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Effects of Indomethacin on the Transcapillary Leakage of Macromolecules and the Efflux of Prostaglandins in the Paw Lymph Following Experimental Scalding Injury

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ABSTRACT

Transport of macromolecules (dextrans and proteins) from blood to lymph and efflux of prostaglandins into lymph were studied in dogs following scalding injury of the paw and treatment with indomethacin. Indomethacin inhibited the efflux of prostaglandins following scalding injury, indicating an inhibition of the biosynthesis of prostaglandins. A pronounced suppression of the increased lymph flow and transcapillary transport of macromolecules following scalding was found after treatment with indomethacin. The increased microvascular permeability in scalded tissue was not significantly altered by indomethacin. These results indicate that the major effect of indomethacin on the microcirculation in the scalded tissue is a reduction of the capillary surface area available for exchange due to a reduced number of capillaries perfused with blood. The results also support the hypothesis that some of the vascular reactions following thermal injury may be mediated by prostaglandins.

INTRODUCTION

Prostaglandins, a group of biologically active lipids (for reviews see 9, 20, 22, 41) have been demonstrated in tissue fluids following chemical inflammation (44, 45), scalding injury (2, 24) and anaphylaxis (16). Recently it was shown that scalding injury in guinea pig is followed by an increased biosynthesis of prostaglandins (18). Prostaglandin E_2 (PGE_2) is the major prostaglandin found in inflammatory fluids. The vascular effects of this compound fulfil many of the criteria as demanded by a chemical mediator of the inflammatory response (37).

Evidence has accumulated for structural changes of the blood-lymph-barrier, i.e. increased microvascular permeability (4, 29), increased capillary surface area due to dilatation of resistance vessels as well as increased tissue osmotic forces in the burn wound (8).

Recently it was shown that drugs like aspirin and indomethacin inhibit the biosynthesis of prostaglandins (33, 39), suggesting that these drugs owe their anti-inflammatory effects to this mechanism.

In the present communication we report effects of indomethacin (47) on prostaglandin efflux in paw lymph and on microvascular reactions following scalding injury.

MATERIAL AND METHODS

Six healthy dogs of the Vorsteh-type, weighing between 17 and 28 kg and of the same breed, were used. All experiments were done using sodium pentobarbital anaesthesia (Nembutal®, Abbott). For induction of anaesthesia a dose of 30 mg per kg of body weight was given. Small additional doses were given when required. The dog was placed on an operating table equipped with electric heating to maintain a normal body temperature; this was controlled throughout the experiment. Free airway was ascertained by endothracheal intubation. Ringer solution was given as a slow intravenous infusion during the experiments in a dose of 5 ml per kg of body weight per hour.

Peripheral lymph was drained from the dog's hind paw via a short PE 50 Intramedic polyethylene cannula (Clay-Adams, Inc., New York). The operative procedures and methodological details have been published elsewhere (4). Lymph flow was facilitated by regular, passive movements of the paw. Lymph was collected in ice-chilled polyethylene tubes for assay of dextran, protein and prostaglandins.

The thermal trauma was inflicted by immersing the animal's paw for 10 sec in water at 100°C or for 20 sec in water at 70°C. This was done about 180 min after clamping of the renal pedicles.

Four dogs were treated with indomethacin (10 or 20 mg/kg body weight) intravenously immediately after scalding and one hour later except one dog which only received a single dose of indomethacin (20 mg/kg body weight). Indomethacin (Merck, Scharp and Dohme, Rahway, N.J., USA) was dissolved in 0.15 M potassium phosphate buffer, pH 7.4, immediately prior to administration. Two dogs served as controls and received no treatment after scalding other than Ringer solution.

Permeability studies

The local microvascular permeability in the scalded tissue was studied before and at different times after the trauma. Dextran was used as test substance. Rheomacrodex®, 10% in 0.9 saline, average molecular weight, $M_w = 40\,000$ (range 5 000–90 000) was given slowly in a dose of 0.5 g/kg body weight. This low dose produces only very small changes in the plasma volume (15). Before the dextran infusion, the renal pedicles were clamped to prevent the rapid elimination of dextran molecules of low molecular weight via the kidneys. After an equilibration period of 120 min the molecular weights of dextran molecules in lymph and plasma were determined and the lymph/plasma concentration ratio (C_L/C_P) of different molecular sizes was calculated.

The total concentration of dextran in plasma and lymph was determined by the anthrone method (23, 40). This method has an error of 1.8% S.D. (40).

The total concentration of protein in lymph was determined according to a modified Folin method (3).

The molecular weight distribution of dextran was estimated by gel chromatography adapted to automated routine and computer analysis (6). The sensitivity, reproducibility and resolving power of the gel chromatography method has been thoroughly investigated (13, 27). The accuracy of the determination of dextran molecular weight distribution is in the range of 3% S.D. (Granath, personal communication).

In all experiments the regional transport of dextran across the blood-lymph barrier was calculated according to:

$$C_L/C_P \cdot v/t = T$$

where C_L and C_P (in mg/ml) is the concentration of dextran in lymph and plasma respectively, and v the volume (in ml) of the lymph collected during the sampling period t (in min). T will then be equal to the net transport of the substance from blood to the collected lymph cannula in mg/min at unit plasma concentration (= 1 mg/ml). The physical dimension of T is actually ml/min. The C_P value used in this calculation corresponded to the plasma concentration at the middle of the lymph sampling period. It was not possible to correct for the time delay between the actual transport through the capillary wall and the collection from the lymph cannula.

Determination of prostaglandins

Lymph was precipitated in absolute ethanol containing 0.1% *d,l* α-tocopherol as antioxidant and stored at −20°C before processing. Lipid extracts were prepared as described by Unger et al. (38) and subjected to silicic acid chromatography (30) prior to bioassay. The silicic acid columns (Unisil, 0.5 g, 100–200 mesh, Clarkson Chemical Corp., Williamsport, Penns.) were made up in ethyl acetate-benzene (1 : 9, v/v) and eluted with 20 ml of ethyl acetate-benzene (1 : 9) followed by 20 ml of ethyl acetate. These fractions were evaporated under reduced pressure and assayed for smooth muscle stimulating activity on colon of the gerbil (42). The recovery was determined in each sample by addition of ^{3}H-PGE_1 (0.1 μC, specific activity 87.3 C/mmole, New England Nuclear Corp., Boston, Mass.), to the lymph-ethanol mixture and determination of radioactivity in aliquots of the ethyl acetate fractions and was about 50% ($x = 49.9$, S.D. = 9.1, $n = 74$). When 50 ng PGE_2 was added to 5 ml human plasma and processed as described the recovery of smooth muscle stimulating activity was similar ($x = 45\%$, S.D. = ±18, $n = 17$). Indomethacin (100 μg/ml plasma) did not affect the recovery of smooth muscle stimulating activity of PGE_2 (35, 39). In all assays the colon of the gerbil responded to 0.5 ng PGE_2 added to the organ bath. Levels below 2 ng per sample could not be detected and were thus regarded as zero values. Smooth muscle stimulating activity was expressed in terms of PGE_2 after correction for recovery of radioactivity.

Radioactivity was determined in a Packard liquid spectrometer (model 3320). 10 ml of Instagel (Packard Instr.) was used as scintillator. Corrections for counting efficiency were made by use of external standardization.

RESULTS

Transcapillary leakage of macromolecules

No significant differences were found in lymph flow, in protein or in dextran concentration of the lymph drained from the different paws before infliction of the thermal trauma.

The changes in sieving ratio (C_L/C_P) in the scalded areas were found to be related to the severity of the burn trauma, being somewhat less pronounced after scalding for 20 sec at 70°C than for 10 sec at 100°C (Figs. 1 and 2). The microvascular permeability and the lymph flow were maximal about 1–4 hours after scalding.

After immersion of a dog's paw for 20 sec in water of 70°C the lymph flow increased about 10 times, the protein concentration of the lymph increased from 0.7 to about 3.0 g/100 ml and the relative amount of dextran transported transcapillary (T-values) increased 20 times (Fig. 1). The lymph plasma dextran concentration ratio (C_L/C_P) for molecular weights of 10 000–80 000 increased immediately after the trauma and reached maximal values 1–3 hours post burn (Fig. 1).

After scalding of another dog's paw for 20 sec at 70°C and treatment with indomethacin (10 mg/kg body weight), repeated twice, immediately and 60 min after the burn, a pronounced decrease of the lymph flow and a slight reduction of the total protein concentration in the lymph compared with non-treated dog was observed (Fig. 1). The relative amount of dextran transported transcapillary from the blood to the extravascular space after scalding was reduced to between 25 and 35% of the amount transported transcapillary in the non-treated dog (Fig. 1). The C_L/C_P ratios for different molecular weights of dextran were about

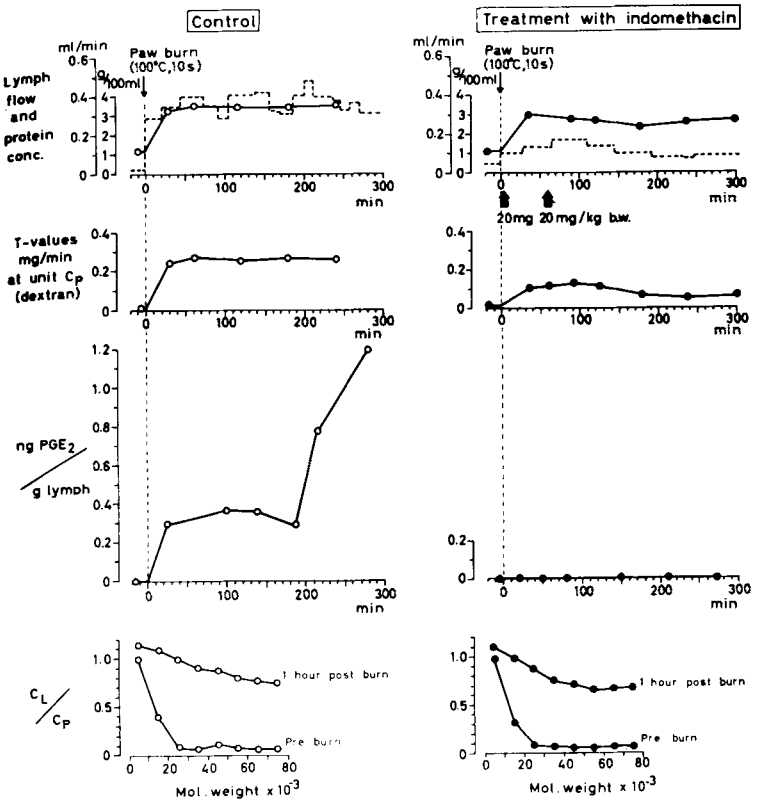

Fig. 1. Lymph flow, total protein concentration of lymph, *T*-values, efflux of prostaglandins and C_L/C_P ratios for dextran in lymph from paws scalded for 20 sec at 70°C in two dogs, one without treatment and one treated with indomethacin 10 mg/kg body weight immediately and one hour after scalding.
-----, lymph flow
O----O, protein conc.

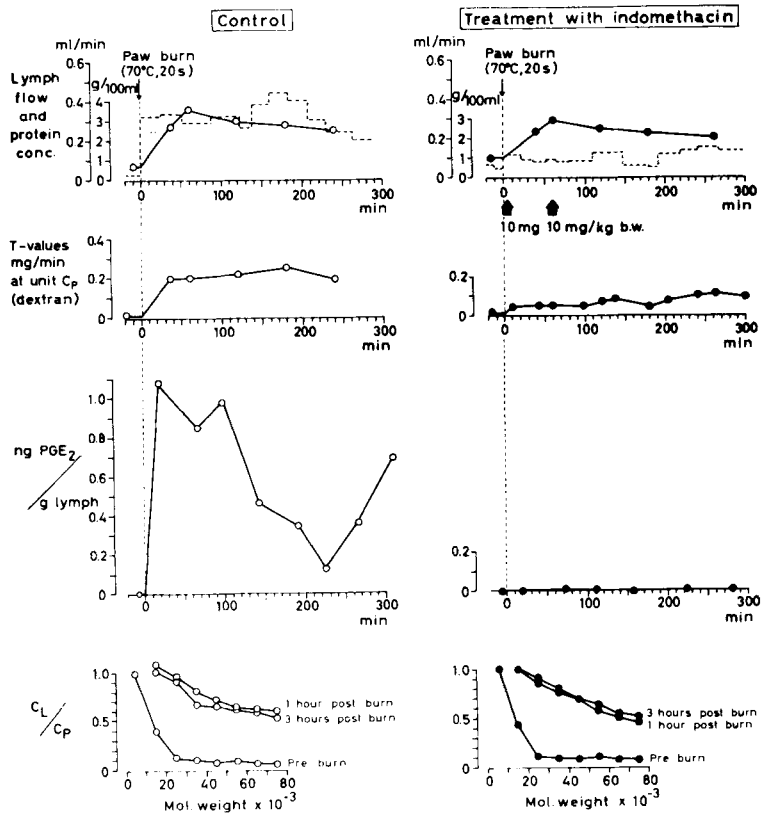

Fig. 2. Lymph flow, total protein concentration of lymph, *T*-values, efflux of prostaglandins and C_L/C_P ratios for dextran in lymph from paws scalded for 10 sec at 100°C in two dogs, one without treatment and one treated with indomethacin 20 mg/kg body weight immediately and one hour after scalding.
-----, lymph flow;
O----O, protein conc.

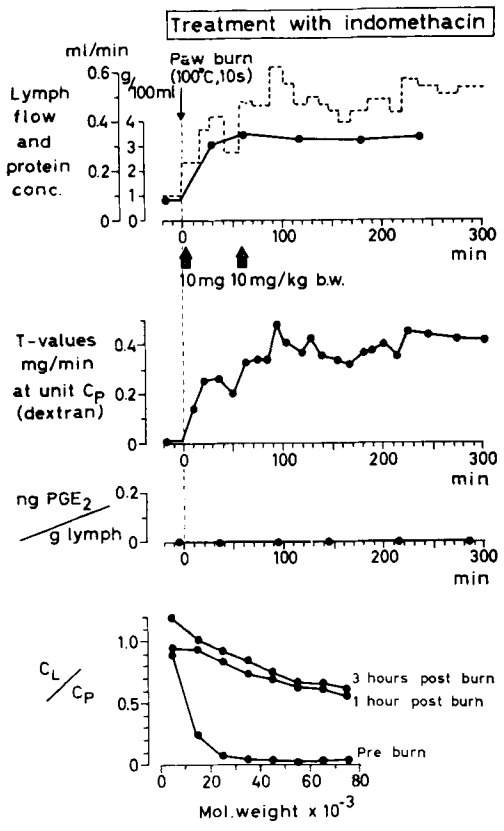

Fig. 3. Lymph flow, total protein concentration of lymph, *T*-values, efflux of prostaglandins and C_L/C_P ratios for dextran in lymph from a paw scalded for 10 sec at 100°C in one dog, treated with indomethacin 10 mg/kg body weight immediately and one hour after scalding. -----, lymph flow; O----O, protein conc.

equally increased in both the scalded non-treated and treated paws (Fig. 1).

Figs. 2 and 3 show results from a comparative study in three dogs of the microcirculation in scalded tissue and with treatment with indomethacin at two dose levels. The scaldings were made in exactly the same way in all three dogs, i.e. immersion of the paws for 10 sec in water of 100°C. The lymph flow, the total protein concentration of lymph and the *T*-values increased immediately after scalding and reached the same level in the non-treated dog and the dog treated with indomethacin in the low dose (10 mg/kg body weight). Following scalding and treatment with indomethacin in a dose of 20 mg/kg body weight the increase in lymph flow and the *T*-values were reduced to 30–40% of those found in the scalded non-treated dog (Figs. 2 and 3). The sieving ratios for dextran molecules of various sizes in lymph/plasma (C_L/C_P) increased following the thermal trauma in a similar way in all three dogs irrespective of treatment or not (Figs. 2 and 3).

A study of the microcirculation in scalded tissue with and without treatment with indomethacin made on one and the same dog is shown in Fig. 4. A burn trauma on the right hind paw resulted in increases in lymph flow, transcapillary transport of dextran (*T*-values) and in the C_L/C_P ratio for all dextran molecules in the preparation. Two hours after the first trauma a second one was made in exactly the same way on the left hind paw and the dog was treated with indomethacin (20 mg/kg). This resulted in less pronounced increases in lymph flow and T-values compared to the scalded non-treated paw (Fig. 4). No significant differences were found in the C_L/C_P ratios obtained from the two paws (Fig. 4).

Efflux of prostaglandins in lymph

After scalding of the dog's paw for 20 sec at 70°C and for 10 sec at 100°C and without treatment there was a rapid increase in the concentration of prostaglandins in the lymph drained from scalded tissue with a decline after about 150 min. This was followed by a second increase of the efflux of prostaglandins in the lymph 3–5 hours after the trauma (Figs. 1 and 2). After treatment with indomethacin in a dose of 10 or 20 mg/kg body weight, administered immediately after scalding and one hour later, prostaglandins were not recovered in the paw lymph (Figs. 1, 2 and 3).

In the study with and without treatment with indomethacin made on one and the same dog total inhibition of the efflux of prostaglandins was found following treatment (Fig. 4). Also on the previously scalded non-treated paw the amount of prostaglandins in the lymph decreased to zero values concomitant with the intravenous administration of indomethacin two hours after the first thermal trauma.

DISCUSSION

In the acute phase of a burn injury there is a rapid loss of intravascular fluid into the burned area. The fluid loss has been ascribed to functional disturbances in the blood lymph barrier, increased effective filtration area and increased tissue osmotic forces (8). By selecting appropriate

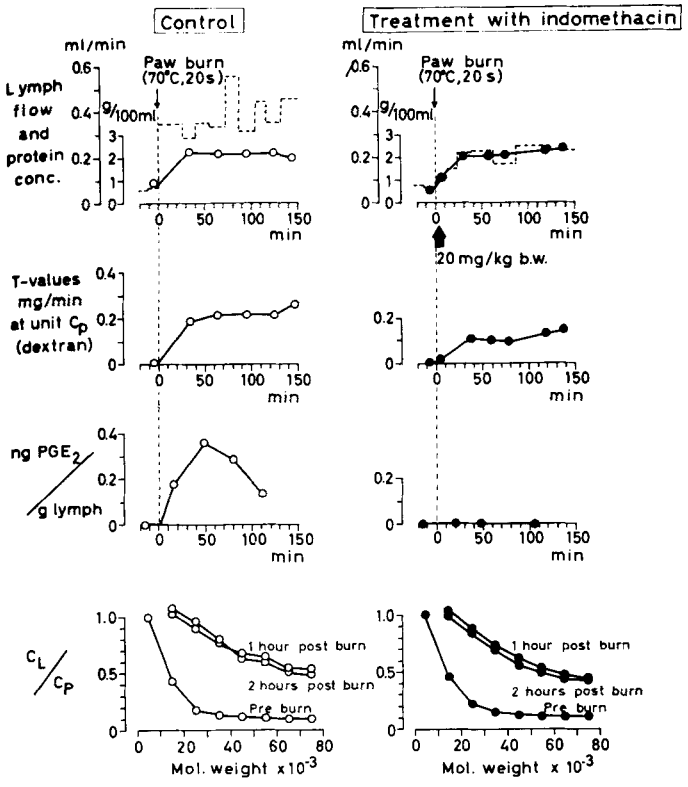

Fig. 4. Lymph flow, total protein concentration of lymph, *T*-values, efflux of prostaglandins and C_L/C_P ratios for dextran in lymph following scalding for 20 sec at 70°C of two paws of a dog, one paw without treatment and one paw treated with 20 mg indomethacin per kg body weight immediately after scalding. The C_L/C_P ratios for dextran molecules of various sizes are plotted pre burn and one and two hours after the trauma, respectively. - - - -, lymph flow; O- - - -O, protein conc.

thermal stimuli to experimental animals two phases of vascular reactions have been discerned, an immediate and a delayed phase (32). Vasoactive-compounds have been suggested to mediate some of these vascular reactions following injury (37, 46).

Attempts have been made to modify the vascular reactions following thermal injury by drugs. Spector & Willoughby (36) demonstrated that by treating rats with mepyramine maleate, a powerful antihistamine drug, prior to moderate thermal injury, the immediate vascular reactions, expressed as leakage of trypan blue and oedema formation, were suppressed. Administration of salicylate suppressed both phases. These observations indicate that histamine is involved only in the immediate response to thermal injury suggesting that histamine may initiate vascular reactions which are maintained by other mechanisms.

The occurrence of PGE-compounds in inflammatory fluids (2, 16, 24, 44, 45) and the vascular effects of these compounds suggest that they participate in the inflammatory response. Parenterally administered PGE-compounds induce hypotension due to vasodilatation and reduced peripheral resistance (11, 14, 28). Intradermally administered PGE-compounds elicit erythema and oedema (10, 25, 34) due to vasodilatation. In rats and guinea pigs, preinjected with protein bound dyes, PGE-compounds induce extravasation of color on intradermal administration (10, 21, 26). This effect has been claimed to be due to increased capillary permeability. In canine adipose tissue PGE_1 is a powerful vasodilatating agent with comparatively small effects on capillary permeability (11).

Recently it was demonstrated that anti-inflammatory drugs like aspirin, sodium salicylate and indomethacin inhibit the synthesis of prostaglandins (12, 17, 33, 39). This was found both in cell-free homogenates and in the whole animal.

In the present investigation the effect of indomethacin on the efflux of prostaglandins in lymph drained from scalded tissue and the effect on the transcapillary leakage of macromolecules into the burn oedema was studied simultaneously. The efflux of prostaglandins in the lymph was assumed to reflect the biosynthesis of prostaglandins in the tissue. The sieving ratio for dextran molecules of various sizes in lymph/plasma (C_L/C_P) was meas-

ured and used as an index of the microvascular permeability. Recently Arturson (5) demonstrated a moderate suppression of the increased microvascular permeability after scalding by 0-(β-hydroxyethyl)-rutosides (HR) a compound with antioedematous effects (48).

In the present investigation the scalding injury resulted in efflux of prostaglandins (cf. 2, 24) concomitant with an increased lymph flow and an increased concentration of both protein and dextran in the lymph drained from scalded tissue (cf. 4). The sieving ratio (C_L/C_P) and the regional transport of dextran across the blood-lymph barrier also increased. This indicates an increase of the microvascular permeability after scalding (cf. 4, 7).

In all dogs except one treatment with indomethacin resulted in a suppression of the increased lymph flow, a slight reduction of the total concentration of protein in lymph and a pronounced reduction of dextran transported from the blood to the extravascular space as compared to scalded non-treated dogs. The sieving ratios for dextran molecules of various sizes in lymph/plasma were unaffected by the treatment with indomethacin. No smooth muscle stimulating activity was found in lymph fluid after scalding and subsequent treatment with indomethacin in any dogs.

The inhibition of prostaglandin efflux indicate an inhibition of prostaglandin biosynthesis by indomethacin. It seems improbable that in the tissue with less rapid lymph drainage there was an increased metabolism of prostaglandin E-compounds into metabolites without biological activity (1). This is supported by the finding that no smooth muscle stimulating activity was recovered after scalding and subsequent treatment with indomethacin in a low dose (10 mg/kg twice) which did not affect the lymph flow (cf. Fig. 3).

The mechanism underlying the effects of indomethacin on the microcirculation in scalded tissue is not clear. It could be either a decreased capillary surface area available for exchange due to a reduced number of capillaries perfused with blood, or to a suppression of the increased microvascular permeability caused by the thermal trauma, or both. The very small changes of the sieving ratios for dextran molecules of various sizes in lymph/plasma (C_L/C_P) and the pronounced suppression of the lymph flow as well as the amount of dextran transported transcapillary following treatment with indomethacin indicate that a reduction of the effective capillary surface area is the most important mechanism for the effect of indomethacin.

Lately a modulatory role of prostaglandins on the sympathetic nervous transmission has been implied (19). Evidence exists to suggest that prostaglandins inhibit the release of norepinephrine (43). Blocking of the local formation of prostaglandins leads to a considerably increased release of norepinephrine on sympathetic stimulation (31). In the same way it may be suggested that after scalding injury and treatment with indomethacin prostaglandins are not formed in sufficient amounts to oppose the increased secretion of catecholamines. However, removal of the vasodilatory effect of prostaglandin E-compounds may also be one explanation. As it has not been demonstrated that indomethacin exclusively inhibits prostaglandin biosynthesis, other effects of the drug itself cannot be excluded.

No suppressive effect on the lymph flow or the transcapillary macromolecular transport was observed in the dog with a paw immersed for 10 sec in water of 100°C and treated with indomethacin in the low dose (Fig. 3) although no PGE_2 was found in the lymph. This might indicate either that the efflux of prostaglandins in lymph is not a satisfactory estimate of the prostaglandin biosynthesis in tissues or that the changes of the microvasculature are not mediated via PGE_2. The fact that clear suppressive effects on both lymph flow and transcapillary transport of dextrans were found following scalding for 10 sec at 100°C and treatment with indomethacin in high dose (Fig. 2) favours the hypothesis that some of the vascular reactions after scalding injury are mediated via the prostaglandin system.

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