocated for the hemorrhage studies, and 22 sheep were used for the endotoxin experiments.

Induction of anaesthesia was accomplished with the intravenous administration of Sodium Pentobarbital (Somnotol 20 mg/kg, MTC Pharmaceuticals, Hamilton, Ontario), with additional smaller doses (2 - 6 mg/kg/hr) given as necessary during surgery and throughout the course of each experiment. All sheep were intubated preoperatively (Portex, 6.5 - 9.0) and allowed to breath unassisted. The animals were placed in the left lateral decubitus position and the operative areas shaved and painted with 2.5% tincture of iodine solution.

Surgical Procedures

"Single Catheter" Cannulation of Mesenteric Lymphatics

Through a right transverse transabdominal incision, the peritoneal cavity was entered and the bowel delivered into the wound. Sterile Evan's Blue dye (1% in phosphate buffered saline) was injected into the mesenteric lymph nodes to clearly delineate the lymphatic vessels. The main mesenteric

efferent lymphatic vessel was identified originating from the terminal lymph node lying within the ileocolic mesentery. The peritoneum overlying the vessel was dissected for a short distance and the surrounding mesenteric fat was cleared. A polyvinyl catheter (Microbore Tygon tubing, O.D. 0.07 in., I.D. 0.04 in., Cole Parmer Instrument Co., Chicago) was flushed with Ringer's Lactate (Travenol Canada Inc.) and inserted into the lymphatic in the direction retrograde to lymph flow. The catheter was secured in place with 4-0 silk ties (Davis and Geck, Cyanamid, Montreal, Quebec) and externalized through the corner of the incision. The bowel was returned into the abdomen and the incision was closed in layers using #1 Dexon (Davis and Geck).

The outflow catheter was positioned at the level of the spine with the animal resting in the decubitus position. This height was assumed to be approximately equal to the point of insertion of the catheter in the lymph vessel.

"Double Catheter" Cannulation of Mesenteric Lymphatics

Changes in lymph flow are ultimately determined by factors that affect either (or both) lymph formation and lymph propulsion. The evaluation of lymphatic pumping activity therefore necessitates a system that would distinguish between the activities of these determinants. The "double catheter" preparation is a modification of an in vivo system developed by Reddy and Staub (1981) and McHale and Thornbury (1984b, 1986) which dissociates lymph

propulsion from lymph formation and thus enables the study of lymphatic pumping activity under conditions where lymph formation may also contribute to flow changes.

Sheep were prepared and the main mesenteric efferent vessel identified as described earlier. A 10 - 15 cm. length of mesenteric lymphatic was selected and cannulated using two polyvinyl catheters; one placed proximally in the direction of flow and the other inserted distally against the direction of flow (fig 1). This segment of lymphatic was then "isolated" from lymph input by ligating all lymph tributaries with 4-0 silk ligatures (Davis and Geck).

Surgical dissection was kept to a minimum in order to preserve local blood and nerve supply to the vessel. Both catheters were externalized and the abdomen closed in layers using #1 Dexon (Davis and Geck).

The proximal-inflow catheter was connected to a reservoir of sterile Ringer's Lactate solution (Travenol Canada Inc.) which provided the only source of fluid input to the vessel segment. The distal-outflow catheter was positioned such that the catheter tip was at exactly the same level as the reservoir fluid level. This ensured that no driving force or hydrostatic gradient was applied to the system (Pi=Po).

A transmural distending pressure (Pt) was applied to the vessel simply by elevating both reservoir and outflow catheter equally above the lymphatic segment. The transmural

distending pressure was increased to a level where the vessel was triggered to contract. As the vessel contracted, fluid was driven in the direction permitted by its valves and out the outflow catheter. As the vessel relaxed, fluid was drawn from the reservoir under conditions where the transmural distending pressure remained constant.

To ensure complete isolation of the vessel segment from extraneous lymph input, the reservoir stopcock was turned off and inspection for persistant flow was made. The failure for flow to stop within 10 minutes signified that a draining lymphatic tributary was left untied and the preparation was not utilized. A check for extraneous lymph input was made before and after each study.

The features of this in vivo preparation can be summarized in that a 10-15 cm segment of lymphatic vessel is completely isolated from lymph input, leaving its nerve and blood supply intact, and where a constant fluid distending pressure is applied. In the absence of a hydrostatic gradient, the only way flow is generated through this system is when the lymphatic vessel actively contracts.

It is important to emphasize the difference between the "single" and the "double" catheter preparations. The "double catheter" preparation permits the measurement of fluid propulsion (lymphatic contractile activity) in the absence of lymph input and is distinguished from the "single catheter" preparation which permits the measurement of lymph flow.

Determination of Lymphatic Pressure and Flow Rates

The outflow catheter from the "single catheter" preparation or from the "double catheter" preparation was connected to a siliconized plexiglass T-piece apparatus which allowed accurate measurements of flow and permitted simultaneous measurements of lymphatic pressures to be recorded (fig 2). The side arm of the T-piece was affixed to a Beckman transducer (model 4-327) coupled to a Beckman dynograph recorder (model R511-A). A 10 cm cannula of slightly smaller diameter than the outflow catheter was connected to the remaining limb of the T-piece. The cannula provided a small arbitrary resistance to flow enabling lymphatic pressures to reflect back and be recorded by the side arm transducer.

The cannula was positioned onto the arm of an isometric tension transducer (Gould Statham Model UC#3) which was coupled to a separate channel on the Beckman dynograph recorder. As a drop of fluid formed on the transducer arm, an increase in tension was recorded. The slope of the tension line reflected flow rate. As the drop enlarged, it fell off the transducer arm and the recording was automatically reset. Flow was calculated using an on-line computer (Medac S100, CPM) and expressed as microlitres per minute (mcl/min).

Measurements of Blood Pressure and Pulse Rate

Blood pressure and pulse rates were obtained by cannulating the right carotid artery using a standard cutdown procedure. The catheter was connected to a pressure transducer (Sanborne 267B) and preamplifier (Hewlett Packard 8805A) coupled to a stripchart recorder (Hewlett Packard 7706). Mean arterial pressures (MAP) were calculated using the formula:

MAP = diastolic pressure + 1/3(systolic - diastolic pressure)

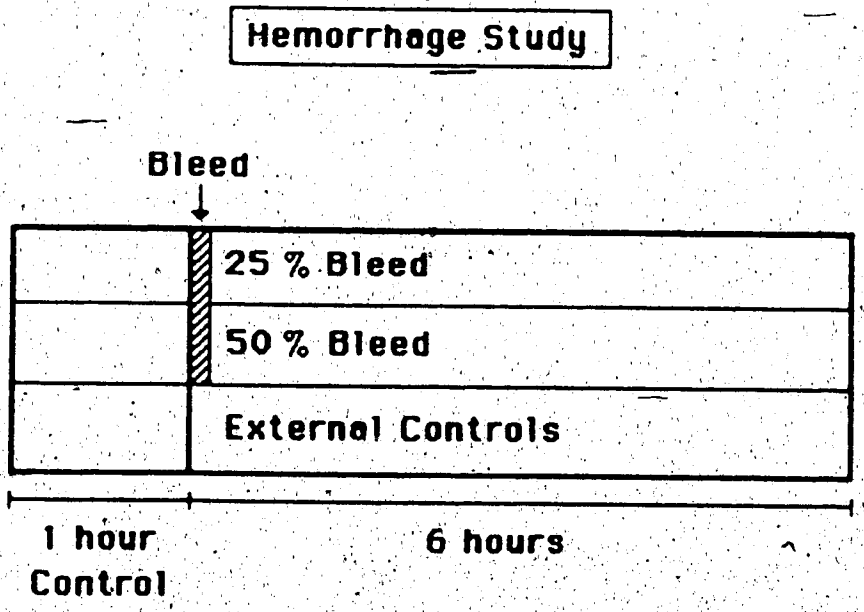
In addition, the right external jugular vein was cannulated using the same incision. All drugs and medications were administered through this line.

Experimental Protocol

In some animals that underwent the "double catheter" procedure, the myogenic properties of the "isolated" vessel were studied. With the sheep in the decubitus position, the isolated vessel lay anatomically close to the vertebral column. Zero transmural distending pressure was therefore arbitrarily chosen to be at the level of the spinous processes. By maintaining the driving pressure (hydrostatic gradient) equal to zero, the transmural distending pressure was varied by 5 cm increments beginning from the spine (Pt=0). After a short period of time was allowed for the vessel to adjust to each new level of distending pressure, contractile activity and fluid output were recorded over the subsequent 10 minutes.

Hemorrhage Study Group

The animals in this study either underwent "single catheter" mesenteric lymphatic cannulation for lymph flow monitoring or "double catheter" cannulation for fluid propulsion determination. Sheep from both preparations were then allocated into 1 of 3 subgroups: 25% hemorrhage, 50% hemorrhage, or an external control group. The experimental protocol is illustrated schematically below.



In the "single catheter" group, lymph flow and lymphatic pressures were monitored for a 60 minute prehemorrhage control period after which blood was withdrawn from the carotid line. Assuming a sheep's total blood volume to be 70 cc/kg (Halmagyi and Gillett, 1967), either 25% (17.5 cc/kg), 50% (35 cc/kg) or 0% (0 cc/kg) of blood was

removed over 7-10 minutes. Lymphatic parameters (lymph flow and lymphatic pressures) were recorded continuously for the next 75 minutes, then for the first 15 minutes of each hour thenceforth.

Blood pressure and pulse rates were recorded every 15 minutes during the control period, immediately after completion of blood withdrawl, every 15 minutes during the first hour of hemorrhage hour, and hourly thereafter. The study was terminated 6 hours after hemorrhage or upon the animal's death.

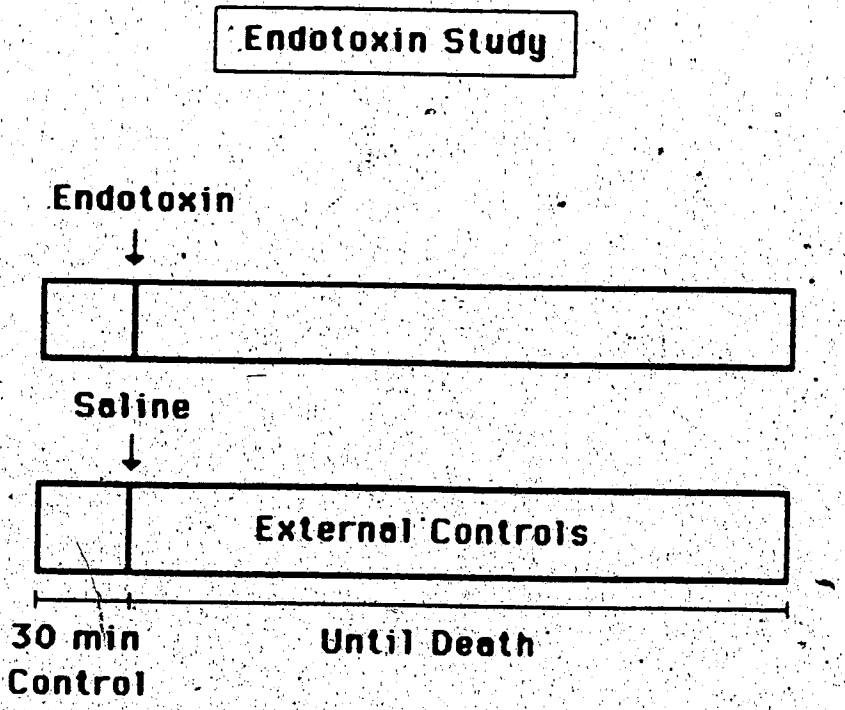
Applying the same hemorrhage protocol to the "double catheter" group, fluid output and lymphatic pressures were continuously monitored during the control period and for the entire 6 hour study period after blood withdrawl. Hemodynamic parameters were recorded every 15 minutes during the control period, immediately after blood withdrawl, and every 15 minutes thereafter.

Endotoxin Study Group

Sheep from both the "single catheter" and "double catheter" preparations were used for this study. Sheep from the "single catheter" preparation were placed into 2 subgroups: those that received intravenous E. coli endotoxin (3.3 mcg/kg, Difco 055:B5, Detroit) and those that received intravenous sterile saline (10 cc of 0.9% NaCl Solution, Travenol Canada Inc.).

Similarily, sheep from the "double catheter" preparation were allocated into a group receiving intravenous endotoxin (3.3 mcg/kg) and those receiving sterile saline (10 cc).

The experimental protocol is schematically represented below.



Lymphatic parameters (lymph or fluid output and lymphatic pressures) were monitored for a 30 minute control period and continuously for 60 minutes after the test drug was injected. Blood pressures were taken every 10 minutes during both the control and study periods.

In both the hemorrhage and endotoxin study groups no attempt was made to resuscitate the animals with blood or intravenous fluids regardless of their hemodynamic status. Each animal was internally controlled (comparisons of flow changes with flows determined during control periods) and were compared with external control animals (sheep that underwent catheter placement but were not bled or injected with endotoxin). The data were assessed using analysis of variance techniques.

RESULTS

Pressure - Flow Recordings from Catheter Preparations

Lymphatic pressures and fluid output have been measured in over 75 sheep after performing either the "single" or the "double" catheter cannulation procedure. A representative example of a pressure-flow recording from each preparation under basal conditions is illustrated in figures 3 and 4. In each figure, the top trace depicts lymphatic pressure fluctuations while the bottom trace is the simultaneous recording of fluid deposition from the outflow catheter onto the transducer arm.

Measurements of Lymph Flow and Pressure from "Single" Catheter Preparations

The recordings from the "single" catheter preparation in figure 3 are representative examples of lymph flow and corresponding lymphatic pressure measurements. In every animal tested, lymphatic pressures were pulsatile with a discernable frequency and amplitude. Pulse pressures were fairly regular in frequency and distributed along a steady baseline. Upon close inspection, the pulse pressure pattern itself was not a smooth curve but a complex of smaller, coarser pressure spikes. There was considerable variation between sheep in the frequency and amplitude of these pulsations.

Similarily, lymph flow showed considerable differences in rate between sheep. In addition, under basal conditions, lymph flow often exhibited minute to minute variations in rate, however, over the course of longer time intervals, lymph flow rate was relatively steady.

In 8 sheep, mean lymph flow and average pulsatile frequencies from the main mesenteric duct or its smaller tributaries were calculated over a one hour period. The results in Table II show average lymph flow was 965.5 microlitres per hour (mcl/hr) while contractile frequency was 9.8 pulses per minute.

There generally appeared to be a positive relationship between the rate of lymph flow and the frequency of pressure pulses. Where pulse pressures were frequent, lymph flow rates were increased, and vice versa. An attempt was made to specifically relate pulse pressure frequency and amplitudes to lymph flow, but the pressure patterns were too complex and precluded quantitative analysis.

In our earlier experiments sheep were not intubated, and occasionally they would vomit and aspirate copious amounts of gastric contents. It was of interest that immediately upon aspiration, a profound depression in contractile activity and lymph flow consistently occurred. An example of this observation is illustrated in figure 5.

Table II

Comparison of basal fluid output and contractile frequency in "Single" and "Double" catheter preparations

	Single Catheter (n=8)	Double Catheter (n=12)
Average Basal Flow (mcl/hr)	965.5	1695.1
Standard Error	376.0	405.6
Range	109.0-3484.9	279.0-3209.0
Average Contractile Frequency (pulses/min)	9.8	14.0
Standard Error	2.0	1.3
Range	5.8-11.9	10.5-17.0

Measurements of Pressure and Flow from "Double" Catheter Preparations

The successful application of the "double" catheter preparation in our experiments requires that several conditions must first be met:

(1) The isolated vessel must demonstrate propulsive activity

(2) The vessel must be completely isolated from lymph input

(3) No hydrostatic gradient can be applied to the system

First, in the absence of a hydrostatic gradient, the "isolated" lymphatic vessel had to be capable of pumping fluid. Failure to demonstrate intrinsic propulsive activity by the lymphatic vessel preparation was encountered more frequently in our earlier studies and likely reflected our early inexperience with the procedure, and our failure to minimize dissection and surgical trauma around the vessel segment. Second, it was imperative to ensure that the lymphatic segment was completely isolated from all lymph input. This was confirmed by turning the stopcock of the inflow reservoir off and watching for fluid output from the outflow catheter to stop (fig 6). In successful preparations, flow usually stopped quickly, often within the first 2-3 minutes. Lymphatic pressures however, continued to be pulsatile but remained nonproductive until the stopcock was turned back on. Unsuccessful preparations, in addition to demonstrating persistant flow with the stopcock off, also showed evidence of cloudy lymph in the expelled fluid. Third, it was necessary to ascertain that no hydrostatic gradient was present. The application of even a slight hydrostatic gradient could potentially drive fluid through the vessel segment despite the absence of intrinsic pumping activity. With the verification of these conditions, flow from this system can only be generated as a result of lymphatic contractile activity (lymphatic pumping).

Failure to satisfy the above criteria led to the rejection of 7 preparations with an overall success rate of about 80% (Table III). In addition, one preparation was rejected because a "leak" in the system developed while monitoring. This was discovered when the volume of fluid in the reservoir fell far more rapidly than the measured flow from the outflow catheter. What presumably occurred was that one of the catheters became dislodged and either pulled out of the vessel segment or punctured the vessel wall.

Table III

Unsuccessful "Double" catheter preparations

Total	8
Failure to demonstrate propulsive activity	5
Continued lymph input	2
Developed leak in the system	1

Using the same pressure-flow monitoring system employed previously, flow rate and pressure patterns were simultaneously recorded from acceptable "double catheter" preparations (fig 4). In all such preparations, the pressure patterns were pulsatile with frequency, amplitude and pressure pulse waveforms resembling those found from the "single catheter" preparations. There was good correlation between contractile frequency and flow rate. The arrows in figure 4 demonstrates each pressure pulse to be coincident

with the stepwise addition of fluid onto the transducer arm. In addition, no flow was generated during intervals between each contractile event.

Flow rate (rate of fluid propulsion) and pulse pressure frequency were recorded for a one hour period in 12 sheep under resting conditions. The mean values are shown in Table II. While both frequency and fluid propulsion remained relatively constant in any given preparation, there was considerable variation between animals. The average flow was 1695 mcl/hr and contractile frequency was 14 pulses per minute. These values were similar to those found in the "single catheter" preparations.

The possibility that respiratory movement could give rise to the observed pressure pulses and fluid output was entertained. In each study, we carefully looked for any relationship between extrinsic forces and the generation of lymphatic pressures and fluid propulsion. Since the animals were anaesthetized, respiration and vascular pulsations were the predominant forces of concern. Resting heart rate and respirations were found to be several times greater than the average contractile frequency measured. No correlation was observed between fluid output and respiration or heart rate since no deflections on the pressure or the flow trace were consistent in time with either of these forces.

Occasionally some animals would give an abrupt, vigorous cough. This was recorded as a brief spike on the pressure

trace and was coincident with the outflow of one or two drops of fluid onto the transducer arm (fig 7). For a brief period after this event, fluid propulsion was decreased, which most likely reflected the time required for the vessel to refill with reservoir fluid. The overall contribution to flow was insignificant.

The rapid injection of 8 mg/kg of pentobarbital (twice the normal maintenance dose) often had immediate effects on lymphatic contractile activity (fig 8). However, in sheep sacrificed with an overdose of pentobarbital, contractile activity and fluid propulsion continued at the same rate for periods of up to 45 minutes after the heart had stopped beating.

The Effect of Varying Transmural Distending Pressure

Due to the nature of the "double catheter" preparation, it was not possible to visualize the isolated vessel while monitoring; however with the animal lying decubitus, the vessel preparation was observed (intraoperatively) to lie along the horizontal plane of the vertebral column. Zero transmural pressure was thus arbitrarily designated to be at the level of the spine. After ensuring that the conditions for utilizing the "double catheter" preparation were met, transmural pressure was increased in 5 cm increments beginning at the spine and the effects on contractile activity and fluid propulsion were studied.

At increasing levels of distending pressure, contractile

frequency and force (reflected by pulse pressure amplitude) showed in general, a progressive increase. An example of the pressure-flow trace obtained in one such experiment where transmural pressure was elevated from 0 to 25 cm H_2O is shown in figure 9. In this study, fluid output was 8.0 mcl/min when transmural pressure was zero. Flow increased to 164.0 mcl/min when distending pressure was 15 cm H_2O, but as distending pressure was increased further, flow declined.

From these results a transmural pressure-flow curve was generated and a composite graph of 4 such experiments is illustrated in figure 10. Although the absolute flow rates from each preparation were diverse, the general pattern was similar. When transmural pressure was zero, flow was equal to or less than 8 mcl/min in all preparations. As distending pressure was increased, flow increased to a maximum then declined. Peak flow averaged 164.3 +/- 34.8 mcl/min at an average transmural pressure of 16.25 cm H_2O. The mean results of these 4 studies are shown in figure 11.

Effects of Shock on Lymph Flow and Fluid Propulsion

The effects of hemorrhagic and endotoxin shock on lymph flow and fluid propulsion were studied in a total of 58 sheep. The numerical breakdown for each subgroup is listed in Table IV.

For all hemorrhage and endotoxin studies utilizing the "double catheter" preparation, it was important that the transmural distending pressure be kept constant throughout the entire study period. We elected to use a standard

transmural distending pressure. From the data above, a submaximal distending pressure of 10 cm H_2O was chosen.

Table IV

Distribution of animals in the Hemorrhage and Endotoxin groups utilizing the "Single" and "Double" catheter preparations.

Total Sheep Studied	58				
"Single" Catheter Gp	23	Hemorrhage Gp	13	Endotoxin Gp	10
		25% Bleed	5	3.3 mcg/kg	4
		50% Bleed	5	Ext Contr	6
		Ext Contr	3		
"Double" Catheter Gp	35	Hemorrhage Gp	23	Endotoxin Gp	12
		25% Bleed	8	3.3 mcg/kg	5
		50% Bleed	4	Ext Contr	5
		Ext Contr	5	Failures	2
		Failures	6		

Effects of 25% Hemorrhage on Lymph Flow

Baseline blood pressure, heart rate and lymph flow rates were determined in each experiment for the initial one hour control period. After 25% of the animal's blood volume was withdrawn, abrupt changes in all parameters were observed. Figure 12 depicts the changes in lymph flow and blood pressure following hemorrhage in one experiment.

All 5 sheep survived the prescribed blood loss. In each experiment, blood pressure, heart rate and lymph flow changes were expressed as a percentage of baseline control. The results are illustrated collectively in figure 13.

Mean arterial pressure (MAP) fell precipitously to below 50% of control after hemorrhage, but recovered to 80% of control by the first hour and was finally restored by the third posthemorrhage hour. Similarily, heart rate dropped in concert with MAP to approximately 75% of control rate, but eventually returned to baseline by the end of the first hour.

In these 5 sheep, mesenteric lymph flow (utilizing the "single catheter" preparation) showed an increase in rate after hemorrhage. Peak rates averaged 365% of control but ranged between 146% and 1253% of control rate. In 2 sheep, lymph flow increased bimodally with maximal flow occurring during the first and fourth hours after blood withdrawl. In other experiments however, lymph flow increased along different time courses. Such variability contributed to the calculation of large standard errors at several time intervals on the graph.

In comparison, figure 14 illustrates lymph flow and blood pressures from 3 external control sheep. These animals were cannulated in the same fashion but not bled. Using the first hour of monitoring to determine baseline values, both lymphatic and hemodynamic parameters displayed little

variation from control over the following 6 hour study period.

Effects of 25% Hemorrhage on Fluid Propulsion

Baseline blood pressure, heart rate and fluid output from lymphatic propulsive activity (using the double catheter preparation) were determined during the initial control period in each sheep; after which 25% of the animal's blood volume was removed.

One animal died at 160 minutes from the profound effects of shock. The lymphatic response was initially increased with a flow rate of 196% of control, but by 60 minutes, pumping activity became depressed and remained less than 5% of control until the animal's death. One other animal died near the completion of the study period from an inadvertent intraarterial injection of pentobarbital.

In the 8 surviving sheep, the responses of blood pressure and heart rate were identical to those found in the previous study group when bled 25% of blood volume. Figure 15 illustrates the effects of 25% hemorrhage on fluid propulsion and blood pressure in one experiment. In this example flow rate during the control period averaged 84.3 mcl/min however after hemorrhage, propulsive activity increased fluid output almost instantaneously to near 200 mcl/min. Flow rates in other studies tended to increase along markedly different time courses as shown in 3 representative examples (fig 16).

Fluid output for each hour period after blood withdrawl was calculated and expressed as a percent of the prehemorrhage control in 8 sheep studies. The collective data is represented in figure 17 and table V. The volume of fluid pumped was better than 120% of the prehemorrhage control during the first 5 hours and more than 260% of control by the sixth hour.

This was quite different when compared with the average fluid output from an external control group consisting of 5 sheep (fig 17 and table V). In this group, sheep were anaesthetized and underwent the same surgical instrumentation as in the hemorrhaged group but were not bled. During the initial control hour, flow was steady but fell to 60% of control over the next 2 hours and gradually declined to approximately 40% of control over the final 4 hours of the study.

The differences between the hemorrhage and the external control groups were significant using an analysis of variance ($p<0.01$). The unpaired T test showed statistical differences at 1,4,5 ($p<0.025$) and 6 hour intervals ($p<0.05$).

Figure 17 also expresses the rate of fluid propulsion in these 2 groups as a ratio (hemorrhage/external control) for each one hour time period. It can be seen that the hemorrhage group pumped fluid at twice the rate during the first two hours and 6 times the external control rate by the sixth hour.

Table V

Comparisons of fluid propulsion in a group of animals bled 25% of blood volume and in an external control group.

Time (hrs)	1	2	3	4	5	6
Flow (% Control)						
25% Bleed (n=8)	143.4	120.8	129.8	157.7	169.2	263.8
standard error	27.6	26.5	45.2	40.2	44.3	95.5
Ext. Contr (n=5)	64.5	62.7	44.8	43.6	44.1	43.6
standard error	10.0	19.5	23.5	22.5	22.1	17.9
Unpaired T test	$p<0.025$	0.1	0.1	0.025	0.025	0.05
Ratio (Hem/Contr)	2.22	1.92	2.89	3.61	3.83	6.05

Lymphatic Pressure Changes Following Hemorrhage

Hemorrhage stimulated lymphatic contractile activity. Lymphatic pressures recorded from the "double catheter" preparation showed pulse pressure frequency and amplitude to increase concomitant with flow after a 25% hemorrhage (fig 18). Similar findings were observed from "single catheter" preparations.

Effects of 50% Hemorrhage on Lymph Flow and Fluid Propulsion

No animal survived the profound effects of shock when bled 50% of total blood volume. The duration of survival was short, and ranged from 26 to 120 minutes. Data collection for this reason was abbreviated (fig 19).

Lymph flow from 5 sheep showed a general decline in rate. In 2 animals, an initial transient increase above control was noted before lymph flow decreased. In one animal, lymph flow remained above control rate for the brief but entire length of the study. Lymph flow from another animal transiently rose above control just prior to death.

Fluid propulsion recorded from 4 other animals showed variable results when bled 50% of blood volume. Lymphatic pumping was initially above control rates in 3 sheep but in 2 of these 3 animals, flow gradually dropped below control prior to death. Fluid propulsion was initially 50% of control in one animal but reached control rates at the time of death.

Effects of Endotoxin (3.3 mcg/kg) on Mesenteric Lymph Flow

The rapid injection of intravenous endotoxin (3.3 mcg/kg) resulted in a dramatic increase in lymph flow in all 4 sheep studied (fig 20). Flow rates peaked temporally between 30 and 40 minutes in all but one study. Peak flow rates ranged between 120 and 277 mcl/min with an average peak flow of 192.3 +/- 42 mcl/min. Near the end of each study, lymph from the outflow catheter was observed to become sanguinous.

When lymph flow was expressed as a percentage of baseline control rates; the collective results of 4 sheep experiments could be illustrated in figure 23. Lymph flow attained an averaged maximal rate of 644% of control at 40 minutes, after which flow began to decline.

In contrast, lymph flow from 6 external control sheep showed little deviation from basal flow rates throughout the entire study period. The difference between these two groups was significant by analysis of variance ($p<0.025$).

Effects of Endotoxin (3.3 mcg/kg) on Fluid Propulsion

Utilizing the "double catheter" preparation, all 5 sheep injected intravenously with endotoxin (3.3 mcg/kg) demonstrated a rapid fall in fluid propulsion (fig 21). The decline in fluid output began 5 - 10 minutes after the administration of endotoxin and continued until the end of the study. Figure 22 illustrates the effects of endotoxin on lymphatic contractile activity in one experiment. Both

frequency and amplitude of the pulse pressures showed marked suppression. Concomittant with this, fluid output progressively decreased.

Figure 23 compares fluid propulsion in 5 sheep injected with endotoxin with 5 external control sheep. The external control sheep underwent the "double catheter" procedure as the endotoxin group but were injected with 10cc of sterile saline instead of endotoxin. In sheep injected with endotoxin, fluid propulsion rapidly dropped to 6.9 +/- 3.5% of baseline control by 50 minutes. Fluid propulsion in the external control group also declined, but to 46.3 +/- 11% of control during the same time interval. The difference in flow rates between these two groups was statistically significant using an analysis of variance ($p<0.01$).

During one "double" cannulation procedure, a "single" catheter was used to cannulate an adjacent lymphatic tributary such that in one animal, both fluid propulsion and lymph flow could similtaneously be monitored. The results of this study mirror the above findings and are shown in figure 24. After endotoxin (3.3 mcg/kg) was injected, lymph flow peaked at 442.3% of control at 40 minutes. In contrast, fluid propulsion fell to below 10% of control by 50 minutes.

No animal survived following the intravenous administration of endotoxin (mortality 100%). Death occurred secondary to pulmonary edema.

DISCUSSION

Experimental Design and Procedure

Several aspects of the experimental design will be discussed under the following subheadings: animal selection and preparation, surgical (technical) considerations and evaluation of the experimental model.

Animal Selection and Preparation

Sheep were selected for our studies. These relatively docile animals were easy to work with and were sufficiently large enough in size to facilitate surgical instrumentation. Halmagyi and Gillett (1967) have reported that the sheep is an excellent model for shock studies. Both ventilatory and metabolic responses during shock share more similarities with man than do other animal species. Because of this, a number of authors including ourselves have used sheep as an experimental model.

Since sheep are ruminant animals, we anticipated a significant incidence of vomiting with aspiration during our experiments. In earlier studies, sheep not intubated occasionally vomited and died shortly afterwards from aspiration. In these animals, the almost immediate suppression of lymph flow and pulsatile lymphatic contractions was an interesting and characteristic feature.

Prior to surgery, food was withheld for 48 hours and both food and water restricted 24 hours preoperatively. After the induction of anaesthesia, all sheep were intubated in attempts to protect their airway from this complication.

The use of anaesthesia during these experiments was an important consideration. Schad and Brechtelsbauer (1977) found that both halothane and pentobarbital reduced Thoracic duct lymph flow by 50% in dogs. Whitwam et al (1984) similarily found that 1% halothane decreased Thoracic duct lymph by as much as 59%. Of the several explanations proposed for these observations, suppression of intrinsic lymphatic pumping was a suggested mechanism (Schad and Brechtelsbauer, 1977; Whitwam et al, 1984). Halothane anaesthesia also requires the use of a ventilator which may in itself affect lymph flow rates and adds yet another variable in studies using general anaesthesia (Browse et al, 1974; Woolverton et al, 1978).

Because of the delicate nature of the cannulation procedure, and the frequency with which these catheters became dislodged while sheep were allowed to awaken in their metabolism cages, we elected to use anaesthesia. In order to minimize the effects of anaesthesia we avoided the use of halothane and assisted ventilation. Rather, pentobarbital was used and administered throughout the study as sparingly as possible to just maintain a level of "light" anaesthesia.

In our studies, it was quite likely that the failure of

some of the "double catheter" preparations to exhibit pumping activity was due to "anaesthetic effects". Bolus injections of pentobarbital given rapidly often had immediate effects on lymphatic pumping activity.

The cumulative effects of prolonged anaesthesia on lymph contractions are not known. The gradual decline in pumping activity in the external control group of our study may be the result of the chronic effects of anaesthesia. Most investigators, including ourselves, would agree that experiments performed on conscious animals would be preferable. As we have become technically proficient, the need for anaesthesia has become less and currently we are now conducting experiments on conscious sheep.

E. coli endotoxin was the agent selected to simulate the conditions of septic shock. Some have argued that neither endotoxin nor live bacterial infusions will completely reproduce the characteristic high flow, low systemic resistance state typical of early human sepsis (Avila et al, 1985). However, Mathison and Ulevitch (1983) claim that several experimental studies have established that endotoxin, and more specifically its Lipid A component, is able to induce most of the pathophysiologic changes observed in septic shock. Observed differences between clinical sepsis and animal models were likely due to species differences and also the fact that most investigators use relatively large doses of endotoxin which ultimately result in short survival

spans. In addition, the natural presentation of clinical sepsis is more often masked by aggressive fluid and antibiotic management such that even hypotension and fever become obscured findings (Gump, 1983; Borzotta and Polk, 1983).

The use of endotoxin has several advantages. A known quantity in a single intravenous injection may be given. Since its effects are reproducible, its administration can therefore be standardized.

Although no animal model will completely reproduce the characteristic features of septic shock, intravenous endotoxin remains the best alternative for the purposes of our study.

Surgical Considerations

Our preference to cannulate the intestinal lymphatics was based on several points. It is commonly known that between 80-90% of total lymph return comes from the Thoracic duct (Cope and Litwin, 1962; Vrein et al, 1977). Most of Thoracic duct lymph is derived from the splanchnic bed with 70% of its volume and 60% of its protein originating from the intestinal tract (Morris, 1956). The physiologic significance of intestinal lymph return therefore becomes obvious. Anatomically, intestinal lymphatic vessels were easily visible within the mesenteric fat and would with patience, accommodate large bore catheters. The main efferent lymphatic was usually single and of acceptable

length for use in the "double catheter" procedure. It was easy to protect these preparations from heat and evaporative losses by simply placing the bowel back into the peritoneal cavity where it could remain within its natural environment.

It was important to adhere to basic surgical principles during surgery. In particular, meticulous attention to hemostasis was important because of its consequences to the study. In addition, traction on the mesenteric pedicle was avoided and dissection around the vessel was minimized to ensure preservation of local blood and nerve supply to the lymphatic.

Evaluation of the Experimental Model:

The "Double Catheter" Preparation

The "double catheter" preparation represents the only true means by which lymphatic contractile activity can be evaluated in vivo. This innovative approach has evolved from the in vitro experiments conducted by McHale and Roddie (1976). Initially, small segments of lymphatic vessel were studied by suspending them in tissue baths to measure contractile activity. But to step from this point to where one could state that the coordinated activity of lymphatic contractions could generate flow, required the development of a new model system.

McHale and Roddie (1976) showed that long segments of lymphatic vessel (8cm) when cannulated at both ends could propel fluid by contractile activity alone. In addition,

they clearly demonstrated the myogenic properties of the lymphatic vessel by showing a positive relationship between transmural distending pressure and flow. As transmural pressure was made to increase, lymphatic pumping activity increased to a maximum then decreased.

The move to in vivo studies using this concept was first reported by Reddy and Staub (1981), and later by McHale and Thornbury (1984b, 1986). Currently this laboratory is the only one to use this model system to study the pathophysiologic states of shock.

It can never be overemphasized that the measurement of lymph flow from an indwelling lymphatic catheter remains a poor method for evaluating lymphatic contractile activity. Measurements of lymph flow alone can not discriminate between changes that occur in lymph formation from those that affect lymph propulsion. This problem becomes exemplified when attempting to evaluate lymphatic contractile activity under the conditions of shock where fluid filtration becomes unsteady.

The "double catheter" preparation however, is a conceptually simple technique which isolates the lymphatic vessel from lymph input and thereby dissociates lymph propulsion from lymph formation. Lymphatic contractile activity is now assessable even in circumstances where lymph input is variable.

We were able to demonstrate that the myogenic properties of the "double catheter" preparation were in fact similar to

the in vitro preparations of McHale and Roddie (1976) and with the in vivo studies of Reddy and Staub (1981) and McHale and Thornbury (1986). Although maximal flow rates occurred at higher transmural distending pressures in our studies, our methods for establishing reference levels of distending pressure were different than those described by the investigators above.

Lymphatics and Hemorrhage

Hemorrhage is often a most devastating complication of trauma. Following hemorrhage, re-expansion of the vascular space with protein and fluid is of paramount importance. With the vast stores known to exist interstitially, many consider the interstitial compartment a potentially valuable reservoir (Lucas and Ledgerwood, 1983).

Several studies have suggested that the interstitium contributes toward the replenishment of plasma volume following hemmorrhage. Shires et al (1960, 1964a,b) found that when dogs were bled using a modified Wigger's protocol, a significant extravascular fluid deficit developed despite the return of all shed blood. Using a similar shock protocol, Hopkins et al (1964) discovered that a 20% difference in plasma volume existed between what was calculated and what actually was found experimentally. The supplemental addition of fluid to the plasma volume in these dogs was attributed to the interstitial transfer of fluid.

It is the popular conception that fluid movement from interstitium into the vascular space comes about through interactions that occur at the microcirculatory level and is predictable by Starling's equation (table I). Utilizing this theory, the destabilizing forces of hemorrhage would favor augmented capillary absorption of fluid and thereby replenish intravascular volume. While Starling's concepts remain

undisputed, Cope and Litwin (1962) have argued that several events still remained unexplained. First, they comment that the spontaneous restoration of plasma volume takes many hours, while the rapid events of microvascular fluid exchange takes place promptly over minutes. Second, the Starling effects offer no provision for the return of interstitial protein back to the circulation. Clearly, from Cope's arguments other mechanisms must also be involved.

Cope and Litwin (1962) recognized the importance of the lymphatic system for maintaining homeostasis and popularized the theory that the lymphatics may draw upon interstitial resources and play a principle role in re-expanding a diminished vascular space following a significant bleed. In their study, dogs were monitored for 24 hours following a sublethal hemorrhage (30% of blood volume withdrawn). A 2-3 fold increase in Thoracic duct flow was noted during the first post-hemorrhage hour. In addition, the amount of protein returned over the 24 hour period was twice what was initially lost from the bleed. Shaffiroff (1943) bled dogs 21-24% of blood volume and found 27 grams of protein (equivalent to 360 cc whole blood) was returned by the Thoracic duct over 27 hours.

Other investigators have also studied lymph flow following hemorrhage. Collectively, their conclusions have provided all but a clear picture. Some studies demonstrated

lymph flow to increase after bleeding (Smith et al, 1965; Berman et al, 1969; Demling et al, 1975; Todd et al, 1978; McHale and Thornbury, 1984a), while others have shown flow rates to decrease (Wessely, 1958; Alican and Hardy, 1961; Glenn and Lefer, 1970; Williams and Clermont, 1973; Hidai et al, 1968; Hayashi et al, 1979). Often an initial transient increase in flow was noted prior to a sustained fall in lymph output (Wessely, 1958; Cope and Litwin, 1962; Hopkins et al, 1964; Hayashi et al, 1979; Power and Brace, 1983). These studies however, differ significantly in methodologic approach making comparisons between each difficult, if not impossible.

Critical analysis of these studies reveals several common flaws to exist. One of the most important of which is the choice of hemorrhage protocol selected. By far the majority of the above studies have used a modification of Wigger's hemorrhage protocol. Animals were bled to a predetermined blood pressure and maintained at this level either by further bleeding or by the return of small amounts of shed blood as required. After an arbitrary period of hypotension, the shed blood was usually reinfused.

One can see immediately that this is a very unsatisfactory protocol for studying the physiologic responses to hemorrhage. With this method, the level and duration of hypotension is artificially determined; animals with superior compensatory mechanisms are therefore bled more

and are, as a result, placed at a greater disadvantage. Posthemorrhage blood pressure should not be dependent upon the experimental protocol, but on the effectiveness of the animal's compensatory mechanisms.

A far better method, employed in our experiments, is to bleed animals according to a percentage of total blood volume. Here hemorrhage becomes the primary disturbance, and hypotension the consequence. The compensatory mechanisms which effect a response to hemorrhage can then be studied.

Another criticism of these studies is the short study period used after hemorrhage. Study periods as brief as 2 hours were used by some investigators. Other experiments have combined several tests using various drugs and fluids with hemorrhage (Power and Brace, 1983). The resulting confusion as to which cause created what effect, severely detracts from the value of their conclusions.

Our experiments tend to support Cope and Litwins' original tenet. In our study, we have investigated the effects of hemorrhage on intestinal lymph flow in the anaesthetized sheep. When sheep were bled 25% of blood volume, lymph flow increased with peak flow rates averaging 365% of the prehemorrhage control. The large error bars signify only that lymph flow increased along different time courses from animal to animal. By extrapolating and applying this data to man, if lymph return increased to twice the basal flow rate, between 2-6 litres of lymph could

theoretically be returned to the plasma pool over 24 hours.

Lymph flow reflects not only the rate at which lymph is formed but also the state of lymphatic function. Since most assume that blood flow and therefore lymph formation is diminished to most "non-vital" organ systems including the gut following hemorrhage (Abel et al, 1965; Seelig et al, 1981; Rosenfeld and Cooper, 1982), the possibility arises that an increase in lymphatic pumping could mobilize interstitial stores and give rise to the observed increase in lymph flow.

It can be argued however, that the increase in lymph flow could have simply been due to microvascular changes which favored increased lymph formation. Increased transvascular filtration during hypotension could possibly occur from a change in capillary permeability (Holcroft and Trunkey, 1974; Todd et al, 1978; Michel et al, 1981) and/or from an elevation of capillary hydrostatic pressure (Pc) secondary to postcapillary venospasm (Berman et al, 1969; Demling et al, 1975, 1980; Hara et al, 1984). Because of this doubt, the significance of lymphatic pumping activity using lymph flow as a criterion, becomes obscured.

The "double catheter" preparation as described in the methods, resolves this controversy by providing conditions where the delivery of fluid to the vessel is controlled. This creates a unique opportunity to evaluate lymphatic pumping activity during shock. In our study, sheep bled 25%

of blood volume demonstrated increased lymphatic contractile activity with a resultant increase in fluid propulsion. No correlation between respiration or heart rate and fluid propulsion was observed.

Our findings of increased lymphatic contractile activity integrate nicely with other related studies. Spontaneous mesenteric lymphatic contractions have been observed to become more frequent and intense following a blood loss (Baez, 1955, 1960; Baez et al, 1957; Zweifach and Prather, 1975). Hopkinson et al (1968), using Guyton's perforated capsule technique found interstitial fluid pressure dropped from -2.03 to -6.64 mmHg after dogs were bled. These findings were confirmed by Ladegaarde and Pedersen (1970) using the "wick" technique. Since Guyton et al (1971) has emphasized the importance of the lymphatics for maintaining negative interstitial fluid pressure, these observations imply that the lymphatics can increase suction forces to obtain fluid and protein from the interstitium following a blood loss.

The severity of hemorrhage is not only an important determinant for survival but also for lymph flow and pumping activity. Animals that were bled 50% of their blood volume demonstrated only a transient increase in lymph flow, however flow usually fell well below control soon after. Similarily lymphatic pumping activity was often increased initially, but it too declined. Mortality in this group of sheep was 100%

and the study period because of this was very short. Because death ensued quickly, definite conclusions about lymphatic contractile activity were difficult to make. Likely a state of "intractable" shock was reached with this massive amount of blood loss whereby all mechanisms for survival, including lymphatic pumping were likely to become overwhelmed or fail from the profound effects of hypoperfusion.

The mechanisms which potentially regulate the lymph pump during hemorrhage are currently being explored by this laboratory. By experimental design, the transmural distending pressure used in the "double catheter" preparation was kept constant. Changes observed were therefore not regulated myogenically or dependent upon factors which influence lymph production.

Since the neurovascular supply to the lymphatic segment was intentionally preserved, both neurogenic and humoral mediators may play an important role in regulating the lymph pump. Hemorrhage classically invokes a strong sympathetic response (Chien, 1967; Bond and Johnson, 1985), it is possible then that adrenergic stimulation is important for pumping activity. Lymphatics are innervated by autonomic fibers (reviewed in the introduction) and can be directly affected by both noradrenalin and adrenalin. Florey (1927) found that locally injected adrenalin caused rat mesenteric lymphatics to vigorously contract and go into spasm. Similarily, Mislin (1967) and Mawhinney and Roddie (1973)

found noradrenalin increased contractile frequency in isolated bovine mesenteric lymphatics.

McHale and Roddie (1976) studied the effects of noradrenalin in a cannulated isolated mesenteric lymphatic preparation (in vitro). While noradrenalin invariably increased contractile frequency, fluid pumped by the vessel was increased only in those with low resting frequencies. Noradrenalin in high doses depressed flow by causing desynchronized "fibrillation-like" contractile activity. Allen et al (1983) demonstrated that in low doses (100 ng/ml), noradrenalin had positive inotropic effects. In high concentrations however, noradrenalin (10 mcg/ml) significantly disrupted lymphatic smooth muscle electrical activity causing increased action potential frequency and disruption of regular spontaneous pacemaking. Separate independent pacemakers spontaneously formed, resulting in desynchronized contractile activity with a reduction of mechanical pumping efficiency.

The effects of catecholamines on lymphatic pumping activity in vivo has recently been studied using techniques similar to the "double catheter" preparation (Reddy and Staub, 1981; McHale and Thornbury, 1984b; 1986). Intravenous infusions of noradrenalin (0.2-2.0 mcg/kg/min) caused an increase in contractile frequency and fluid propulsion but isoprenaline (1 mcg/kg/min) caused the opposite effect. In addition, McHale and Thornbury (1986) observed a

"noradrenalin-like" effect on sheep lymphatic contractions after instigating a "fright" stimulus.

Many other biochemical and endocrine factors including prostaglandins (Lefer, 1985) are released and circulate during hemorrhage. These factors may reach the lymphatic by their vasa vasorum and could potentially affect contractile activity. In addition, complex metabolic changes that result from hemorrhage (hypoxia, acidosis, hyperglycemia etc.) gives each animal a very different biochemical profile which in itself may affect lymphatic function. These issues are complex and will remain an area for active research.

In summary, hemorrhage stimulates lymphatic contractile activity independent of changes in lymph formation. It appears that despite a suspected decrease in transvascular filtration of fluid that results from hemorrhage, the lymphatics may mobilize interstitial stores of fluid and protein in efforts to restore a diminished plasma volume.

Lymphatics and Endotoxin Shock

The development of massive generalized interstitial edema remains a most alarming clinical feature encountered in the management of septic patients. Characteristically an enormous quantity of crystalloid infusion is necessary just to maintain adequate intravascular volume and tissue perfusion (Robin et al, 1972).

The distributional disturbances that occur between interstitial and intravascular fluid compartments have largely been attributed to increased microvascular permeability which allows protein rich fluid to leak from capillary into the interstitium (Tranbaugh and Lewis, 1984; Kinnebrew et al, 1982; Brigham et al, 1979; Robin et al, 1972). Endotoxin infusion, either directly or by activation of cellular or humoral factors including complement, leads to the loss of capillary endothelial integrity and a marked increase in vascular permeability (McGrath and Stewart, 1969; Heideman et al, 1979; Mathison and Ulevitch, 1983). Anas et al (1968) found that a significant increase in interstitial fluid pressure and a significant reduction in plasma volume had occurred in dogs when injected intravenously with E. Coli endotoxin (2 mg/kg). The changes in capillary permeability are seemingly reflected by a concomittant increase in lymph flow as noted by several investigators (Avila et al, 1985, Brigham et al, 1979; Alican and Hardy,

1961) and as suggested by a 7 fold increase in intestinal lymph flow in our study.

The development of interstitial edema, however, depends upon an imbalance between the extravasation of protein and fluid and the removal of these elements from the interstitium (Hamilton et al, 1986). Either the fluid load must be excessively high or the lymphatics significantly compromised before edema becomes manifest (Johnston, 1985).

There is evidence to suggest that the lymphatics can easily return fluid loads several times their usual capacity in efforts to prevent the development of interstitial edema. Several investigators have employed Starling's concepts in order to study fluid filtration and interstitial fluid dynamics. Demling et al (1982, 1980, 1979a;b), Kramer et al (1983, 1982, 1981) and Harms et al (1981) in related studies reduced plasma oncotic pressure by a variety of methods to experimentally increase transcapillary fluid filtration in sheep. Hypoproteinemia was induced by rapid plasmaphersis, fluid overloading with Ringer's Lactate, or after acute hemorrhage with crystalloid resuscitation, and the effects were assessed. While pulmonary lymph flow invariably increased several fold in these studies, it was very difficult to document an increase in interstitial lung water.

Gibson and Gaar (1970) studied the effect of varying interstitial fluid pressure on lymph flow in dogs that were

purposely made edematous. While infusing enormous amounts of crystalloid solution intravenously, interstitial fluid pressure rose from -7 mmHg to +1 mmHg and lymph flow rapidly increased up to a maximum rate of 30 times normal. Once interstitial fluid pressure became positive, edema began to develop, as lymph flow reached a plateau and did not increase further.

The ability for the lymphatics to function several times in excess of their normal duties provides a major contribution toward the maintenance of a "margin of safety" against edema formation. Thus while there is little doubt that protein and fluid extravasation is prerequisite, the compromise of the normally efficient lymphatic transport system is a factor forgotten in determining the pathogenesis of edema.

The inhibition of lymphatic pumping during endotoxin shock is an entity not recognized previous to this study. Only with the use of the "double catheter" preparation was this finding made possible. The injection of endotoxin (3.3 mcg/kg) intravenously profoundly suppressed lymphatic contractile activity in all sheep studied by this method. Although pulsatile lymphatic pressures were clearly demonstrated to become inhibited, the experimental model precluded the direct visualization of the vessel. Because a number of humoral mediators, both stimulatory and inhibitory for lymphatic contractile activity, are known to circulate

during endotoxin shock, it was thus impossible to tell whether the inhibition of lymphatic propulsion was due to diffuse vasospasm or perhaps discoordinated contractile activity from mediators which hyperstimulate or due to atonic dilatation from mediators that inhibit contractile activity. While fluid output fell significantly to less than 15% of control, lymph flow (measured from the "single" catheter preparation), reached peak rates of 6.4 times control rate during the same time interval. This dissociation was even observed in experiments where lymphatic pumping and lymph flow were simultaneously monitored in the same sheep. This was surprising since normally the lymphatic vessel responds to an increased fluid load by increasing its contractile activity. It is once again obvious that lymph flow represents only the end result of complex interactions between lymph formation and lymph propulsion and does not in itself give a true picture of the pathophysiologic changes of either determinant.

By demonstrating an increase in lymph formation and a decrease in lymphatic pumping, these studies provide a plausible mechanistic explanation for the genesis of sepsis induced edema. The marked suppression of lymphatic contractile activity could compromise lymphatic function and render this system incapable of handling the flood of extravasated protein and fluid. These elements would therefore be forced to accumulate interstitially as the rate

of lymph formation would greatly overwhelm lymph propulsion.

Although purely speculative, quite possibly this phenomena could prove beneficial for host defense. Limited foci of infection or inflammation could alter lymphatic function in the above manner to produce localized edema and allow inflammatory cells to aggregate and humoral mediators to concentrate their actions. It would be easy to predict however, that these effects would be detrimental for host survival if they were to become expressed systemically.

The factors responsible for the suppression of lymphatic contractile function (lymph pump) were not addressed in this study, however, several possibilities might be entertained. Although endotoxin is important for the simulation of shock, it does not act directly upon the lymph vessel to inhibit contractile activity (Elias and Johnston, unpublished observations).

Humoral mediators including histamine, leukotrienes, serotonin, bradykinin, and numerous arachidonate metabolites are produced in response to an inflammatory challenge (Saldeen, 1976; Johnston et al, 1979; 1980). They are found in significant amounts locally in tissues and in lymph draining inflamed sites. Each of these compounds have characteristic stimulatory or inhibitory effects on lymphatic contractions when studied in vitro (Sinzinger et al, 1984a,b; Azuma et al, 1983; Johnston et al, 1983; Ohhashi et al, 1978). Prostaglandins of the E type (PGE_1, PGE_2) have been

found to be potent inhibitors of spontaneous contractions in isolated lymphatic vessel preparations (Johnston et al, 1983). Lymphatic smooth muscle relaxation and contractile inhibition were also noted with adenosine and adenine nucleotides (ADP, ATP, AMP) (Ohhashi et al, 1978).

Endotoxin has been found to increase plasma catecholamine levels experimentally (Hardy and Alican, 1961). Catecholamines given in high concentrations have been shown to inhibit lymphatic contractility in vitro (McHale and Roddie, 1983; Allen et al, 1983).

Other factors considered important in the pathogenesis of septic shock include acidosis, hypoxia, imbalances in glucose, and calcium metabolism and numerous drugs including inotropes, steroids and antibiotics (Wilson et al, 1979; Wilson, 1985). The precise effects of this "broth" of metabolic and inflammatory mediators on the "lymph pump" is presumably complex and presently unknown.

Future Considerations

The primary intention of this study was to challenge the previous established concepts in lymphatic physiology and to focus attention on the dynamic aspects of the lymphatic system. No longer is it permissible to regard the lymphatic system as simply a passive receptacle for microvascular fluid exchange.

This is the first report where the responses of the lymph vessel to shock have been addressed. The development and implementation of the "double catheter" preparation has allowed significant progress to be made in this field of study. The unique qualities of this model now make it possible to investigate the effects of many physiologic and pathophysiologic conditions on lymphatic pumping. The potential applications in future research are bounded only by one's imagination.

One drawback with the "double catheter" preparation has been that it is not possible to visualize the isolated lymphatic segment during the course of each experiment. Only by visualizing the lymphatic's response to shock and simultaneously monitoring lymphatic pressure and flow, would an accurate interpretation of the pulse pressure recordings be possible. Such a system would provide a clearer understanding of the pumping characteristics of the lymph vessel during shock.

Despite this drawback, observations that lymph vessels play a dynamic role in shock has stimulated interest in determining the specific factor(s) that regulate lymphatic contractile activity. This problem may be approached in several different ways, however the initial objective would be to determine the relative significance of neurogenic and humorally based mechanisms.

The "double catheter" preparation would be extremely useful in this regard since it provides a unique opportunity to study both systemic and direct effects of lymph and blood borne mediators and many pharmacologic agents on lymphatic pumping. With the addition of these agents (endotoxin, catecholamines, prostaglandins, and other mediators of inflammation) to the reservoir, the direct effects on lymphatic function could be tested.

Hemorrhage stimulates, while endotoxin inhibits lymphatic contractile activity. These findings were determined using a standard transmural distending pressure of 10 cm H_2O. In future shock studies, it may be useful to define the complete profile of contractile activity over the entire range of distending pressures (0-25 cm H_2O).

At least one investigator has noted a similarity between the Transmural distending pressure - Flow curve and Starling's ventricular function curve of the heart (McHale and Roddie, 1976). The Starling curve provides valuable information for the intensivist, as it enables him to

therapeutically maximize cardiac function in the critically ill. In much the same manner it would be of interest to apply this concept experimentally to the lymphatics. Studies could be designed which would attempt to pharmacologically manipulate contractile activity in efforts to define and establish optimal lymphatic function using the Transmural distending pressure-Flow curve as a guide.

Hemorrhage and sepsis are not mutually exclusive events. The application of the "double catheter" preparation to a Hemorrhage-Sepsis model would extend and clarify our knowledge of this often lethal combination of shock.

The ultimate goal of this research is to eventually apply the acquired knowledge clinically. While our invasive techniques are quite suitable for animal studies, they remain outside of the ethical boundaries for clinical experimentation. The development of less invasive methods which accurately correlate with the experimental data, will hopefully influence future management and favorably alter the prognosis of the critically ill.

Conclusions

The lymphatic system still remains a largely unexplored area of research. This is perplexing since the lymphatics provide important homeostatic functions. Considering the profound implications of shock, the adaptive mechanisms of the lymphatic system were investigated.

This thesis represents the first time that the effects of shock on lymphatic function have been studied. Specifically, we have studied lymph propulsion (lymphatic contractile activity) in response to hemorrhagic and endotoxin shock.

The myogenic regulation of lymph propulsion is a system totally dependent upon the rate of lymph formation and volume of lymph input. While this is a perfectly adequate system for maintaining homeostasis under resting conditions, we have found this relationship no longer holds true during shock. It has been demonstrated that the lymphatics can function independent of changes in lymph formation. Hemorrhage stimulates lymphatic contractile activity despite conditions where lymph formation is presumably diminished.

In contrast, endotoxin shock inhibits lymphatic contractile activity whereas lymph formation is augmented. This maladaptive response has not been previously recognized and is likely a significant factor for the promotion of sepsis related interstitial edema.

The "double catheter" preparation has allowed us to make these observations and represents a powerful tool for the study of lymph propulsion in vivo. In addition, this model has provided the opportunity to study lymphatic contractile activity in relation to a host of physiologic and pharmacologic disturbances which, in the future, may positively influence our management of the critically ill.

Summary

From our studies we conclude that:

(1) The lymphatic vessel is intrinsically capable of pumping fluid. It responds myogenically to varying levels of distending pressure. An increase in distending pressure leads to a proportionate increase in contractile activity and a concomittant increase in flow.

(2) Under the conditions of shock, the regulation of lymphatic contractile activity is independent of lymph formation, and the events that affect fluid filtration within the microvascular beds.

(3) Hemorrhage effectively stimulates lymphatic contractile activity.

(4) Endotoxin shock significantly inhibits lymphatic contractile activity.

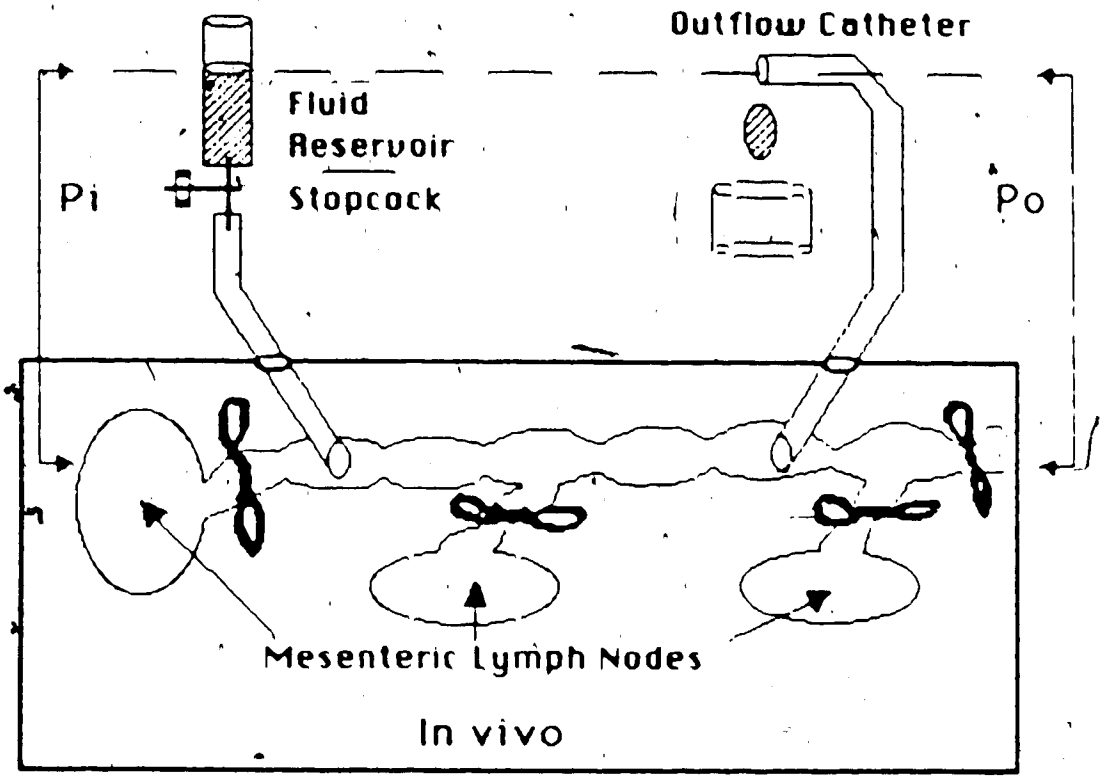


Figure 1. Schematic representation of the "Double Catheter" preparation for monitoring fluid propulsion in vivo. Pi and Po represent the inflow and outflow pressures (cm H_2O) respectively. The height of the reservoir (Pi) was set equal to the outflow catheter (Po) to ensure no hydrostatic gradient (driving pressure) was applied to the system (Pi=Po). By simply elevating both reservoir and outflow catheter equally above the vessel segment, a transmural distending pressure (cm H_2O) was applied, triggering the lymphatic vessel to contract spontaneously. In the absence of a hydrostatic gradient, flow generated from this system occurs only from lymphatic contractile activity.

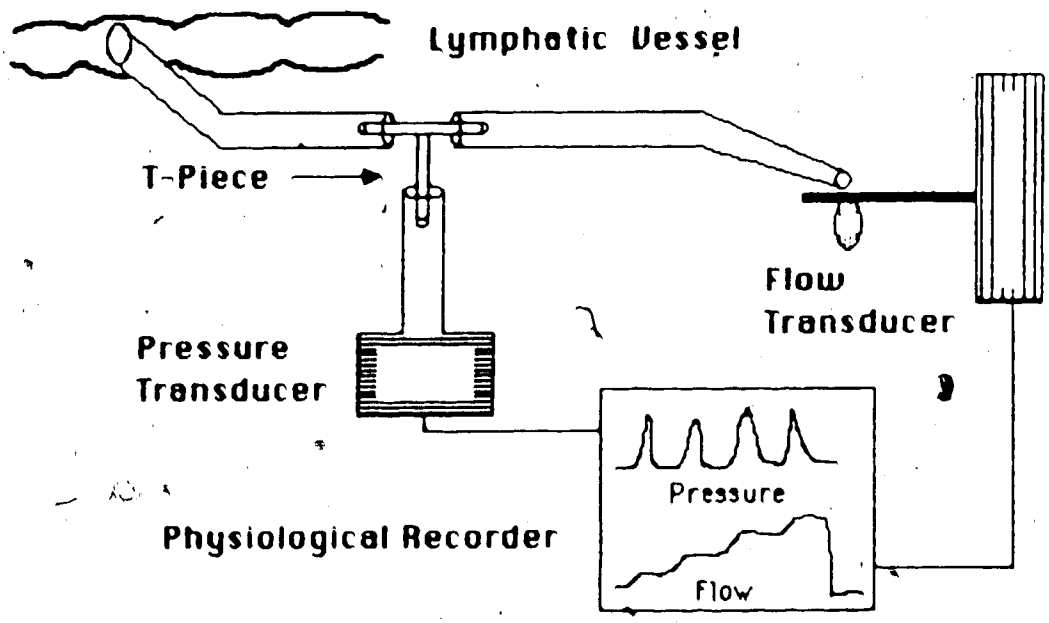


Figure 2. Schematic representation of the system used to measure lymphatic pressure, and flow rates in the anaesthetized sheep (see text for details).

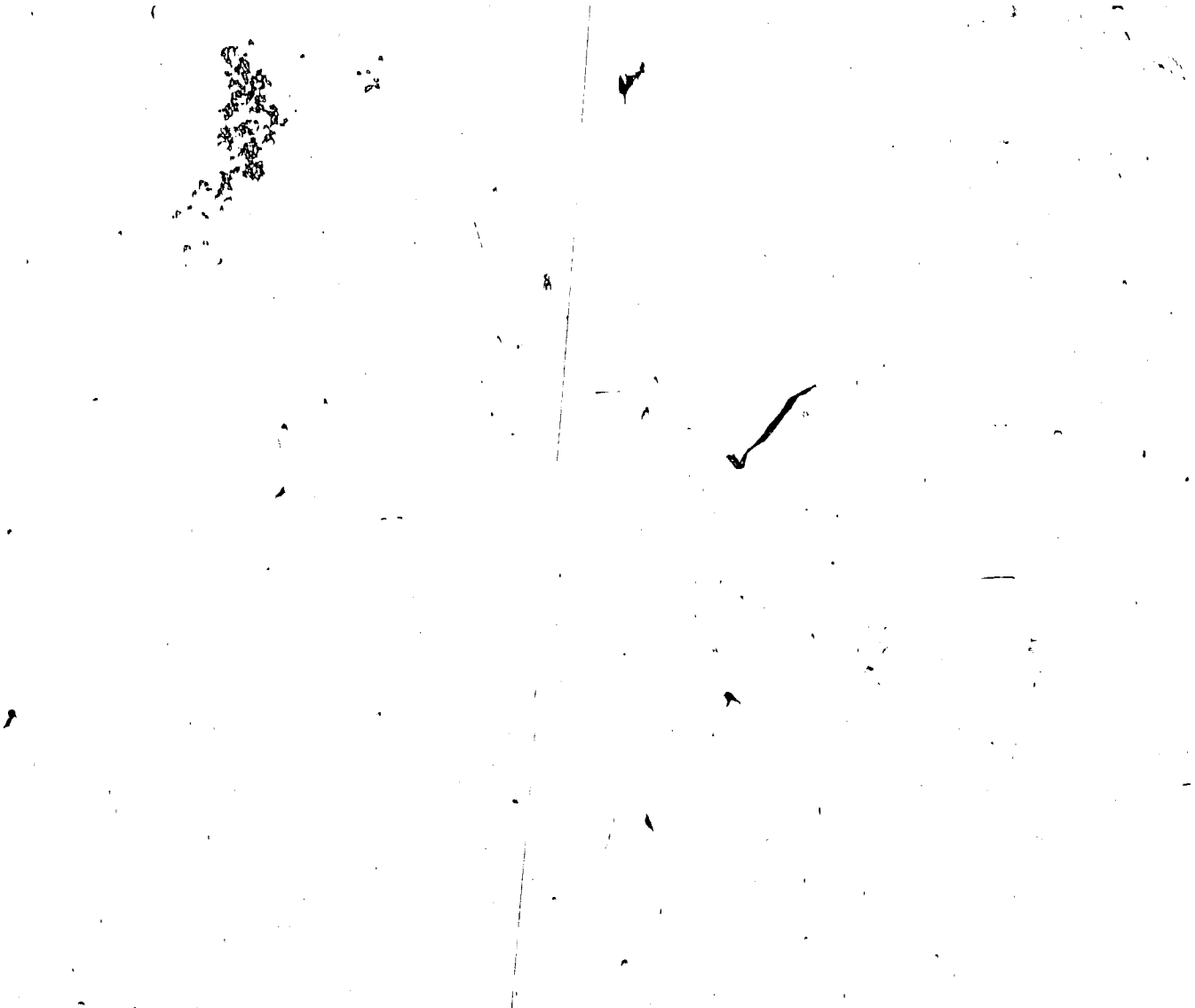
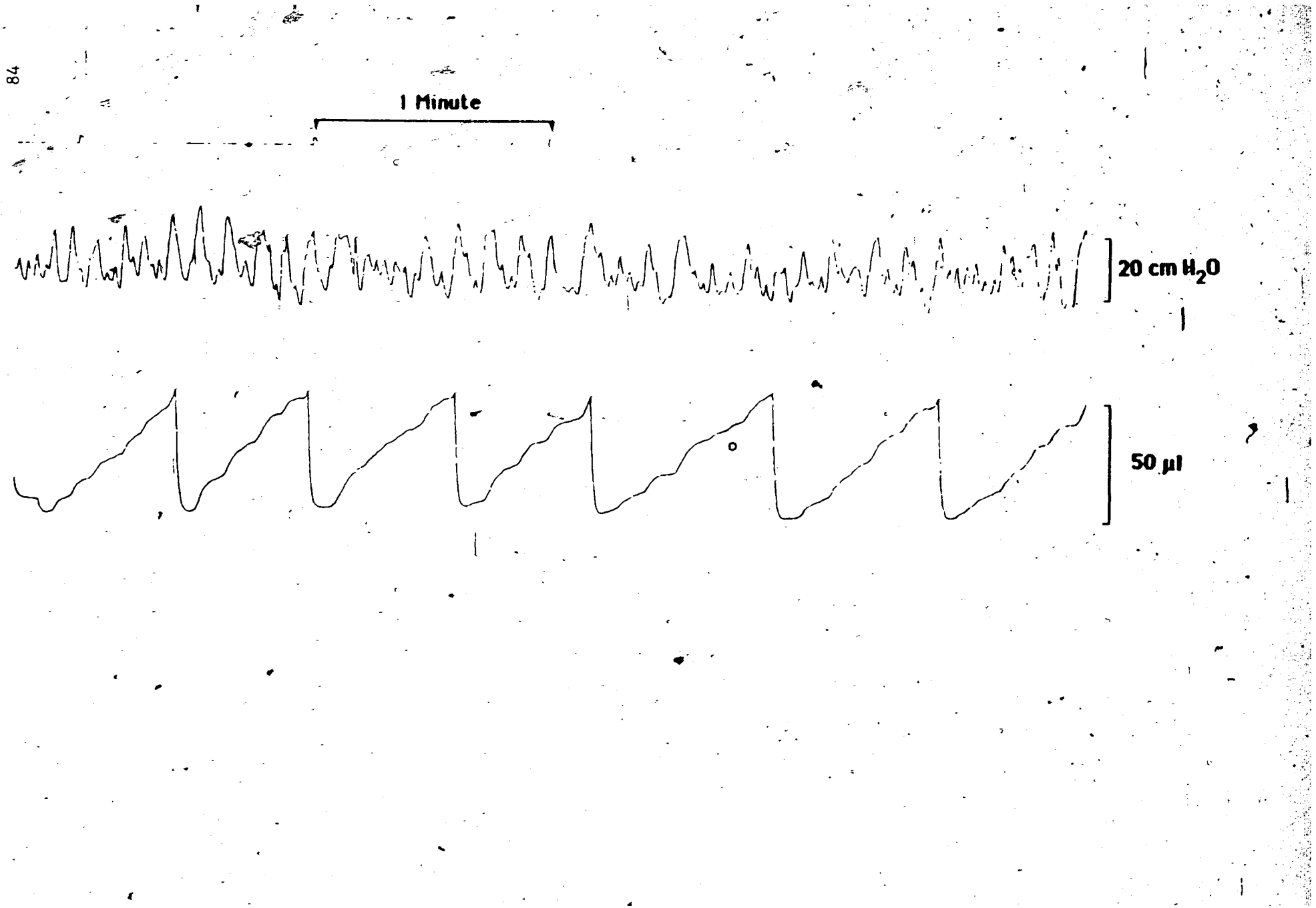


Figure 3. Example of the pressure-flow recording monitored from the main mesenteric lymphatic vessel using the "Single Catheter" preparation. The top trace depicts the pulsatile lymphatic pressures characteristically observed. The bottom trace is the simultaneous record of lymph flow rate. The slope of the flow trace represents the rate of accumulation of lymph on the arm of the flow transducer. As each drop enlarges and falls, the pen is automatically reset to record the next drop.


1 Minute
20 cm H_2O
50 µl

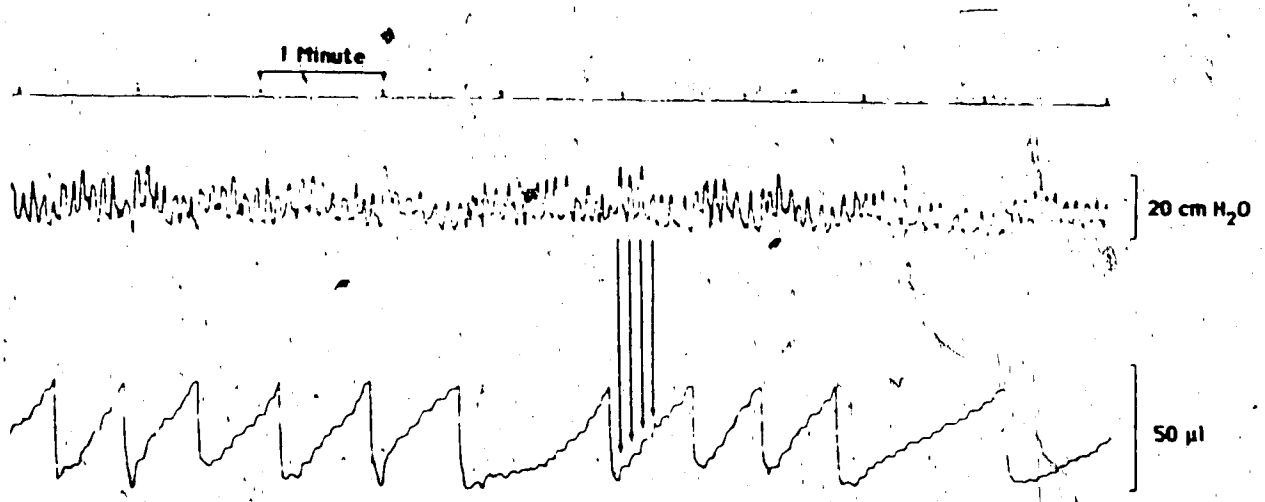


Figure 4. Example of the pressure-flow relationship monitored from an "isolated" mesenteric lymphatic vessel using the "Double Catheter" preparation. With transmural distending pressure set at 10 cm H_2O and with no hydrostatic pressure, the top trace illustrates the pulsatile lymphatic pressures obtained from this preparation. The bottom trace is the simultaneous record of fluid output. The arrows illustrate each pressure pulse to closely correspond with the addition of fluid onto the flow transducer arm, indicating that propulsion of fluid occurs by lymphatic contractions.

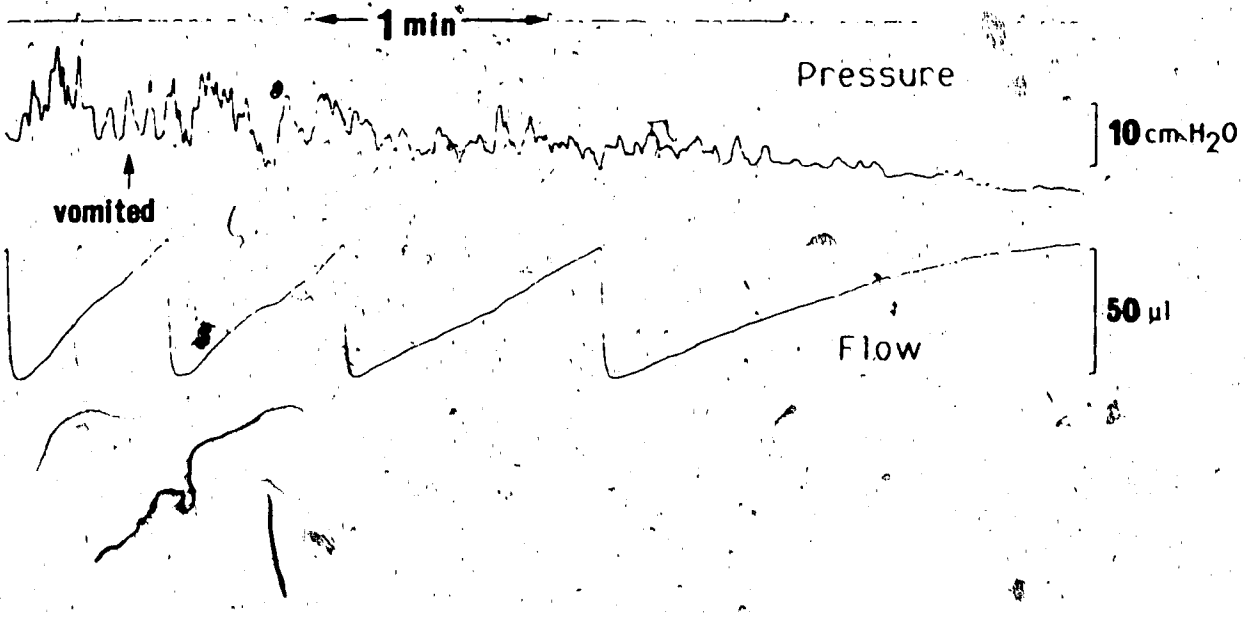


Figure 5. Effects of aspiration on lymph flow and pressure. While monitoring lymph flow and pressure from a mesenteric lymphatic using the "Single Catheter" technique, the animal vomited (arrow) and aspirated copious amounts of gastric contents. A profound depression of pulsatile lymphatic pressure and lymph flow was subsequently observed.

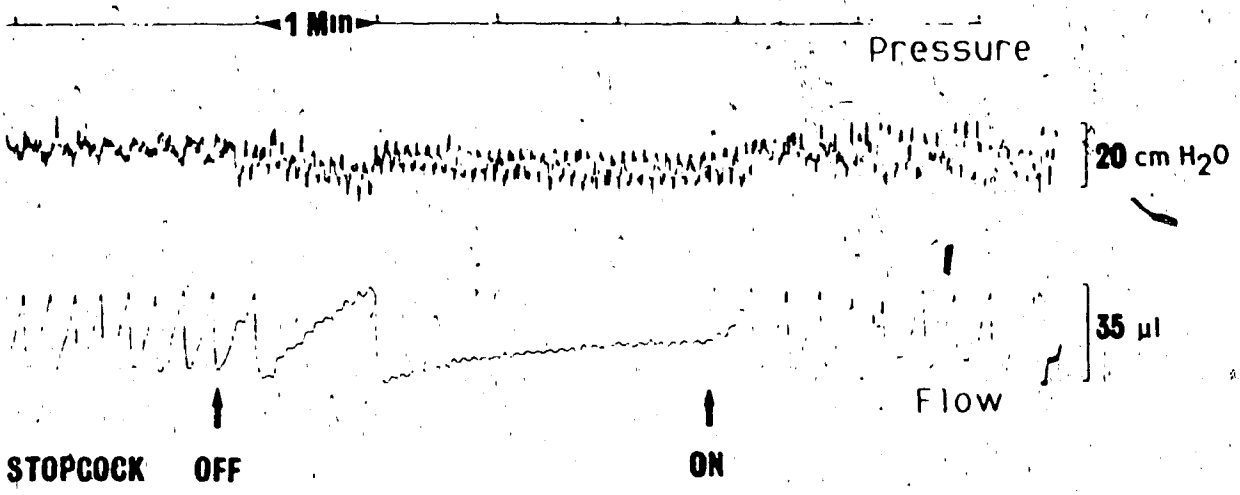


Figure 6. The "Double Catheter" Preparation. Testing for complete isolation from lymph input. After the reservoir stopcock was turned off (arrow), fluid output (bottom trace) was observed to stop within 3 minutes. Once the stopcock was turned back on, flow gradually returned to basal rate. Lymphatic pressures (top trace) continued to be pulsatile even while the stopcock was off.

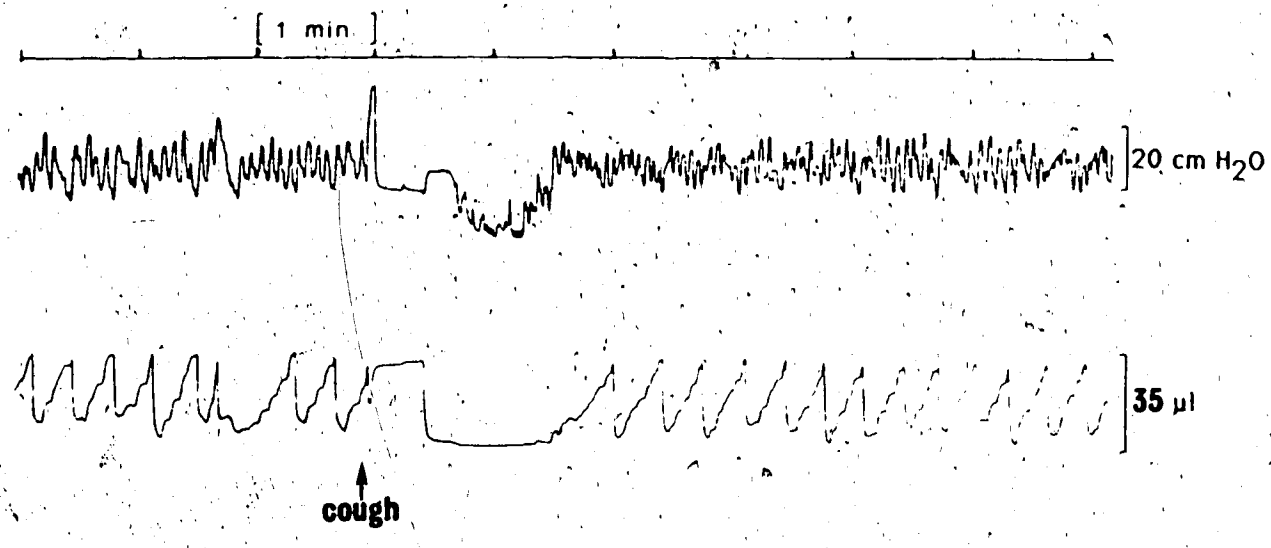


Figure 7. The effects of coughing on fluid output. Utilizing the "Double Catheter" preparation, abrupt, vigorous coughing (arrow) was recorded as sharp spikes on the pressure trace (top trace). Concomittantly, 2 drops of fluid were expressed onto the transducer arm (bottom trace). Following the coughing spell, a brief pause in fluid propulsion was observed, reflecting the time required for the vessel to refill with reservoir fluid. The overall contribution of this event to flow was insignificant. Neither normal respiration, nor vascular pulsations were observed to alter flow rate.

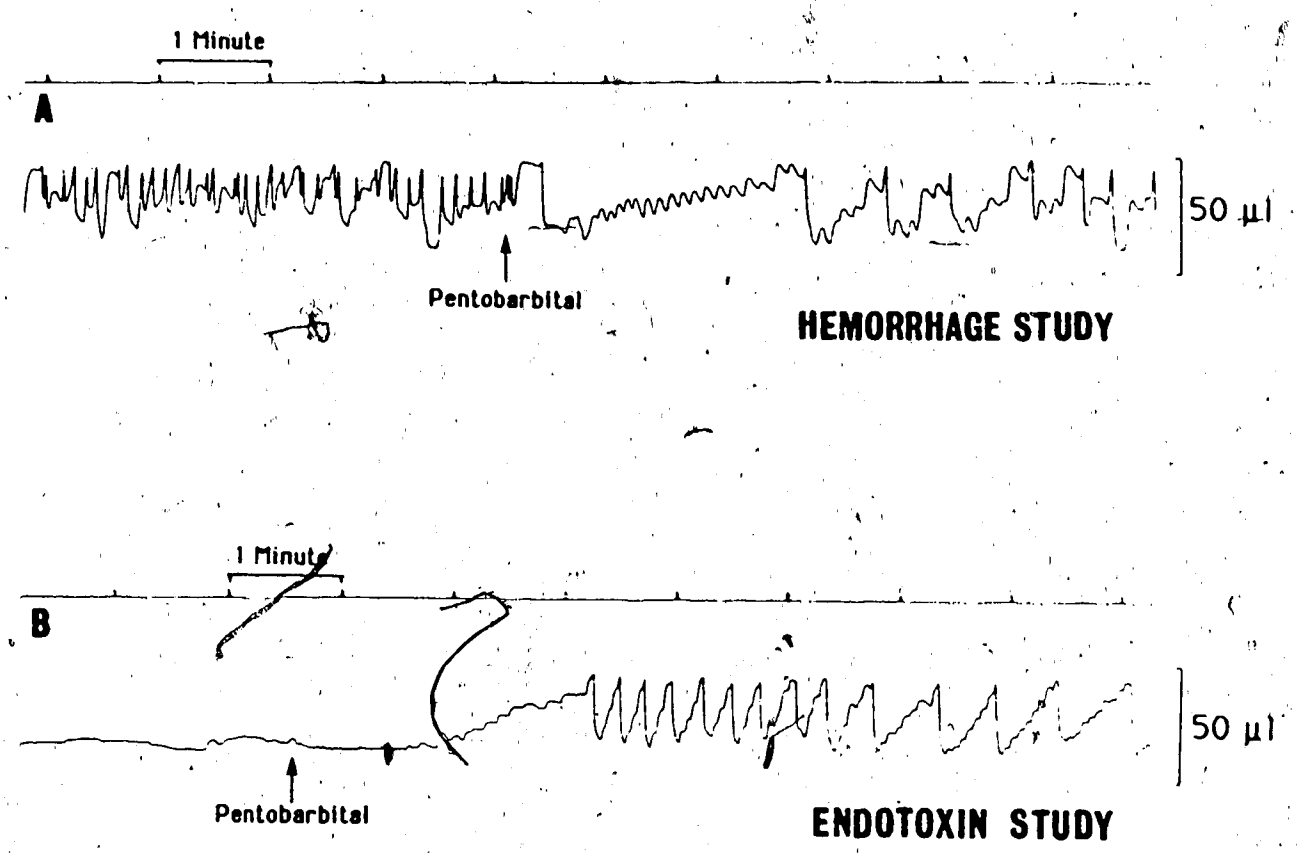
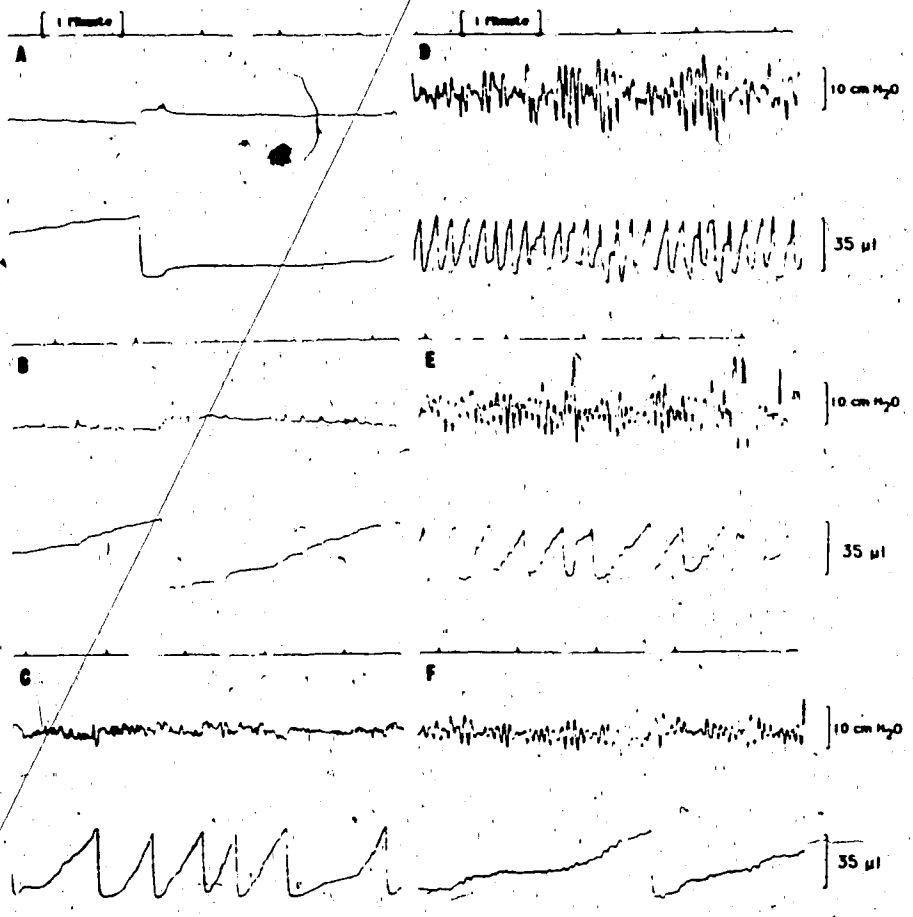


Figure 8. The effects of pentobarbital on lymphatic contractile activity in the "Double Catheter" preparation. The rapid intravenous injection of twice the maintenance dose of pentobarbital (8 mg/kg) (arrow), caused immediate effects on pulse pressures and fluid output. The top trace shows contractile activity to become depressed after pentobarbital was given during a hemorrhage experiment 40 minutes after blood loss. The bottom trace was taken from an endotoxin experiment. The endotoxin had markedly suppressed contractile activity and flow in this study, but after the administration of pentobarbital, lymphatic pumping transiently resumed for a period of 7 minutes.

Figure 9. Examples of pressure-flow recordings from a "Double Catheter" preparation when transmural distending pressure (TDP) was varied from 0 to 25 cm H_2O. In each case the pressure trace is on the top and flow rate recording on the bottom. The flow rates for each trace are tabled below.

TRACE	A	B	C	D	E	F
TDP (cm H_2O)	0	5	10	15	20	25
FLOW (mcl/min)	8.0	12.0	64.0	164.0	88.0	12.0


1 Minute
A
D
10 cm H2O
35 µl
B
E
10 cm H2O
35 µl
C
F
10 cm H2O
35 µl

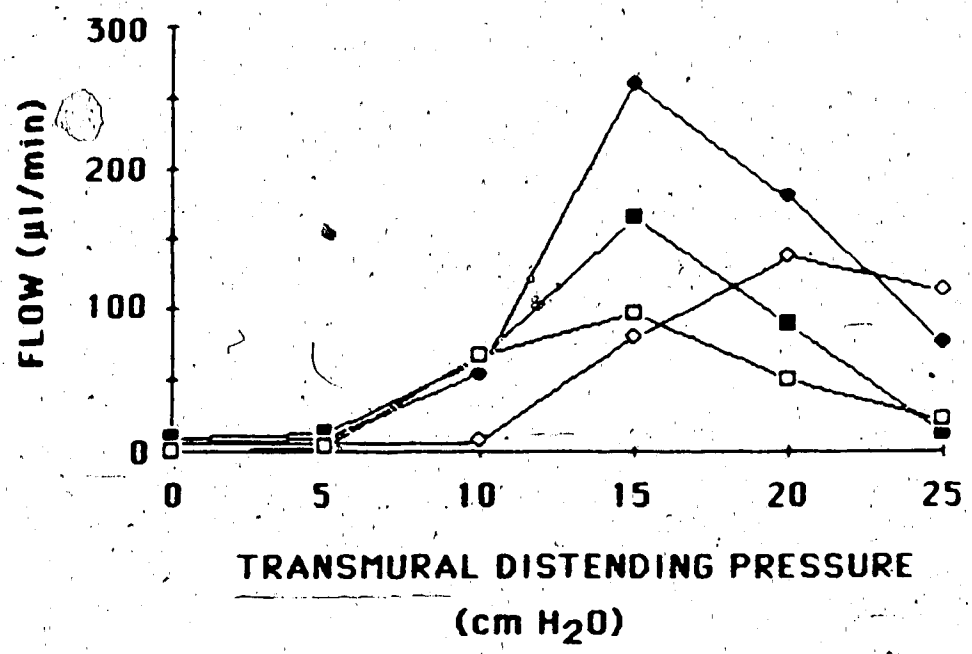


Figure 10. Effects of varying transmural pressure on fluid propulsion. Utilizing the "Double Catheter" preparation, the relationship between transmural distending pressure and fluid output (flow) was determined in 4 experiments.

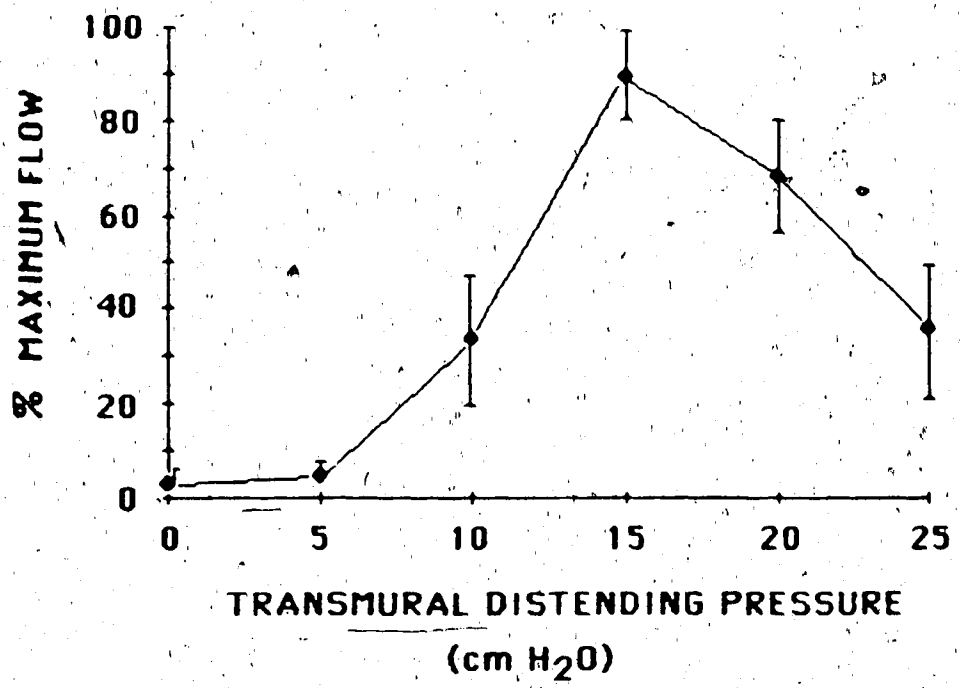


Figure 11. Mean results (+/- SEM) of 4 experiments where transmural distending pressure was increased from 0 to 25 cm H_2O in 5 cm increments. In each experiment, all values were expressed as a percent of the maximum flow rate.

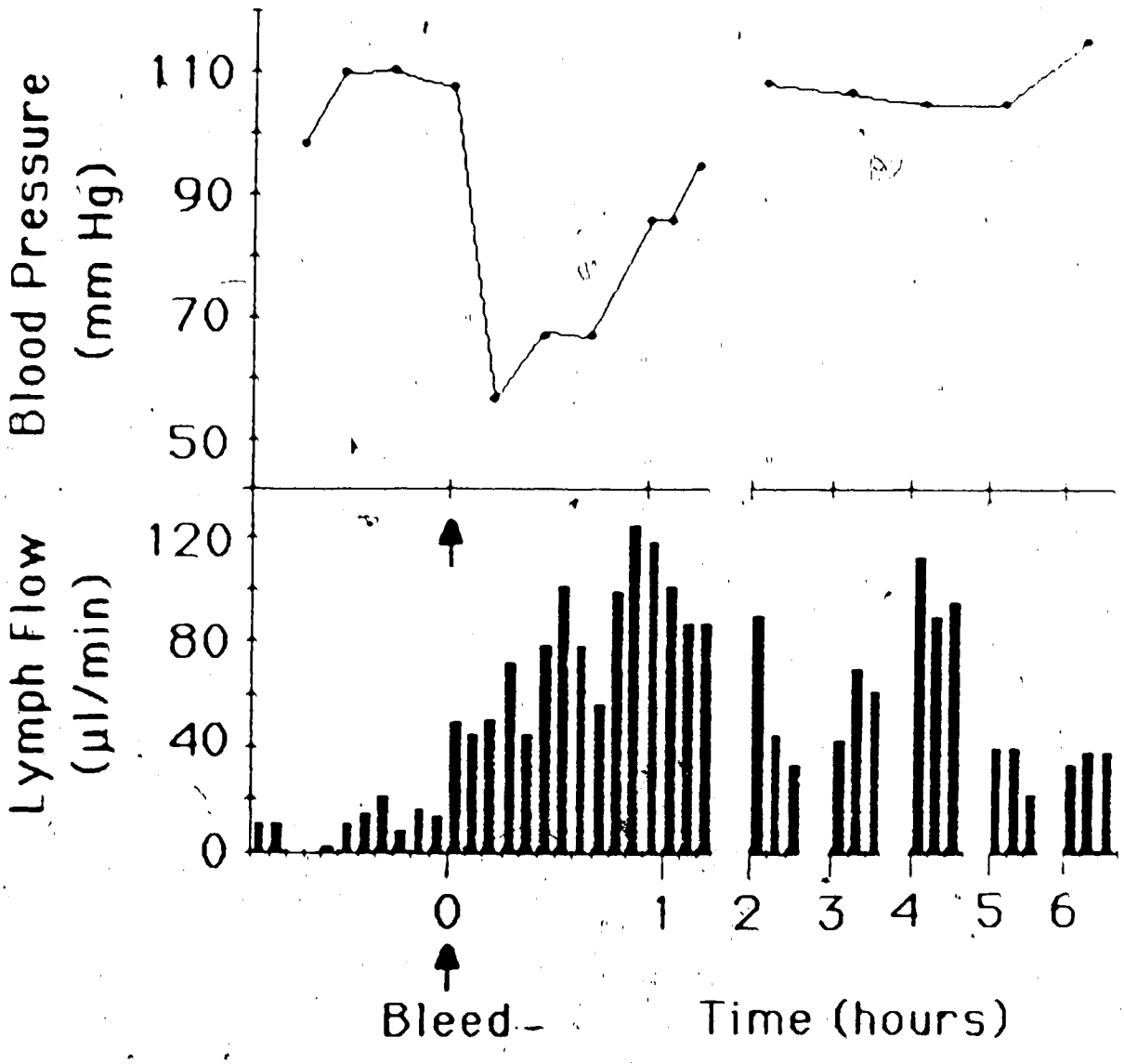
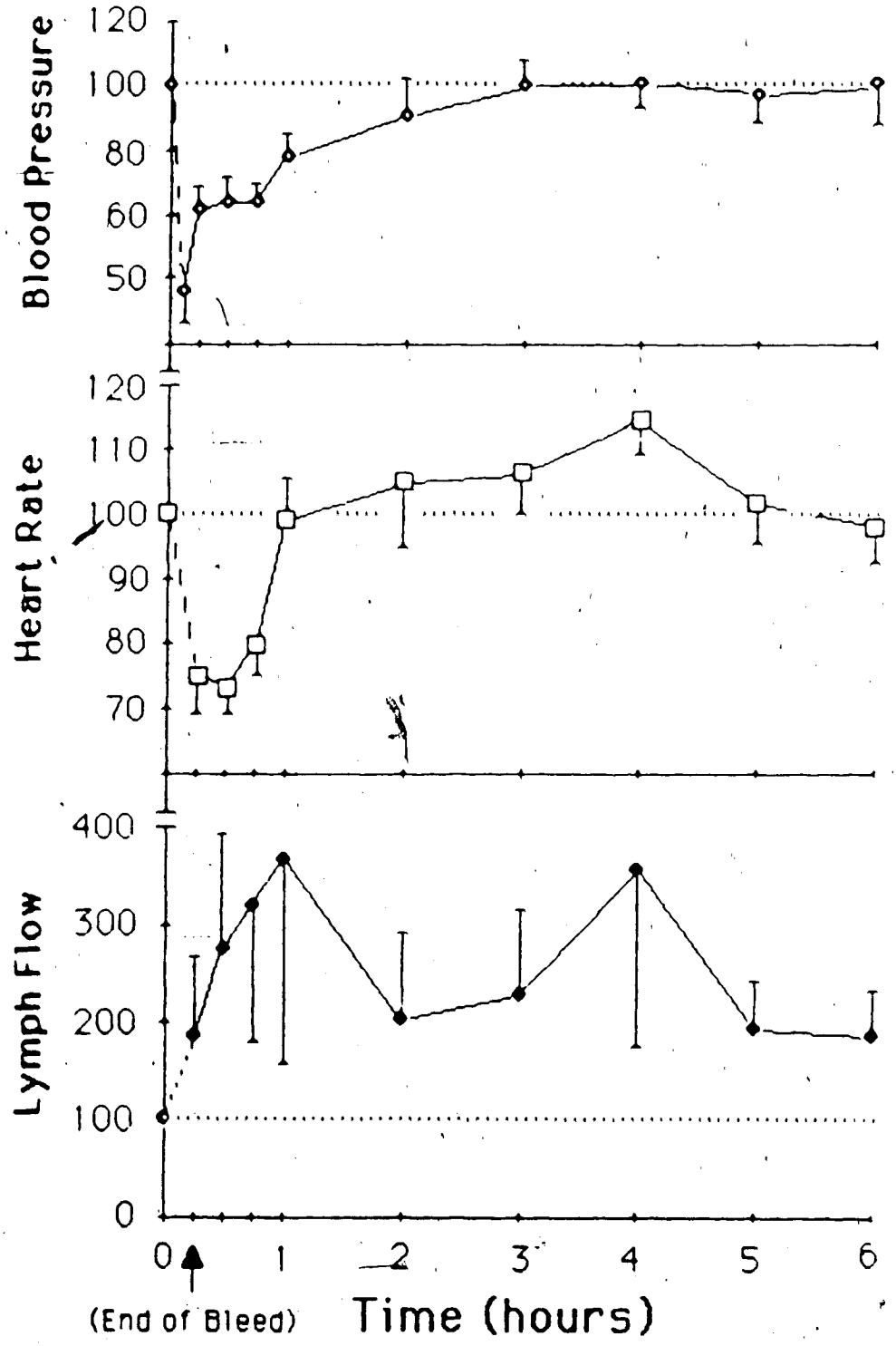


Figure 12. A representative experiment illustrating lymph flow (bottom trace) and mean arterial pressure (top trace) during the one hour prehemorrhage control period, and after 25% of the animals' blood volume was removed (arrow). In this study, control lymph flow averaged 9.1 mcl/min and increased bimodally to 125 mcl/min and 110 mcl/min, at one and four hours respectively after hemorrhage.

Figure 13. Effect of 25% hemorrhage on mesenteric lymph flow (bottom), heart rate (middle) and mean arterial pressure (upper trace). For each experiment, baseline levels for the parameters above were obtained during the one hour prehemorrhage control period. All values following blood withdrawl were then calculated as a percent of the prehemorrhage control level. The graph depicts the mean (+/- SEM) of 5 sheep studies.


Blood Pressure
120
100
80
60
50
Heart Rate
120
110
100
90
80
70
Lymph Flow
400
300
200
100
0
0
1
2
3
4
5
6
(End of Bleed)
Time (hours)

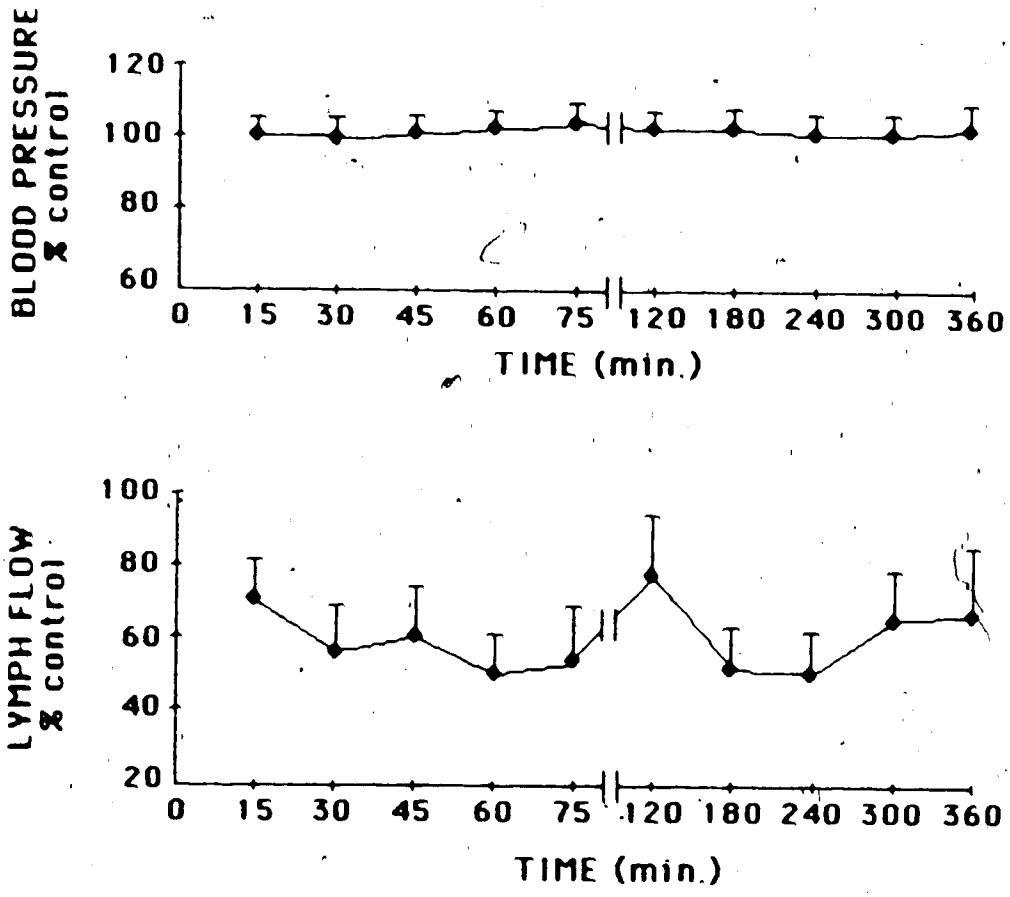


Figure 14. Lymph flow and mean arterial pressure in an external control group of sheep. 3 sheep underwent mesenteric lymphatic cannulation ("single catheter" procedure). The animals were not bled but lymph flow and mean blood pressures were monitored for 7 hours. Designating the first hour as the control period, all values over the next 6 hours were expressed as a percent of control. Each point on the graph represents the mean (+/- SEM) of 3 experiments.

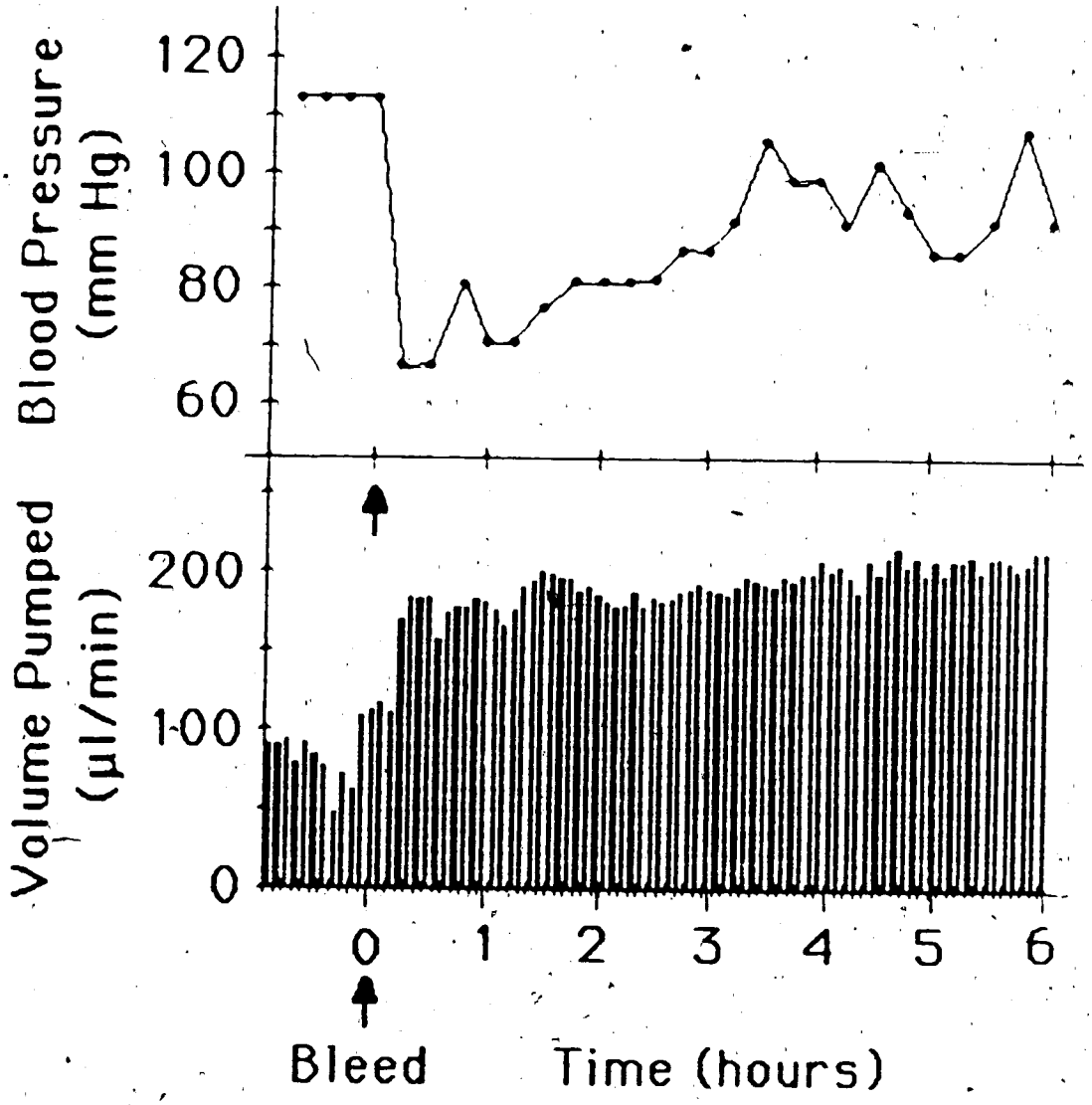


Figure 15. A representative example of fluid output (bottom) and mean arterial pressure (top trace) before and after a 25% hemorrhage (arrow) in an experiment utilizing the "Double Catheter" preparation. Flow averaged 84.3 mcl/min during the one hour prehemorrhage control period and increased to almost 200 mcl/min following blood withdrawl.

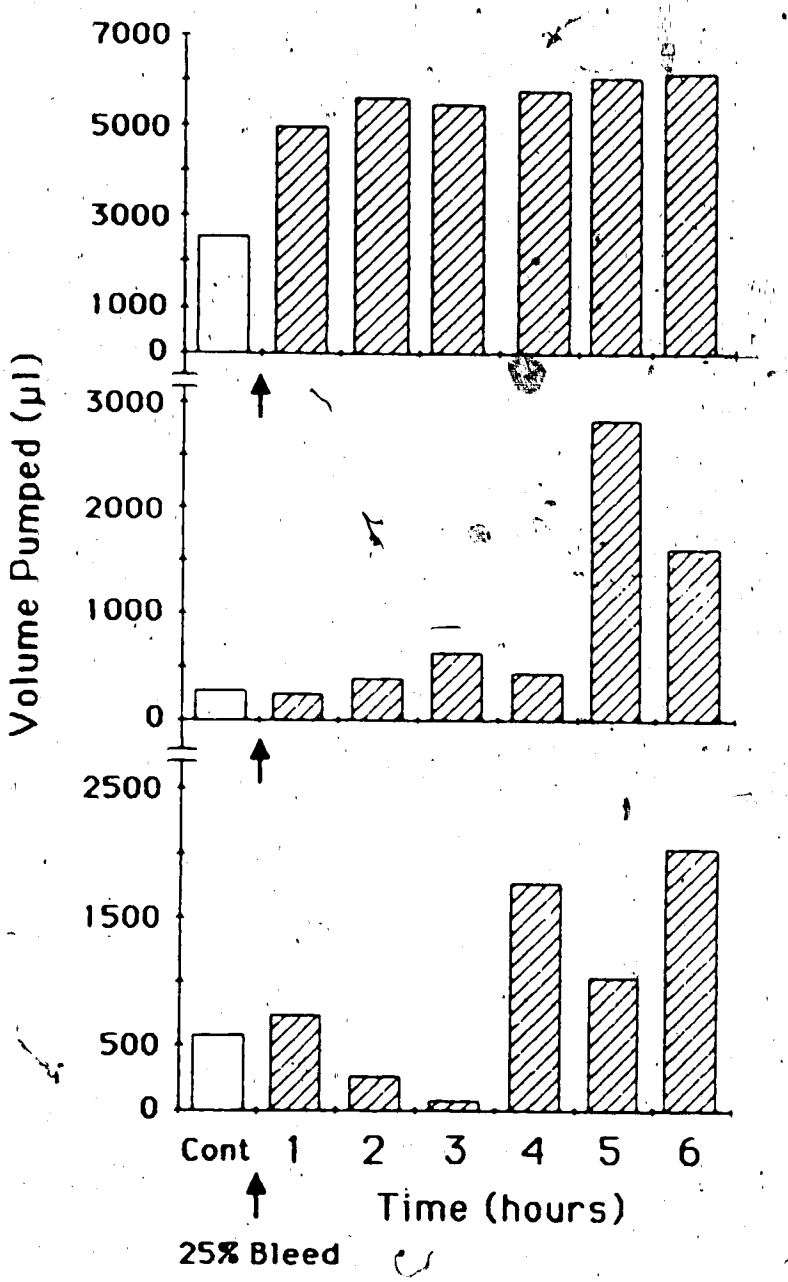
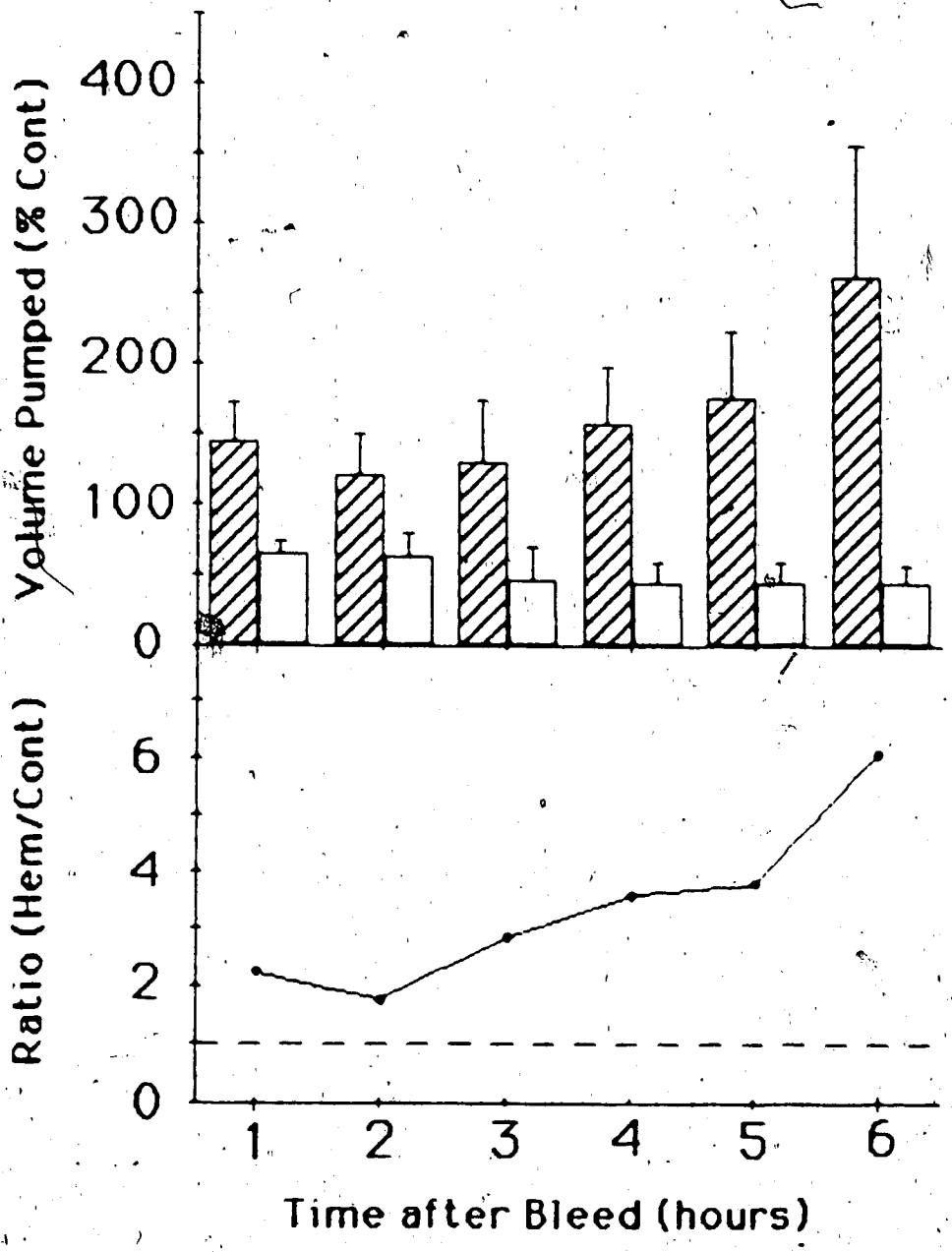


Figure 16. Effect of a 25% blood loss on fluid output (volume pumped) in 3 representative examples utilizing the "Double Catheter" preparation. The open bars represent the volume of fluid pumped (mcl) for each experiment during the one hour prehemorrhage control period. The cross hatched bars represent the fluid output collected during each subsequent hour after hemorrhage. These histographs demonstrate the variable time courses with which flow rates increase after hemorrhage.

Figure 17. Comparison of fluid propulsion in animals bled 25% of blood volume with an external control group utilizing the "Double Catheter" preparation. The histograph (top graph) compares the mean fluid output (+/- SEM) in 8 sheep bled 25% of blood volume (crossed hatched bars) with 5 sheep cannulated in the same fashion but not bled (open bars). Each bar represents the hourly fluid output collected and is expressed as a percent of the basal control level. In the hemorrhage group, basal flow was determined during the first hour preceding hemorrhage. For the external control group, each animal was monitored for 7 hours and the first hour of the study was designated as the control period. Analysis of variance determined a significant difference between the hemorrhage and external control groups ($p<0.01$).
The bottom graph expresses the differences in fluid propulsion of these two groups as a ratio (Hemorrhage gp/External control gp) for each hour-interval.


Volume Pumped (% Cont)
400
300
200
100
0
Ratio (Hem/Cont)
6
4
2
0
1
2
3
4
5
6
Time after Bleed (hours)

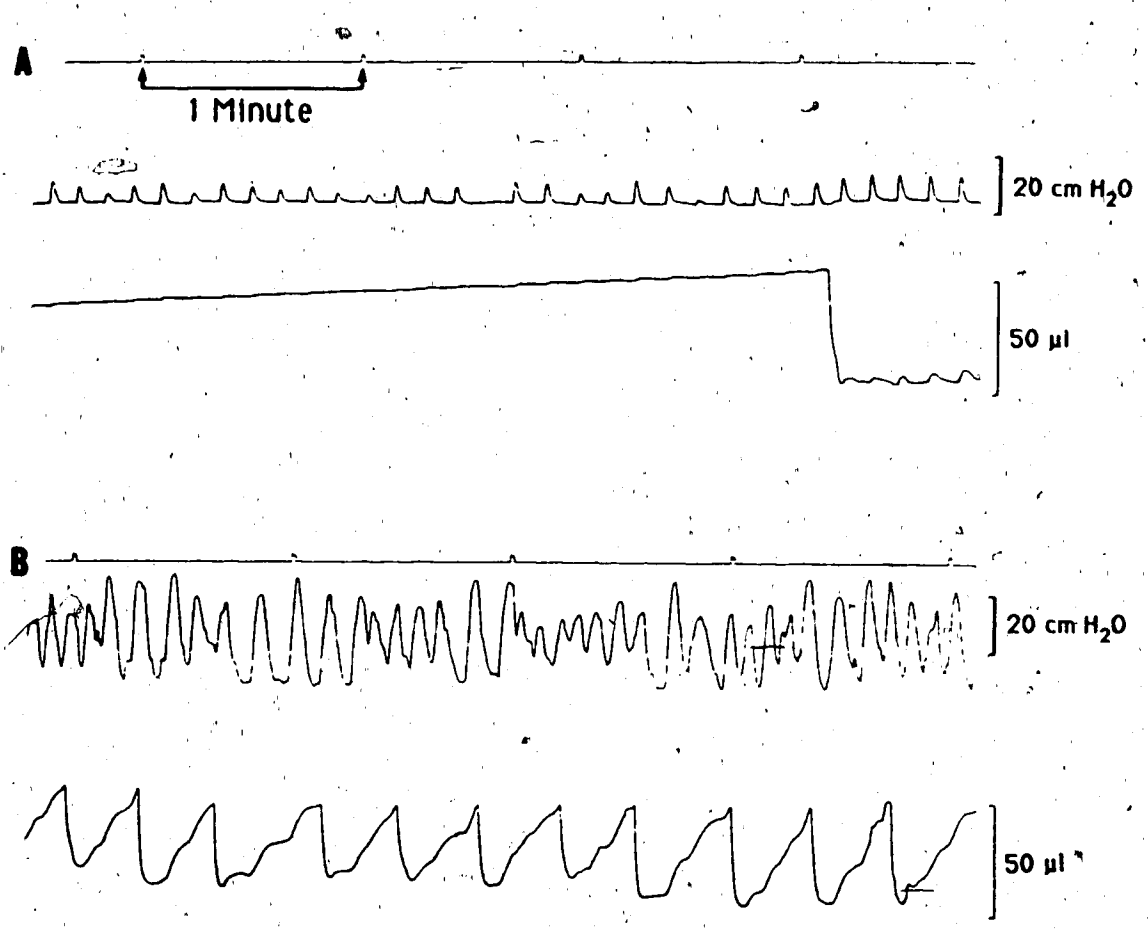


Figure 18. An example of a pressure-flow recording from the "Double Catheter" preparation before and after a 25% hemorrhage. In each case, the pressure trace is on the top and flow rate recording on the bottom.
(A) recording taken during the prehemorrhage control period
(B) recording taken 2 hours and 30 minutes after blood loss

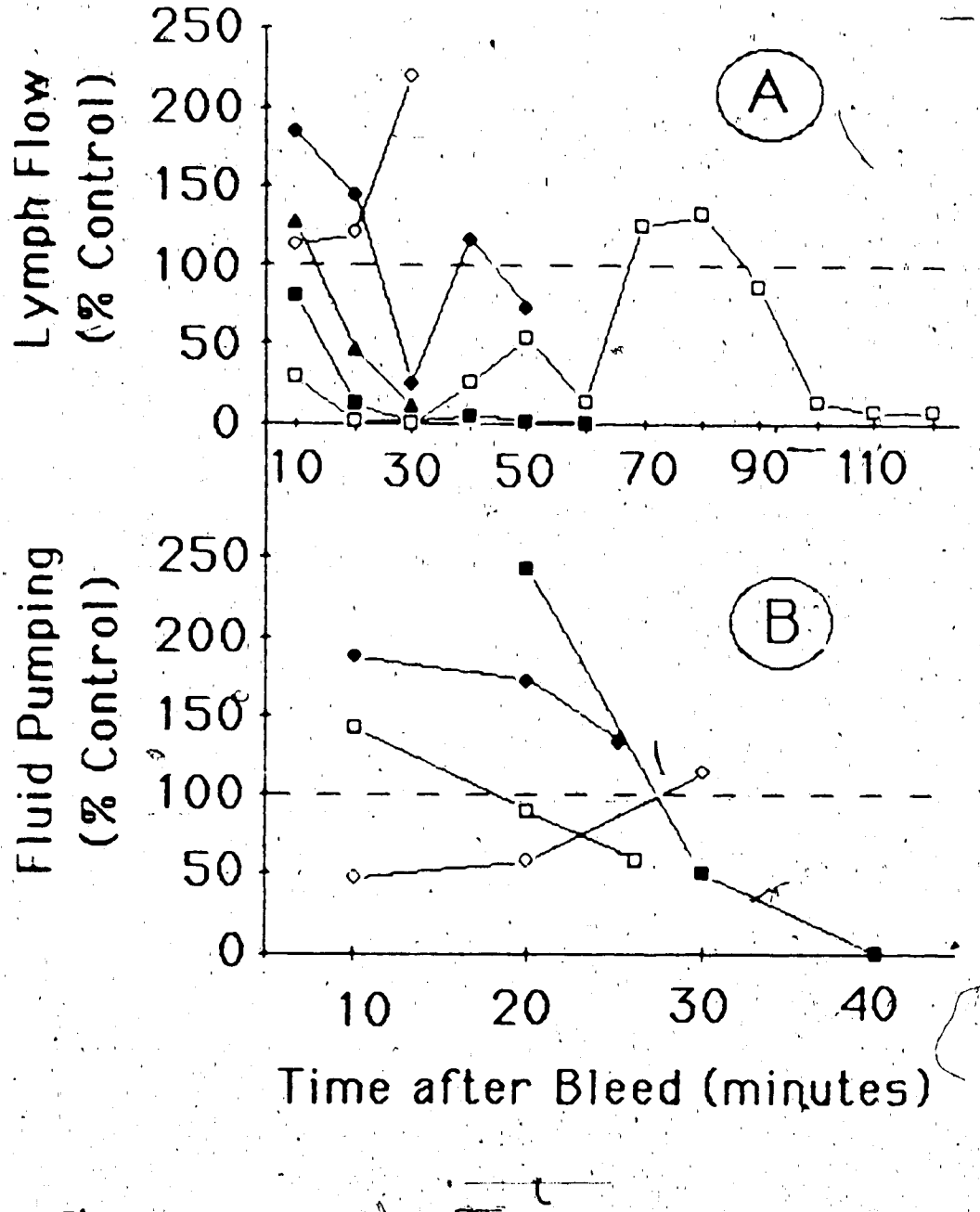


Figure 19. Effect of a 50% hemorrhage on lymph flow and fluid propulsion. Following blood withdrawl, flow rates from the "Single" and "Double" Catheter preparations were calculated for each 10 minute interval and expressed as a percent of the prehemorrhage control rate. The upper graph (A) depicts the changes in lymph flow from 5 experiments (utilizing the "Single Catheter" preparation) and the lower graph (B) shows the results of fluid propulsion from 4 studies (utilizing the "Double Catheter" preparation) after a 50% bleed.

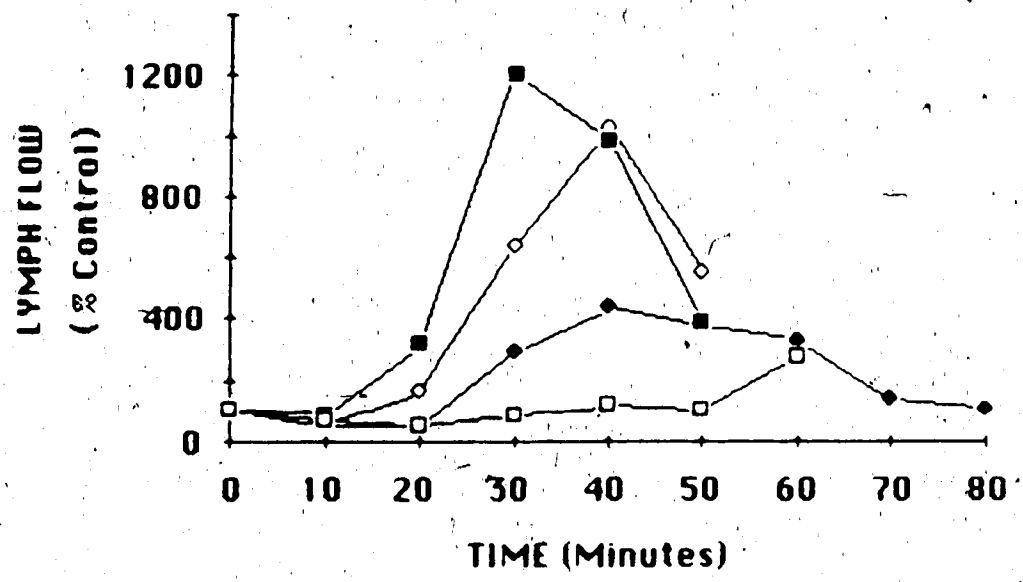


Figure 20. Effect of endotoxin on mesenteric lymph flow. Following the intravenous administration of endotoxin (3.3 mcg/kg), lymph flow rates over 10 minute intervals were determined and the results expressed as a percent of basal control rate. The graph depicts the results of 4 separate experiments. The last point on each trace denotes the time of death for each animal.

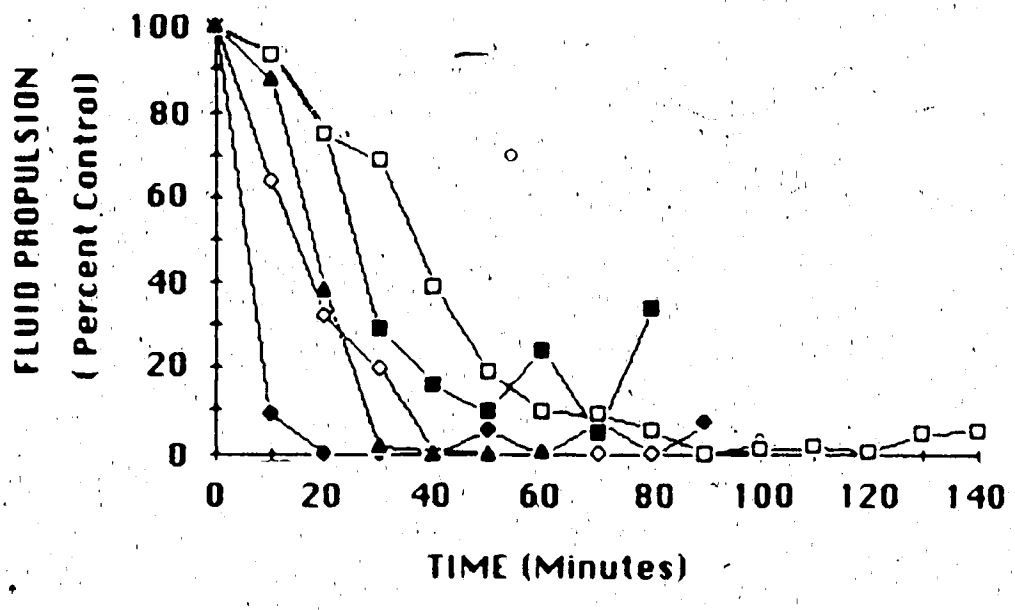


Figure 21. Effect of endotoxin on fluid propulsion. Following the intravenous injection of endotoxin (3.3 mcg/kg), flow rates utilizing the "Double Catheter" preparation were calculated over 10 minute intervals and expressed as a percent of the basal control rate. The graph depicts the results of 5 separate experiments. The last point on each trace denotes the time of death for each animal.

Figure 22. Effect of endotoxin on lymphatic pressures and fluid propulsion recorded from a "Double Catheter" preparation in an anaesthetized sheep. The pressure-flow traces (A,B,C,D) were taken during the control period, and 30, 60, and 110 minutes after the injection of 3.3 mcg/kg of endotoxin. In each example, the pressure trace is on the top and flow rate recording on the bottom. The flow rates for each trace are tabled below.

TRACE	FLOW (mcl/min)
A	193.8
B	132.7
C	19.2
D	0.0

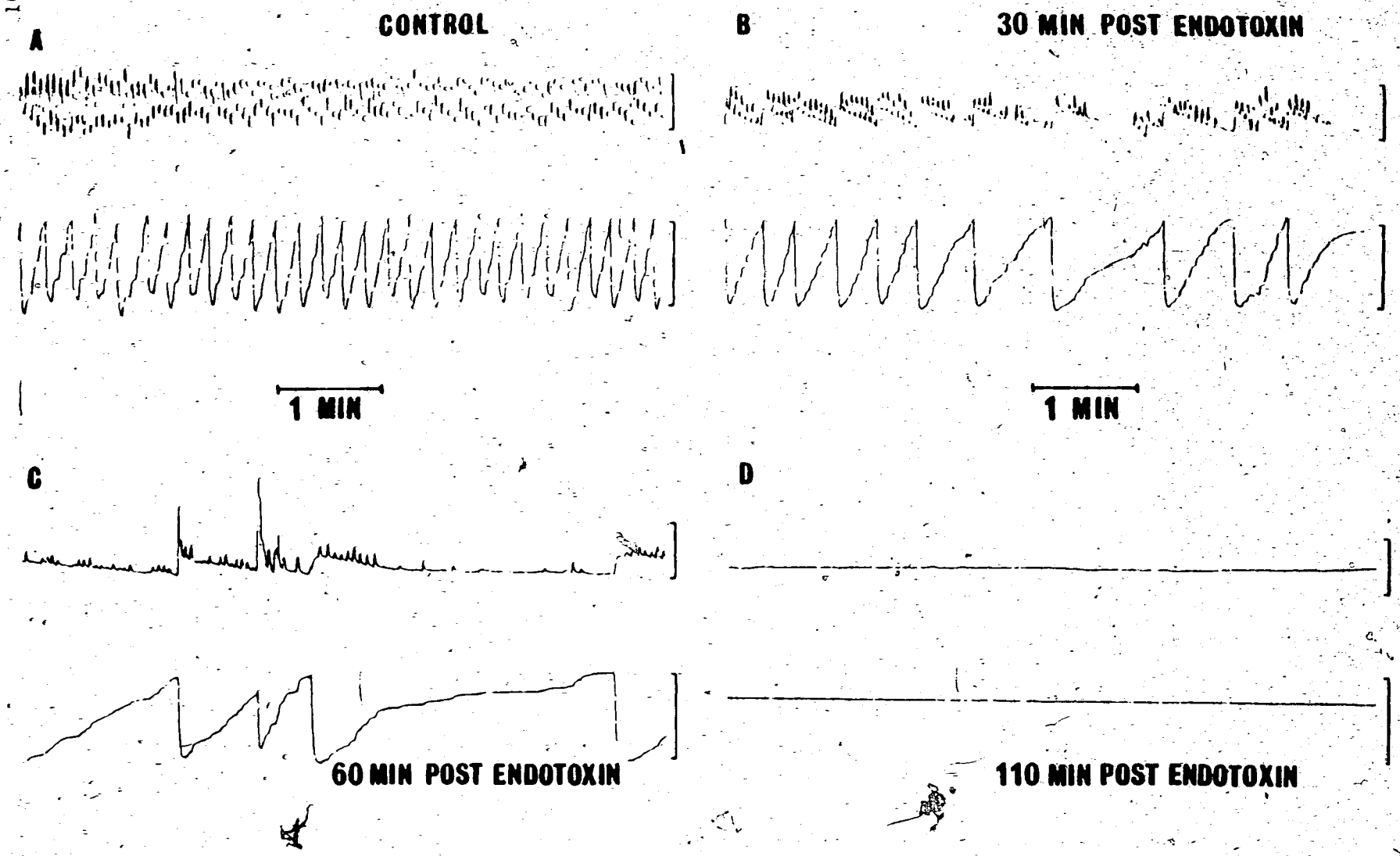

A
CONTROL
B
30 MIN POST ENDOTOXIN
1 MIN
1 MIN
C
D
60 MIN POST ENDOTOXIN
110 MIN POST ENDOTOXIN

Figure 23. Effect of endotoxin on fluid propulsion (lower trace), mesenteric lymph flow (middle trace) and mean arterial pressure (upper trace). Each value was calculated as a percent of the basal control rate and the plotted values on the graph represent the mean (+/- SEM) in each group. The lower trace compares fluid output from "Double Catheter" preparations in 5 sheep intravenously injected with endotoxin (3.3 mcg/kg) with 5 injected with saline (external controls). The differences were significant (ANOVA $p<0.01$).
The middle trace compares lymph flow rates from "Single Catheter" preparations in 4 sheep injected with 3.3 mcg/kg of endotoxin with 6 external control sheep. The differences were once again statistically significant by analysis of variance ($p<0.025$).

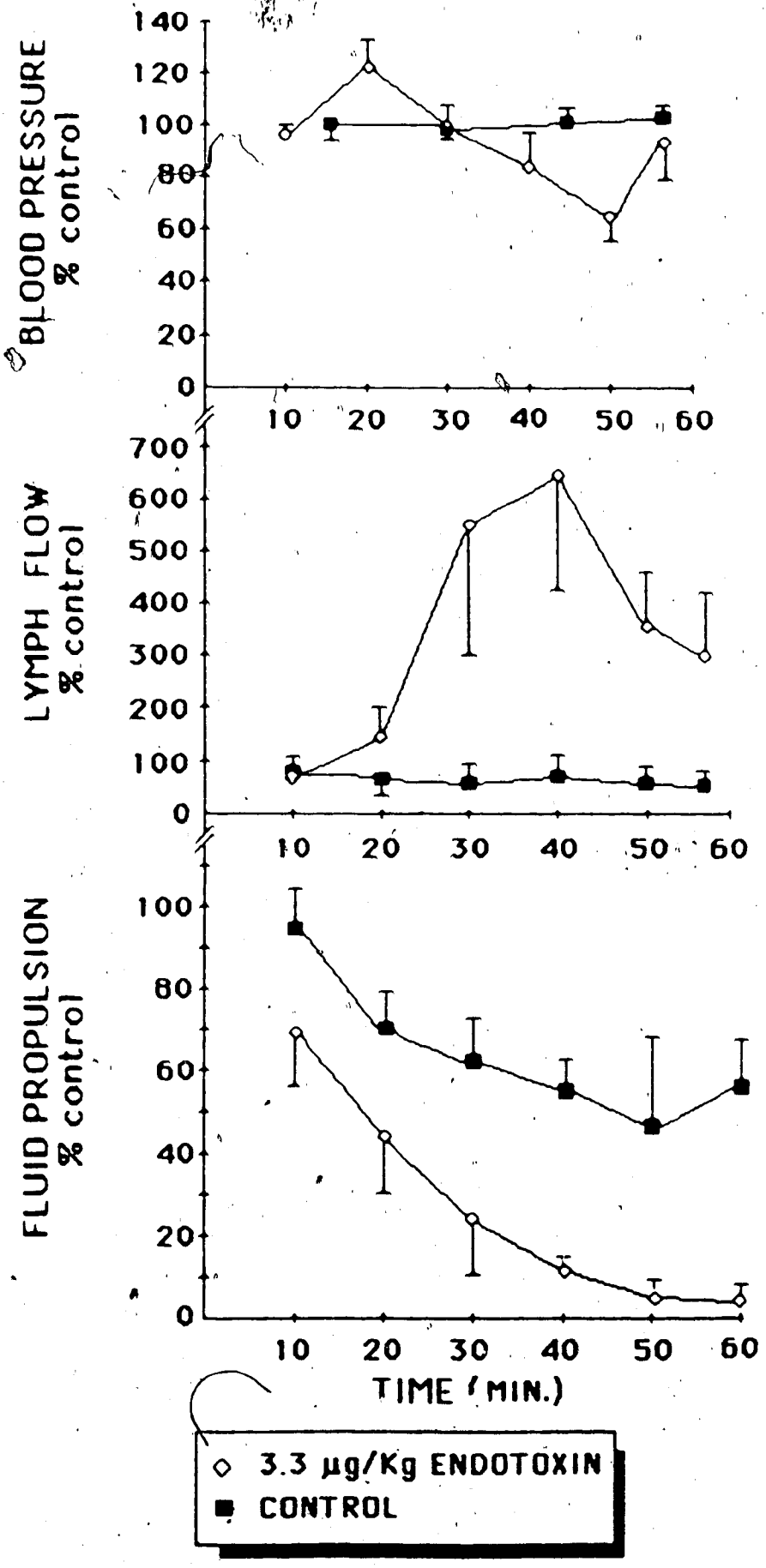
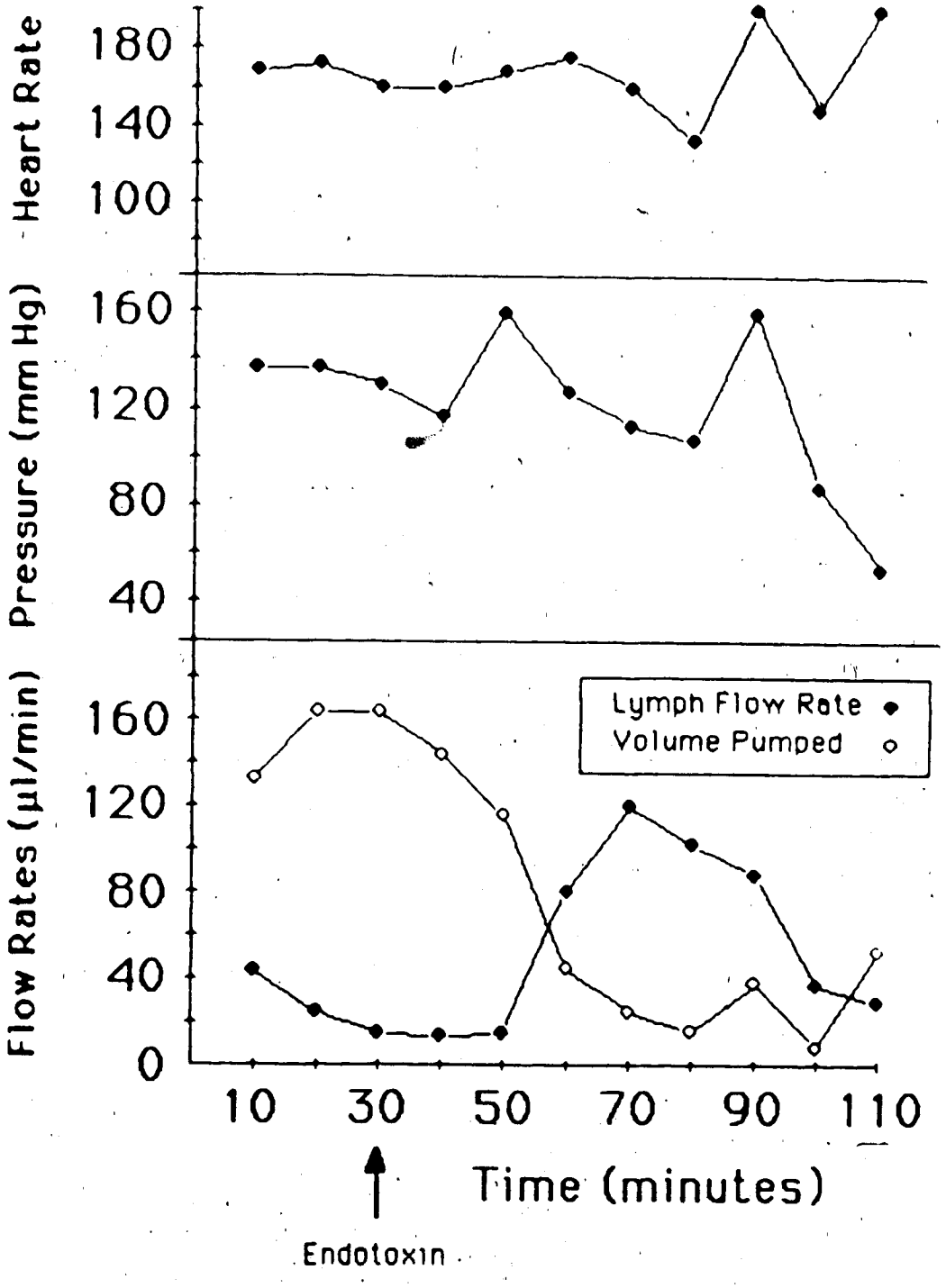

BLOOD PRESSURE
% control
140
120
100
80
60
40
20
0
10
20
30
40
50
60
LYMPH FLOW
% control
700
600
500
400
300
200
100
0
10
20
30
40
50
60
FLUID PROPULSION
% control
100
80
60
40
20
0
10
20
30
40
50
60
TIME (MIN.)
3.3 µg/Kg ENDOTOXIN
CONTROL

Figure 24. Simultaneous measurements of fluid propulsion and lymph flow in an anaesthetized sheep injected with endotoxin. In the same animal, both "Double" and "Single" catheter procedures were performed on the mesenteric lymphatics. The top and middle traces illustrate heart rate and mean arterial pressure respectively. The lower trace depicts the record of fluid propulsion (volume pumped) and lymph flow during the 30 minute control period and after 3.3 mcg/kg of endotoxin (arrow) was intravenously injected. The animal died at 110 minutes (80 minutes after the administration of endotoxin).


Heart Rate
180
140
100
Pressure (mm Hg)
160
120
80
40
Flow Rates (µl/min)
160
120
80
40
0
Lymph Flow Rate
Volume Pumped
10
30
50
70
90
110
Time (minutes)
Endotoxin

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