Erythromelalgia: studies on pathogenesis and therapy.

ABSTRACT

Erythromelalgia (EM) is a rare condition characterized by red, hot and painful extremities. Local skin cooling provides relief. Warmth, exercise and dependency of the extremity intensify the discomfort.

Shunt hypothesis: Clinical observations and pathophysiological findings are compatible with microvascular arteriovenous shunting of blood as a final common pathway of pathogenesis of erythromelalgia in affected skin. The hypothesis postulates that available blood is maldistributed. An insufficient proportion of blood is directed through nutritional capillaries, leading to skin hypoxia, while a large proportion of blood is shunted through microvascular anatomical or functional arteriovenous anastomoses. The skin hypoxia induces arteriolar dilatation and hyperemia, resulting in elevated skin temperature and accelerated metabolism. Because the hyperemic perfusion is still maldistributed, the hypoxia is not revealed. This vicious cycle may be triggered by different mechanisms related to hemorrheological problems, defects in prostaglandin metabolism, dysfunction of endothelial cells or the autonomic nervous system.

Aim of the studies: The aim of this thesis was: to challenge the shunt hypothesis, to characterize the mechanisms underlying the vascular dysfunction in erythromelalgia, and to test if therapy, based on this understanding of the pathogenesis, would improve clinical and objective outcome measures in patients with erythromelalgia.

Materials, methods and study design: Patients were selected from a database of EM patients built up over nearly 20 years (n=160). The patients were characterized by demographic and clinical parameters (cooling and pain scores, global assessment). Skin perfusion was assessed using laser Doppler flowmetry (LDF), laser Doppler perfusion imaging (LDPI) and computer assisted video microscopy (CAVM). LDPI assesses global (thermoregulatory and nutritive) skin perfusion; CAVM evaluates skin capillary morphology. Perfusion was assessed following vasoconstrictory and vasodilatory stimuli to characterize central and local neurogenic reflexes as well as vascular smooth muscle and vascular endothelial cell function and following central body heating to provoke EM symptoms in patients and healthy controls. In a double blind, crossover, placebo controlled clinical trial, EM patients were treated with misoprostol, a prostaglandin E1 analogue with vasodilatory and thrombocyte inhibitory effects.

Results: In EM patients, LDF assessed skin microvascular perfusion was significantly reduced during basal conditions. Vasoconstrictor responses involving central sympathetic reflexes were attenuated in affected skin. Central body heating induced a significant
increase in LDPI-assessed perfusion and reduction in capillary density in affected EM skin containing many anatomical arteriovenous shunts, as compared to asymptomatic patients and controls. In areas with no or few shunts, the groups did not differ. We demonstrated beneficial clinical effects of misoprostol and redistribution in skin microcirculation in favor of nutritive perfusion.

**Conclusion:** Increased thermoregulatory flow and decreased capillary density during EM attacks, as well as clinical improvement and redistribution of the skin microcirculation in favor of the nutritive perfusion after misoprostol treatment give support to the shunt hypothesis. Erythromelalgia was associated with neuropathy, which may be one underlying mechanism for the disturbance in the vascular dynamics. On the other hand, vasculopathy with hypoxia may cause neuropathy.

**LIST OF PAPERS ASSOCIATED WITH THIS THESIS**

This thesis is based on the following papers, referred to in the text by their Roman numerals:


**PART ONE: INTRODUCTION**

Erythromelalgia (EM) is a clinical syndrome characterized by heat, redness and pain. EM symptoms are commonly intermittent, though constant in some patients. The duration of episodes or "flare-ups" varies from minutes to hours. The attacks occur more frequently during evenings and nights. Doctors therefore rarely observe the episodes unless the patients are hospitalized. As many doctors are not familiar with the disease, most patients have symptoms for many years before they get a diagnosis.

A short introduction to the present understanding of erythromelalgia is given in Appendix I (Mørk & Kvernebo, 2000a).

**1.1 History and nomenclature**

Graves reported in 1834 a young lady who suffered from a "hot and painful" leg, and the associated increase in temperature was found to be "so disagreeable" (Graves, 1834). In 1878, Silas Weir Mitchell reported 16 cases with painful affections of the feet of various types and severity and introduced the term "erythromelalgia" by combining the three Greek words erythros (red), melos (extremities) and algos (pain) to illustrate the triad of presenting symptoms and signs of the condition (Mitchell, 1872; Mitchell, 1878). He also described erythromelalgia as a `vascular storm." Erythromelalgia has also been named Mitchell's or Weir Mitchell's disease. Little notice was taken of the condition for
many years (Collier, 1898; Cassirer 1912; May & Hillemand, 1924).

In the literature, there has been confusion and inconsistency regarding the nomenclature, classification, prevalence, and pathophysiology. In 1932, Brown proposed five criteria for the diagnosis of erythromelalgia: (1) Attacks of bilateral or symmetrical burning pain in the hands and/or feet, (2) During attacks, the affected parts are flushed and congested and exhibit increased local temperature, (3) The attacks are initiated or aggravated by standing, exercise or exposure to warmth (limb temperature >34 °C), (4) Relief is obtained by elevation or exposure to cold, and (5) The condition is refractory to treatment (Brown & Giffin, 1930; Brown, 1932). The term "erythermalgia" was introduced by Smith and Allen to emphasize the rubor, calor, and dolor, and they proposed a subdivision into a primary group, with no associated disease, and a secondary group, particularly hyperviscosity syndromes (Smith & Allen, 1938). The term "erythralgia" has also been used (Lewis, 1933; Lewis & Hess, 1933). In German literature "erythroprosopalgia" (prosopon-face) describes the condition in the face (Regli, 1969). The term "erythromelalgia" is a more complete description of the clinical features, but this unwieldy label is not in general use (Zoppi et al, 1985). Other less common terms are acromelalgia, erythrothermia, red neuralgia, vasomotor paralysis of the extremities and Gerhardt’s syndrome (Gerhardt, 1892). Lazareth et al proposed three major criteria (paroxysmal pain, burning pain, redness of affected skin) and four minor criteria (typical precipitating factors (heat exposure, effort), typical relieving factors (cold, rest), elevated skin temperature in affected skin, response of symptoms to acetylsalicylic acid) and demanded three major and two minor criteria for the diagnosis (Lazareth et al, 1988). Erythromelalgia and erythermalgia have been used interchangeable for many years, but Michiels et al proposed to restrict the term erythromelalgia to aspirin-responsive disease associated with thrombocytosis related to myeloproliferative disorder, and used erythermalgia for aspirin-resistant idiopathic cases or conditions secondary to other diseases (Michiels et al, 1995).

Some authors subgroup their patients into "early-onset" and "late-onset" cases irrespective of etiology (Kurzrock & Cohen, 1991; Kurzrock & Cohen, 1995). "Pediatric erythromelalgia" has also been subdivided into a primary form with early debut, often severe, resistance to treatment and sometimes with a family history, and a secondary form associated with hypertension (Finley et al, 1992; Drenth et al, 1995). The term "epidemic erythromelalgia" has been used for outbreaks in China (Zheng et al, 1988). Some authors use a combination of the above terms to sub-classify cases based on the underlying pathophysiology. A modified classification of erythromelalgia, type I erythromelalgia ("classical") and type II erythromelalgia, lacking the burning nature of the pain, and lacking relief by cooling or limb elevation, has also been used (Littleford, 1997; Littleford et al, 1999a,b).

Like most authors, we use the term erythromelalgia, and in our work we have used the following inclusion criteria for this clinical syndrome (Thompson et al, 1979; Mørk & Kvernebo, 2000a):

- Burning extremity pain
- Pain aggravated by warming
- Pain relieved by cooling
- Erythema of affected skin
Increased temperature of affected skin
Each criterion is dependent on individual clinical judgment.

1.2 Erythromelalgia in Norway
Einar Hval (1901-1958) at the Department of Dermatology, Rikshospitalet, reported the first case of erythromelalgia in Norway (Hval, 1928). A renewed interest for erythromelalgia in Norway started in 1983 when a patient with a medical history typical for erythromelalgia was referred to Knut Kvernebo. Kvernebo was then working at the Department of Surgery, Aker University Hospital in Oslo with a vascular laboratory for examination of arterial and venous extremity blood flow as well as skin microvascular perfusion. The patient had cooled her feet in a bucket of iced water for up to 20 hours a day over 16 years. Together with Egil Seem, Kvernebo investigated the microvascular hemodynamics in the affected EM skin. Her situation was mentioned in a national radio program (Asa Rytter Evensen, "Saran er livet", NRK, Oslo), resulting in contact with a large number of frustrated patients with similar symptoms. 39 patients (50% of the respondents) suffered from erythromelalgia. Hemodynamic studies were performed in 1984-89, and a more thorough understanding of the pathophysiological and thereby pathogenetic mechanisms was obtained, resulting in the hypothesis that erythromelalgia is a condition caused by microvascular arteriovenous (AV) shunting. This work was rewarded with the King Olav V's Gold Medal in 1990. Kvernebo's work is presented in a monography (Kvernebo, 1998).

Kvernebo contacted the Department of Dermatology, Rikshospitalet University Hospital, and presented his work, resulting in initiation of the scientific work presented in this thesis. An Erythromelalgia Study Group (ESG) was established with focus on epidemiology, pathogenesis and therapy. ESG is organized as a research network, so far involving the following seven units: Department of Biomedical Engineering, University Hospital, Linköpings Universitet, Sweden; Department of Cardiothoracic Surgery, Ullevaal University Hospital; Rikshospitalet University Hospital with the following departments: Institute of Laboratorium of Clinical Neurophysiology, Department of Neurology, Department of Psychosomatic Medicine, Department of Pathology, and Department of Dermatology; and The Erythromelalgia Association (TEA; www.erythromelalgia.org). Patients have continuously been referred to Department of Dermatology, Rikshospitalet University Hospital, and we have over a period of almost 20 years established a database of 160 patients, probably the largest EM cohort in the western world. All patients have been examined by one of three physicians in the group (Kvernebo, Kalgaard, Mork). Patients from Norway, Sweden, Denmark, UK, and USA were included.

1.3 Clinical features
The EM diagnosis is based on the medical history and findings, and no objective laboratory criteria are available. Erythromelalgia is heterogeneous with regard to severity, localization, age at onset, etiology and prognosis.

1.3.1 Symptoms
Before erythromelalgia is diagnosed, heat intolerance and symptomatic relief from cooling should be demonstrated. These features are hallmarks of erythromelalgia. The patients report major adjustments to their lifestyle to avoid precipitation of an EM attack. A prickling or itching sensation may herald the pain. All patients find self-induced
measures to control their burning pain or deep aching. Patients typically seek relief by cooling of affected skin on cold floors, wet sand, wet towels, immersion in cold water, or by applying cold objects. The patients frequently sleep with the affected extremities outside the bed cover. Partial relief can be obtained by elevation of the affected extremity. Some patients show peculiar behavior, like walking barefoot in the snow, sleeping with the feet out of a window, putting the feet into a refrigerator or continuously living with a bucket with iced water at their side day and night.

Cold and immersion may induce tissue damage, like immersion feet, infections, reactive flaring, and severe ulcers that can take many months to heal. Beneficial effects of aspirin is rarely seen, except for cases secondary to myeloproliferative conditions. Analgesics have limited effect. Warm environments make the EM symptoms more severe. Aggravating or inducing factors often reported are warm rooms, floors, water, heating appliances, bed covers, shoes, and gloves. Walking, physical activity, placing the limb in a dependent position (hanging down), and application of skin pressure may also intensify or precipitate the symptoms. Many patients report an increase in severity and frequency of erythromelalgia in the summer period. In addition, some patients have found that foods, beverages (particularly alcohol), spices (monosodium glutamate), and some drugs aggravate EM symptoms.

Some doctors may find the patients' stories bizarre and may not believe that the patient is suffering from a genuine somatic condition. Some of the patients have significant complaints without objective signs when seeing the doctors.

1.3.2 Signs

Affected skin in erythromelalgia is red to purple in color and may be warm or hot on touching compared to the adjoining asymptomatic skin. After anemisation of affected skin by diascopy, there is a short refilling time. Initially, the patients do not always notice this redness and local heat. Skin temperature and color are often normal at the time of physical examination, as the patients frequently are asymptomatic at the time of examination. The skin may even be white/pale or blue/cyanotic, which is suggestive of low basal skin blood flow in the asymptomatic skin.

The patients should be instructed to look for changes in skin temperature and color during EM symptoms. Photography and measurements of skin temperature with and without EM symptoms may be of help in assessing changes in skin color and temperature. The affected limb can occasionally be oedematous.

Trophic skin changes of affected skin areas have been described (Belch, 1996). Nail dystrophy and slow-healing skin ulcers of the feet without obvious signs of trauma or infection has been observed in severely affected patients (Kvernebo, 1998). Biopsies from affected skin may also heal slowly. Gangrene of the affected extremity, in spite of open limb arteries, has also been observed.

1.3.3 Impact on quality of life

Erythromelalgia may have a severe impact on normal life. Diminished scores in quality of life questionnaires have been reported, primarily due to the pain associated with erythromelalgia (Davis et al, 2000a). Disabilities described in this study from USA
include inability to walk long distances (50%), stand for long periods (49%), having to give up a job (12.5%), inability to drive (12.5%), use of wheel chair (3%), and being bed bound (2%). Patients avoid warm weather and limit their activities to cool or air conditioned locations. Some have to move to cooler climates. They cannot wear socks or closed shoes even in winter. Work and social functioning may be disrupted. Sometimes the patients avoid leaving their homes, leading to disablement and isolation. Restless sleeping patterns affects family functioning. Over a third of a large cohort have reported clinical depression or "depressed" states (Littleford, 1997).

Like most chronic pain conditions, psychological mechanisms probably play an important part in the experience and coping of pain in many EM cases. Compared with an age- and sex-matched population, an increased mortality rate, including suicide, has been found (Davis et al, 2000a). No specific psychological profile has been found in the EM patients compared to patients with other chronic disorders (Kalgaard et al, in manuscript). Contact with The Erythromelalgia Association (TEA; www.erythromelalgia.org) may be helpful for frustrated EM patients "shopping" from treatment to treatment and from doctor to doctor in an effort to get answers and help for their problems.

1.3.4 Clinical classification

The severity of the symptoms varies widely, from mild discomfort to erythromelalgia with gangrene that require amputation of the affected limb. In daily clinical work the terms mild, moderate and severe may be used. Erythromelalgia can be regarded as the antithesis to Raynaud’s phenomenon representing an extreme condition on a continuous spectrum from cold to warm hands and feet (Fig 1). A severity score scale has been introduced based on the needs to cool the affected skin.

1. Feeling uncomfortably warm in periods. No active cooling.
2. Feeling uncomfortably warm in periods. Walking barefoot on cold floors etc.
3. Feeling burning pain in periods. Immersion in cold water <1 hour/d
4. Feeling burning pain in periods. Immersion in cold water 1-3 hours/d,
5. Feeling burning pain in periods. Immersion in cold water 3-6 hours/d,
6. Feeling strong burning pain. Immersion in cold water 6-12 hours/d
7. Feeling very strong burning pain. Immersion in cold water >12 hours/d
8. Feeling very strong burning pain continually. Continuous need for cooling or plexus/epidural anesthesia
Based on clinical findings and etiology a classification has been proposed to characterize the wide spectrum of erythromelalgia (Fig 2). The term "erythromelalgia syndrome" is used when there is a family history of erythromelalgia, and when the symptoms start during childhood or adolescence and gradually increase in intensity. The disturbances are associated with structural changes in the microvascular architecture of the skin. The term "erythromelalgia (Kvernebo, 1998) phenomenon" is used for all other cases. Erythromelalgia may be primary (idiopathic) or secondary when considered to be caused by a primary disease or condition. The term "acute" is used when the symptoms increase and reach maximum severity within one month. Cases with persistent symptoms over long periods are termed "chronic." Prognosis depends on the clinical subgroup: erythromelalgia syndrome gradually gets worse, acute erythromelalgia gets better, while chronic erythromelalgia seems to remain stable (Kalgaard et al, 1997).

In all new cases, underlying causes should be looked for, and a variety of etiological factors may be involved in secondary erythromelalgia. Cases initially classified as primary should be reclassified when an underlying condition has been diagnosed. Several hematological, metabolic, neurologic, connective tissue, musculoskeletal, cardiovascular, and infectious disorders as well as pharmacological substances have been described associated with erythromelalgia (Table 1).

When erythromelalgia coexist with another disease or condition, the question arises whether the relationship is a coincidental or causal. A high incidence of co-occurrence, parallel and synchronous course of both conditions, remission of erythromelalgia after treatment of the underlying condition, and pathogenetic or etiological evidence of causality are all indications of a causal relationship. Due to the low prevalence of erythromelalgia, statistical or epidemiological evidence for a causal relationship is difficult to prove.

A beneficial effect on the EM symptoms after successful treatment of the primary condition indicates a causal relationship. An understanding of the pathogenetic mechanisms in the primary condition as in myeloproliferative diseases may indicate a cause-and-effect linkage between the conditions. Many of the accompanying conditions reported in erythromelalgia may produce physiological disturbances in the vessel wall (blood/tissue diffusion barrier), in the composition of the blood (hemorrheological properties), or in blood flow (microvascular maldistribution), which seems to be important pathogenetic or etiological evidence for a linkage.

The temporal relationship between erythromelalgia and an underlying disease varies. Since secondary erythromelalgia is caused by an underlying disease, the primary condition by definition must precede the development of erythromelalgia, but the primary disease may be undiagnosed for many years. The symptoms and signs of erythromelalgia usually affect the extremities, most frequently the lower extremities, and usually bilaterally and symmetrically. Most frequently the acral parts of hands and/or feet are involved but more proximal parts of the extremities may also be affected Rarely,
Erythromelalgia may appear in the face and ears. Primary erythromelalgia is localized to skin areas distal to the ankle or wrist areas, where anatomical AV anastomoses have been described. In secondary erythromelalgia and in erythromelalgia syndrome, symptoms may occur in other skin areas.

Based on the follow-up of 112 patients from our EM cohort, the clinical and demographic characteristics are presented in figure 3 and tables I-III. Twelve patients had a family history of erythromelalgia. Four legs in three patients are amputated, in spite of open limb arteries.

Table 1. Accompanying diseases, conditions and pharmacological substances described to be associated with erythromelalgia. The number in brackets are the relevant number in our cohort of 112 patients.

Metabolic conditions (n=12)

- Diabetes mellitus (Babb et al. 1964; Vendrell et al. 1988; Belch, 1996; Kalgaard et al, 1997; Littleford 1997; Davis et al, 2000a)
- Hyperlipidaemia (Kalgaard et al, 1997; Davis et al, 2000a)
- Gout (Markel, 1938; Babb et al, 1964; Belch. 1996)

Blood dyscrasia (n=8)

- Chronic myeloid leukemia (Kurzrock & Cohen, 1993: Belch, 1996: Kalgaard et al, 1997; Davis et al, 2000a)
- Pernicious anemia (Mehle et al, 1990: Rauck et al, 1996)

Autoimmune diseases (n=6)

- Lichen sclerosus et atrophicus (Hammar, 1978)
- Sclerodenna (Daroczy. 1973)
Neurological diseases (n=3)

- Cerebral vascular accident (Thomas. 1985; Littleford 1997)
- Neurofibromatosis (Kikuchi et al, 1985)
- Migraine (Michiels et al, 1996: Koudstaal & Koudstaal, 1997)
- Astrocyroma (Levine & Gustafson, 1987)
- Multiple sclerosis (Cendrowski, 1988: Rauck et al, 1996; Littleford, 1997)
- Parkinson's disease (Littleford, 1997)
- Carpal tunnel syndrome (Kalgaard et al, 1997)

Pharmacological substances (n=3)

- Norepinephrine (Wagner et al, 1993)
- Pergolide (Monk et al, 1984)
- Ticlopidine (Yasipovitch et al, 1999)
- Cyclosporine (Thami & Bhalla, 2003)
- Iodide contrast injection (Kalgaard et al, 1997)
- Vaccines (hepatitis and influenza) (Confino et al, 1997: Rabaud et al, 1999)
- Mushroom (Saviuc et al, 2001; Saviuc et al, 2002)
- Mercury (Martin et al, 1994)

Musculoskeletal disorders (n=3)

- Peritendinitis (Kalgaard et al, 1997)
- Osteoarthritis (Kvemebo,1998)

Infections (n=2)

- Bacterial infections (Kalgaard et al, 1997)
- Herpes zoster (Jabs & Druke, 1994)
- Mononucleosis (Clayton & Faden, 1993)
- Syphilis (Collier, 1898; Hval, 1928)
Cancer (n=1)

- Internal malignancy (Lantrade et al, 1980; Levine et al, 1987; Mørk et al, 1999; Davis et al, 2000a)

Cardiovascular diseases (n=1)

- Atherosclerosis (Kalgaard et al, 1997)
- Cholesterol emboli syndrome (Kalgaard et al, 1997)

Miscellaneous (n=5)

- Frostbite (Kalgaard et al, 1997)
- Nephritis (Cross, 1962)
- Small bowel disease (Littleford, 1997)
- Psychiatric disorders (Munford, 1929; Kalgaard et al, 1997; Kvernebo, 1998)

1.4 Epidemiology of erythromelalgia

Based on the different nomenclature and definitions of erythromelalgia, it is difficult to estimate the prevalence and incidence of erythromelalgia (Belch & Mackay, 1992). Literature reports are biased by different referral patterns and severity. As erythromelalgia can predate the development of an associated disease by many years, the prevalence of secondary erythromelalgia is even more difficult to estimate (Babb et al, 1964; Michiels et al, 1995). Many doctors are not aware of cases, and persons with mild erythromelalgia may never consult a doctor.

The EM entity was controversial some years ago (Snapper & Kahn, 1967; Housley,

The prevalence of moderate to severe erythromelalgia has been estimated to 2.09 per 100,000 in the Norwegian population, and the incidence 0.33 per 100 000, assuming that we see 50% of EM patients with severity group plus or equal to 3 (Mork & Kvernebo, 1997).

1.5 Skin vasculature and blood flow regulation
1.5.1 Morphology and function of skin microcirculation

The main functions of the skin circulation are tissue nutrition, thermoregulation and being a reserve for repair following injury. The skin vessels are arranged in a delicate network for ready transfer of heat from the blood to the external environment. The cutaneous microcirculation is organized as two horizontal plexa (Braverman et al, 1992; Braverman, 2000). The superficial plexus is situated in papillary dermis 1.0-1.5 mm below skin surface. The deep plexus is at the dermal-subcutaneous junction 3.0-5.0 mm below skin surface, depending on the skin region. Most vessels are found in the upper plexus, which is composed of terminal arterioles, capillary loops, and postcapillary venules, the latter being the most numerous. Thus plexus may be called the "radiator of the skin." The lower plexus is formed by perforating vessels (with diameter 50-100 gm) from the underlying muscle and subcutaneous tissue. Ascending arterioles and descending venules are paired as they connect the two plexa. The deep plexus is the source of perpendicular ascending arterioles (diameter 25-30 gm), which divide into four to five arterioles. The ascending arterioles are 2-7 mm apart in almost all areas of the skin surface, closer at the toe and fingertips, and supply a specific number of capillaries. The sympathetic fibers to the arterioles run along the adventitial media border, ending in small swellings or simply fades out as free endings.
**Fig 3.** Epidemiology and clinical characteristics of 112 EM patients.

*Clinical subgroup (number of patients).*

**Females (%) in the clinical category.**

***Age (years, mean with total range) at the time of onset of EM symptoms.*

****Time from initial symptoms to diagnosis (mean with total range).*

EPPA = erythromelalgia-phenomenon-primary- acute
EPPC = erythromelalgia-phenomenon-primary-chronic
EPSA = erythromelalgia-phenomenon-secondary-acute
EPSC = erythromelalgia-phenomenon-secondary-chronic

(Levick, 1995). The arterioles may be heat sensitive or non-heat sensitive. The terminal arterioles, with their strong muscular coating, are important resistance vessels in the microvasculature and play a key role in the regulation of blood flow to the capillaries. A smooth muscle encircles the entrance of the capillary, the capillary sphincter. The capillaries are organized in loops, which are composed of an ascending limb, an intrapapillary loop with a hairpin turn, and a descending venous limb to the postcapillary venule. Each dermal papilla is supplied by a single capillary loop. The loops are perpendicular to the skin surface, except those in the nail beds, which run parallel to the surface of the skin. The capillary density is about 50 per mm$^2$, but varies at different locations (Rothman, 1954; Pasyk et al, 1989). Exchange of respiratory gases, fluids and metabolites takes place mainly during the passage of the capillaries by filtration, diffusion, osmosis and pinocytosis. The nutrients migrate out into the interstitial space towards the cells in the tissue and waste products in the opposite direction. The capillary is 0.5 $\mu$m thick, consisting of only a single layer of endothelial cells, and is surrounded by a basement membrane. The diameter is 49 $\mu$m, and the length 1-2 mm. As there is no nerve endings and nor smooth muscles in the vessel wall, the size of the capillaries are dependent on the arterial pressure and the tonus of the precapillary sphincter (Nelms, 1963). The disc shaped erythrocytes, with an average diameter of 8 $\mu$m, have to fold in order to pass through the capillary. The erythrocytes move with a velocity of 0.4-0.8 mm/s. The endothelial cells are active multifunctional cells and are important in the evolution of inflammatory disorders.
Eight to ten postcapillary venules converge to the descending venule, running alongside the ascending arteriole, which connect the venous side of the upper plexus to the lower horizontal plexus. At the lower plexus, collecting veins with two-cusped valves prevent retrograde flow of blood. An umbrella-like vascular unit could be defined, where the ascending arteriole and the descending venule represent the handle, and the umbrella proper is formed by branching arterioles in the center, and venules in the periphery.

The skin vasculature largely exceeds what is necessary to meet the metabolic demands of the skin. To regulate the heat loss, the blood can be passed through microvascular AV anastomoses, shunt vessels that connect arterioles with venules (Fig 4). AV anastomoses are, as the arterioles, important resistance vessels, but with a more specialized function. The diameter of the AV anastomoses in the skin range from 35 to 165 μm. Their locations are somewhere above the level of the sweat glands, deep in dermis. The anastomotic channel may be single or multiple, straight or curved. The intermediate segment, called Suquet-Hoyer canal, between the arterial and the venous part of the AV anastomoses, has a lumen diameter that varies between 20 and 70 μm, three to ten times the size of an erythrocyte (Johnson et al, 1986). The vessel wall of this canal consists of a thick layer of smooth muscle cells with a "muff" of nerve fibers (Nelms, 1963; Ijima & Tagawa, 1976; Molyneux, 1977; Morishima & Hanawa, 1978). They can rapidly redistribute blood in the skin to the deep layer and thereby depriving the capillary loops of blood flow and simultaneously directing the flow into the venular bed giving increased skin temperature. Heat from the core of the body is conducted to the skin surface where it is lost to the surroundings by radiation, conduction, convection or evaporation.

AV anastomoses are confined to apical or acral skin, i.e. distal part of the limbs, the nose and the external ear. The highest concentration of shunts is found in the nail beds, about 500/cm². The fingertips and toe tips have about 50% of the number of shunts found in the nail bed; the palmar and plantar surfaces about 20-25%. None or few AV anastomoses are found on the dorsal surfaces of hands and feet, and it is generally assumed that they are not found in the skin of the thorax and abdomen (Suquet 1862; Hoyer 1877; Grosser, 1902; Grant & Bland, 1931; Popoff, 1934; Mescon et al, 1956; Sherman, 1963).

1.5.2 Morphology in erythromelalgia

No diagnostic structural diagnostic criteria for erythromelalgia have been described. In many cases, no pathological findings are described, while other authors report non-specific histological changes of skin vessels (Cross, 1962; Eisler et al, 1981; Vendrell et al, 1988; Kvemebo, 1998). Perivascular inflammation, fibromuscular intima proliferation, thickened blood vessel basement membranes, perivascular edema, endothelial swelling, as well as thrombotic occlusion have been reported in...

1.5.3 Regulation of skin blood flow

Regulation of skin blood flow is complex and is dependent on perfusion pressure, blood viscosity, and diameter of the blood vessels. The skin blood flow range from 0.3 to 150 ml/100 g tissue/min (Sejrsen, 1971). In steady state conditions, about 250 ml/min goes to healthy skin and up to 6-81/min during hyperthermia. Diseases in the arteries (atherosclerosis) and arterioles (Buerger's disease) may reduce the perfusion pressure. Viscosity of the blood and thereby changes in the hemoreological properties of the blood may be influenced by the concentration, aggregation and flexibility of blood cells (thrombocytes, erythrocytes, leukocytes), plasma proteins, clotting factors, and environmental temperature.

The vessel diameter is controlled by the muscle tone in the arterial tree, the contractile activity of the pericytes and endothelial cells. Nervous stimuli, local pH, oxygen tension, and vasoactive mediators control the vascular tone of the arterioles, AV anastomoses and precapillary sphincters (Table IV; Strand, 1983).

Vasomotor neurons are mainly activated by thermoregulatory, arousal and emotional reactions. Acral, non-glabrous skin vasculature is under strong central nervous control and is innervated by sympathetic vasoconstrictor nerves, whereas non-acral skin is regulated by both vasoconstrictory and vasodilatory nerves governed by local and central factors (Burton, 1939; Thoresen & Walloe, 1980; Levick, 1995; Johnson & Poppe, 1996; Morris & Gibbins, 1997). Axon reflexes in afferent C-fibers mediate vasodilatation.

1.5.4 Regulation of skin blood flow in erythromelalgia

Few reports have been published on regulation on skin vasculature in erythromelalgia. Affected EM skin is hyperemic (Kvernebo, 1998), while reduced perfusion have reported between EM attacks compared to controls (Littleford et al, 1999a). The normal ability to post-ischemic hyperemia is impaired in affected skin, but intact in the underlying muscle (Kvernebo, 1998). Reduced vasoconstriction in response to inspiratory gasp and contralateral cold challenge has been described (Littleford et al, 1999b).

1.5.5 Temperature regulation

Skin microcirculation regulates the temperature of the body, and the skin vessels are under central control. The central control of thermoregulation is located in the preoptic/anterior hypothalamus, which co-ordinates the different efferent nerves in response to changes in core temperature. In the thermoneutral zone, the core temperature is controlled solely by increased or reduced vasoconstrictor nerve impulses. During cold
exposure, vasoconstriction, and heat production (shivering) protect against hypothermia. The arterioles and AV anastomoses constrict, and the blood is shifted from the superficial to the deep circulation, bringing the venous blood closer to the arteries, and the countercurrent flow of heat from the warm arterial blood to the returning venous blood is facilitated.

The first response to heat stress is cessation of vasoconstrictory nerve impulses to the arterioles and AV anastomoses. Through the AV anastomoses the blood can pass directly to the superficial veins without being precooled when passing through the capillaries. If the core temperature continues to rise, the arterioles of non-acral skin dilate further by activation of the active cutaneous vasodilatation system. Active cutaneous vasodilatation and sweating occur simultaneously. Local warming induces vasodilatation via antidromic peptidergic afferent C-fibres and nitric oxide (Charakoudian, 2003; Fig 5).

1.6 Pathogenesis of erythromelalgia
1.6.1 Clinical findings indicating coexistence of skin hypoxia and hyperemia

In a few patients, the following clinical findings indicate skin hypoxia: nail growth disturbances following periods with severe EM distress, spontaneous and/or slow healing skin ulcers, gangrene and amputation of the lower extremities in spite of open limb arteries (four extremities in three patients; Kvernebo, 1998) and trophic skin changes (Belch, 1996). The pain in erythromelalgia is relieved by cooling, but difficult to treat by opiates, which is typical of pain induced by hypoxia. On the other hand, red and warm skin, as well as reduced capillary refilling time after anemisation, indicate hyperemia.

1.6.2 Pathophysiological findings indicating coexistence of hypoxia and hyperemia

Few physiological studies of the circulation have been performed in EM patients. Increased content of oxygen of venous blood from the affected extremity has been demonstrated (Brown et al, 1932). A correlation has been found between skin temperature and EM distress, and a critical temperature of 32-36 °C for eliciting EM symptoms has been claimed (Lewis, 1930; Lewis, 1933; Lewis & Hess, 1933; Babb et al, 1964). Elevated skin temperature increases skin metabolism and oxygen consumption.

Severely disturbed hemodynamics has been demonstrated in severely affected EM patients (Kvernebo, 1998). Using laser Doppler flowmetry (LDF), affected EM skin is found to be hyperemic compared to unaffected skin. The normal ability to post-ischemic hyperemia is impaired in affected skin, but intact in the underlying resting muscle. These findings are interpreted as nearly maximal dilatation of the skin vessels in patients with EM symptoms before arterial cuff occlusion. Furthermore, the microvascular abnormalities are confined to the skin. Ultrasound flow velocity measurements demonstrate that skin hyperemia coincide with hyperemic flow velocity profiles in the
arteries (posterior tibial artery) supplying the affected skin area. This pattern is typical of low peripheral resistance. Using strain gauge plethysmography, increase in calf blood flow has also been recorded. Transcutaneous oxygen tensiometry measurements demonstrate critical hypoxia in affected skin in some patients, and normal values in unaffected skin. In severe erythromelalgia, measurement of high cardiac output and reduced core temperature secondary to continuous limb cooling has been reported.

### 1.6.3 Histological findings indicating hypoxia

Increased number of clustered, small and dilated vessels have been demonstrated in erythromelalgia syndrome (Kvernebo, 1998). Capillary proliferation has been shown in 12 out of 36 patients with primary erythromelalgia (Kalgaard, personal communication).

### 1.6.4 The shunt hypothesis

Clinical observations and pathological findings indicate a coexistence of hyperemia and hypoxia in affected EM skin areas. Based on these observations, Kvernebo proposed the shunt hypothesis for the pathogenesis of erythromelalgia (Kvernebo, 1998):

Skin hypoxia is postulated to be a key to the initiation of EM symptoms. Oxygen tension in affected skin varies considerably, and with normal levels next to perfused vessels, if the blood-tissue barrier is not disturbed, and with lower levels between the vessels. As the oxygen diffusion capacity is poor, areas relatively far from the nearest perfused vessels may be hypoxic. If there is a distribution mismatch, there will be a
considerable variation in the oxygen tension offered to different skin cells. Some cells may have adequate supply, while others may be severely hypoxic (Fig 5). Hypoxia is caused by maldistribution of skin perfusion, with high levels of microvascular arteriovenous shunting of blood resulting in increased thermoregulatory and insufficient nutritive perfusion. The maldistribution of blood flow may be caused by an opening of acral microvascular AV anastomoses and, at the same time, increased tone in the precapillary sphincter. In primary erythromelalgia, the symptoms are located only in areas with anatomical microvascular AV anastomoses, i.e. hands and/or feet. In erythromelalgia syndrome, the shunting may occur through proliferated microvessels. With plugging of capillaries, the density of perfused capillaries may be reduced, and the tissue hypoxic in certain areas due to increased diffusion distances and increased blood flow velocities in the perfused micro-vessels (as in myeloproliferative disorders). The passage time of the erythrocytes may be too short for equilibration of oxygen with the surrounding tissue. The oxygen diffusion may also be disturbed in case of pathologically increased blood-tissue diffusion barrier (as in vasculitis and diabetes mellitus). Shunting through capillaries serving physiologically as "through-fare channels" may occur in secondary erythromelalgia.

The consequence of tissue hypoxia is arteriolar dilatation and hyperemia (Fig 6). As the increased perfusion is once more maldistributed, the skin temperature increase and accelerate the skin metabolism, causing an increased oxygen consumption, and a further deterioration of the skin nutrition. A vicious circle is maintained by maldistribution of the perfusion. This shunt hypothesis for the pathogenesis of erythromelalgia can explain the effect of cooling, the effect of treatment of the primary disease in secondary erythromelalgia, as well as the beneficial effect of vasodilatory treatment (Özsoylu et al, 1979; Özsoylu & Coskun, 1984; Belch, 1992; Kvernebo, 1998), and deterioration caused by vasoconstrictor treatment (Kvernebo, 1998).

1.7 Therapy of erythromelalgia

Erythromelalgia has for many years been described as difficult to treat (Brown, 1930; Smith & Allen, 1938), and a long list of different types of medications and treatments have been used, most of which give no or partial symptomatic relief (Cohen, 2000; Davis
The wide spectrum of approaches (oral and parenteral medications, topical treatment, and parenteral, invasive, nonmedicational approaches) in the management of erythromelalgia reflects that the treatments are based on anecdotal evidence and heterogeneity in etiology of erythromelalgia.

A recent review of the literature on the treatment of erythromelalgia is given in Appendix II (Mørk & Kvernebo, 2002).

PART TWO: AIM OF THESIS

The objectives of these studies on EM patients were:
1. To challenge the shunt hypothesis of the pathogenesis of erythromelalgia
2. To characterize the mechanisms underlying the vascular dysfunction
3. To treat patients based on the present understanding of the pathogenesis

PART THREE: METHODS USED IN THE STUDIES

3.1 Laser Doppler flowmetry

Laser Doppler flowmetry (LDF) is based on a bio-optical technique studying the interaction mechanism between monochromatic laser light and living tissue. When laser light penetrates a tissue, some photons are absorbed, while others are scattered by moving particles (erythrocytes) in the skin tissue or in the microvascular network, so that the light changes wavelength (frequency and amplitude) according to the Doppler principle (Doppler-broadened or shifted light). The back-scattered Doppler-broadened light is detected by recording optical fibers. A signal-processing unit converts the light intensity into an electrical output signal. The signal equals the product of average velocity ($v$) and the concentration of the moving blood cells (CMBC) within the sample volume ($\text{perfusion} = v \times \text{CMBC}$). In in vitro models this signal is proportional to perfusion. The perfusion is expressed in arbitrary units (AU). A probe must be attached to the tissue during recording. LDF exhibit spatial variation in its measurements, explained by the random distribution of the ascending arterioles at 1.5-7.0 mm intervals. The method is more suited for stimuli-response experiments, than for mapping of the blood flow distribution over a specific tissue area. The laser beam illuminates the tissue volume to a depth of a few hundred gm, but the exact measuring volume cannot be predicted precisely. The measuring depth and volume are influenced by the wavelength of the laser light, the size of the probes, the number, diameter and separation of the optical fibers, and optical properties (coefficients for light absorption) of the illuminated tissue (Wardell et al, 1993).

We used a Helium-Neon laser operating at 632.8 nm (Periflux, Perimed, Sweden). In all measurements (paper III) an output circuit time constant of 0.2 s was chosen. The cut-off filter was set to 12 kHz. The optical zero of the instrument was obtained by holding the probe against a white reflecting surface. The biological zero value was recorded following tourniquet pressure above systolic blood pressure. The difference between the optical and the biological zero value can be explained by Brownian movements of blood cells, vaso-motion activity and "rolling" of cellular aggregates which are formed as the
blood velocity approximates zero. The biological zero value was lows (< 5% of the measured LDF value) in our experiments and was therefore not extracted from measured values.

3.2 Laser Doppler perfusion imaging

Laser Doppler perfusion imaging (LDPI) is based on the LDF-principle. LDPI is a non-invasive, non-touch technique recording perfusion by two-dimensional beam scans of a tissue area (Wardell et al, 1993). In the LDPI system (PIM, Lisca AB, Sweden) a computer-controlled optical scanner was used. The laser light (Helium-Neon 632.5 nm) was directed sequentially in a rectangular pattern over the skin surface giving two-dimensional mapping of tissue blood flow. The backscattered Doppler broadened light was detected by a photo-detector positioned in the scanner head and processed to form a measure of the blood perfusion within the illuminated volume of the complex network of vessels in the microcirculation of the skin. To eliminate interference from ambient light, measurements were performed in a dark room. A full format image includes 64 x 64 pixels corresponding to a 12 x 12 cm area and is captured in 4.5 minutes. When all measurement values had been recorded, processed, and stored, a color coded perfusion image, showing the spatial heterogeneity of the tissue perfusion, was displayed on a monitor. The average perfusion and spatial heterogeneity expressed as coefficient of variation of the recordings were determined.

In our experiments the resulting estimates, comprising 20 x 20 pixels, represented the blood perfusion within a skin area of approximately 3.75 x 3.75 cm. Resolution was set to high. The test subjects were positioned such that gross movement artifacts were avoided. The measurement area was marked on the skin surface, and these marks, which were depicted gray on the color coded image (zero perfusion), were used as fixed points. The scanner head was adjusted parallel to the measurement area.

3.3 Capillary assisted video microscopy

Capillary assisted video microscopy (CAVM) makes possible in vivo analysis of capillary density in a tissue surface (Zhong et al, 2000). CAVM combines modern microscopic techniques and information technology. The hardware comprises a PC and a portable video capillary microscope consisting of a video signal control unit and a handheld mini-video camera. A computerized imaging system was used to extract the individual capillaries for objective and accurate analysis of the capillary ensemble (topology heterogeneity). Approximately 100 capillaries can be investigated in a field of view of 1.7 mm² if the magnification is set to 200. The captured color images show dark red spots with comma-like shapes, which correspond to the apex of the capillary loops perpendicular to the skin surface. The following parameters were extracted: capillary density, capillary size, distance to the neighboring capillaries and heterogeneity in the distribution of capillaries (mutual distance).

PART FOUR: SUMMARY OF PAPERS

4.1 Paper I

Aim of the study: Anatomical AV anastomoses are located in acral areas, mainly on the plantar and palmar aspects of feet and hands. By relating the perfusion increase in plantar and dorsal aspects of the feet in EM patients and controls during central body heating with the occurrence of EM symptoms, we wanted to test the hypothesis of AV shunting.
**Materials, methods, and experimental design:** LDPI-assessed skin perfusion and skin temperature were recorded in plantar and dorsal aspects of the feet in primary EM patients with moderate severity (n=14) and healthy controls (n=11) following central body heating. LDPI record global skin perfusion, i.e. nutritive and thermoregulatory perfusion, and the main part of the output signal originate from the thermoregulatory part of the perfusion. Two measurement sites were chosen at the left foot: (1) the anterior section of the foot arch, where the epidermis is thin compared to the weight bearing parts of the foot (many AV anastomoses), and (2) at the dorsal region of the foot just proximal to the first and second toe (no or few AV anastomoses). To provoke EM symptoms, central body heating, which increase skin perfusion through reduced sympathetic action on the AV anastomoses, was used. The patients were covered with twelve duvets, except for the head and left foot, in order not to disturb the LDPI recordings. The heat provocation was terminated when skin temperature of the plantar surface of the first toe reached 32 °C ("critical" temperature), patients reported pain (cut-off value for VAS score >50) and 100 min heating time (logistical reasons).

**Results:** EM symptoms were induced in eight patients. Following the same heat provocation, the increase in perfusion was significantly higher in symptomatic EM patients in areas with many AV anastomoses (plantar area) compared to asymptomatic patients and controls. In areas with no or few AV anastomoses, no such difference was demonstrated between the same groups. The skin temperature recordings were in accordance with the results of the perfusion studies.

**Interpretation:** The increased LDPI-assessed perfusion in symptomatic EM patients compared to asymptomatic EM patients and controls can be interpreted as increased thermoregulatory AV shunt flow in symptomatic erythromelalgia support the shunt hypothesis.

### 4.2 Paper II

**Aim of the study:** The shunt hypothesis implies inadequate nutritional skin perfusion as compared to needs in affected skin of patients with erythromelalgia during attacks. To challenge this hypothesis we analysed the capillary bed before and after heat provocation in EM patients and healthy controls in areas with different density of AV anastomoses.

**Materials, methods, and experimental design:** We analyzed the capillary bed morphology using CAVM in primary EM (n=14) and controls (n=10). CAVM recordings were performed at two sites with thin epidermis on the left foot: (1) just proximal to the nail bed of the first toe where the capillary loops were perpendicular to the skin surface and with a high density of AV anastomoses, and (2) in the foot arch with fewer AV anastomoses. The same heating protocol as in paper I was chosen, except for a prolonged maximum heating time (120 min).

**Results:** In EM patients, capillary density was significantly reduced after central body heating in areas with many AV anastomoses (nail bed area), in contrast to skin areas with fewer AV anastomoses (foot arch). This fording was most pronounced in symptomatic patients. In healthy controls and in EM patients who did not develop symptoms after heat provocation, no changes were demonstrated. In symptomatic EM patients the change in capillary density was significantly larger in the capillary bed compared to asymptomatic patients and controls, in contrast to the foot arch. Symptomatic patients showed a significantly higher increase in skin temperature following central body heating, as
compared to asymptomatic patients and controls.

**Interpretation:** AV anastomoses stay permanently open in symptomatic EM patients after central warming, causing a steal phenomenon of blood from the capillaries, resulting in a relative lack of capillary perfusion. The consequence of a larger increase in temperature in affected skin is higher metabolism of the skin and thereby higher nutritional needs. Tissue hypoxia is therefore likely in affected skin during EM attacks, and is maintained by a maldistribution of skin perfusion.

### 4.3 Paper III

**Aim of the study:** The smooth muscle activity of the arterioles and the precapillary sphincters regulate the distribution of blood flow. This muscle tone is under control of the local metabolism, neurogenic and endothelial activity, and transmural hydrostatic pressure. To characterize the mechanisms underlying the vascular dysfunction in EM, the response in skin microcirculation to local and central vasoconstrictory stimuli was studied. In addition, endothelial function was tested indirectly by studying the post-ischemic hyperemic response.

**Material, methods, and experimental procedure:** Microvascular skin perfusion at rest and during well-known physiologic provocation tests were assessed by LDF at the pulp of the first toe in primary EM patients (n=14) with moderate severity and compared to healthy controls. The patients were asymptomatic during the measurements. Vasoconstriction in response to forced expiration against closed glottis (Valsalva's maneuver), contralateral cooling (water temperature 15°C), venous cuff occlusion and change in position from supine to standing were recorded. Vasodilator response to local heating (probe temperature 28, 32, 36, 40, and 44°C) and hyperemic response to 3 min arterial occlusion were recorded.

**Results:** EM patients had a significantly reduced skin microvascular perfusion during basal conditions compared to healthy control subjects. Attenuated vasoconstrictor responses involving central sympathetic reflexes (Valsalva's maneuver and cold challenge) were found in patients with erythromelalgia compared to control subjects. Local neurogenic vasoconstrictor regulation (venoarteriolar reflex and venous cuff occlusion test), vasodilator response to local heating and hyperemic response to ischemia were maintained in the EM patients.

**Interpretation:** The finding of impaired Valsalva's maneuver and cold challenge reflexes with different afferent and common efferent pathways imply a postganglionic sympathetic dysfunction. This neuropathy may be primary or secondary to tissue hypoxia. The vasoconstrictor tendency seems to be a paradoxical finding, but may indicate denervation supersensitivity. Local neurogenic and endothelial function is unaffected, which is in favor of a primary sympathetic dysfunction.
4.4 Paper IV

Aim of the study: According to the shunt hypothesis, substances that improve capillary perfusion and/or improve hemoreological properties of the blood, may enhance nutrition and thereby improve skin oxygenation and induce relief of EM symptoms. Prostanoids have dilatory effects on cutaneous circulation as well as thrombocyte inhibitory effects. Beneficial clinical effect has been reported using parenteral treatment. The present study was designed to determine whether treatment with misoprostol, an oral prostaglandin E1 analogue, leads to improvement in EM symptoms and redistribution of skin microcirculation in favor of nutritive perfusion, compared to placebo treatment.

Material, methods and experimental procedure: Inclusion/exclusion criteria and study design (double-blind, crossover, placebo compared) were chosen based on number of available patients and a pre-study definition of clinical significant improvement of the primary efficacy variable (pain VAS). 21 EM patients were recruited (Table V). Secondary efficacy variables included change in cooling score and patient’s global assessment (PGA). Six weeks treatment with placebo was followed by six weeks treatment with misoprostol, as randomization could result in a hangover effect of misoprostol. The patients were also evaluated at three months follow-up. Skin microcirculation was assessed using LDPI and CAVM following central body heating at baseline, and after placebo and misoprostol treatment, and compared to healthy controls (n=11). The heating protocol was the same as in paper I, but with a fixed 30 min heating time. Pain VAS was recorded following heat provocation.
**Results:** Change in pain and cooling scores were significantly better following misoprostol treatment compared to placebo treatment and after three months follow-up without treatment. The number of responders were significantly higher during treatment with misoprostol (n=14), compared to placebo (n=4) and at follow-up (n=0). Based on PGA, treatment with misoprostol was superior to placebo, but this difference did not persist at follow-up. The number of symptomatic patients and the increase in pain score after central body heating were significantly lower after misoprostol treatment compared to baseline and after placebo treatment. After misoprostol treatment, the increase in LDPI assessed skin perfusion following heating did not differ from the response in the control group. This was in contrast to assessments at baseline and after placebo treatment, where the increase in skin perfusion was significantly larger as compared to assessments after misoprostol treatment and in controls. After central body warming, CAVM-assessed capillary density was reduced in EM patients at baseline, after placebo and after misoprostol treatment, in spite of the significant increase in temperature and perfusion. This was in contrast to findings in the controls where capillary density was unchanged in response to heat provocation. The average distance between neighboring skin capillaries, an estimate of diffusion distances for oxygen and nutrients, increased in patients after warming, but not in controls.

**Interpretation:** to our knowledge, this is the first report of a properly designed, placebo-controlled clinical trial for treatment of erythromelalgia. A statistical significant and clinically relevant reduction in EM symptoms was demonstrated after misoprostol treatment compared to placebo. The mechanism of action may be reduced microvascular AV shunting in affected skin based on quantitative measurements of skin microcirculation.

**PART FIVE: DISCUSSION**

5.1 Is skin with erythromelalgia hypoxic during attacks?

The coexistence of hypoxia and hyperemia was first proposed in 1987 based on transcutaneous oxygen tension measurements and quantification of skin microvascular perfusion with LDF in a severely affected patient (Kvernebo & Seem, 1987). The transcutaneous measurements showed an average value for a measurement area of around one cm². In EM affected skin, there is, however, probably a varying oxygen tension throughout the tissue with excellent oxygenation close to perfused vessels, while tissue cells further away from perfused capillaries may live in a hypoxic environment. Current transcutaneous oxygen tension measurement techniques average oxygen tension values of an area that may be too large to detect any heterogeneity in oxygen levels. Implanted probes that measure oxygen levels in smaller areas induce trauma and bleeding that may disturb recordings.

The following evidence indicate hypoxia in erythromelalgia:

In patients with primary erythromelalgia, we have demonstrated during EM attacks following central body warming (paper II):

1. Significant reduction in CAVM-assessed capillary density in areas with many AV
2. Temperature in plantar toe, an area with many AV anastomoses, increased significantly more as compared to asymptomatic EM patients and control subjects. Since the temperature increased by the warming provocation, the local skin metabolism will accelerate. To account for the increased need for oxygen a maintained or increased capillary density could be expected. This was the case in asymptomatic EM patients and controls, although an actual reduction was observed in symptomatic EM.

Further evidence for hypoxia in erythromelalgia is:
Clinical symptoms (pain relieved by cooling and relatively resistant to opiates) and signs (trophic skin changes, nail growth disturbances, spontaneous and/or slow healing ulcers and gangrene) indicative of skin hypoxia has been described (Belch, 1992; Kvernebo, 1998). Increased content of oxygen in venous blood from the affected extremity has been demonstrated (Brown, 1932). Oxygenation of the affected areas during symptomatic periods using transcutaneous oximetry pressure has been found to be decreased or unchanged, despite a marked increase in blood flow and temperature (Kvernebo, 1998; Sandroni et al, 1999; Davis et al, 2003). Histological examinations have demonstrated capillary proliferation in affected skin, which may be interpreted as a response to hypoxia (Kalgaard, personal communication).

5.2 Is arteriovenous shunting a common final pathway of the pathogenesis of erythromelalgia?
In favor of microvascular AV shunting in the pathogenesis of erythromelalgia, we have the following observations:
• In primary EM patients with central warming induced EM attacks (paper I), we demonstrated: Significantly higher increase in overall or global skin perfusion (LDPI) from baseline perfusion in areas with many AV anastomoses compared to EM patients without symptoms and healthy control subjects. No such difference between the groups was demonstrated for global skin perfusion in areas with no or few AV anastomoses. Plantar toe temperature increased significantly more as compared to asymptomatic EM patients and control subjects.
• We found reduced increase in LDPI-assessed skin perfusion after central body warming following treatment with misoprostol, a prostaglandin El analogue as compared to values with after placebo treatment (paper IV). This effect coincides with a significant treatment effect of misoprostol as also assessed by clinical outcome variables.

LDPI was used to indirectly demonstrate the occurrence of increased shunt flow during EM symptoms. Despite an increase in global perfusion of affected skin, the EM symptoms are accompanied by a decreased number of perfused superficial capillaries in areas with the highest density of AV anastomoses. The observations support the occurrence of a steal phenomenon away from the superficial nutritive vascular plexus to the deeper thermoregulatory shunt perfusion.
Other observations in favor of AV shunting are:
• The clinical findings of hyperemia (red and warm skin with rapid refilling time after anemisation).
• Hyperemia is demonstrated in affected EM skin by LDF measurements, in calf blood
flow by strain gauge plethysmography, and in the arteries supplying the affected skin by ultrasound Doppler (Kvernebo, 1998). The post-ischemic hyperemia response is impaired in symptomatic skin, but intact in the underlying muscle (Kvernebo, 1998). Increased cardiac output and decreased core temperature has also been demonstrated in severe cases (Kvernebo, 1998). The demonstrated increase in blood flow indicates low vascular resistance in the skin. The low resistance is most probably caused by opening of the AV shunts in acral skin.

- Histological examination has demonstrated proliferating vessels in erythromelalgic syndrome that may serve as shunts (Kvernebo, 1998).

5.3 Is erythromelalgia associated with neuropathy?

In patients with primary erythromelalgia we induced EM attacks during central warming (paper III). Under basal conditions asymptomatic EM patients had reduced skin blood flow and reduced perfusion in response to local heating. We also demonstrate diminished vasoconstrictor responses to central sympathetic reflexes (Valsalva's maneuver and contralateral cooling). Local neurogenic and endothelial function were unaffected. Valsalva's maneuver and contralateral cooling have different afferent pathways and common efferent pathways, implying an impaired efferent limb and consequently a postganglionic sympathetic dysfunction. Denervation supersensitivity may explain the vasoconstriction demonstrated between attacks.

In the literature there are further support for neuropathy in patients with erythromelalgia:

- There have only been a few reports on beneficial effect of sympathectomy (Bonica, 1953; Telford & Simmons, 1940; Zoppi et al, 1985; Shiga et al, 1999; Seishima et al, 2000), while others have found aggravation of EM symptoms following sympathetic blocks (Rosati & Verga, 1955; Cross, 1962; Smith & Allen, 1938; Kvernebo, 1998).

- Pharmacological substances used for the treatment of neuropathy (gabapentin, antidepressants and serotonin reuptake inhibitors) have been reported to have beneficial clinical effects in erythromelalgia (Herskovitz et al, 1993; McGraw & Kosek, 1997, Rudikoff & Jaffe, 1997; Maldonova et al, 1999; Moiin et al, 2002; Pandey et al, 2002; Rey et al, 2003b). Improved sympathetic dysfunction and clinical outcomes following intravenous treatment with iloprost in a double-blind pilot study in 12 patients with erythromelalgia is recently published (Kalgaard et al, 2003).


- Attenuated vasoconstriction in response to inspiratory gasp and contralateral cold challenge and reduced basal skin perfusion has been reported in EM patients (Littleford et al, 1999b).

- Axonal neuropathy and abnormal sudomotor function have been demonstrated in EM patients (Sandroni et al, 1999). The authors suggest the presence of efferent small fiber neuropathy.

- Abnormal sympathetic skin responses have been described in patients with erythromelalgia in a controlled study. The authors conclude that C fibers may be involved in the pathogenesis of EM (Kazemi et al, 2003).
Pathological C-fibers have been described by Ørstavik in our group (Ørstavik et al, 2003). Recently, further neurophysiological laboratory tests of our patients have demonstrated tactile hyperalgesia and involvement of afferent small fibers (Ørstavik et al, in press). No changes were demonstrated in the efferent fibers in this study.

5.4 Is vasculopathy or neuropathy the primary event?

Reduced perfusion between EM attacks (paper III) is in accordance with previous findings (Littleford, 1997; Littleford et al, 1999a) and clinical observations that many EM patients may have pale/white or blue/cyanotic skin color, reduced toe temperature and concomitant Raynaud’s phenomenon between attacks. The EM patients seem to be cold between attacks and warm during attacks. The reduced perfusion may be explained by structural microvascular changes, increased adrenergic vasomotor activity or increased adrenergic receptor sensitivity, as well as dysfunction of endothelial cells with imbalance in secretion of vasoactive mediators (endothelin, nitric oxide). Structural changes can be caused by reduced vascular density, endothelial swelling, thickened vessel basement membrane, intimal proliferation, perivascular edema or microthrombi with occlusion of microvessels as a result of changes in blood viscosity, platelet aggregation, fibrinolytic activity and erythrocyte deformability.

Capillary density is not reduced in asymptomatic erythromelalgia (paper II) and endothelial function, as assessed by postocclusive hyperemia response, is maintained (paper III). An elevated sympathetic vasomotor activity in erythromelalgia seems unlikely, based on our findings in paper II. Structural changes in the vessel walls, perivascular inflammation and edema, as well as thrombi, have been described (Michiels et al, 1985; Drenth et al, 1996; Kvernebo, 1998). We postulate, like other authors, that increased adrenergic receptor sensitivity may be involved in erythromelalgia (Uno & Parker, 1983; Littleford et al, 1999b; Mørk et al, 2002b).

We have demonstrated that erythromelalgia is associated with both vasculopathy and neuropathy (Fig 12). Dysfunctional AV anastomoses and precapillary sphincters are probably essential elements of the vascular dysfunction in erythromelalgia. The shunting of blood in the skin is probably triggered by a number of mechanisms related to hemorrheological problems, defects in prostaglandin metabolism, or defects in sympathetic or endothelial function (Littleford et al, 1999a,b; Sandroni et al, 1999; Ørstavik et al, 2003; Drenth et al, 1996; van Genderen et al, 1996; Jørgensen & Søndergaard, 1978; Michiels et al, 1997; Chan et al, 2002). Vasculopathy and neuropathy may therefore coexist, like microangiopathy and neuropathy in diabetes mellitus (Tesfaye et al, 1993).

We believe that both phenomena are important in the pathogenesis of erythromelalgia. Vasculopathy may cause skin and neural hypoxia resulting in neural dysfunction. On the other hand, neuropathy may underline the microvascular dysfunction. A vascular and a neurological pathophysiology do not exclude each other. Our group has demonstrated improvement in neuropathy and clinical outcome measures following treatment of the vasculopathy with iloprost (Kalgaard et al, 2003). On the other hand, medications used for the treatment of neuropathy have been reported to have a beneficial clinical effect (McGraw & Kosek, 1997; Rudikoff & Jaffe, 1997; Maldonova et al, 1999; Moiin et al,
Neuropathy has been demonstrated only in some patients with erythromelalgia (Ørstavik et al, submitted; Davis et al, 2003), but EM has been reported following neuropathy (Herskovitz et al, 1993).

Further studies are needed to determine if erythromelalgia is primarily a vasculopathy leading to neuropathy, or vice versa (Sandroni et al, 1999; Davis et al, 2000b; Mark & Kvernebo, 2000b; Davis et al, 2003).

5.5 Is erythromelalgia a separate disease entity?

From a clinical point of view it is appropriate to regard EM as a disease or syndrome (Kvernebo M, 2003). A pathologist will hesitate to regard erythromelalgia as a specific disease, as there is no common etiological factor, and as pathognomonic structural changes are difficult to find. The shunt hypothesis implies that erythromelalgia is not a separate disease entity, but rather a condition with a specific pathophysiological response pattern of skin circulation with a common final pathway of increased thermoregulatory shunt flow and insufficient nutritive perfusion. No single treatment is effective in erythromelalgia. Therapy must be tailored for each patient based on the underlying conditions. This view is analogous to the concept that inflammation is not a specific disease, but a condition (rubor, tumor, calor, dolor, functio laesa) with a specific physiological response to stimuli such as infection, trauma, or tumor.

5.6 Therapy with misoprostol
Improvement in clinical outcome measures and redistribution of skin microcirculation in favor of nutritive perfusion following treatment with misoprostol was demonstrated (paper IV).

The study was based on the shunt hypothesis and experiences from vasodilator treatment of EM patients. According to the shunt hypothesis, the aim of therapy is to increase skin oxygen supply and/or reduce oxygen consumption. This can be achieved by cooling, by treatment of underlying conditions or by redistribution of skin microcirculation in affected skin. The rationale of misoprostol treatment is vasodilatation and reduction in the formation of microthrombi. Enhancement in nutritional blood flow and improvement in tissue oxygenation will break the vicious cycle that maintains erythromelalgia (Fig 8). Theoretically, dilatation in the precapillary sphincter with concurrent constriction of the AV shunts would be the optimal intervention in primary erythromelalgia, but currently no single pharmacological substance or combination of drugs, according to our knowledge, have this targeted effects on skin vasculature. The success of a treatment is dependent on redistribution of skin perfusion in favor of the capillaries, improving the relative hypoperfusion in the nutritive vessels. A vasodilator that predominantly increases the shunt flow, may deteriorate erythromelalgia, as the steal phenomenon may increase.

The study design was determined by the number of available patients (Table V). A parallel group study would require more patients, but the crossover design used in our study had sufficient power to detect a statistically significant change in key parameters. An additional effect after placebo treatment support the efficacy of misoprostol. Blinding was necessary to reduce bias, but blinding may have been disturbed by adverse events associated with misoprostol. A very long washout period (months to years) would be required in a randomized trial based on long-term effects after PGE1 infusions (Kvernebo, 1998). Pain VAS was defined as the primary endpoint as thus parameter has been widely used in other studies (including paper I and II) and validated in pain assessments. The effect of misoprostol could be called statistical and clinical significant in terms of difference in change from placebo and number of responders (success rate). Cooling score and PGA was chosen as secondary efficacy variables. Cooling score has previously been used in the characterization and monitoring of EM severity (Kalgaard et al, 1997; Kalgaard et al, 2003), and global response to treatment is well known in clinical trials. A statistical correlation between the clinical outcome measures supports our interpretation.

We focused on pathogenetic mechanisms, using hard endpoints. The effect of misoprostol on skin microcirculation was studied using LDPI and CAVM and heat provocation. The selection of measurement sites and heat provocation test was based on experience from the studies reported in paper I and II. The reduction in heat-induced pain corresponds with the physiological assessments of skin microcirculation following treatment. Bonferroni adjustments of data were made to avoid misleading false positive results. The recorded changes in the clinical outcome measures were larger than the pre-study definition for treatment success. Evidence for the clinical effectiveness of prostanoids is presented in paper IV from a physiological point of view. The results give further support for the shunt hypothesis.
PART SIX: CONCLUSIONS

- In this thesis, increased AV shunting and reduced nutritive capillary density in affected skin of patients with primary EM were demonstrated. These findings are compatible with skin hypoxia during EM attacks. These studies support the shunt hypothesis in the pathogenesis of erythromelalgia, i.e. skin microvascular arteriovenous shunting as a final common pathway leading to tissue hypoxia, a uniform vascular response to different etiological factors.

- In primary EM patients, we demonstrated attenuated central sympathetic vasomotor reflexes, which indicate autonomic dysfunction. Local reflexes and endothelial function were intact. This neuropathy may be an underlying mechanism for the dysfunctional vascular dynamics. Vasculopathy with hypoxia may also cause neuropathy.

- Furthermore, we found improvement in clinical outcome measures and changes in the skin vasculature in favor of reduced microvascular AV shunting following misoprostol, an oral prostaglandin E1 analogue.

PART SEVEN: REFERENCES

Bonica JJ: The management of pain. Lea and Febiger, Philadelphia 1064-1065, 1953
Cassirer R: Die vasomotorisch-tropischen Neurosen. Handbuch der Neurologie, 182-274, 1912
Davis MD, Rooke TW, Sandroni P: Mechanisms other than shunting are likely contributing to the pathophysiology of erythromelalgia. *J Invest Dermatol* 115:1166-1167, 2000b
Drenth JP, Michiels JJ, van Joost T, Vuzevski VD: Verapamil-induced secondary
Gerhardt C: Über Erythromelalgie. Berliner klinische Wochenschrift 29:1125, 1892
Graves RJ: Clinical lectures on the practice of medicine. Frannin & Co, Dublin, 1834
Housley F: What is erythromelalgia and how should it be treated? *BMJ* 293:117, 1986
Hoyer H: Über unmittelbare einmundung kleinster arterien in Gefassaste venosen charakterts. *Arch Mikros Anat* 13, 603-644, 1877
Hval E: Erythromelalgia cum polyglobulia megalosplenica. *N Mag Lcegev* 89:3136, 1928
Jabs HU, Druke P: Recurrent burning pain, erythema, cutaneous edema and hyperthermia
of both lower legs after herpes zoster thoracalis. *Internist (Berl)* 35:392-394, 1994
Lazareth I, Priollet P: Coexistence of Raynaud’s syndrome and erythromelalgia. *Lancet*
Lewis T: Observations upon the reactions of the vessels of the human skin to cold. *Heart* 15:177-208, 1930
Lewis T: Clinical observations and experiments relating to burning pain in the extremities and so-called "erythromelalgia" in particular. *Clin Sci* 2:175211, 1933
Martorell F, Martorell A: Erythromelalgic syndrome in a hypertensive patient rapidly cured with the new adrenolytic drug, 688A. *Angiologica* 5:120122, 1953
May E, Hillemand P: L'erythromelalgie; contribution a l'etude de la pathologie du sympathique. *Ann de coed* 16:51-83, 1924


Mitchell SW: On a rare vasomotor neurosis of the extremities, and on some maladies with which it may be confounded. *Am J Med Sci* 76:2-36, 1878


Popoff NW: The digital vascular system. *Arch Path* 18:295-330, 1934


Redding KG: Thrombocytopenia as a cause of erythromelalgia. *Arch Dermatol* 113:468-471, 1977


