The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle[1]

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Schwartzkopf-Genswein, K. S. and Stookey, J. M. 1997. **The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle**. Can. J. Anim. Sci. **77**: 577–583. Infrared thermography was used to compare differences in extent and duration of inflammation observed on hot-iron and freeze brand sites as an indicator of tissue damage and the associated discomfort to the animals. Thirty beef heifers of mixed breed were assigned to either hot-iron (H) or freeze (F) branding treatments according to a predetermined randomized branding order. Ten animals were branded each day over a 3-d period. On the day prior to branding, animals were clipped to expose two patches of skin; one to be used for the branding treatment and the other for a control. Thermographic images of control and treatment sites were made at 0.08 h (5 min) prior to branding, immediately after the brand was completed (0 h), as well as 0.08, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h after branding. Control site temperatures were subtracted from treatment site temperatures for each individual animal. Both F and H brand sites were consistently warmer (1.9 ± 0.3 and 1.6 ± 0.3°C, respectively) than their corresponding control sites between 2 and 168 h after branding. Treatment differences were obtained at 0, 0.08, 2, 8, and 144 h after branding ($P < 0.001$, 0.05, 0.005, 0.001, and 0.01, respectively). Freeze brand sites were warmer at 2 and 8 h after branding while H sites were warmer at 144 h after branding. The thermographic evaluation of hot-iron and freeze brand sites indicated that both methods caused tissue damage. However, H brand sites remained significantly warmer than F sites at 168 h after branding. In addition, H sites were significantly warmer than control sites while F sites were not warmer than control sites at 168 h. The prolonged inflammatory response observed in H animals indicates that more tissue damage and perhaps more discomfort are associated with H branding.

Key words: Thermography, branding, cattle, animal welfare, pain

Schwartzkopf-Genswein, K. S. et Stookey, J. M. 1997. **Emploi de la thermographie infrarouge pour évaluer l'inflammation causée par le marquage à chaud et à froid des bovins**. Can. J. Soil Sci. **77**: 577–583. Nous avons observé par thermographie infrarouge les différences affectant l'étendue et la durée de l'inflammation causée au point de marquage à chaud et à froid des bovins, dans le but d'utiliser ces valeurs comme indicateurs des lésions causées aux tissus et de la douleur résultante pour les animaux. Trente génisses de race à viande ont étaient affectées, soit au marquage au fer rouge (à chaud C) soit au marquage à froid (F). Dix animaux étaient marqués chaque jour pendant un intervalle de 3 jours. La veille, ils étaient tondus de manière à mettre à découvert deux placettes de peau, l'une destinée au marquage, l'autre servant de témoin. Des images thermographiques des deux types de placettes étaient prises 5 mn avant, tout de suite après (0 h) puis 5 mn et 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 et 168 h après le marquage. Pour chaque animal, la température au site témoin était soustraite de celle observée au site de marquage. Les plages de marquage (C et F) étaient plus chaudes (respectivement, 1,9 ± 0,3 et 1,6 ± 0,3°C) que les plages témoins correspondantes de la 2[e] à la 168[e] h après l'opération. Des différences entre les 2 types de marquage étaient observées à 0 h, 5 mn, 2, 8, et 144 h après le marquage (respectivement P<0,001, 0,05, 0,005, 0,001 et 0,01). Les emplacements marqués à froid étaient plus chauds à 2 et à 8 h après le marquage alors que les plages marquées au fer rouge l'étaient à 144 h après. La thermographie des plages marquées à chaud et à froid révèle que les 2 méthodes provoquent des lésions tissulaires. Les plages de marquage C demeuraient significativement plus chaudes que celles de marquage F 168 h après l'opération. En outre, à ce moment-là seuls les emplacements C étaient significativement plus chauds que les emplacements témoins. La durée de l'inflammation observée dans le cas du marquage à chaud porte à conclure que ce mode de marquage provoque plus de lésions aux tissus et éventuellement, une douleur plus vive.

Mots clés: Thermographie, marquage, bovin, bien-être des animaux, douleur

Hot-iron and freeze branding are presently the only ways to produce a permanent visible mark on cattle. However, temperature-associated injuries have long been recognized as painful (Provost 1992) and public concerns for animal welfare have drawn attention to the practise of branding. Previous branding studies (Lay et al. 1992a,b,c; Schwartzkopf-Genswein et al. 1997a,b) have shown both methods of branding produce behavioural and physiological indicators of pain in the first 3 h after branding. However, little information is available regarding the tissue damage and inflammation associated with either of the methods.

Abbreviations: **ADG**, average daily gain; **C**, control treatment; **EWL**, evaporative water loss; **F**, freeze branding treatment; **H**, hot-iron branding treatment; **IRT**, infrared thermography

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A permanent brand is a result of tissue damage that occurs when excessive heat or cold is applied on the skin at a rate that exceeds the tissue's ability to dissipate it fast enough to avoid cell destruction (Pope 1993). One way to investigate the impact of branding on surface tissue is through the use of infrared thermography (IRT). Thermography has been used successfully as a diagnostic tool to assess burn severity in humans (Cole et al. 1990, 1991) and rodents (Anselmo and Zawacki 1977; Lepenye et al. 1978). In addition, thermography has been used to document and quantify tissue inflammation associated with a number of medical conditions such as neoplasia, blood flow disorders, and arteritis as well as tissue damage (Yang and Yang 1992).

The objective of this study was to use IRT to document differences in the extent and duration of inflammation following hot-iron and freeze branding in beef cattle. Comparing treatment differences in the inflammatory response at the brand site may help to determine which method causes the least amount of tissue damage and perhaps the least discomfort to cattle.

MATERIALS AND METHODS

Experimental animals consisted of 30 yearling heifers of mixed breed (Angus, Hereford, Charolais) from the University of Saskatchewan, Goodale Research Farm. These heifers were kept under feedlot conditions and had not been previously branded. Animals were provided with ad libitum hay and water during the 10-d test period which took place in early September.

One day prior to the experiment, two patches (approximately 25 cm^2) were clipped on the right thigh of all animals. One patch was located on the upper portion of the thigh and the second was positioned directly below it (approximately 5 cm) on the mid-thigh. The higher patch was allocated the treatment site (hot-iron or freeze brand) and the lower patch was designated the control or brand-free site.

All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care.

Branding

Heifers were assigned to either hot-iron (H) or freeze branding (F) treatments according to a predetermined, randomized order (n = 15 per treatment). Animals were taken from their pen, moved through a chute, and captured in a manual, self catching headgate and squeeze chute (W & W Manufacturing Inc., Dodge City, KA) to minimize movement. All brands were applied on the shaved portion of the right thigh by an experienced District Livestock Brand Inspector. Cattle assigned to H treatments were branded with an electric branding-iron (L & H Electric Brand Co., Mandan, ND) that formed the University of Saskatchewan's registered "US" brand occupying an area 15 × 17 cm. The electric iron was allowed a minimum of 10 min to heat before the first application when it was held on the animal until the hide turned a light tan colour, usually 3 to 5 s (Alberta Agriculture 1988). Heifers assigned to freeze branding treatments were branded with a single copper iron, shaped to form the same characters and dimensions described for hot-iron branding. The shaved area was saturated with 95% methyl hydrate (vol/vol) just prior to the application of the branding iron. The freeze iron was applied to the clipped patch for 25 s before being removed. Prior to branding, the freeze iron was immersed and maintained in liquid nitrogen.

Ten animals were branded each day over a 3-d period. An equal number of H and F treatments were imposed on each of the 3 d of the experiment. Animals were branded at approximately the same time each day. The time of day was recorded so that a data collection schedule could be maintained.

Thermography

An infrared thermography camera (Thermovision® 470, Agema Infrared Systems AB, Danderyd, Sweden) with the ability to measure temperature to within ± 0.1°C, was used to obtain skin surface temperatures of the treatment and control sites from all animals. The camera was calibrated by Agema Infrared Systems (Burlington, ON) and had a measurement accuracy within 2°C and a repeatability of 98%. Thermographic images were taken at 0.08 h (5 min) before and 0, 0.08, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h after branding. All thermograms were acquired while the animals were restrained in the chute where they had been branded. Animals were brought into the chute (within a barn) approximately 10 min prior to sampling. This allowed for the reduction of the effects of direct sunlight, wind or rain on skin temperature. Animals were maintained in a single feedlot pen with access to shade and shelter whenever they were not being used for data collection.

Appropriate adjustments were then made on the camera to ensure the correct sensitivity (1–3 on dial setting), emissivity (0.9 on dial setting) thermal level (30–35°C) and focus required to obtain a clear image (Academy of Infrared Thermography 1994). Sensitivity refers to the adjustment which limits the range of temperatures observed on a thermogram so that better resolution of an image may be obtained. Emissivity is defined as the ratio of radiation emitted by a surface to that emitted by a black body (perfect absorber and emitter of radiation) at the same temperature and wavelength. The thermal level refers to the temperature setting that will allow the object being evaluated to be seen on the thermogram. For example, to evaluate skin temperature a thermal level between 29 and 35°C would be used.

When the test animal was stationary, a thermogram was taken and stored onto a floppy disk within the camera. Stored thermograms were retrieved from the floppy disks with software (Irwin, Agema Infrared Systems AB, Sweden) designed to analyze the thermographic images.

Skin temperatures were obtained by using the software to superimpose a computer-generated square onto the treatment site of each thermographic image. The square was sized and positioned such that the entire brand (including skin between each character and symbol of the brand) was centred within its boundaries. The average temperature within that area was automatically calculated and recorded for the entire area enclosed by the outside edges of the

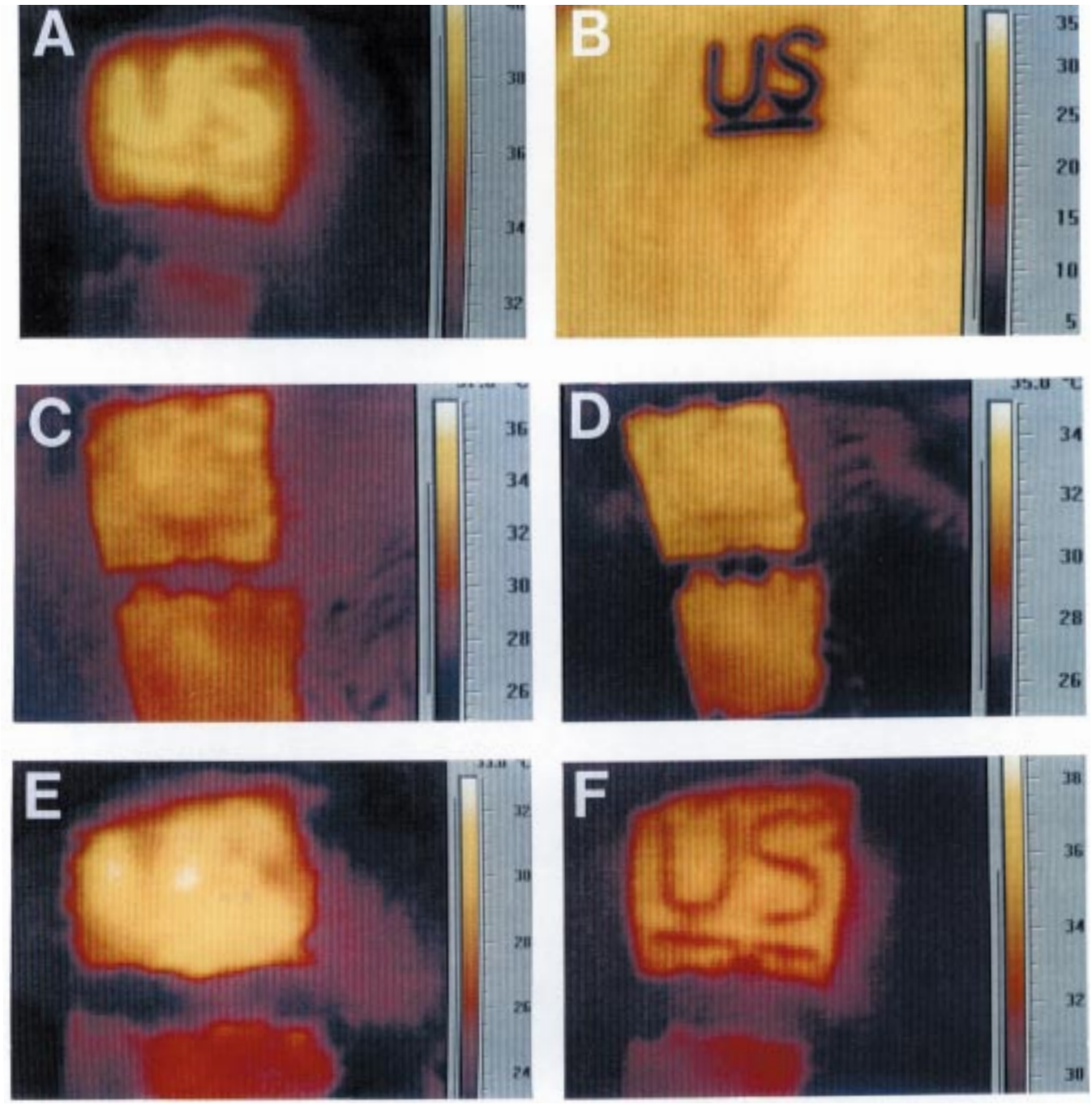

Fig. 1. Thermographic images of brand and control sites on cattle taken immediately after hot-iron (A) and freeze branding (B); 48 h after hot-iron branding (C) and freeze branding (D); 96 h after hot-iron (E) and freeze branding (F).

brand. The square was moved, within the same thermogram, to the control site so that the average temperature of an area equivalent to the size of the brand site could be obtained for comparison. The dimensions of the square positioned over the brand varied slightly on each animal due to the placement of the irons on the hide at the time of branding.

Examples of thermograms taken immediately after branding irons were taken off of the skin as well as at 48 and 96 h after hot-iron and freeze branding are shown in Fig. 1. Within each thermogram the differential skin temperature between the brand site (top patch) and control site (bottom patch) can be easily seen.

Statistical Analyses

Two statistical comparisons were made in this study; one on the skin temperature differences between control and brand sites and the second on skin temperature differences between H and F branding treatments. Differences between

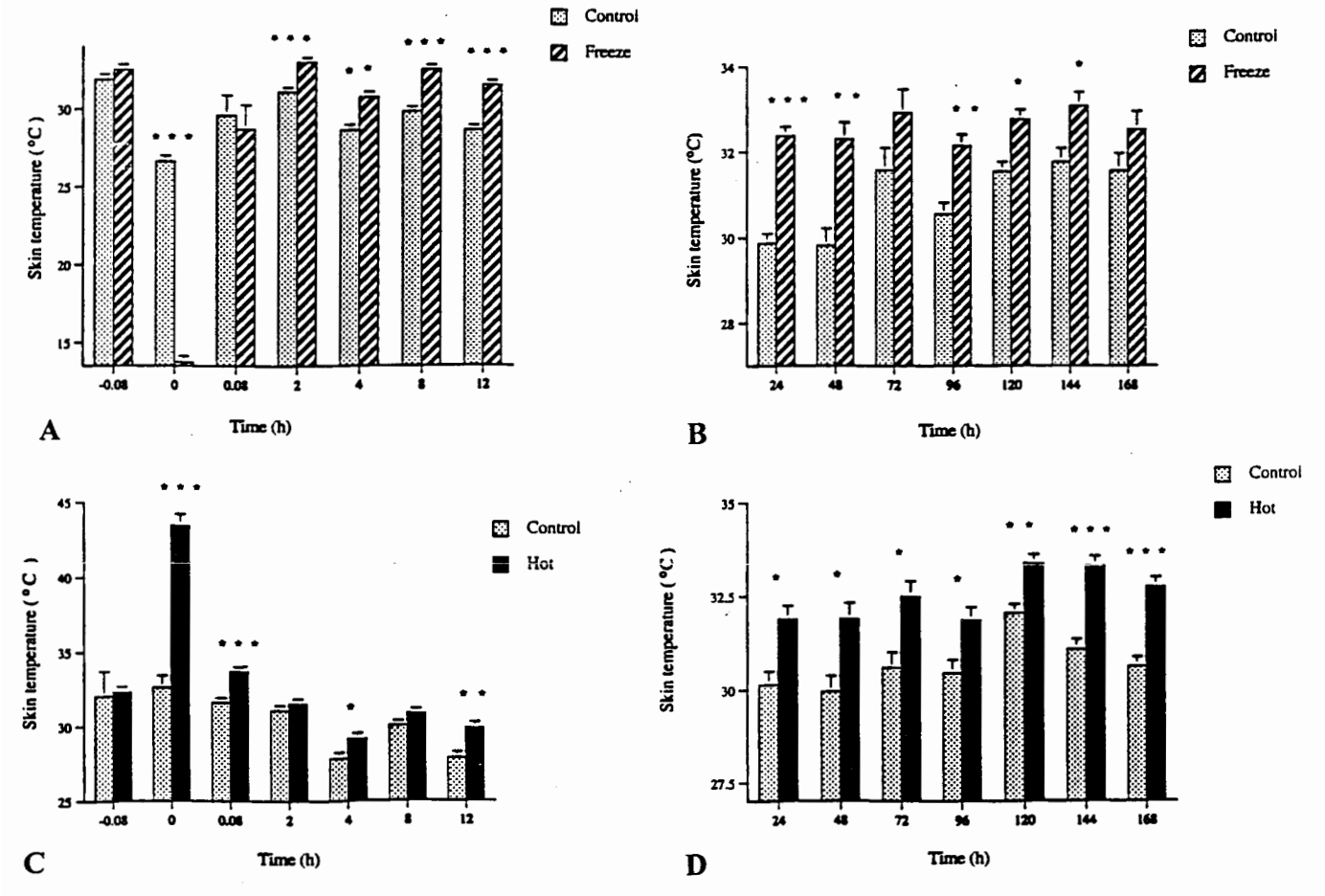

Fig. 2. Mean skin temperature (± SE) of brand and control sites in the first 12 h after branding on freeze branded animals [A] and hot-iron branded animals [C], and 24 to 168 h after branding on freeze branded animals [B] and hot-iron branded animals [D]. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

brand and control sites on both H and F animals were compared at each sampling time using a general linear models ANOVA (SAS Institute, Inc. 1990) with the site (brand or control) and the day the animals were tested as main factors in the model. Fourteen thermographic images were taken of each animal which included the brand site and the control site (total of 28 temperature readings per animal). A repeated measures ANOVA was attempted but it discarded 15 animals (420 data points) because 15 of the thermographic images were missing. Therefore, a repeated measures ANOVA was unacceptable due to its inability to handle missing data points.

Before comparing differences between branding treatments the data were corrected for individual variation in skin temperature. This was done by subtracting the average temperature of the control site from the average temperature of the treatment site for each animal at each sampling time. The values were subjected to ANOVA (SAS Institute, Inc. 1990). Due to the small number of heifers tested each day ($n = 10$) the heifer and day in which they were tested were combined to form a single variable (replicate) (within treatment). Treatment and (replicate) were used as main factors in the model.

RESULTS

No differences were observed between the brand and control sites on H or F animals 0.08 h before branding (Fig. 2A and C). However, F brand sites were different from control sites at 0, 2, 4, 8 and 12 h ($P < 0.001$) after branding (Fig. 2A). Similarly, H brand sites were different from control sites at 0, 0.08, 4, and 12 h after branding ($P < 0.05$) (Fig. 2C). Freeze brand sites were cooler than control sites immediately after branding while H brand sites were warmer. All other differences obtained in the first 12 h after branding indicated that the brand sites (H and F) were warmer than control sites (Figs. 2A and C).

Brand sites were also found to be warmer than control sites in F heifers at 24, 48, 96, 120 and 144 h ($P < 0.01$) and marginally warmer at 72 h ($P < 0.08$), however, no difference was observed at 168 h after branding (Fig. 2B). Similar results were obtained in H heifers with brand sites being warmer from 24 to 168 h ($P < 0.01$) (Fig. 2D).

The comparison between branding treatments indicated that F animals had larger average differences (2.4 ± 0.4°C) in skin temperature between brand and control sites than H animals (1.4 ± 0.4°C) from 2 to 48 h after branding.

However, F sites were only higher than H sites at 2 and 8 h after branding ($P < 0.01$, and 0.001, respectively) (Fig. 3A). Freeze brand sites were cooler than H sites at 0 and 0.08 h ($P < 0.05$) (Fig. 3A).

Differential temperatures in both H and F animals declined between 48 and 144 h, however, there was a substantial increase in H brand site temperatures compared with F sites at 144 h ($P < 0.01$) (Fig. 3B).

DISCUSSION

The scar left on the hide of animals after hot-iron branding and after some freeze branding is what makes branding useful as a permanent method of identification. The presence of a scar provides some evidence to support that both branding methods may cause a second or third degree burn. Second and third degree burns are known to result in tissue scarring while less severe first degree burns do not (Johnson 1994). Consequently, we were not concerned with diagnosing the severity of burns caused by branding, but rather the extent and duration of the inflammation associated with both branding techniques.

The inflammatory response associated with tissue damage usually persists until a new collagen matrix and blood supply are produced at the site of the injury (Greenhalgh and Staley 1994). This implies that the duration and extent of the inflammatory response would be proportional to the severity of the tissue damage and the amount of repair required.

Although a direct measure of pain associated with the brands was not obtained in this study, it is well documented that inflammation is painful. The inflammatory response is the first phase of tissue repair following a burn injury. It is characterized by swelling, redness, and pain which peaks between 8 to 12 h after the trauma (Pope 1993). The redness and heat observed are caused by local vasodilation and edema resulting from increased permeability of the damaged tissue. Pain is caused by the release of local mediators such as histamine, kinins and prostaglandins that activate pain nerve fibres in the area of the burn (Johnson 1994). Therefore the assessment of inflammation may provide some information on the duration of discomfort experienced by the animal.

Differences between sites (brand and control) and treatments (hot-iron and freeze) obtained at 0 and 0.08 h after branding were expected as the skin temperatures would reflect the respective temperatures of the hot and freeze irons applied. The differences are the result of the inability of the skin to dissipate excessive heat or cold quickly enough to maintain normal temperature (Pope 1993).

Both H and F brand sites were consistently warmer than the control sites from 2 to 168 h after branding and were significantly warmer than the control sites in the majority of the sampling times (Fig. 2A to D). The brand sites of F and H animals were on average warmer (1.9 ± 0.3 and 1.6 ± 0.3°C, respectively) than the control sites from 2 to 168 h after branding. The increased temperatures at the brand sites are indicative of inflammation as a result of tissue injury. In contrast, no differences were observed between control and treatment sites before branding. This implies that increased skin temperatures recorded on brand sites were a result of branding and the associated changes caused by the branding heat or cold.

Comparing differential skin temperatures between branding treatments, F animals were found to have higher skin temperature differences than H animals in the first 48 h after branding. However, the differences were not significant except at 2 and 8 h after branding (Fig. 3A). The differences observed in the first 12 h after branding may not be a useful comparison because of changes in dermal capillaries (Wyllie and Sutherland 1991). Pope (1993) indicated that changes are caused by the immediate reduction in blood flow to the damaged tissue followed by a pronounced vasodilation of the blood vessels which results in swelling of the burn wound. The capillaries become highly permeable facilitating the movement of large volumes of electrolytes and proteins from the vascular to the extracelluar spaces, a process which peaks 8 to 12 h after an injury and may not return to normal until 18 to 36 h later.

Differential temperatures in both H and F animals peaked at 48 h after branding but gradually started to decline after that point (Fig. 3B). The decline may be due in part to the return of normal circulation patterns and the reduction of inflammation and swelling associated with it, which is part of the healing process.

There were no differences in the extent of the inflammatory response between H or F branding sites until 144 h at which point H animals had substantially elevated skin temperatures compared with F animals (Fig. 3B). The higher surface temperatures obtained from H brand sites after 144 h may be due in part to the fact that the hot-iron burns were more severe than freeze burns in terms of tissue damage and pain. These results support previous studies on branding which indicate that H animals experience a more acute sensation of pain than F animals based on a slightly prolonged cortisol response (40 versus 20 min after branding, respectively) (Schwartzkopf-Genswein et al. 1997a) and higher frequencies of behaviours during branding, indicative of pain (Schwartzkopf-Genswein et al. 1997b). However, it was also reported that although H branding causes a more acute response, there appears to be no long term effects on animal production. Schwartzkopf-Genswein et al. (1997c) found that the ADG and antibiotic treatment rates of H and F branded steers were not different from one another when compared over a 28 d period after branding.

Although thermographic evaluations were not made beyond 7 d after branding the inflammatory phase of wound healing persisted longer on hot-iron brands than freeze brands. This is supported by the fact that no temperature differences were observed between freeze and control sites at 168 h (Fig. 2D); however, hot-iron sites were still significantly warmer than control sites at 168 h (Fig. 2B). The prolonged inflammatory response observed in H animals indicates that the repair of the collagen matrix and blood supply were not complete by 7 d after branding. This implies that the amount of tissue damage and repair required for H burn wounds was greater than that of F burn wounds.

Although the thermographic assessment of burn wounds has proven to be a valuable tool, caution must be taken when interpreting results. A factor that can greatly affect all burn

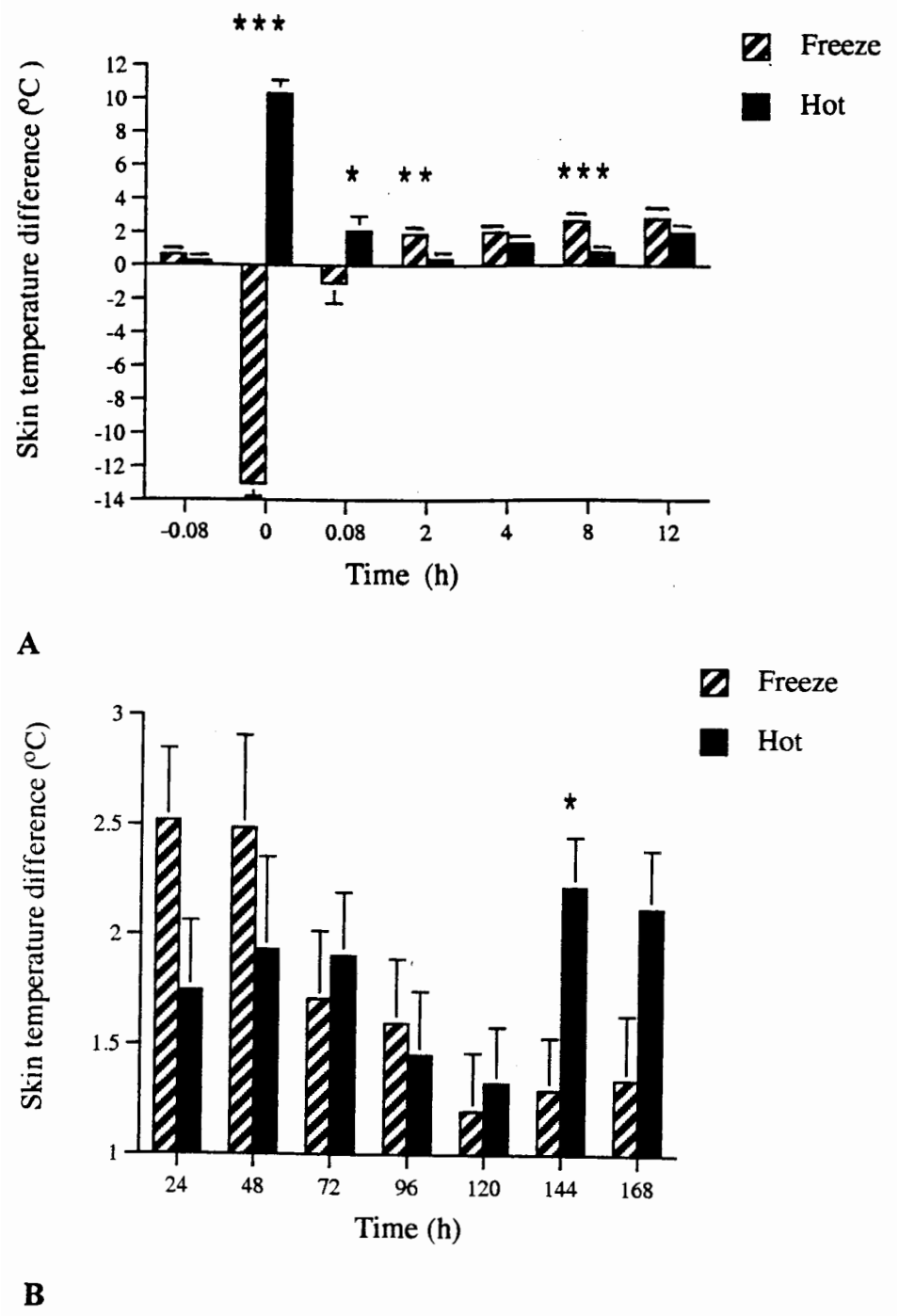

Fig. 3. Mean skin temperature differences (treatment site minus control site) (± SE) of freeze and hot-iron branded animals in the first 12 h after branding [A] and 24 to 168 h after branding [B]. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

surface temperatures is cooling caused by evaporative water loss (EWL). Anselmo and Zawacki (1977) indicated that scar formation occurring 3–4 d after burn trauma allows the surface temperature to be measured without the complication of EWL. Other researchers have found that the effect of EWL may be eliminated by placing a non-permeable clear membrane (cellophane wrap) over the burn before thermographic images are taken (Cole et al. 1991). Since the effect of EWL was not accounted for in our study, the thermographic surface temperatures obtained within 72 h of branding may have been lower than the actual temperatures obtained. However, there was no reason to believe that the effect of EWL was not consistent across branding treatments and therefore should not have interfered with the comparison of the inflammatory response.

CONCLUSIONS

The thermographic evaluation of H and F brand sites indicated that both methods cause an inflammatory response consistent with tissue damage and perhaps pain. Based on

the prolonged inflammation observed in H animals it is probable that H branding causes a more intense and prolonged pain than F branding.

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