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# Effects of Ischemia on Forelimb Weight and Lymph Protein Concentration[1] (38856)

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The aim of this study was to determine if significant edema develops after relief of prolonged ischemia. While many investigators have attempted to determine the effects of local and systemic hypoxemia on fluid filtration across capillary membranes, there is almost no quantitative data on the effects of ischemia per se on the store of interstitial fluid. This is somewhat surprising since this problem has concerned physiologists since at least 1894. Lazarus-Barlow (1) demonstrated that the relief of complete "anemia" (ischemia produced by tightly bandaging the limb to stop blood flow) of a dog limb for 1 hr does not lead to the development of edema (judged by changes in limb circumference) after the relief of the anemia. On the other hand, Pochin (2) occluded the base of the rabbit ear for 2 to 24 hr and noted an increased thickening over the central ear artery proportional in magnitude to the time of occlusion. Clearly marked edema developed in the rabbit ears occluded for 18–24 hr. Additionally, Brown and Garrett (3) occluded the rat hindpaw with a constrictor band for 2–60 min and noted edema (measured plethysmographically) after the relief of the occlusion. There is little convincing data from humans germane to this issue. Most clinical studies are complicated by trauma, shock, or preexisting vascular atrophy (4, 5). These sparse but conflicting observations prompted the present study.

In this study, the gravimetric method was used to estimate the development of edema in the dog forelimb after relief of complete ischemia of 2-hr duration. Lymph flow and protein concentration were also measured since such data are not available in the literature.

*Methods.* Male mongrel dogs were anesthetized with sodium pentobarbital and intubated with a cuffed endotrachial tube. All the animals were artificially ventilated with room air via a positive-pressure respirator (Harvard Apparatus Company).

*Collateral-free forelimbs.* The collateral-free, innervated canine forelimb was utilized as the test organ to study the effects of the relief of complete ischemia on weight, vascular pressure, blood flows, lymph flow rate, and lymph protein concentration (6–12, 14, 15).

The following cannulations were performed to measure intravascular pressures: (1) large skin vein pressure from the cephalic vein via a side branch, (2) large muscle vein pressure from the brachial vein via a side branch, and (3) systemic pressure from the femoral or carotid artery. The cannulae were connected to a low-volume displacement transducer (Statham P23G-b). The transducer output was recorded on a direct writing oscillograph (Hewlett-Packard). Both skin and muscle venous outflows were quantified by timed collections of the brachial and cephalic venous outflows. Additionally, the forelimb was also suspended on a sensitive I-beam balance with a strain-gauge transducer located at the fulcrum to record changes in weight.

Ischemia was produced by clamp occlusion of the brachial artery upstream to the collateral-free forelimb. The clamp was placed on the artery after an isogravimetric control period of about 15 min, and after 2 hr the clamp was removed. All responses were then

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followed up to 60 min after relief of the ischemia.

A second series of experiments was conducted in which the experimental preparation was identical to that described above except that the brachial artery was cannulated upstream to the isolated organ. The cannula was connected to a constant-volume pump which was supplied with arterial blood from the carotid or femoral artery. The pump was adjusted to produce a brachial artery pressure slightly less than the systemic arterial pressure. Perfusion pressure was measured from a side branch of the brachial artery just below the outlet cannula. Ischemia was produced by turning off the pump for 2 hr after the control period. All parameters were followed up to 1 hr after restarting the pump at the same rate of blood flow.

In a third series of experiments (collateral-free, innervated, naturally perfused forelimbs), a lymphatic vessel was isolated just distal to the elbow, running in close proximity to the cephalic vein (12). This vessel as well as a small vein in the skin (second superficial dorsal metacarpal vein) of the paw area were cannulated. The catheter of the skin vein was connected to a pressure transducer. The outflow of the lymph vessel was directed into a small-bore, graduated, collecting tube. The collecting tubes were changed at regular intervals (10 min). The collecting tubes were immediately sealed to prevent water loss. The fluid was analyzed for total protein content by the spectrophotometric (Beckman DC spectrophotometer) method of Waddell (13). Electrophoresis was used to measure albumin and globulin fractions in each sample. To facilitate the isolation and cannulation of the lymphatic vessel, 500 ml of a 0.9% sodium chloride solution was given via the saphenous vein prior to the isolation of the forelimb. Occlusion in this series was carried out by clamping the brachial artery upstream to the test organ. The ischemic and post-ischemic periods were the same as in the previous two series.

*Intact forelimb.* Two additional series of experiments were conducted utilizing nearly intact canine forelimbs. Occlusion in these experiments was produced by inflation of a cuff secured around the limb above the elbow. The cuff was rapidly inflated to about 500 mm Hg pressure. In the first series, intact forelimbs were placed on the I-beam balance to measure relative changes in forelimb weight. The paw and forearms were placed on the pan with the shoulder acting as the fulcrum. After an isogravimetric control period the cuff was inflated 2 hr. After relief of the cuff occlusion, all responses were followed up to 60 min. There were no measurements of intravascular pressure via catheters and the animals were not anticoagulated. In a second series of intact forelimbs a lymphatic vessel which lies in close proximity to the cephalic vein just below the elbow, was isolated and cannulated as previously described. In several of these experiments a small skin vein was also isolated and cannulated (second superficial dorsal metacarpal vein) as an indicator of the change in intravascular pressure. After a control period, the cuff occlusion responses were followed up to 60 min.

Student's *t*-test, modified for paired replicates, was used to determine differences between experimental and control values.

*Results. Forelimb weight.* In the collateral-free limbs, interruption of arterial inflow produced a rapid (within 2 min) 12.5-g weight loss as the vascular bed emptied into the reservoir and weight was 18 g below the control level by 120 min. Release of the arterial clamp in the naturally perfused limbs caused a rapid increase in weight to a level 8 g in excess of the control value (Table I). The average absolute weight of the limbs was 474 g. Thus, weight increased 1.7% and weight then returned to the control level within 15 min. Restarting the pump in the limbs perfused at contant flow increased weight to a level not different from that in the control period, where it remained for the entire 60-min period (Table I). In the intact forelimbs ($n = 7$), neither cuff occlusion nor relief of the cuff occlusion significantly affected limb weight (not shown). Inspection and palpation failed to reveal edema in any experiment.

*Pressure.* Interruption of inflow produced a sharp decline in brachial and cephalic vein pressures in both series of collateral-free innervated, forelimbs. Restoration of blood flow immediately produced large, transient

TABLE I. AVERAGE FORELIMB WEIGHT CHANGE, VASCULAR PRESSURES, BLOOD FLOWS, AND VASCULAR RESISTANCES IN COLLATERAL-FREE, INNERVATED FORELIMBS IN RESPONSE TO 2 HR OF ISCHEMIA (MEAN ± SE).[a]

| Minutes | | Control | | Postocclusion | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | −5 | 0 | 2 | 5 | 10 | 15 | 30 | 45 | 60 |
| Net weight change (g) | N | 0.0 | 0.0 ± 0.4 | 8 ± 2[b] | 6 ± 3[b] | 3 ± 3 | 2 ± 3 | 1 ± 3 | −1 ± 3 | −2 ± 3 |
| | C | 0.0 | 0.2 ± 0.4 | −3 ± 2 | −1 ± 2 | −1 ± 2 | −1 ± 3 | −1 ± 4 | −2 ± 4 | −2 ± 4 |
| Systemic arterial pressure (mm Hg) | N | 136 ± 3 | 136 ± 3 | 131 ± 6 | 135 ± 7 | 139 ± 6 | 139 ± 7 | 139 ± 7 | 139 ± 7 | 139 ± 6 |
| | C | 126 ± 8 | 126 ± 8 | 139 ± 9 | 141 ± 9 | 138 ± 7 | 134 ± 6 | 131 ± 6 | 131 ± 6 | 131 ± 6 |
| Perfusion pressure (mm Hg) | C | 107 ± 7 | 107 ± 7 | 54 ± 3[b] | 57 ± 3[b] | 71 ± 7[b] | 91 ± 12 | 103 ± 11 | 107 ± 12 | 116 ± 10 |
| Brachial vein pressure (mm Hg) | N | 7 ± 1 | 7 ± 1 | 23 ± 1[b] | 17 ± 1[b] | 10 ± 1[b] | 8 ± 1 | 7 ± 1 | 7 ± 1 | 7 ± 1 |
| | C | 5 ± 2 | 5 ± 2 | 9 ± 2[b] | 9 ± 2[b] | 8 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 |
| Cephalic vein pressure (mm Hg) | N | 7 ± 1 | 7 ± 1 | 21 ± 2[b] | 10 ± 2[b] | 7 ± 1 | 6 ± 1 | 6 ± 1 | 6 ± 1 | 6 ± 1 |
| | C | 5 ± 2 | 5 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 | 7 ± 2 |
| Brachial vein blood flow (ml/min) | N | 50 ± 7 | 50 ± 7 | 141 ± 14[b] | 105 ± 10[b] | 71 ± 7[b] | 53 ± 7 | 45 ± 7 | 44 ± 7 | 41 ± 7 |
| | C | 56 ± 11 | 56 ± 11 | 58 ± 6 | 54 ± 7 | 52 ± 9 | 48 ± 9 | 48 ± 9 | 48 ± 9 | 48 ± 9 |
| Cephalic vein blood flow (ml/min) | N | 74 ± 10 | 74 ± 10 | 160 ± 18[b] | 92 ± 12[b] | 74 ± 8 | 67 ± 7 | 65 ± 7 | 61 ± 7 | 57 ± 7 |
| | C | 48 ± 8 | 48 ± 8 | 46 ± 5 | 49 ± 5 | 52 ± 6 | 55 ± 6 | 55 ± 6 | 55 ± 6 | 55 ± 6 |
| Total forelimb flow (ml/min) | N | 124 ± 15 | 124 ± 15 | 314 ± 30[b] | 200 ± 19[b] | 145 ± 10 | 120 ± 11 | 110 ± 12 | 105 ± 12 | 99 ± 12 |
| | C | 105 ± 9 | 105 ± 9 | 104 ± 7 | 103 ± 8 | 104 ± 8 | 104 ± 9 | 104 ± 9 | 104 ± 9 | 104 ±9 |
| Muscle resistance (mm Hg/ml/min) | N | 3.0 ± 0.6 | 3.0 ± 0.6 | 0.8 ± 0.1[b] | 1.2 ± 0.2[b] | 2.0 ± 0.3[b] | 2.8 ± 0.5 | 3.5 ± 0.7 | 3.5 ± 0.7 | 3.9 ± 0.8 |
| | C | 2.6 ± 0.7 | 2.6 ± 0.7 | 0.8 ± 0.1[b] | 1.0 ± 0.2[b] | 1.6 ± 0.3[b] | 2.2 ± 0.5 | 2.5 ± 0.5 | 3.0 ± 0.8 | 3.3 ± 0.8 |
| Skin resistance (mm Hg/ml/min) | N | 2.1 ± 0.4 | 2.1 ± 0.4 | 0.7 ± 0.1[b] | 1.5 ± 0.2[b] | 1.9 ± 0.3 | 2.1 ± 0.3 | 2.3 ± 0.4 | 2.6 ± 0.5 | 2.6 ± 0.5 |
| | C | 2.4 ± 0.4 | 2.4 ± 0.4 | 1.1 ± 0.1[b] | 1.2 ± 0.1[b] | 1.4 ± 0.2[b] | 1.8 ± 0.4 | 2.1 ± 0.5 | 2.4 ± 0.7 | 3.0 ± 0.7 |
| Total forelimb resistance (mm Hg/ml/min) | N | 1.2 ± 0.2 | 1.2 ± 0.2 | 0.4 ± 0.1[b] | 0.6 ± 0.1[b] | 0.9 ± 0.1[b] | 1.0 ± 0.2 | 1.3 ± 0.2 | 1.4 ± 0.2 | 1.5 ± 0.2 |
| | C | 1.1 ± 0.1 | 1.1 ± 0.1 | 0.3 ± 0.1[b] | 0.4 ± 0.1[b] | 0.6 ± 0.1[b] | 0.8 ± 0.2 | 1.0 ± 0.2 | 1.1 ± 0.3 | 1.3 ± 0.4 |

[a] N = natural inflow ($n$ = 7), average limb weight = 474 ± 30 g. C = constant inflow ($n$ = 7), average limb weight = 500 ± 25 g.
[b] Indicates statistical significance at the 0.05 level or less compared to control.

TABLE II. LYMPH FLOW, SMALL SKIN VEIN PRESSURE, AND LYMPH PROTEIN RESPONSES TO 2 HR OF ISCHEMIA INDUCED BY CLAMP OCCLUSION OF THE BRACHIAL ARTERY IN COLLATERAL-FREE, INNERVATED, NATURALLY PERFUSED FORELIMBS ($n$ = 6) (CF) OR BY INFLATION OF A PRESSURE CUFF FOR 2 HR IN INTACT FORELIMBS ($n$ = 6) (I).[a]

| Minutes | | Control | | Postocclusion | | | |
|---|---|---|---|---|---|---|---|
| | | −20−−10 | −10-0 | 0–10 | 10–20 | 20–30 | 50–60 |
| Lymph flow rate (ml/10 min) | CF | 0.02 | 0.02 | 0.01 | 0.04 | 0.02 | 0.02 |
| | I | 0.08 | 0.07 | 0.12 | 0.07 | 0.07 | 0.07 |
| Pressure small skin vein[b] mm Hg | CF | 11.5 | 11.5 | 14[c, d] | 13 | 13 | 11 |
| | I | —[e] | —[e] | —[e] | —[e] | —[e] | —[e] |
| Total protein (g/100 ml) | CF | 1.9 | 1.9 | 2.3 | 2.2[d] | 2.1 | 2.1 |
| | I | 0.9 | 1.0 | 1.3 | 1.3 | 1.3 | 1.3 |
| Albumin fraction (g/100 ml) | CF | 1.1 | 1.0 | 1.3 | 1.2 | 1.1 | 1.0 |
| | I | 0.5 | 0.5 | 0.8 | 0.8 | 0.8 | 0.8 |
| Globulin fraction (g/100 ml) | CF | 0.8 | 0.9 | 1.0 | 1.0 | 1.0 | 1.1 |
| | I | 0.4 | 0.4 | 0.5 | 0.6 | 0.5 | 0.5 |

[a] CF, collateral-free forelimb; I, intact forelimb.
[b] The small vein pressure reported is the pressure at the end of the collection period.
[c] Pressure much higher over 0–3 minute time interval.
[d] Indicates statistical significance $P \leq 0.05$ compared to control.
[e] —, not regularly measured.

increases in large-vein pressures relative to control in the naturally perfused forelimbs (Table I). In the forelimbs perfused at constant inflow, reinstitution of flow produced a small transient increase in brachial vein pressure but had no effect on cephalic pressure. Systemic arterial pressure did not significantly change from the control level during the postischemic period. However, a statistically significant increase to 153 mm Hg relative to control was observed during the last hour of the occlusion period in the animals perfused at natural inflow (not shown).

*Blood flow.* In the collateral-free, innervated, naturally perfused forelimbs, flows greatly exceeded control levels immediately after release of the brachial artery clamp (Table I). They then returned to control levels within 15 min. In the collateral-free, innervated, forelimbs perfused at constant inflow, there was no significant shift in flow between brachial and cephalic veins upon relief of the ischemia.

*Vascular resistance.* Total forelimb resistance was markedly decreased after the relief of the ischemia (Table I). Within 15 min, vascular resistance returned to control. The transient decline in the total forelimb resistance was due to a decrease in both skin and skeletal muscle vascular resistances.

*Lymph flow.* Compared to control period, the rate of lymph flow did not change significantly after the relief of 2 hr of ischemia in either the collateral-free, innervated, naturally perfused (Table II), or the intact (Table II) forelimbs.

*Concentration of protein in lymph.* Total protein concentration increased slightly but significantly in the second 10-min sample after relief of ischemia in the collateral-free innervated, naturally perfused limbs (Table II). Albumin and globulin did not change significantly. None of the three variables was significantly affected in the intact limbs (Table II).

*Discussion.* Release of occlusion in the collateral-free, innervated, naturally perfused preparation produced an 8-g increase in weight which disappeared within 15 min. This increase in weight likely had two components: increased vascular volume and increased interstitial fluid volume. Vascular resistance was decreased and venous transmural pressures were increased, suggesting increased vessel caliber and hence blood volume. Venous pressures were increased to levels equal to the colloid osmotic pressure of dog plasma, suggesting capillary hydrostatic pressure in excess of colloid osmotic pressure. The relative contributions of the

two components to the increased weight is is difficult to evaluate but even assuming all of it resulted from increased interstitial fluid volume, the amount of excess fluid is still very small. Limb weight increased only 1.7%, visual and palpatory signs of edema were absent and lymph flow did not increase. By contrast, intra-arterial infusion of histamine or bradykinin for 30 min in this preparation can produce an 18% increase in weight, lymph flows nine times normal, and all the classic signs of gross edema (12, 14, 15). Furthermore, increased weight and decreased resistances disappeared within 15 min, indicating normal interstitial fluid volume at this time and thereafter. By contrast, weight remains elevated once an infusion of histamine or bradykinin is discontinued (14, 15).

Increased weight was not seen in the collateral-free, innervated, constantly perfused preparation despite the fact that vascular resistance decreased. Thus, both components of the weight gain were abolished when inflow was not allowed to exceed control levels. Apparently capillary hydrostatic pressure and venous vascular volume did not increase. This is suggested by the essentially normal venous pressures. Weight also failed to increase in the intact preparations in which the cuff occluded all vessels including the lymphatics. Lymph flow increased transiently in only three of six preparations. Clearly, in the dog forelimb, relief of 2 hr of ischemia does not produce more than very slight and transient edema. This observation is consistent with that of Lazarus-Barlow in the dog limb (1) but contrary to that of Brown and Garrett in the rat hindpaw (3).

The absence of significant edema in the dog forelimb is apparently related to the fact that the hemodynamic changes disappear very quickly and the microvascular membrane does not become grossly permeable to plasma proteins. Total lymph protein concentration, which probably reflects interstitial fluid protein concentration (12), transiently increased only 16% in the collateral-free natural flow preparation and did not change significantly in the intact limbs. By contrast, intra-arterial infusion of histamine in the dog forelimb can increase total lymph protein concentration by 215% (to 6.9 g/100 ml), almost equaling that in plasma (12). Intra-arterial infusion of bradykinin can almost double lymph protein concentration (15).

Our conclusion, that permeability to protein is not greatly affected in the dog forelimb by 2 hr of ischemia, is similar to that of Diana and Laughlin in the dog hindlimb (16). They calculated pore radius 20–40 min after relief of 30-, 60-, and 180-min periods of ischemia and found no change except in a few limbs subjected to 180 min of ischemia. They did not present weight in the free-flow situation during the period immediately after reperfusion.

Effects of hypoxemia are also difficult to demonstrate in the dog limb. Nairn (17) perfused the dog hindlimb with venous blood from the femoral vein of the opposite hindlimb (oxygen saturation = 50%) for 6–7 hr and edema did not develop. Scott *et al.* (18) perfused dog forelimbs at constant flow with hypoxemic blood ($P_{O_2}$ = 10 mm Hg, pH unchanged) for 15 min and failed to observe changes in weight. Increased $CO_2$ tension is also without effect on weight in this preparation (19). Studies in man also suggest a minor effect of hypoxemia on protein permeability (20, 21).

On the other hand, Landis (22), studying single capillaries in the frog mesentery, sometimes observed filtration even in the face of a low capillary hydrostatic pressure 30–60 sec after occlusion of the capillary when the mesentery was bathed with oxygen-free Ringer's solution. In other experiments, the effective colloid osmotic pressure across single capillaries was decreased 1 min after the relief of 3 min of ischemia of the entire intestine when the intestine was bathed with the same solution. The effect disappeared within 15 min and did not occur when the intestine was bathed with oxygenated Ringer's solution. Carbon dioxide had little effect on filtration or protein permeability.

Furthermore, certain studies suggest that the rat limb may be more sensitive to ischemia (3) and hypoxemia (23, 24). Obviously, much more study is needed before the findings of the present study can be extended to other tissues and species.

*Summary.* The aim of this study was to determine if edema develops in the canine

forelimb after relief of prolonged ischemia. Two hours of ischemia was induced either by interrupting brachial artery inflow in collateral-free, innervated, naturally and constantly perfused preparations or by applying a pressure cuff in intact preparations. Changes in extravascular fluid volume were inferred from changes in limb weight. Relief of ischemia produced a transient 1.7% increase in weight in the collateral-free, naturally perfused preparation but had no effect on weight in the collateral-free, constantly perfused or intact preparations. Skin lymph flow failed to change significantly and total lymph protein concentration increased only slightly. Thus, relief of 2 hr of ischemia does not produce marked edema or a large increase in microvascular permeability to plasma proteins in the dog forelimb.

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