Nine species of endoparasitic insects, the number of individuals and loci analyzed electrophoretically per species together with the enzymes that show variation, the mean number of alleles per locus ($\bar{A}$), mean proportion of loci polymorphic per species ($\bar{P}$) and heterozygous per individual (H)

Species	Family	Number of individuals ♂	♀	Loci	Polymorphic enzymes**	$\bar{A}$	$\bar{P}$	H***
Hymenoptera								
Ageniaspis fuscicollis	Encyrtidae	13*		25	k	1.080	0.040	0.019****
Diadegma armillata	Ichneumonidae	9	8	19	g, h, k	1.158	0.158	0.048
Itoplectis maculator	Ichneumonidae	5	-	20	g, h, i	1.150	0.150	0.056
Mesochorus vittator	Ichneumonidae	2	1	3	-	1.000	0.000	0.000
Pimpla turionellae	Ichneumonidae	-	12	29	d, e, g, i	1.138	0.138	0.038
Triclistus yponomeutae	Ichneumonidae	-	11	20	a, g	1.100	0.100	0.020
Trieces tricarinatus	Ichneumonidae	14	-	14	-	1.000	0.000	0.000
Tetrastichus evonymellae	Eulophidae	-	10	20	c, h, j	1.150	0.150	0.055
Diptera								
Discochaeta hyponomeutae	Tachinidae	-	5	16	b, f, g, h	1.250	0.250	0.096

*All larvae; 13 clones of 20–30 individuals each. **A, malate dehydrogenase; b, malic enzyme; c, NADH dehydrogenase; d, aldehyde oxidase; e, glucose oxidase; f, hexokinase; g, phosphoglucomutase; h, esterase; i, alkaline phosphatase; j, leucine aminopeptidase; k, phosphoglucose isomerase. ***Values computed from field populations except for *P. turionellae* (see text). ****Computed assuming a 1:1 sex ratio and no differentiation in allozyme frequencies between the sexes[8].

dehydrogenase in *D. armillata*. Assuming identical larva-adult patterns, all other parasites were not detected in *Yponomeuta* larvae (routinely electrophoresed as L4 or L5) due to their rare occurrence and probably to the fact that they parasitize in general later stages of *Yponomeuta* than do *D. armillata* and especially *A. fuscicollis*. As larvae were opened and investigated prior to electrophoresis, at least those parasite larvae that have reached a size at which parasite-specific bands may be intense enough to interfere with the host bands were detected. In *A. fuscicollis Pgi* occurs with 3 alleles, whereas all other polymorphic enzyme systems listed in the table have 2 alleles (so $\bar{A} = 1 + \bar{P}$). Moreover, *Pgi* appears to be a monomer in this species as only 1 or 2 banded patterns occcur; normally this enzyme is a dimer (e.g. in *Diadegma* and *Yponomeuta*[3,10–12]).

It is possible to compute percentages of parasitization in a population from analysis of the zymograms of a sample of individual host-larvae. A total of 456 specimens were analyzed from a population of *Y. cagnagellus* in a dune area near The Hague[11]. *A. fuscicollis* was found to parasitize 10.5%. In a restricted part of this area intensive sampling and subsequent rearing resulted in 7.8 and 13.1% by direct count of the emerging parasites (N = 795 and 703 respectively), whereas based on interpretation of the zymograms of hosts from this area this value was 12.9% (N = 54).

Parasites, analyzed from more than 1 sympatric host (e.g. *A. fuscicollis* from *Y. cagnagellus* and *Y. rorellus*) showed similar variation patterns, probably indicating that they belong to interbreeding populations. The *T. yponomeutae* population is apparently composed of genetically different clones as all 11 ♀♀ originated from 1 single population of *Y. vigintipunctatus*.

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Adrenergic nerves and mast cells after skin freezing. A hypothesis based on fluorescence microscope observations in the rat*

T. Waris and K. Kyösola

4th Department of Surgery, University Central Hospital, Kasarmikatu 11, SF-00130 Helsinki (Finland), and Department of Thoracic and Cardiovascular Surgery, University Central Hospital, SF-00290 Helsinki (Finland), 5 February 1982

Summary. After freezing and thawing of rat skin, degranulation and disappearance of mast cell fluorescence became apparent in the skin up to 1 h after thawing. Gradual disappearance of catecholamines from the adrenergic nerves of the injured area occurred during the 1st 24 h. Both mast cells and adrenergic nerves may play a role in tissue destruction after freezing injury.

The pathogenesis of tissue injury caused by freezing and cold is still not fully understood. Many factors such as direct cell injury and resultant biochemical changes and the development of microthrombi are likely to be involved[1,2]. Sympathectomy has been used in the treatment of frostbite[3–5] and intra-arterial reserpine, which pharmacologically depletes catecholamines, has been put forward as possible treatment of frostbite[6,7]. However, there is no direct

evidence on the possible role played by adrenergic nerves in freezing injury. Although mast cells are known to take part in the tissue response to injury[8], no morphological evidence of their role in cold injury is available at present. Fluorescence microscopic methods now permit the specific histochemical demonstration of noradrenaline-containing adrenergic nerves and 5-hydroxytryptamine (5-HT) containing mast cells[9-11]. The aim of the present study was to investigate the changes in the adrenergic nerves and mast cells produced by experimental skin freezing in the rat. A combined trypan blue catecholamine fluorescence method[12] was adopted in order to correlate these to changes in the blood flow and blood vessel permeability.

30 rats of the Spraque-Dawley strain were shaved and depilated 4 days prior to the experiment. On the right side the dorsal skin of the 30 rats was frozen for 30 sec (in a preliminary study, the skin of 6 rats was frozen for 60 sec) with a copper cylinder (diameter 3 cm) precooled in liquid nitrogen (−196 °C). During shaving, freezing and collection of specimens the rats were under thiopentone sodium ('Nembutal') anesthesia (35 mg/kg). The skin biopsies were studied using a combined trypan blue-catecholamine fluorescence method at 0.5, 1, 4, 8, 16 and 24 h or at 2, 4, 7, 9 and 14 days after freezing. A 2% solution of trypan blue in 0.9% NaCl was injected (15 ml/kg) into a hind limb vein. 10 min later specimens from the injured skin and contralateral control side were taken and processed as follows: 1. The specimens were rapidly frozen in liquid nitrogen, freeze-dried and processed for the demonstration of formaldehyde-induced fluorescence of catecholamines[9]. 2. Specimens of the s.c. fascia were immersed in 2% glyoxylic acid solution (in 0.1 M phosphate buffer, the pH of the solution was 7.2). After 5–30 min, immersion in the glyoxylic acid solution the specimens were blotted dry, stretched on microscope slides and dried in the hot air of a hair dryer.

The specimens were then either formaldehyde-gassed for 1 h at 80 °C or processed for the demonstration of glyoxylic acid-induced fluorescence of catecholamines by heating for 6 min at 100 °C[8]. Two additional rats were injected with reserpine (Serpasil®, Ciba) 20 mg/kg 24 h before freezing the skin. These rats were killed 1 h after freezing. The specificity of the catecholamine fluorescence was confirmed by the disappearance of catecholamines in these specimens. Tissue sections or stretch preparations were mounted in xylene or Entellan (Merck) and studied with a Leitz Ortholux microscope. The UV-light source was a HBO 200 mercury vapor lamp. The filter combination employed consisted of BG 38, BG 12, BG 3, TAL 405 and K 470 filters.

General observations. The hard frozen area of skin thawed quickly and spontaneously at room temperature, becoming soft and flexible. After thawing the area became edematous. The distribution of i.v.-injected trypan blue was uneven. In the middle there was a weakly staining, scantily-perfused area, which was annularly surrounded by strongly blue-staining tissue.

Fluorescence microscope observations. Trypan blue has a red fluorescence when absorbed onto proteins. In normal skin this stain in reddish inside blood vessels and is seen as a reddish hue in dermal tissues. Blue-green fluorescent adrenergic nerves were seen in erector pili muscles and around arteries and arterioles. The fluorescence of mast cells was yellow. In the specimens taken 0.5 and 1 h after skin freezing, some decrease in the fluorescence intensity of adenergic nerves was noted but the fluorescence in these nerves appeared otherwise unchanged. In the middle of the injured area only slight trypan blue fluorescence was seen. This ischaemis area was surrounded by an area characterized by increased red fluorescence, indicating extravasation of trypan blue. An almost complete disappearance of mast cell fluorescence from the middle of the injured area was noted. In the surrounding tissue, mast cells undergoing degeneration and degranulation were frequently seen (fig. 1). Large numbers of mast cells, some of which were undergoing degranulation, were often accumulated at the margins of the injured area. This area often correlated roughly with increased trypan blue extravasation. As early as 1 h after freezing, some accumulation of catecholamines could be seen in the non-terminal nerve trunks at the margins of the injured area. In the specimens obtained 4, 8 and 16 h after freezing, the central ischaemic area gradually grew and the trypan blue fluorescence in this area diminished further. This tissue was surrounded by an area of increased trypan blue fluorescence and extravasation.

The figures are fluorescence photomicrographs from stretch preparations of the s.c. fascia of rat dorsal skin. The specimens have been processed for the combined demonstration of trypan blue and catecholamine fluorescence.

Figure 1. 0.5 h after freezing. The morphology of adrenergic nerves appears in the fluorescence microscope similar to control side. Some degranulating mast cells can be seen (arrowhead). The rather light view indicates trypan blue extravasation. × 160.

Figure 2. 16 h after freezing. A slightly diffuse catecholamine fluorescence is seen in the adrenergic nerves around the artery (a). Note the spasticity of the artery and its branches. The rather dark view indicates diminished trypan blue fluorescence and ischaemia. × 160.

Severe vasospasm was noted in the arteries and arterioles. An increasing number of degenerative changes were seen in the adrenergic nerves 8 and especially 16 h after freezing (fig. 2). This led to the total disappearance of the catecholamines from the central ischaemis area 24 h after freezing. Moreover, no mast cell fluorescence was seen in this area. The accumulation of catecholamines in nerve trunks at the margin of the injured area was also clearly apparent. In the present study, the disappearance of 5-HT fluorescence from the mast cells of the skin and the degranulation of mast cells were observed after experimental skin freezing. Since 5-HT and histamine are located in the same granules of rat mast cells[13], it may be assumed that both these granular components are liberated following the present experimental cold injury. The present observations provide morphological evidence corroborating Lewis's[14] theories concerning the possible role played by histamine-like substances in cold injuries.

The catecholamines liberated from degenerating adrenergic nerves provide a considerable stimulus to the effector cells[15]. The catecholamines liberated from the degenerating adrenergic nerves in frozen skin during the 1st 24 h after thawing, as observed in the present study, may thus increase the vasospasm of the injured tissue. The vasospasm in the cold injured tissue might further be increased by catecholamines accumulating in the adrenergic nerves at the margins of the cold-injured area. The present skin-freezing experiment leads us to emphasize the important role of mast cells and andenergic nerves in cold injury. The freezing method adopted for the present study is commonly used for tissue destruction in cryosurgery. In slow freezing lesions caused by clinical cold injury, icecrystals are formed predominantly extracellularly[1].

Further studies are needed to evaluate whether slow freesing also causes secondary effects on adrenergic nerves and mast cells. The following suggestions are made to stimulate further experimental and clinical testing.

Hypothesis. It is proposed that in addition to direct cell injury and injury to the vascular wall, vasoactive substances liberated from the mast cells may also in part be responsible for the increase in blood vessel permeability beginning during the acute phase following freezing injury. During the 1st 24 h after freezing of the skin, the catecholamines liberated from the degenerating adrenergic nerves of the frozen skin, as well as those catecholamines beginning to accumulate at the margin of the cold-injured area, may further increase the vasospasm and thus diminish the blood flow in the injured area.

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Sensitivity of follicular melanoblasts in newborn mouse skin to tritiated thymidine: Evidence for a long term retention of label[1]

C.S. Potten

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX (England), 4 May 1982

Summary. Injection of tritiated thymidine into newborn mice results in a progressive greying of hair that does not begin until after the first hair coat is grown. After a year the depigmentation is appreciable (about 60% of the hair are white). The effect cannot be simulated by external irradiation of newborn mice or by the administration of radioactive uridine or methionine. The effect can best be explained by a long-term retention of radioactivity in the DNA of melanocyte stem cells (melanoblasts) in spite of several rounds of cell division. This could be achieved by labelling the strands of DNA destined to act as templates throughout life by being selectively retained in the stem line as described in Cairns' hypothesis.

Cairns[2] has recently suggested that stem cell populations might have evolved a mechanism for selectively segregating the new from the old DNA strands at division. If the older, template, strands were conserved in the stem line this would protect the stem cells from DNA replication-induced errors. The evidence in support of this hypothesis has been reviewed elsewhere[2-5]. If the template strands of DNA in stem cells were labeled at the time when they were undergoing their terminal developmental cell divisions (e.g. in the skin of a newborn mouse) then cells might persist as labeled cells for a long time since they would not halve their radioactivity at each successive division. The only way in which the radioactivity might be reduced is via sister chromatid exchanges. A selective retention of a 'permanently' labeled complement of DNA strands (chromatids) has been suggested as the explanation for the persistence of labeled cells within the stem cell region of crypts of the small intestine that were labeled during post-irradiation regeneration[6].

I present here a series of experiments where newborn mice were labeled with tritiated thymidine (^{3}HTdR) which was assumed to be incorporated into the stem cells for the hair follicle pigments cells, the melanoblasts, during their establishment in newly forming hair follicles. These melanocyte precursors gradually died over a period of 1-3 years suggesting that the label was retained over the many cell generations required for the successive hair growth cycles over this period i.e. observations in accord with the Cairns' hypothesis.

Hair follicles are formed by downgrowths from the epider-