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Effect of Transient Intestinal Ischaemia on the Thoracic Duct Lymph Absorption of Endotoxin

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Summary

One-hour intestinal ischaemia by clamping the superior mesenteric artery and interrupting the intramural collaterals was produced in 8 dogs. Thoracic duct lymph, mesenteric venous blood and arterial blood were examined before and during intestinal ischaemia and after revascularization of the small bowel. Venous blood was the major route of transport of lactic acid and lactate dehydrogenase from the ischaemic bowel. Endotoxin activity determined by the limulus test was found in the lymph before the induction of intestinal ischaemia in 4 of 6 dogs while it was absent in arterial blood. After one hour lasting revascularization endotoxin was present in the lymph of all dogs and in 3 and 4 dogs in arterial and mesenteric venous blood respectively. It was demonstrated that limulus-positive substances escape from the intestine both via the lymph and blood.

The small intestine is very sensitive to ischaemia. Functional changes develop as early as ten minutes and irreversible changes two hours after the interruption of blood flow (12). The intestine is incapable of active resorption after one hour of acute ischaemia (11). *Ackerman* (1) found in dogs with ligated mesenteric artery the lymph flow from the ischaemic intestine to decrease to 38 % of the control value but not to stop completely. Lymph vessels may be of a considerable importance for the transport of toxic substances produced by or resorbed from the ischaemic intestine. In haemorrhagic shock the absorption of endotoxin increases dramatically and may be the cause of death in this condition (14).

We studied changes of the thoracic duct lymph flow and composition attending intestinal ischaemia and following intestinal revascularization.

Methods

The experiments were carried out on 8 mongrel dogs of 18 to 28 kg in sterile conditions. The thoracic duct and the femoral artery were cannulated under general pentobarbital anaesthesia (25 mg/kg) to collect the lymph and arterial blood, respectively. After opening the abdominal cavity, the small intestine was severed proximally at the level of the second part of the duodenum and distally at the ileocaecal valve. To obtain venous blood of the small intestine a great branch of the mesenteric vein was cannulated. One hour intestinal ischaemia was produced by clamping the superior mesenteric artery and interrupting the intramural collaterals. Samples of thoracic duct lymph, venous blood from the ischaemic intestine and arterial blood were collected before and 30 and 60 min after the interruption of intestinal blood flow and then 1, 2 and 4 hours after revascularization. Lymph was allowed to flow freely throughout the experiment. Thoracic duct lymph flow was measured and haematocrit and the concentration of total protein (biuret), lactic acid and lactate dehydrogenase – LDH (Boehringer test combination) were determined in the lymph and blood plasma. The lymph and plasma of 6 dogs was analyzed qualitatively for the endotoxin activity by the limulus test (8). This method is based on the observation that endotoxin induces gelation of the lysate obtained from amebocytes of the horseshoe crab, Limulus polyphemus. The formation of a firm gel was interpreted as a positive test (3+). The test proved to be reproducible with a sensitivity for the lipopolysaccharide used as standard of 1 ng of endotoxin per 1 ml of plasma. Bacteria were cultured from the distal ends of the small bowel. Gram-negative microorganisms were found in all dogs.

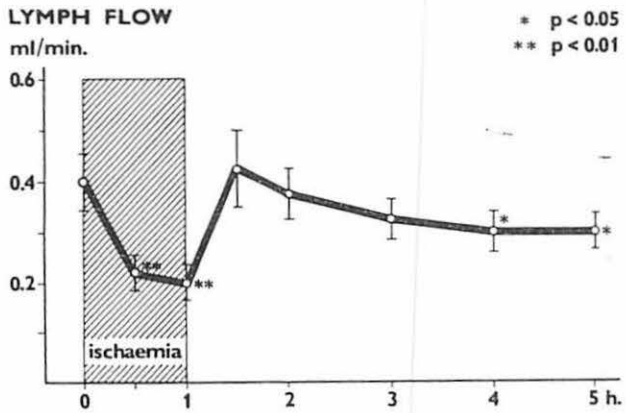

Fig. 1: Mean ± SD of the thoracic duct lymph flow.

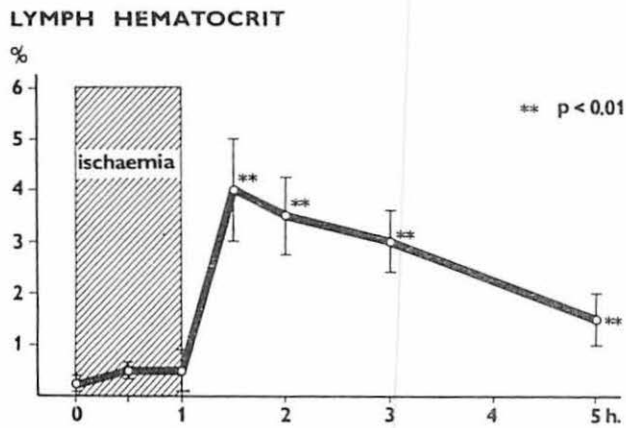

Fig. 2: Thoracic duct lymph hematocrit.

Results

Intestinal ischaemia was associated with a statistically significant decrease in thoracic duct lymph flow; after revascularization of the bowel it rose above the control value (Fig. 1). The significant increase of the lymph haematocrit shows that erythrocyte penetration into the lymph occurred after the restoration of intestinal blood flow (Fig. 2). The lymph/plasma ratio for the total protein increased significantly during intestinal ischaemia and then decreased significantly after revascularization (Fig. 3). This was due to changes in the lymph protein; its levels in blood plasma did not change significantly, only at the end of the experiment the plasma protein was lower than at the beginning. Changes in lactic acid are shown in figure 4. Intestinal ischaemia was associated with a rapid increase in lactic acid only in mesenteric vein blood. After revascularization its levels in mesenteric vein blood and in the lymph were almost uniform. LDH activity exhibited similar pattern, i.e. increase in intestinal venous blood during ischaemia and in the lymph after revascularization (Fig. 5). The slow increase in LDH in arterial blood might have been caused by the simultaneous external lymph drainage.

Before the induction of intestinal ischaemia the presence of endotoxin in thoracic duct lymph was established in 4 of 6 dogs and in the mesenteric vein blood in one dog (table 1). The limulus test in arterial blood was always negative. At the end of ischaemia and after one hour lasting revascularization endo-

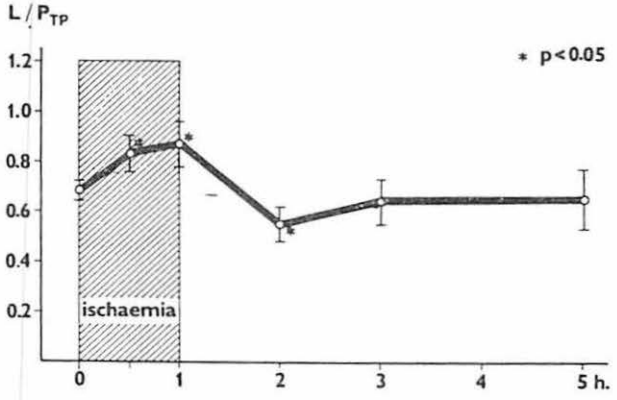

Fig. 3: Lymph/plasma ratio for total protein.

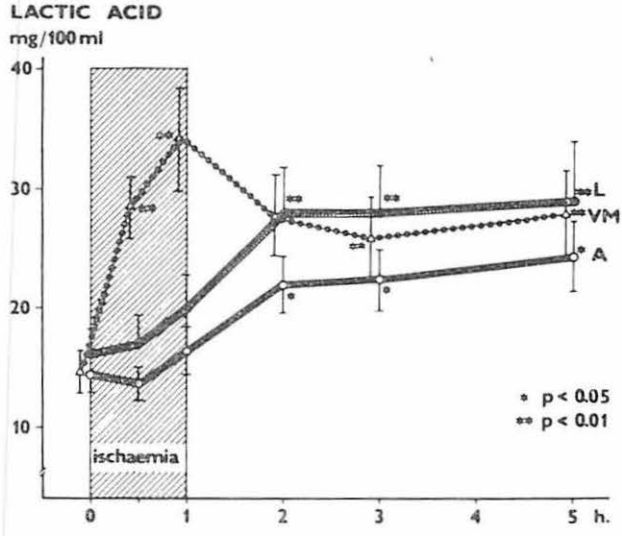

Fig. 4: The levels of lactic acid in the lymph (L), mesenteric venous blood plasma (VM) and arterial blood plasma (A).

Table 1 Number of dogs with positive endotoxin activity

n = 6	before ischaemia	1 hour ischaemia	1 hour revascul.
Thoracic duct lymph	4	6	6
Intestinal venous blood	1	1	4
Arterial blood	0	0	3

toxin was present in the lymph of all experimental animals. Venous and arterial blood displayed a higher positivity rate only after intestinal revascularization.

Discussion

Examination of mixed thoracic duct lymph permits only an indirect evaluation of intestinal lymph changes on the basis of the protein level. Protein changes show that during intestinal ischaemia the thoracic duct contains more liver lymph while intestinal lymph predominates after revascularization. Because intestinal hypoperfusion elicits an increase in tissue fluid hydrostatic pressure (7) and vigorous contractions of the bowel (6) a complete arrest of intestinal lymph flow is unlikely. However, veins are the major routes of transport from the ischaemic intestine even for substances of a higher molecular weight such as LDH. Intestinal revascularize-

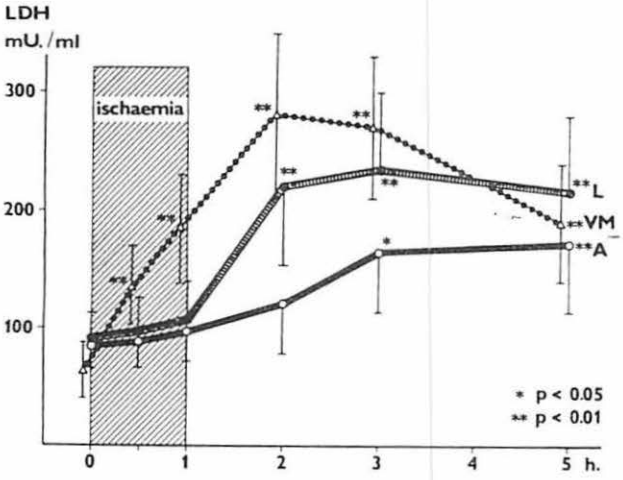

Fig. 5: LDH activities of the lymph (L), mesenteric venous blood plasma (VM) and arterial blood plasma (A).

tion which results in an elevation of the capillary hydrostatic pressure permits an increased intestinal lymph production and an increased transport of metabolic and breakdown products via the intestinal lymph.

The abrupt increase of lactic acid in the mesenteric vein blood militates against the opinion that the gastrointestinal tract is unable to produce lactate (9). This metabolite may originate in the lymphoid tissue which comprizes up to 1/4 of the intestinal mass (4). Hypoxia is known to cause the release of high amounts of lactate from lymphoid cells. Some of our results correspond with the findings of *Ahonen* et al. (2). In a similar study on 4 dogs, they found that occlusion of the superior mesenteric artery had not changed thoracic duct lymph flow and the lactic acid level in the lymph had gone up 50 %.

While there has been some dispute in the past about the absorption of endotoxin from the gut, there seems little doubt that it can and does pass through the intestinal wall (10). Owing to their high molecular weight (0.5 to 2.0 million) lipopolysaccharides could be hardly transported by unimpaired blood vessels. Endotoxin injected into the peritoneal cavity was demonstrated to travel via the thoracic duct lymph (3). Thus far, there is only indirect experimental evidence of the transport of endotoxin via the intestinal lymph (5). Our study produced a direct evidence that a certain amount of endotoxin is absorbed via lymphatics despite negative findings in mesenteric vein blood. It is still impossible to decide whether this is true under physiological conditions or whether the escape of endotoxin into the lymph was made possible by anaesthesia. Permeability of the intestinal wall for the transport of endotoxin via the lymph and in part also via mesenteric blood seems to increase with intestinal ischaemia. Endotoxin could also get access into the lymph from the liver. *Tice* and *Dumont* (13) found lethal substances in the thoracic duct lymph of dogs following long-term superior mesenteric artery ligation when the liver was responsible for most of the lymph flow. Yet it cannot be ruled out that the limulus test could be positive also in the presence of substances other than endotoxin.

We can conclude that during intestinal ischaemia lymph transport from the gut is low but can be sufficient for the transport of endotoxin. Transport via the intestinal lymph significantly increases after revascularization. We have demonstrated that limulus-positive substances escape from the ischaemic intestine both via the lymph and blood.

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