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DISTRIBUTION OF ENDOGENOUS ALBUMIN WITHIN THE PERI-LYMPHATIC CAPILLARY REGION.

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The distribution of endogenous albumin on the abluminal side of lymphatic endothelium was evaluated by immunoelectron microscopy with the protein-A-gold technique. Lymphatic capillaries from the guinea-pig epicardium and myocardium were selected and the thin sections were labeled. The distribution of albumin within the periphery of the lymphatic capillary is heterogenous. Albumin is distributed uniformly within collagen bundles with a loose arrangement; however, the protein is partially excluded from the narrower spaces of packed collagen. A prominent feature near the lymphatic capillary is the presence of albumin concentrations (A.C.) which are clearly identified by a high density of labeling. Of low frequency A.C. are mostly observed as elongated structures, of variable diameters (between .08 and 2μm), with parallel or perpendicular orientations to the lymphatic endothelium. Parallel A.C. can cover several μm of the endothelial surface and are often seen in direct continuity with perpendicular A.C. Some A.C. were observed to link the surface of myocardial cells and the intercellular contacts of lymphatic endothelium. These results suggest that the access of albumin to the lymphatic capillary is through loosely arranged collagen bundles or within pools of proteins. It also supports the concept that proteins are drained by preferred pathways within the interstitium.

THE ISOTOPE CLEARANCE TECHNIQUE (I.C.T.) FOR MEASURING SKIN LYMPH FLOW.

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The measurement of skin lymph flow, by monitoring clearance of an intradermal injection of radiolabeled protein, has been investigated in pig and human skin. 123 lymph flow experiments were completed in the skin of anesthetized Large White Pigs to ascertain: the reproducibility of the I.C.T., the most suitable tracer, the influence of injection depth, and the effect of massage. Results demonstrated 99mTc-colloid (TCK 17, CIS) performed similarly to ^{131}I-HSA, but provided less radiation risk. Subepidermal injections cleared faster and more consistently than either subcutaneous or deep dermal injections ($P<0.001$). Local massage enhanced clearance ($P<0.005$). 56 lymph flow experiments were performed in normal human subjects and patients with lymphedema. The effect of massage, heating and gravity were studied. Subepidermal injections were administered into the dorsum of the foot. Nodal uptake was measured concomitantly. At rest there was no significant difference in 1/2t between normal skin ($n=10$) and lymphedema ($n=18$). Lymph massage resulted in enhanced skin clearance and marked nodal uptake ($P<0.001$)

only in normals. Heating (skin temperature 40°C) produced faster clearance than at a skin temperature of 30°C ($P<0.05$). Gravity (horizontal vs. vertical position) did not influence clearance but nodal uptake increased in the vertical position ($P<0.05$). The I.C.T. using a dual channel scintillation detector system with simultaneous measurement of depot clearance and nodal uptake provides a mobile and low radiation method for the functional assessment of the lymphatic system.

LYMPHATIC ABSORPTION IN PERITONEAL DIALYSIS IN THE RAT.

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Since the contribution of lymphatic absorption to loss of ultrafiltration (UF) in peritoneal dialysis (PD) has been ignored, we evaluated the role of lymphatic flow in UF kinetics in PD in a rat model. Net UF was directly measured each hour during a 6-hr exchange using 15% anhydrous glucose dialysate ($n=6$). Concurrent hourly lymphatic reabsorption was calculated from the rate of disappearance of rat albumin added to the dialysate ($n=3$). The same studies were repeated using Ringer's lactate instead of hypertonic glucose ($n=6$). With hypertonic glucose dialysate lymphatic reabsorption increased linearly at a mean rate of 4.7ml/hr and, at the end of the 6-hr dwell, had reduced actual UF by 52% (Actual UF 54ml, net UF 26ml). Cumulative net UF (intraperitoneal volume) was maximal at 210 min dwell time. Thereafter the lymphatic reabsorption rate exceeded the UF rate. Peak intraperitoneal volume preceded osmotic equilibrium (240 min) which in turn preceded glucose equilibrium (after 360 min). With Ringer's lactate the added albumin concentration remained constant throughout the dwell and cumulative directly measured lymphatic absorption correlated with cumulative lymphatic absorption via the albumin method ($r=0.98$, $p<0.01$). Cumulative lymphatic flow (ml/100g body weight) was similar in both groups or rats. We conclude that cumulative lymphatic absorption significantly reduces net UF in rats and is likely to similarly influence UF kinetics in PD in man.

ON LYMPH, LYMPHATICS, LYMPH GLANDS AND LYMPHOCYTES.

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Claude Bernard emphasized that lymph constitutes the plasma in blood, as well as the liquid medium which enables intercellular communication and nourishment throughout the body. In the invertebrates only lymph circulates to perform such homeostatic functions. In the vertebrates lymph is formed largely by capillary ultrafiltration of the circulating blood plasma, but originates as a result of the oxidative combustion of food in all body cells. As per the equation $6O_2 + C_6H_{12}O_6 \rightarrow 6H_2O + 6CO_2 +$ Energy, lymph H_2O arises in proportion to the rate O_2 burns combustible food to produce CO_2 and the energy essential for cell growth or movement. The lymph H_2O becomes a universal solvent which dissolves the CO_2, enables intracellular metabolism and which carries away the cell products in solution or in suspension, so that the products can be reutilized for growth in other cells, or be excreted conveniently. Lymphatics develop from mesenchyme proportional to the rate lymph flows from respiring tissue cells into central veins where efficient mixing proceeds with circulation. Lymph glands develop along the course of lymphatics to filter peripheral lymph emanating from all parts of the body. The glands use some of the filtrates for modifying the production of central lymph rich in dissolved globulins and migrant small cytoplasm-depleted lymphocytes. The dissolved globulins and suspended lymphocytes, in turn, carry very complex nutrients and lymphokines, as well as immunologic protection back to all respiring tissues.

A COMPARATIVE ANALYSIS OF

MORPHOMETRIC TECHNIQUES USING AN IMAGE ANALYSIS SYSTEM.

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Morphometric analysis is becoming a commonly used tool in light and electron microscopic studies of intraorgan lymphatic systems. If results are to be compared across published studies, information on reproducibility of measurements is needed for the various methods. In order to provide this information, 5 individuals used the Bioquant Image Analysis system with an IBM XT computer to carry out studies involving the measurement of (A) 25 high-contrast geometric diagrams of known size and (B) 25 structures in a histologic section of thyroid tissue. The measurements were made using the three most often found input methods. These involve the use of a digitizing tablet (1) alone, (2) in combination with a video camera and (3) in combination with both a microscope and a video camera. With each method reproducibility was found to be dependent both on the contrast of the subject and on the distance that the cursor traversed on the digitizing tablet during the measurement process (the traced area). Measurements that involved a traced area of less than $3 \times 10^8 \mu m^2$ displayed a high coefficient of variance. Variation among the different observers and across the different techniques was not significant. It was concluded that the technique of choice was #3, because the magnifications and contrast can be changed to suit each structure. This is particularly true if the capability for computer digitization and storage of video images is available, for then the time and cost of photography can be avoided.

INTRAGLANDULAR LYMPHATICS OF THE RAT THYROID.

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In order to investigate the possible role of intrathyroid lymphatics in the transit of T3 and T4 a combined light and electron microscopic study and morphometric analysis were performed. Eighty-two lymphatics with valves were identified by light microscopy and confirmed by electron microscopy. These vessels were traced in serial sections by light microscopy and a total of 291 cross-sections were analyzed. They were classified as capsular, interlobular, and interglandular (between thyroid and parathyroid). The majority were interlobular and only a few of their tributaries could be traced into the lobules. The mean maximum diameter of all the lymphatics was $17.87 \mu m \pm 0.33$ (S.E.), the profile density was $5.68/mm^2$, and the volume density about 0.007. 46% of the interlobular and 26% of the interglandular vessels were closely related to follicles and associated blood capillaries but were always separated from them by occasional collagen fibers and fibroblasts. Endothelial vesicles had a mean maximum diameter of 96nm and were equally distributed between the luminal and abluminal surfaces and cytoplasm. The volume density and numerical density of the vesicles was 0.069 and 57.19, respectively. The ultrastructural appearance of these lymphatics resembled that of lymphatics in other organs where significant protein transport occurs.

LYMPH NODE TRANSPLANTATION FOR THE STUDY OF LYMPHATIC METASTASIS OF TUMOR.

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Ideal model to study the whole phenomenon of lymphatic metastasis of tumors does not presently exist. Earlier models involved direct intralymphatic injection of tumor cells or the injections of tumor cells in situations in which direct intralymphatic injection is likely. Ideal model would involve

reproducible metastasis of a primary tumor to a defined lymph node that drained only that tumor, metastasizing further to kill the animal. Such an ideal tumor would further be transplantable in syngeneic strain and its effluence could be easily studied. Vascularized lymph node transplantation using microvascular anastomotic technique can be such an ideal tool. Inbred strain-2 guinea pigs and transplantable line-10 tumor cells were used as a model. An autotransplantation of vascularized superficial inguinal lymph node was performed to various anatomic sites. Either the nodes containing micrometastatic foci or the nodes carrying large burden of tumor cells acting as a new primary can now be transplanted with functional and architectural integrity and studied.

INHIBITION OF LYMPHATIC PUMPING AS A MECHANISM CONTRIBUTING TO EDEMA IN ENDOTOXIN SHOCK.

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The lymphatic vessel contributes to lymph propulsion through its ability to contract and push the lymph in the direction allowed by the valves. In this study we have determined the effects of endotoxin on lymph formation and its possible effects on lymphatic contractile activity *in vivo* using a new model system. A segment of intestinal lymphatic vessel, cannulated at both proximal and distal ends, was surgically "isolated" from all lymph input and supplied with fluid from a reservoir. With no hydrostatic gradient, flow generated from this preparation could only occur from lymphatic pumping. The intravenous administration of 3.3μg/kg (anesthetized sheep) or 33μg/kg endotoxin (in conscious sheep) resulted in reductions in fluid propulsion relative to internal controls (pre-endotoxin pumping) or external controls (animals that had not received endotoxin). In addition to the systemic inhibitory effect of endotoxin, lymph samples collected from preparations in which input was permitted contained a factor(s) that suppressed the contractile activity of isolated bovine mesenteric lymphatic vessels suspended in tissue baths. Despite the reductions in the activity of the lymph pump, measurements of lymph flow rates from intact intestinal catheters revealed marked increases following endotoxin administration. We speculate that this imbalance between the increased transvascular flux of fluid and protein into the tissue spaces and the impaired ability of the lymphatics to carry this material from the interstitium, contributes to the edema associated with septicemia.

CARDIAC LYMPH: COMPOSITION AND ALTERATION WITH MYOCARDIAL ISCHEMIA.

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Macromolecules gain access to the cardiac lymphatic drainage from the vascular space or from myocardial cells. The rate of appearance of macromolecules increases significantly during reperfusion following myocardial ischemia. Cannulation of the cardiac lymph duct in dogs and subsequent lymph collection in the conscious state reveals that reperfusion lymph has: 1) intracellular enzymes, glycogen phosphorylase, and creatine kinase, in significantly increased quantities from control state; these levels occur after occlusions as short as 10 minutes; 2) increased concentration of thromboxane B_2 accompanied by a decrease in platelets; and 3) additional proteins capable of binding to the subunit of the first component of complement (Cl_q). Analysis of cardiac lymph from post-ischemic states demonstrates the presence of macromolecules which is indicative of cell damage during short-term coronary occlusions. Some of these molecules may promote vasoactivity and some may promote leukocyte infiltrates; both of these processes may affect the severity of ischemic injury.