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FLOW AND COMPOSITION OF SKIN AND MUSCLE LYMPH OF THE HIND LIMB OF THE RABBIT AFTER INJURY

CATHERINE S. BACH & G.P. LEWIS[1]
CIBA Laboratories, Horsham, Sussex

1 Collection of skin lymph separately from muscle lymph has enabled us to repeat earlier experiments in which lymph was collected from the whole hind limb to determine whether the changes then observed were the result of changes occurring in the skin or the muscle or both.

2 After thermal and chemical injury, it appeared that most of the changes were due to leakage of enzymes from the muscle; after freezing, changes occurred in both skin and muscle lymph while ischaemia caused no significant changes in either skin or muscle lymph.

3 After mild thermal injury it took longer for the enzyme leakage to reach a maximum in muscle lymph than in skin lymph. It seems likely that the changes were buffered by the large tissue space of muscle.

4 Lactic dehydrogenase (LDH) activity appears to diffuse from muscle into skin since although intramuscular dimethyl sulphoxide (DMSO) causes the release of LDH into skin lymph, subcutaneous DMSO does not.

5 That proportion of muscle LDH released during injury might represent the unbound or 'active' portion since no matter how severe the injury only about 1% of the total muscle LDH was released into the lymph.

Introduction

In previous work from this laboratory, lymph collected from the femoral lymphatic has been examined after various injuries such as thermal (Lewis, 1967, 1969), chemical (Boyles, Lewis & Westcott, 1970) and immunological (Jasani & Lewis, 1971). This lymph drained both skin and muscle and all of it was thought to pass through the popliteal node. However, when comparing changes in pre- and post-nodal lymph, it was found that lymph draining the muscles did not pass through the node and that muscle lymph could be collected separately from skin lymph (Lewis & Yates, 1972). The technique has been used to compare changes in lymph flow and protein concentration after thermal injury (Bach & Lewis, 1973) and now chemical injury and ischaemia have been studied. The experiments were also designed to compare the changes in the concentration of intracellular enzymes which occur in muscle and skin lymph after injury. The enzymes examined have been chosen as markers of different intracellular compartments as in previous studies. It was hoped that the present examination would enable us to determine whether the changes previously observed were the result of skin or muscle cell injury.

Methods

Experiments were performed on New Zealand White rabbits (2.6 to 3.2 kg) anaesthetized with intravenous pentobarbitone sodium. The dose was about 40 mg/kg but was adjusted according to the depth of respiration and corneal and other reflexes. It was usually found necessary to give additional anaesthetic each 0.5-1 hour.

Collection of lymph

Muscle lymph was collected by cannulating the main femoral lymphatic and ligating the post-nodal lymphatic close to the node as described by Lewis & Yates (1972). Skin lymph was collected by cannulating one of the lymphatics in the lower leg before it entered the popliteal node. One of the

[1] Present address: Department of Pharmacology, Royal College of Surgeons, Lincoln's Inn Fields, London, WC2A 3PN

most prominent of these runs close to a vein which branches off the large saphenous and runs superficially along the dorsal aspect of the popliteal fossa. The limb was moved passively to facilitate the collection of lymph as described by Lewis & Westcott (1968).

Injuries

The hind limb was subjected to thermal injury by immersion in water at 60°C for 1 min or 80°C for 15-20 s or frozen by covering with dry ice/acetone mixture for 3 minutes. Chemical injury to skin or muscle was produced by injecting a total of 0.6 ml dimethyl sulphoxide (DMSO) in 4-6 injection sites either intradermally or intramuscularly. Injury by ischaemia was obtained by arresting the circulation through the leg for 4 h by means of clamps on the femoral artery and vein and a ligature tied around the remainder of the limb to avoid circulation through collateral vessels.

Biochemical Methods

The activities of the following enzymes and protein were estimated by the methods described by Lewis (1967, 1969): glutamic oxaloacetic transaminase (L-aspartate: 2-oxoglutarate aminotransferase E.C.2.6.1.1); glutamic pyruvic transaminase (L-alanine:2-oxoglutarate aminotransferase E.C.2.6.1.2); acid phosphatase (orthophosphoric monoester phosphohydrolase E.C.3.1.3.2); β-glucuronidase (β-glucuronide glucuronohydrolase E.C.3.2.1.31). Cathepsin (cathepsin D, E.C.3.4.4.23) was determined by a modification of the method of Anson (1938). Lactic dehydrogenase (L-lactate: NAD oxidoreductase E.C.1.1.1.27) was determined by the method of Wroblewski & LaDue (1955) using the Boehringer Mannheim test combination for the LKB Reaction Rate Analyser.

Results

Lymph flow and protein concentration

In earlier experiments chemical injury produced by subcutaneous DMSO caused an increase in lymph flow with a corresponding fall in protein concentration (Boyles *et al.*, 1968). The present results given in Table 1 show that the increase in flow occurred only with respect to muscle lymph and no change occurred in skin lymph flow. However, there appeared to be a small decrease in protein concentration in skin lymph although, unlike that in muscle lymph, the change did not appear to be significant. None of these changes occurred when an equal volume of saline (0.9% w/v NaCl solution) was injected.

In previous experiments in cats (Lewis, 1967), it was shown that after arresting the blood flow for 1-2 h there was a three to six-fold increase in lymph flow and a fall in lymph protein concentration after the circulation was re-established. In the present experiments in which the blood flow was occluded for 4 h, there was a sustained two to three-fold increase in muscle lymph flow whereas the skin lymph flow increased only transiently immediately after re-establishing the circulation. At the same time there was a decrease in muscle lymph protein but no change in skin lymph protein.

Biochemical composition

The concentrations of six intracellular enzymes, lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), acid phosphatase (acid phos), β-glucuronidase (β-gluc) and cathepsin were measured in lymph draining skin and muscle.

With all the enzymes except LDH, there was no significant difference between the control levels

Table 1 Protein concentration and flow of skin and muscle lymph before and 2 h after chemical injury caused by (a) intramuscular and (b) intradermal injection of dimethyl sulphoxide

	Skin		*Muscle*	
(a) *Intramuscular*	*Control*	*2 h*	*Control*	*2 h*
Prot (mg/ml)	21.7 ± 2.3 (3)	15.5 ± 3.3 (3)	27.6 ± 5.5 (3)	11.7 ± 0.6 (3)
Flow (μl/min)	3.2 ± 0.59 (5)	2.4 ± 0.5 (5)	3.3 ± 0.3 (6)	10.2 ± 1.5 (6)
(b) *Intradermal*				
Prot (mg/ml)	23.8 ± 3.4 (4)	15.4 ± 1.4 (4)	23.0 ± 2.3 (4)	17.9 ± 0.9 (4)
Flow (μl/min)	4.7 ± 0.7 (6)	3.7 ± 1.2 (6)	3.3 ± 0.7 (8)	3.0 ± 0.6 (8)

Values are means with s.e. The number of experiments is given in brackets

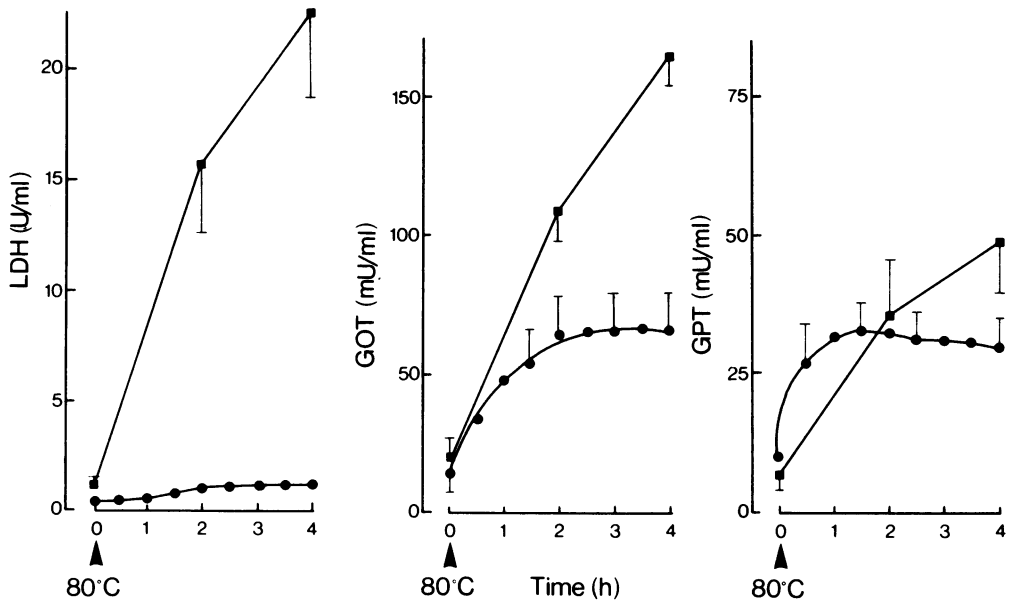

Figure 1 The concentrations of lactic dehydrogenase (LDH) in u/ml, glutamic oxaloacetic transaminase (GOT) in mu/ml and glutamic pyruvic transaminase (GPT) in mu/ml in samples of lymph draining the muscle (■) and skin (●) of the hind limb. At the arrow the limb was immersed in water at 80° C for 15-20 seconds.

nor were they different from the femoral lymph and plasma levels reported earlier (Lewis, 1969). However, the LDH activity in muscle lymph (0.74 ± 0.11 u/ml, $n = 6$) was higher ($P < 0.01$) than that in skin lymph (0.29 ± 0.09 u/ml, $n = 6$).

In general the enzyme changes observed earlier (Lewis, 1969; Boyles *et al.*, 1970) in femoral lymph after thermal and chemical injury were found to be derived mainly from the muscle rather than skin. Figure 1 illustrates the increases in LDH, GOT and GPT in skin and muscle lymph after the limb had been injured at 80°C for 15 seconds. Similar changes occurred after intramuscular (Fig. 2) or intradermal injection of DMSO.

Table 2 gives the mean increases of LDH activity in skin and muscle lymph obtained after scalding the limb at 60°C for 1 min, at 80°C for 15-20 s, after freezing or intradermal or intramuscular injection of DMSO compared with the

Table 2 Lymph concentration of lactic dehydrogenase in u/ml before and after injury

		60° C 1 min	*80° C 15-20 s*	*Freezing*	*i.d.*	*DMSO s.c.*	*i.m.*
Mixed femoral lymph	Before	*0.26	*0.37	*0.32		**0.095	
	After	14.98	11.81	24.42		6.49	
Skin lymph	Before	0.28	0.40	0.14	0.26		0.36
	After	0.30	1.15	5.00	0.28		3.70
Muscle lymph	Before	0.79	0.94	0.58	0.46		1.3
	After	11.18	15.75	32.58	1.33		30.7

DMSO, dimethyl sulphoxide
* From Lewis (1969)
** Boyles *et al* (1970)

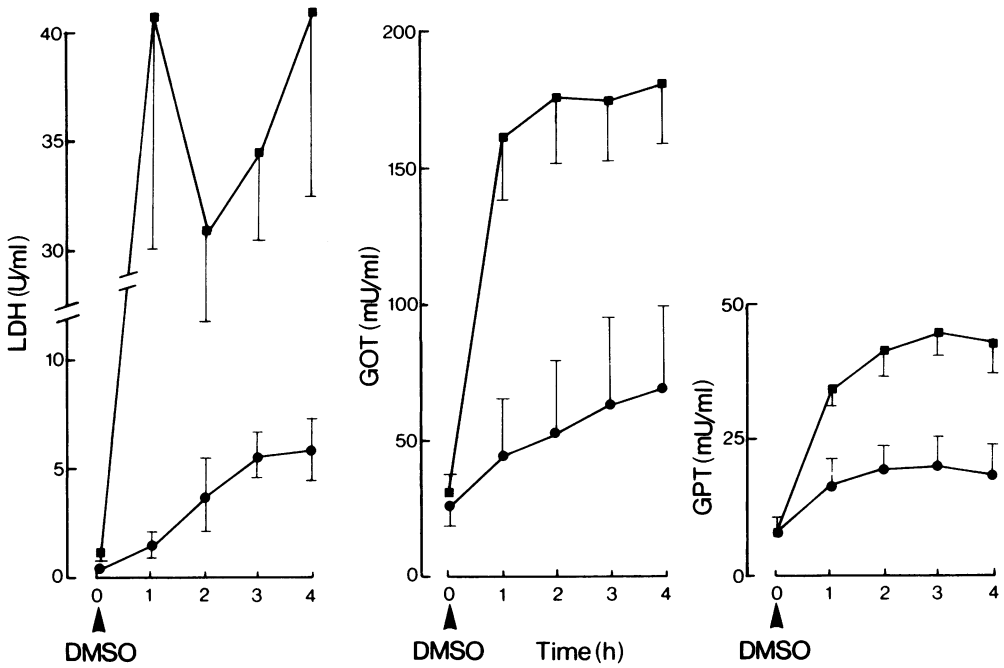

Figure 2 The concentrations of lactic dehydrogenase (LDH) in u/ml, glutamic oxaloacetic transaminase (GOT) in mu/ml and glutamic pyruvic transaminase (GPT) in mu/ml in samples of lymph draining the muscle (■) and skin (●) of the hind limb. At the arrow, 6 x 0.1 ml injections of dimethyl sulphoxide (DMSO) were made intramuscularly.

results obtained earlier when the changes were examined in mixed femoral lymph.

The changes in concentrations of GOT and GPT in skin and muscle lymph after thermal injury at 60°C or 80°C or injection of DMSO were similar to those found earlier in mixed femoral lymph although generally they were somewhat higher in muscle lymph than in skin lymph.

After freezing as shown in Table 3 the increases in concentrations of the lysosomal enzymes were found to be greater in skin lymph than in muscle, whereas GOT and GPT activities again were greater in muscle lymph. Although there was a considerable increase in the LDH in skin lymph after the leg had been frozen, the total activity was still only about 15% of that in muscle lymph.

After arresting the blood flow in cats for 1-2 h, no changes in enzyme levels in femoral lymph

Table 3 Concentration of intracellular enzymes in skin and muscle lymph before and 2 h after freezing

	Skin		*Muscle*	
	Control	*2 h*	*Control*	*2 h*
LDH (u/ml)	0.14 ± 0.06 (3)	5.0 ± 0.46 (3)	0.58 ± 0.08 (3)	32.58 ± 11.8 (3)
GOT (mu/ml)	11 ± 7 (3)	91 ± 16 (3)	11 ± 4 (3)	171 ± 5 (3)
GPT (mu/ml)	2 ± 1 (3)	24 ± 4 (3)	8 ± 2 (3)	74 ± 7 (3)
β-gluc (u/100 ml)	134 ± 49 (3)	7015 ± 2606 (3)	426 ± 344 (5)	3918 ± 918 (5)
Acid phos (u/100 ml)	5 ± 1.6 (3)	32.4 ± 8.9 (3)	6.7 ± 1.9 (3)	21.2 ± 5.3 (3)
Cathepsin (u/ml)	4.7 ± 0.2 (3)	33.2 ± 3.2 (3)	2.8 – (2)	21.1 – (2)

Values are means with s.e. The number of experiments is given in brackets
LDH, lactic dehydrogenase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; β-gluc, β glucuronidase; Acid phos., acid phosphatase

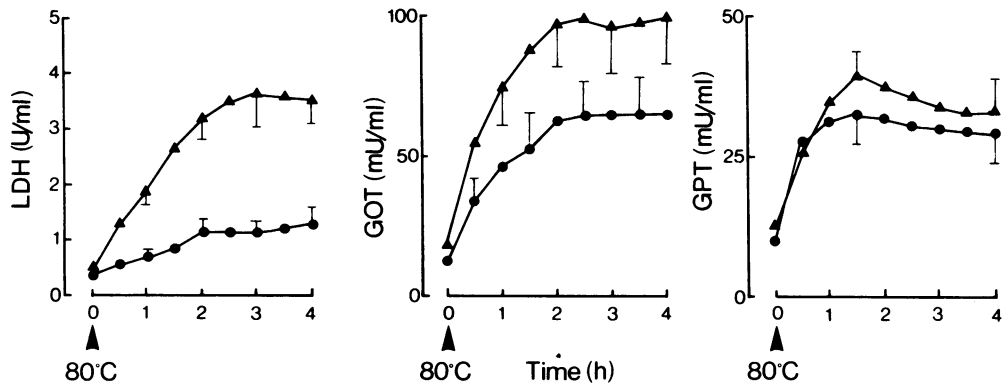

Figure 3 The concentrations of lactic dehydrogenase (LDH) in u/ml, glutamic oxaloacetic transaminase (GOT) in mu/ml and glutamic pyruvic transaminase (GPT) in mu/ml in samples of lymph collected before (pre nodal, ●) and after (post-nodal, ▲) passing through the popliteal lymph node. At the arrow the limb was immersed in water at 80° C for 15-20 seconds.

were detected (Lewis, 1967). In the present series, ischaemia for 4 h produced no significant increases in either skin or muscle lymph.

In some experiments the post-nodal lymphatic was cannulated close to the point where the vessel leaves the popliteal node. It was therefore possible to compare the composition of pre-nodal lymph draining directly from the skin with post-nodal skin lymph having passed through the popliteal node but not having mixed with muscle lymph. Figure 3 illustrates that after an 80° C burn there was no significant difference between the pre- and post-nodal levels of GOT and GPT thus showing that there was no filtration or inactivation of enzymes by the node. In the case of LDH, there was an increase in activity in post-nodal lymph. This was probably due to lymphocytes which have a high LDH content (Jasani & Lewis, 1971) entering the lymph as it passed through the node.

Discussion

In the present study, we have been able to collect skin and muscle lymph separately. This has enabled us to repeat the earlier experiments in which we examined changes of flow and composition of femoral lymph after injury, to find out whether the changes reflected those occurring in the skin or the muscle or both.

After thermal injury to the hind limb there was a two to four-fold increase in flow and about a two-fold increase in the protein concentration of lymph collected in the femoral lymphatic (Lewis, 1967, 1969). Bach & Lewis (1973) later found that when lymph was collected separately from skin and muscle, neither protein nor flow changed in muscle lymph whereas both increased in skin lymph and these differences were explained as being due to the different behaviour of the skin and muscle vascular beds.

When the hind limb was subjected to a mild chemical injury, on the other hand, such as treatment with DMSO, a different pattern emerged. When the lymph was collected from the femoral lymphatic there was an increase in flow and a decrease in protein concentration (Boyles *et al.*, 1970). In the present experiments, we have shown that these findings reflect changes in muscle lymph rather than changes in skin lymph. Similar changes occurred after the limb was subjected to ischaemia or when the muscles were maximally stimulated electrically (Bach & Lewis, 1973) although in these experiments the decrease in muscle lymph protein was small and not statistically significant. The experiments with DMSO emphasize even further the difference in the behaviour of the skin and muscle vascular beds. After intramuscular injection, the decrease in protein concentration in muscle lymph could be explained by dilution since there was a corresponding increase in lymph flow. However, this was not the case in skin since there was no increase in the flow but there was, nevertheless, a small decrease in protein concentration although this was not statistically significant. Perhaps DMSO causes vasodilatation in muscle with a reduced reabsorption or an increased filtration.

On injection DMSO caused the release of a high concentration of LDH into muscle lymph but little or none into skin lymph. After intradermal injection of DMSO, there was no increase in LDH

concentration in skin lymph. The small increase in LDH in muscle lymph after intradermal injection was probably due to the DMSO recirculating into the muscle and subsequently releasing the enzyme. On the other hand, after intramuscular injection of DMSO, besides the high concentration of LDH in muscle lymph there was a smaller but significant increase in LDH in skin lymph.

This increase in the LDH activity in skin lymph appears to be due to a diffusion of the enzyme from the muscle into the skin since DMSO does not release the enzyme into the lymph when injected into skin nor does it cause an increase in circulating LDH (Boyles *et al.*, 1970). The diffusion of LDH from the large pool in the muscle into the skin might have partly accounted for the apparent leakage of LDH into skin lymph during other types of injury. For example, after the leg was frozen and even after a severe thermal injury, the amount of LDH appearing in skin lymph was proportional to that appearing in muscle lymph.

Diffusion of LDH from damaged muscle into skin might explain another finding. Lewis, Peters & White (1971) found that 4-6 h after a 60°C thermal injury there was an increase in LDH present in the skin and that this increase was not prevented by pretreatment with inhibitors of protein synthesis. The increase could be accounted for by the gradual accumulation of the enzyme in the skin after prolonged diffusion from the injured muscle.

However, it seems unlikely that the diffusion of LDH from muscle into skin lymph takes place via a direct lymphatic connection, because firstly, Bach & Lewis (1973) have shown that dye injected into the muscle does not enter skin lymph and *vice versa*. Secondly, after thermal injury the considerable increases in the protein in the skin lymph do not appear in the lymph draining muscle as would be expected if there was a direct connection.

After the mild thermal injury of burning at 60°C for 1 min, the only significant change in enzyme activity was the release of the cytoplasmic enzymes LDH and GOT into muscle lymph. After a more severe injury, burning at 80°C for 20 s there was an increase in both muscle and skin lymph of LDH and GOT and in addition of the mitochondrial enzyme GPT. It would seem reasonable to assume that not all of the enzyme activity appearing in skin lymph diffused from muscle but at least a part originated in the skin cells themselves. Nevertheless, it is still not surprising that the total amount of LDH in skin lymph was considerably smaller than in muscle lymph, since the muscle fibres contain much more LDH activity than skin cells. In the case of lysosomal enzymes, however, there appears to be greater activity in skin than in muscle since after freezing the concentration of lysosomal enzymes leaking into skin lymph was higher than that into muscle lymph.

Since the amount of cellular and tissue damage produced after the leg had been frozen and thawed was so much greater than that after injection of DMSO or thermal injury, it was surprising to find that the amount of LDH released into the muscle lymph was about the same after each type of injury. This finding raises the possibility that the maximum available LDH activity in the muscle was released during each injury. However, when compared with results obtained from studies of homogenates of skeletal muscle, the amount released into the lymph appears to be only a fraction (about 1%) of the total enzyme activity. The question then arises, does the proportion of LDH activity released represent the unbound fraction or is it possible that the proportion not released is normally present in an inactive form and is only activated on homogenization?

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