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10. Rats were anesthetized with 2 percent halothane and a ventral midline incision was made in the neck. The nodose ganglion was removed unilaterally after transection of the vagus nerve immediately rostral and caudal to the ganglion. The wound was closed, anesthesia stopped, and the animal returned to its cage.
11. The rats were decapitated, and the brainstems were removed and placed on a glass plate over ice. A 1-mm transverse slice was removed with the caudal edge of the slice at the calamus scriptorius. Regions were removed from the fresh unfrozen slice with a microbore punch (inside diameter, 1 mm), by the method of Palkovits (*12*), and were homogenized in 0.32*M* sucrose. The homogenate was centrifuged for 10 minutes at 1000*g*. The high-affinity uptake of 1.0 μM [^{3}H]glutamate or 1.0 μM [^{3}H]GABA was determined (*13*) in the homogenate containing 5 to 15 μg of protein for 1 minute at 30°C in 250 μl of freshly oxygenated buffer (Krebs-Phosphate) whose [Ca^{2+}] was halved to 1.2 m*M*. Uptake was terminated by vacuum filtration through filters (GF/B). Nonspecific uptake was determined in parallel incubations in Na^+-free medium (osmolarity maintained with sucrose). Separate experiments showed that uptake was linear with protein concentration under these conditions.
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Histamine-Mediated Delayed Permeability Response After Scald Burn Inhibited by Cimetidine or Cold-Water Treatment

Abstract. *Scald injury to one ear of the hairless mouse induced significant ($P < .05$) delayed edema formation in remote, uninjured skin. This remote edema formation was completely inhibited by immediate cold-water treatment of the scalded ear. Cold-water treatment significantly reduced histamine loss from the scalded ear, and the edema-inhibiting effect of the treatment could be mimicked by treating the animal prior to injury with the H_2-histamine receptor antagonist cimetidine or a drug that causes histamine depletion. These observations suggest (i) that a histamine-mediated, delayed permeability response occurs after thermal injury that causes remote edema formation and (ii) that one mechanism of remote edema inhibition by cold-water treatment is the prevention of histamine release from thermally injured tissues.*

Scald injury (54°C water for 20 seconds) to a single ear of the hairless mouse significantly ($P < .05$) increased the water content of the skin (edema) of the uninjured ear and the abdomen for up to 2 to 6 hours. This remote edema formation was completely abolished after immediate cold-water treatment (CWT) of the burned ear for 5 minutes in 8° to 10°C water. Chemical reproduction of this effect could be achieved by treating the animals prior to injury with cimetidine, or a drug that causes histamine depletion. Tissue histamine loss from ears subjected to scald injury and CWT was significantly decreased when compared to untreated burns. Ultrastructural features of the microvasculature in uninjured skin displaying increased water content at 2 hours after injury included the presence of endothelial vacuoles thought to be associated with increased permeability. These findings suggest that a histamine-mediated, delayed systemic permeability response occurs after scald injury, and that this response causes remote edema formation that can be chemically inhibited with the H_2-histamine receptor antagonist cimetidine. It has been generally accepted that after mild or moderate thermal injury the mediated effects of histamine are limited to "immediate, transient" local permeability responses of the burn wound not observed beyond 0.5 hour after injury (*1*). Our study suggests that histamine may also mediate delayed increases in vascular permeability. In addition, we verify the phenomenon of remote burn edema and suggest that burn wound histamine release is related to both postburn hypovolemia and the protective influence of CWT in preventing burn shock.

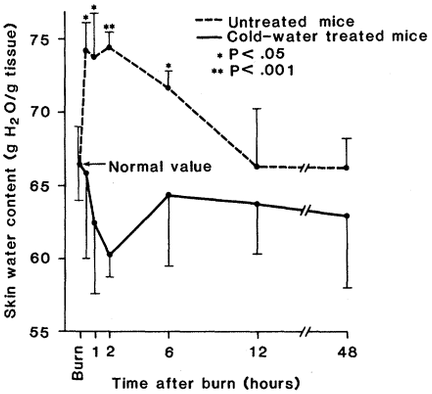

Fig. 1. Comparison of remote edema formation in the unburned ears of burned mice versus burned mice that received CWT for 5 minutes. Cold-water treatment of the burned ear completely abolished remote edema formation in the unburned ear. The skin water content of the unburned ears of animals that received CWT as depicted here, is not significantly different from time-matched controls that received only anesthesia. The *P* values compare the water content of skin from unburned ears of mice that were given CWT. Each data point represents six animals.

For experimental observations we used male, homozygous (*hr/hr*) hairless mice (Jackson Laboratories; 28 to 35 days old) anesthetized with Diabutol (50 mg/kg). Their left ears were exposed to 54°C water for 20 seconds. This resulted in a reproducible burn injury covering about 6 percent of the total body surface area (*2*). To determine the extent of edema in the contralateral, unburned ears of the injured mice we measured the difference between the wet weight and dry weight of the skin (Fig. 1). In the unburned right ear the skin water content was increased by 12 percent (maximum value) at 0.5 and 2 hours after burn injury of the left ear. The increased skin water content of the unburned ears was significant between 0.5 and 6 hours after injury ($P < .05$, Student's *t*-test). At 6 hours after injury to the left ear the edema in the unburned ear began to decrease gradually, with the water content approaching normal values by 9 hours. Mouse abdominal skin obtained at 2 hours after scald injury to the ear showed a similar increase in water content. Such increases in the skin water content at uninjured remote sites following moderate scald injury has not to our knowledge been reported previously.

We also conducted morphological studies of unburned ear skin which demonstrated significant increases in water content after the opposite ear had received a burn injury. Ultrastructurally, the skin of these unburned ears displayed two changes that may be related to increased vascular permeability. The intercellular clefts of capillaries and small venules sometimes contained small dilatations (but not complete patencies in any single section) similar to those seen by Casley-Smith and Window (*3*) in directly burned sites or after application of histamine. Also, in capillaries and, especially, venules, we observed numerous small endothelial vacuoles. These vacuoles were always distinctly larger than micropinocytotic vesicles and, when attached either to the luminal or abluminal cellular membranes, they were closed by a diaphragm (Fig. 2). We studied similar specimens from the ears of mice injected intravenously with colloidal carbon (~250 to 300 Å) and were unable to demonstrate vacuolar inclusion of carbon; however, transport of ferritin (~110 Å) in ultrastructurally similar vacuoles

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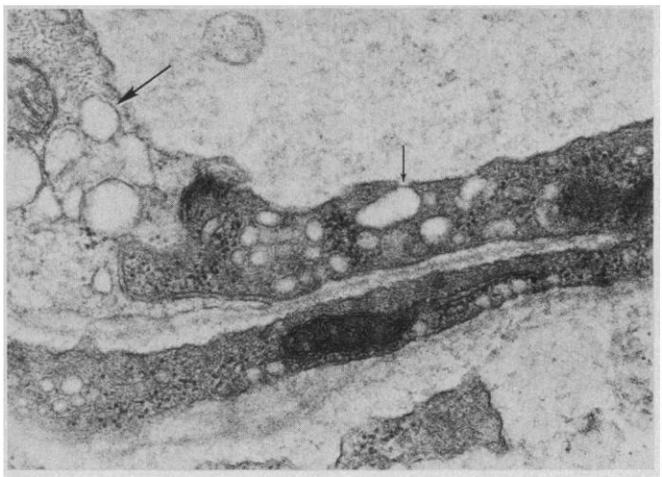

Fig. 2. Electron micrograph of part of a middermal capillary wall in unburned ear skin 2 hours after the opposite ear was burned. A normal intercellular cleft is shown, although small dilatations of the clefts were observed in other vessels. The capillary and venular endothelial cells contain numerous vacuoles (large arrow) which, when attached to either the luminal or abluminal cellular membranes, are closed by a diaphragm (small arrow). Tissue was fixed by immersion in 2 percent paraformaldehyde and 2.5 percent glutaraldehyde in 0.1*M* phosphate buffer and postfixed for electron microscopy in 2 percent osmium tetroxide in 0.1*M* phosphate buffer. (Uranyl acetate and lead citrate stain; ×56,000)

has been demonstrated by others (*4*).

We also studied the effects on remote edema formation in burned mice following short-term CWT or the prior administration of anti-inflammatory drugs. The left ear of the mouse was subjected to a routine scald burn and was immediately bathed in cold water (8° to 10°C) continuously for 5 minutes. This CWT completely abolished edema formation in the unburned ear (Fig. 1) throughout a 48-hour observation period. When we compared the water content of ear skin from control mice that received only anesthesia with that from the unburned ear of animals that received scald injury and CWT we found no significant difference. Anesthesia alone caused an early transient decrease and increase in the ear skin water content of control animals. Also, we found no significant difference in water content between the abdominal skin of animals receiving ear scald burns and CWT and control mice receiving only anesthesia.

Groups of mice (N = 6) were treated with the following drugs before they received scald burn injuries: (i) indomethacin, 5.0 and 12.5 mg/kg, intraperitoneally 1 hour before injury; (ii) mepyramine, 6.0 and 60 mg/kg, subcutaneously and intraperitoneally, respectively, 1 hour before injury; (iii) cimetidine, 50 and 150 mg/kg intraperitoneally, 10 to 20 minutes before injury; (iv) a combination of mepyramine and cimetidine; and (v) cyproheptadine (an antihistamine, antiserotonin compound), 0.8 mg/kg intraperitoneally once daily for 2 days (*5*). An additional group of mice (N = 6) received polymyxin-B (4.0 mg/kg, intraperitoneally once daily for 3 days prior to burn injury); this drug causes histamine depletion (*5*). Treatment of mice with compound 48/80 has been unsuccessful in causing relative histamine depletion (*6*). After treatment with those drugs all the mice were subjected to scald burn of one ear and then killed 2 hours later with an overdose of Diabutol. Table 1 shows that while indomethacin and, to a greater extent, the combination of mepyramine and cimetidine partially reduced remote burn edema formation, only the high dosage of cimetidine alone (150 mg/kg) reduced the water content of skin from the unburned ear to levels not significantly different from those of control mice that received no burn injury. The effectiveness of cimetidine is consistent with previous work showing that increased cutaneous microvascular permeability after histamine stimulation can be selectively inhibited by cimetidine (*7*). Cyproheptadine was also effective in partially reducing remote burn edema formation, but no more so than mepyramine alone. Histamine depletion with polymyxin-B prior to injury inhibited remote burn edema formation as effectively as CWT or high doses of cimetidine. Animal recovery was unremarkable after all treatments including burn injury, and no animals died before they were killed for examination.

Table 1. Water content of skin from unburned right ear 2 hours after the left ear received a scald burn.

Treatment	Dosage (mg/kg)	Water content* (g H_2O/ 100 g tissue)	P†
None (untreated burn)		74.5 ± 1.2	.001
CWT		59.8 ± 1.3	N.S.
None (control, no burn)		56.3 ± 1.4	
Indomethacin	5.0‡	66.8 ± 3.0	.05
Indomethacin	12.5‡	65.5 ± 1.4	.05
Mepyramine	6.0‡	66.2 ± 0.8	.001
Mepyramine	60.0‡	65.0 ± 2.3	.05
Cimetidine	50.0§	68.1 ± 1.5	.001
Cimetidine	150.0§	60.8 ± 2.7	N.S.
Mepyramine and cimetidine	6.0‡ plus 150.0§	61.8 ± 1.7	.05
Mepyramine and cimetidine	60.0‡ plus 150.0§	64.4 ± 1.2	.05
Mepyramine and cimetidine	6.0‡ plus 50.0§	67.0 ± 2.8	.05
Mepyramine and cimetidine	60.0‡ plus 50.0§	65.6 ± 2.1	.05
Cyproheptadine	0.8‖	66.1 ± 2.4	.05
Polymyxin-B	4.0‖	60.2 ± 3.1	N.S.

*Mean ± standard error (N = 6). †Compared to controls without burn; N.S., not significant. ‡Drug administered 1 hour before animal received burn injury. §Drug administered 10 to 20 minutes before burn injury. ‖Dose given once daily for 2 days (cyproheptadine) or 3 days (polymyxin-B).

Histamine concentrations in minced, homogenized ears were determined by a fluorometric technique (Bio-Science Laboratories). At 2 hours after burn injury the histamine content of untreated burned ears was 18 μg per gram of tissue; in burned ears given CWT the histamine content was 25 μg per gram of tissue. Thus CWT significantly reduced tissue histamine loss after scald injury. The histamine concentration in ears from control mice receiving no scald injury was 29 μg per gram of tissue.

Histamine released from burned skin is primarily supplied from mast cell stores by increased spontaneous degranulation of heat-stimulated mast cells (*8*) and by complement-mediated degranula-

tion triggered at the site of injury by heat-modified tissue proteins (*9*). Complement-mediated release of histamine from induced mast cells appears to follow enzymatic pathways for degranulation (*6*); heat-stimulated histamine release has not been proved to proceed enzymatically (*10*). Fredholm and Hagermark (*8*) have demonstrated a delayed histamine release with heating of rat peritoneal mast cells above 52°C. At higher temperatures the histamine release became more prompt and was almost instantaneous as the surrounding temperature exceeded 60°C. A similar pattern of delayed histamine release was observed by Horakova and Beaven (*11*) in animals subjected to mild thermal injury (53°C); however, increased histamine was measured almost immediately after more severe (56°C) thermal injury. Conversely, cold (5°C) has been shown to cause prompt, complete, and reversible inhibition of histamine release from rat peritoneal mast cells induced by antigen and compound 48/80 (Burroughs Wellcome Co.) (*10*). Histamine release caused by lytic agents (Triton X-100) was not inhibited by cold. It appears, therefore, that heat-stimulated mast cells not undergoing lysis, or complement-stimulated mast cells in burned tissues, can be "stablilized" by cold (CWT) and the resultant histamine release substantially inhibited. Our observations support this hypothesis.

Thus we conclude that the histamine released from a burn wound is the predominant vasoactive substance that mediates a delayed systemic permeability response and leads to remote edema formation after moderate scald injury, apparently by stimulation of the H_2-histamine receptor. Immediate CWT of scald burns may inhibit heat-stimulated and complement-induced degranulation of viable mast cells, preventing increased histamine release from burned tissues. Cold has also been demonstrated to inhibit kininogenase activator in human serum (*12*). This enzyme inactivation effectively inhibits kinin formation. It appears likely, then, that CWT properly applied to burned surfaces or, perhaps, to any traumatized or inflamed tissues, might effectively inhibit kinin formation as well as histamine release.

The clinical implications of our findings should signal the need for a reevaluation of the pathophysiology and treatment of burn shock. It is apparent that the use of cold-water therapy (or cooling) in the early treatment of burn victims should be reexamined. Aside from its use for pain reduction and burn edema suppresion, the potential of CWT as an effective means of promoting cardiovascular stability after burn injury appears promising. Such therapy in cases of major burns could prevent significant intravascular losses, hypovolemia, and "burn shock." The use of combined H_1- and H_2-histamine receptor antagonists in the early treatment of burns also appears to warrant investigation.

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Liver Tumors Induced in Rats by Oral Administration of the Antihistaminic Methapyrilene Hydrochloride

Abstract. *The antihistaminic over-the-counter drug methapyrilene hydrochloride, mixed with food at a concentration of 0.1 percent, was administered to 50 male and 50 female Fischer rats. A second group of 50 male and 50 female rats was given the same treatment together with 0.2 percent of sodium nitrite added to the food. Almost all of the rats in both groups developed liver neoplasms, mainly hepatocellular carcinomas and cholangiocarcinomas. The first rat died with a liver neoplasm at the 43rd week. Over 50 percent of the rats in both groups had metastases from the carcinomas of the liver to distant organs. Control rats treated with nitrite only, or untreated, did not develop liver neoplasms. There was no discernible effect of nitrite on the carcinogenicity of methapyrilene hydrochloride.*

In a previous study (*1*) the possibility was investigated that the common antihistaminic methapyrilene, used in many over-the-counter sleep aids in the United States, might form carcinogenic nitrosamines by reaction with nitrite in the mammalian stomach. Of 30 rats given methapyrilene hydrochloride in their drinking water together with sodium nitrite for 18 months, nine developed liver tumors (*1*). Although a nitrite-treated control group (which did not develop liver tumors) was included in the study, no group of animals was given methapyrilene alone. We have therefore repeated the study using groups of rats giv-

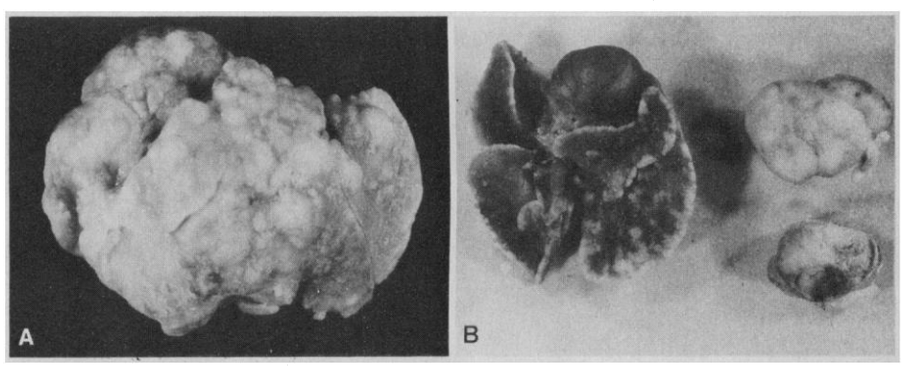

Fig. 1. (A) Carcinoma of the liver. The liver is replaced by firm, white carcinoma which is invading and replacing the adjacent liver tissue. (B) Carcinoma of the liver has metastasized to the lungs and portahepatic lymph nodes.

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