The Effect of Ambient Temperature and Type of Wound on Healing of Cutaneous Wounds in the Common Garter Snake (*Thamnophis sirtalis*)

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ABSTRACT

The effects of ambient temperature (13.5° C, 21° C, 30° C) and type of wound on healing of skin wounds were evaluated in common garter snakes (*Thamnophis sirtalis*). Linear unsutured incisions and circular excisional wounds were evaluated grossly and microscopically in three snakes held at each temperature at each of two, five and ten days after surgery. Linear sutured and unsutured incisions and circular and square excisional wounds were similarly evaluated three and six weeks after wound production in groups of six snakes held at each temperature. The rates of stabilization of wound margins, scab formation, migration and maturation of the regenerating epithelium, resolution of dermal inflammation, and fibroplasia varied directly with temperature. The inflammatory reaction to wounding was similar in character and intensity in snakes held at all three temperatures two days after surgery. Unsutured linear incisions, compared to sutured incisions, tended to have more rapid epithelial maturation and a less intense inflammatory response. Healing of square and circular excisional wounds was similar; contraction of round wounds was slightly more irregular and, at a few observations, dermal maturation was slower and inflammation more widespread. It was concluded that healing of skin wounds can be accelerated by holding reptiles at the upper end of their voluntary temperature range. Wounds, if possible, should be created along the axis of lines of skin tension. Suturing small incisional wounds may not be advantageous.

RÉSUMÉ

Cette expérience consistait à évaluer les effets d'une température ambiante de 13,5° C, 21° C et 30° C, ainsi que du genre de plaie, sur la guérison des plaies cutanées, chez des serpents à collier (*Thamnophis sirtalis*). Les auteurs effectuèrent à cette fin l'évaluation macroscopique et microscopique d'incisions linéaires non suturées et de plaies d'excision circulaires, chez trois serpents gardés à chacune des trois températures précitées, au bout de deux, cinq et dix jours après la chirurgie. Ils évaluèrent aussi de la même façon des incisions linéaires, suturées ou non, et des plaies d'excision, rondes ou carrées, au bout de trois et six semaines après la chirurgie, chez d'autres serpents également gardés à chacune des trois températures précitées. Les taux de stabilisation du pourtour des plaies, de la formation de la croûte, de la margination et de la maturation de l'épithélium en régénération, de la résolution de l'inflammation dermique et de la fibroplasie, varièrent directement avec la température. Le caractère et l'intensité de la réaction inflammatoire consécutive à la production des plaies se révélèrent similaires, chez les serpents gardés aux trois températures précitées, deux jours après la chirurgie. Les incisions linéaires non suturées tendaient à présenter une maturation épithéliale plus rapide et une réaction inflammatoire moins intense que les suturées. La guérison des plaies d'excision rondes et carrées s'effectua de façon analogue; la contraction des plaies rondes s'avéra un peu plus irrégulière et, à la suite de quelques observations, la maturation dermique s'avéra plus lente et l'inflammation, plus étendue. Les auteurs concluent qu'on peut accélérer la guérison des plaies cutanées des reptiles en les gardant à la limite supérieure de l'éventail des températures qu'ils aiment. Autant que possible, on devrait pratiquer les incisions cutanées dans l'axe des lignes de tension de la peau. Il n'apparaît pas avantageux de suturer de courtes incisions cutanées.

INTRODUCTION

The body temperature of reptiles, which are ectotherms, is variable and dependent upon the availability of external sources of heat. Chemical reaction rates in reptilian tissues generally increase with rising body temperature, up to a maximum value at the optimum reaction temperature,

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and then decline (1). These rate-temperature interactions can be affected by acclimation to a specific temperature, by time-independent enzyme shifts which prevent changes in metabolic rate within certain specific temperature ranges, by photoperiod, and by season of the year (2). More complex processes, such as digestion (3), also follow this pattern, with different optimum temperatures existing for different functions and in various species (4). The rate of wound healing may also be related to ambient temperature within the voluntary temperature range (the thermal range within which the animal is voluntarily active) for a reptilian species. Exploitation of this relationship should assist in providing optimal postsurgical care.

Wound healing in mammals is strongly influenced by surgical technique (5,6). The effects of different methods of incision (7), wound shapes (8), and suture materials (9) have been extensively evaluated to improve postsurgical healing. The influence of these factors has not been studied in reptiles.

In a previous paper (10) we described qualitative observations on the healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). In this report the effects of different ambient temperatures, suturing of linear incisions, and shape of excisional wounds (round or square), on wound healing are compared quantitatively. The effects of certain topical medications on healing skin wounds in snakes are described in a subsequent paper (11).

MATERIALS AND METHODS

Common garter snakes (*Thamnophis sirtalis parietalis*) were held at ambient temperatures of 13.5°C, 21°C and 30°C. These temperatures were chosen to span the voluntary temperature range of this species (12). Details of experimental design and methods have been described (10) and are summarized briefly here. In one series, three snakes held at each temperature were killed at each of two, five and ten days after surgery. Pairs of unsutured linear incisions and round excisional wounds were evaluated by gross and histological examination. In a second experiment, six snakes were held at each temperature. Each of these snakes had two sets of four wounds: one linear incision sutured with 5-0 proline (Davis & Geck, Montreal, Quebec), one linear incision left unsutured, and one round and one square excisional wound. One set of wounds was three weeks and one was six weeks of age at the time the snakes were killed for histological examination. The wounds on these snakes were examined grossly at weekly intervals following surgery.

Paraffin-embedded tissue sections through the center of each wound, (a total of 252 sections) were coded and examined by light microscopy without knowledge of the treatment. The epithelium and dermis were given numerical scores based on the stage of healing (Table I). These scores were tabulated, but not statistically analyzed. The distance which the regenerating epithelium had migrated from the wound margin was measured on each side of the wound using a calibrated ocular micrometer. Sections of wounds of two, five and ten days duration were further examined for the presence or absence of 11 characteristics relating to inflammation and epithelial and dermal repair (see 10 and Table II). The prevalence of each of 13 similar characteristics was determined in sections of wounds of three and six weeks duration (Table III) from the second experiment. To evaluate the effects of temperature, duration and type of wound, the prevalence of each characteristic was analyzed by binary stepwise logistic regression using PLR of the BMDP (13). Where interactions between these three factors were not evident ($P > 0.1$) treatment groups were combined to examine main effects. Groups were also compared by chi-square or Fisher's exact tests (14) (if group size < 36), and differences were considered significant if $P < 0.05$.

The area containing inflammatory cells surrounding each wound was measured using a camera lucida and computerized image analyzer (MOP-3, Carl Zeiss Inc., Toronto, Ontario). The total numbers of heterophils and of macrophages in this area were counted directly and the densities of the cells were calculated. The values for area of inflammation and density of heterophils and macrophages were log transformed to stabilize error variances. Multiple-way analyses of variance were performed, using proc ANOVA of SAS (15), to examine the influence of temperature, duration, and type of wound as major factors. Where interactions among these three factors were not evident ($P > 0.1$), treatment groups were combined and mean effects examined. Group means were compared by least significant difference t tests using proc ANOVA of SAS (15), and were considered significantly different if $P < 0.05$.

RESULTS

Snakes held at 30°C were extremely active and fed voraciously. Those at 21°C were obviously less active but continued to feed readily, while snakes held at 13.5°C were lethargic and fed infrequently.

Wound margins moved freely over the exposed epaxial muscle immediately after surgery, but exudate gradually caused them to adhere to each other or to the underlying tissue. The rate of fixation of wound margins was slowest in snakes held at 13.5°C, most rapid in snakes held at 21°C, and was faster in linear incisions than in circular excisional wounds (Table IV). Between two and five days after surgery the adhesive bond in several incisional wounds separated, but reformed.

Superficial scabs did not form over excisional wounds within the ten day experimental period in snakes held at 13.5°C. In snakes held at 21°C, scab formation was noted on 5/12 (42%) wounds five days after surgery, and was present on 4/6 (67%) wounds of ten days duration. At 30°C superficial scabs were present on 2/18 (11%) wounds two days after surgery, on 4/12 (33%) wounds five days after surgery and on 4/6 (67%) wounds ten days after surgery. One snake held at 30°C underwent ecdysis between five and ten days after wounding, removing the scab and exposing smooth, dark tissue bridging epithelial margins. In contrast, ecdysis had occurred within the first five days after surgery in two snakes at 13.5°C and 30°C and in one snake held at 21°C without an

TABLE I. Numerical Scores Assigned to Categorize the Appearance of the Epithelium and the Dermis in Healing Skin Wounds on Garter Snakes

Score	Epithelium (range 0-5)	Score	Dermis (range 1-3)
0	Inactive		
1	Migrating across wound	1	Primarily fibrin and inflammatory cells
2	Completely across wound but epithelium indistinct from underlying dermis	2	Active fibroplasia
	+ 1 if epithelium distinct from dermis but basal layer unorganized or + 2 if basal layer organized	3	Fibroblasts, fibrocytes and collagen oriented parallel to wound surface
	+ 1 if superficial cells rounded and keratinizing		

TABLE II. Prevalence of Morphological Characteristics Associated with the Healing of Skin Wounds of Two, Five and Ten Days Duration in Snakes Held at Each of 13.5° C, 21° C and 30° C[a]

		Unsutured Incisional Wound			Round Excisional Wound		
		Days Duration			Days Duration		
Characteristic	C	2	5	10	2	5	10
Superficial scab	13.5	5	5	6	6	6	6
	21	6	4	4	6	6	6
	30	5	6	5	6	6	6
Epithelial migration[b,c]	13.5	0	0	4	1	1	2
	21	2	4/5	6	0	5	6
	30	5	6	6	1	5	6
Complete reepithelialization of wound[b,c]	13.5	0	0	0	0	0	0
	21	0	0	3	0	0	0
	30	0	1	6	0	0	5
Migration of heterophils through the epithelium	13.5	6	6	6	6	6	6
	21	6	6	6	6	6	6
	30	6	6	4	6	6	6
Dermal edema[b,c]	13.5	6	6	5	6	6	4
	21	6	3	2	5	5	5
	30	6	2	1	6	6	3
Dermal fibrin	13.5	4	4	4	6	6	6
	21	5	4	5	6	6	6
	30	4	6	3	6	6	6
Perivascular lymphoid cuffs[b]	13.5	1	1	0	0	0	0
	21	1	2	3	0	4	4
	30	5	2	1	3	6	1
Diffuse increase in density of mononuclear inflammatory cells in dermis[b]	13.5	2	1	3	0	2	4
	21	5	3	5	2	5	6
	30	6	3	2	4	6	3
Foci of inflammatory cells in dermis	13.5	6	6	6	6	6	6
	21	6	6	5	6	6	6
	30	6	6	2	6	6	6
Active fibroblasts[b,c]	13.5	0	0	1	0	0	1
	21	0	1	0	0	0	0
	30	0	1	4	0	2	6
Horizontally oriented fibroplasia[b,c]	13.5	0	0	0	0	0	0
	21	0	0	1	0	0	0
	30	0	0	4	0	0	5

[a]Evaluated by light microscopy in sections of a total of 108 wounds; n = 6 except where noted
[b]Differences in prevalence between groups held at 13.5°C and 30°C significant ($P < 0.05$)
[c]Differences in prevalence in groups with wounds of two and ten days duration significant ($P < 0.05$)

obvious effect on the gross appearance of wounds.

Ambient temperature had no effect on the evolution of the shape of circular wounds during the first ten days of healing. There was usually a reduction in both dorsoventral and anteroposterior dimensions, although a few wounds actually increased in anteroposterior dimension. Resultant shapes included a variety of ovals, rectangles and irregular triangles, with the long axis oriented anteroposteriorly. There was some decrease in the area of wounds over the ten day period, but an individual wound might decrease, then increase in size on subsequent observations.

In the second experiment all linear wounds had closed and were visible only as pale lines one week after surgery. Some incisions, especially in animals held at 30°C, became less visible with time. There was no difference in the gross appearance of sutured and unsutured incisions.

In snakes held at 30°C, square and round excisional wounds measured 1-2 mm dorsoventrally and were covered by a scab one week after surgery. There was little change in the anteroposterior length of the square wounds; that of the round ones remained the same, increased, or decreased. The square biopsies resulted in rectangular or linear lesions; the eventual shape of the round wounds was much more variable. The surface scab varied little over the six weeks of observation except in the snakes which underwent ecdysis. In these it was removed with the sloughed epithelium, leaving a stellate area of pink or grey smooth and unscaled tissue confluent with the surrounding skin.

In the group held at 21°C, square wounds had become rectangular and measured 1-2 mm dorsoventrally one week after surgery. After two weeks a superficial scab covered all the lesions. Dorsoventral contraction and anteroposterior shortening in round wounds were slower and less complete than in the square ones, resulting in longer, oval lesions 1-3 mm in width. One of 12 round wounds was open one week after surgery with wound edges still mobile over the underlying muscle.

The excisional wounds in snakes held at 13.5°C remained open for two

TABLE III. Prevalence of Morphological Characteristics Associated with the Healing of Skin Wounds of Three and Six Weeks Duration in Snakes Held at Each of 13.5° C, 21° C and 30° C[a]

		Unsutured Incisional Wound		Sutured Incisional Wound		Square Excisional Wound		Round Excisional Wound	
		Week		Week		Week		Week	
Characteristic	C	3	6	3	6	3	6	3	6
Disrupted scale pattern	13.5	6	5	6	6	6	6	6	6
	21	5	3	6	6	6	6	6	6
	30	4	3	5	6	6	6	6	5
Overlap of wound margins[b]	13.5	2/5	0	5	1	0	0	0	0
	21	0	0	1	0	1	0	0	0
	30	0	0	2	1	0	0	1	0
Superficial scab	13.5	6	6	6	6	6	6	6	6
	21	6	6	6	6	6	6	6	6
	30	4	4	4	5	5	4	5	4
Complete reepithelialization of wound[b]	13.5	3/5	4	1	4	2	4	2	3
	21	6	6	6	6	4/4	6	3/4	6
	30	6	6	6	6	6	6	6	6
Epithelium distinct from underlying dermis[b]	13.5	3	3	0	3	0	1	0	0
	21	6	6	4	6	3	6	1	6
	30	6	6	6	6	6	6	6	6
Organized basal cell layer[b,c]	13.5	0	0	0	0	0	0	0	0
	21	2	6	0	6	1	5	0	5
	30	5	5	5	5	6	6	4	6
Rounded superficial epithelial cells[b,c]	13.5	0	0	0	0	0	0	0	0
	21	3	5	1	2	0	4	0	4
	30	4	4	4	4	6	5	3	6
Migration of heterophils through the epithelium[b]	13.5	6	6	6	6	6	6	6	6
	21	6	6	6	6	6	6	6	6
	30	2	0	3	3	5	2	5	1
Dermal edema[b,c]	13.5	5	5	6	6	6	6	6	6
	21	0	0	2	1	5	1	5	0
	30	0	0	0	1	0	0	0	0
Dermal fibrin[b,c]	13.5	4	5	6	5	6	6	6	6
	21	2	0	6	0	6	1	6	1
	30	0	0	0	0	0	0	0	0
Foci of inflammatory cells in dermis[b,c]	13.5	4/5	5	6	6	6	6	6	6
	21	4	0	5	2	6	3	6	2
	30	0	0	0	1	1	0	0	0
Active fibroblasts[b]	13.5	2/5	4	5	5	6	4	2	5
	21	6	5	6	6	6	6	6	6
	30	4	2	5	3	5	2	6	6
Horizontally oriented fibroplasia[b,c]	13.5	0/5	1	0	0	0	0	0	0
	21	3	6	2	6	0	6	0	6
	30	6	6	6	5	6	6	6	6

[a]Evaluated by light microscopy in sections of a total of 144 wounds; n = 6 except where noted
[b]Difference in prevalence between snakes held at 13.5°C and 30°C significant ($P < 0.05$)
[c]Difference in prevalence between wounds of three and six weeks duration significant in snakes held at 21°C ($P < 0.05$)

to four weeks, exposing moist epaxial muscles over which the wound edges moved freely. The wound edges eventually adhered to a thin grey opaque layer which developed over the muscle. Superficial scabs were apparent on only 3/12 wounds of three or more weeks of age. Square wounds became rectangular with the long axis oriented anteroposteriorly, and circular lesions became oval, triangular, or stellate.

The rate of migration of the regenerating epithelium appeared directly related to ambient temperature; many linear wounds in snakes held at 21°C and 30°C were completely reepithelialized by ten days after surgery (Table V). Epithelial maturation (Table VI) was also accelerated by increasing ambient temperature. Dermal maturation also appeared to be promoted by increasing temperature, the effect being most evident in wounds of greater duration (Table VII).

The prevalence of certain morphological characteristics associated with healing in snakes held at various temperatures is presented in Table II for wounds of two, five and ten days duration, and in Table III for wounds of three and six weeks duration. Trends are summarized below.

In wounds on snakes held at 30°C, migration of epithelial cells had begun

TABLE IV. The Effect of Temperature, Duration and Type of Wound on the Prevalence of Mobility of Wound Margins in Snakes

	Unsutured Incisional Wound			Circular Excisional Wound		
	Days Duration			Days Duration		
C	2[a]	5[b]	10[c]	2[a]	5[b]	10[c]
13.5	3(17)[d]	0	0	11(61)	7(58)	4(67)
21	0	1(8)	0	4(22)	1(8)	0
30	6(33)	0	0	7(39)	4(33)	0

[a]n = 18
[b]n = 12
[c]n = 6
[d]Percent prevalence in parentheses

by two days after surgery in unsutured incisional wounds and was consistently present by five days of age in round excisional wounds. All linear wounds and 5/6 excisional wounds were reepithelialized ten days after surgery. By three weeks after surgery the epithelium had crossed all 24 wounds of all types, and the majority of wounds had an organized basal cell layer and maturing, rounded superficial epithelial cells. A scab was still present over most wounds of six weeks duration, although heterophil transmigration of the epithelium was much reduced.

At 21°C epithelial migration was occurring in the majority of wounds five days after surgery, but in only half of the incisions had it covered the wound by ten days after surgery. All incisional and square excisional wounds were reepithelialized three weeks after surgery; the round excisional wounds had reached this point six weeks after surgery. At this time an organized basal layer was present in all incisional, and most excisional wounds, and in many wounds the superficial epithelial cells were also maturing.

In snakes held at 13.5°C epithelial migration was first observed in many incisional wounds and in a few excisional wounds ten days after surgery. Only 15/24 wounds of all types were reepithelialized six weeks after surgery.

At 30°C dermal edema began to decline in incisional wounds after two days, and in excisional wounds of greater than five days duration. Foci of inflammatory cells, especially of macrophages, were reduced in number in incisional wounds of greater than five days duration and in excisional wounds over ten days old. Fibrin had disappeared from all wounds by three weeks after surgery. The number of excisional wounds with large numbers of mononuclear inflammatory cells and perivascular lymphoid cuffs in the adjacent dermis rose during the first five days, then began to decline. The appearance and disappearance of these cells was more rapid in incisional wounds. Fibroblasts and horizontally oriented areas of fibroplasia were common ten days after surgery; by three weeks dermal scars were forming in all wounds and the number of fibroblasts began to decline.

In snakes held at 21°C the number of linear wounds with edema decreased after two days. Three weeks after surgery a few sutured incisions and most excisional wounds still contained fibrin and edema, but these findings were rare by six weeks. At this time foci of macrophages were still present in 1/3 of the sutured incisional wounds and 1/2 of the excisional wounds. Perivascular lymphoid cuffs and large numbers of mononuclear inflammatory cells were still present in the dermis adjacent to the wound ten days after surgery. Fibroblasts were present in all wounds of three weeks duration, and scar formation had begun in incisional wounds. Six weeks after surgery all types of wound contained fibroblasts and horizontally oriented fibrous tissue.

Six weeks after surgery all wounds in snakes held at 13.5°C still contained edema, fibrin, and focal accumulations of macrophages. There were no perivascular lymphoid cuffs, but there was a slow increase in the number of mononuclear inflammatory cells in the dermis adjacent to excisional wounds during the first ten days. Fibroblasts had entered most wounds by three weeks after surgery, but there was horizontal orientation of new connective tissue in only one unsutured incisional wound even after six weeks.

Although the sequence of cellular events in wound healing was similar for all treatment groups, the rate of appearance of characteristics associated with healing, and of disappearance of those reflecting inflammation was more rapid in snakes held at high temperatures. When animals held at 13.5°C and 30°C were compared, significant differences in prevalence occurred in 7/11 characteristics examined in wounds of two to ten days duration (Table II) and in 11/13 characteristics examined in wounds of three and six weeks duration (Table III). Significant differences in prevalence of these characteristics were also frequently present between wounds in animals held at 13.5°C and 21°C or 21°C and 30°C (D.A. Smith, DVSc thesis, University of Guelph, 1984).

During the early phase (two to ten days) of healing, the area containing inflammatory cells around the wound was less in snakes held at higher

TABLE V. The Effect of Temperature, Duration and Type of Wound on the Mean Distance (μm) from Each Wound Margin Covered by Migrating Epithelium, Two to Ten Days after Surgery

	Unsutured Incisional Wound			Circular Excisional Wound		
	Days Duration[a]			Days Duration[a]		
C	2	5	10	2	5	10
13.5	0	0	51.1	3.7	3.7	22.1
21	6.5	80.5[b]	232.0[c]	0	64.0	356.0
30	52.4	183.0[d]	[e]	2.9	482.0	1165.0[d]

[a]n = 6 wounds except as indicated
[b]5 wounds (1 reepithelialized)
[c]3 wounds (3 reepithelialized)
[d]1 wound (5 reepithelialized)
[e]All 6 wounds reepithelialized

TABLE VI. The Effect of Temperature, Duration and Type of Wound on Mean Epithelial Score (see Table I for Explanation; Range = 0-5, n = 6)

	Unsutured Incision			Sutured Incision			Round Excision			Square Excision		
	C			C			C			C		
Day	13.5	21	30	13.5	21	30	13.5	21	30	13.5	21	30
2	0	0.3	0.8	—[a]	—	—	0.2	0	0.2	—	—	—
5	0	1.0	3.2	—	—	—	0.2	0.8	0.8	—	—	—
10	0.7	2.0	4.5	—	—	—	0.3	1.0	3.5	—	—	—
21	1.8	3.8	4.5	1.2	2.8	4.5	1.3	1.7	4.2	1.3	2.3	4.8
42	2.2	4.8	4.5	2.2	4.3	4.5	1.5	4.5	5.0	1.8	4.5	4.8

[a]Not examined

temperatures (Fig. 1), but there was no effect of temperature on heterophil or macrophage density (D.A. Smith, DVSc thesis, University of Guelph, 1984). At three and six weeks after surgery, the density of heterophils (Fig. 2) and macrophages (Fig. 3), and the area of inflammation (Fig. 4) varied inversely, and often significantly, with the temperature at which snakes were held.

Progression of healing and evolution of inflammation with time was reflected in significant changes in prevalence of 5/11 characteristics examined in wounds, between two and ten days duration (Table II), and of 6/13 characteristics between weeks 3 and 6 in snakes held at 21°C (Table III). Between three and six weeks the area of inflammation and density of heterophils decreased significantly in snakes held at 30°C (Table VIII) as did the density of macrophages in snakes held at 21°C (Table VIII).

The effect of suturing incisional wounds, and the influence of shape of excisional wounds on healing were compared at three and six weeks duration (Tables III, VI, VII, IX). Unsutured incisional wounds had significantly less disruption of scale pattern and overlap of wound margins (Table III) and less intense inflammatory infiltrates (Table IX). There was little difference between square and round excisional wounds, though at six weeks postsurgery, in snakes held at 30°C, there was a significantly smaller area of inflammatory infiltrate around the square wound (Table IX).

DISCUSSION

Healing of cutaneous wounds in snakes was accelerated by increasing ambient temperature, based on observations on reepithelialization, dermal fibroplasia, and the initiation and resolution of inflammation. Therefore, it appears that the effect of temperature on metabolic rate and on chemical reaction rates at the cellular level is reflected in the complex process of wound healing.

Contrary to what might be expected, shortly after wounding, more wounds remained mobile in snakes held at 30°C than in those held at 21°C (Table IV). This was probably due to mechanical disruption of early adhesions due to the increased physical activity of snakes held at the higher temperature. A similar detrimental mechanical effect on wounds of increased activity also has been noted in lizards (16) and in fish (17) held at higher temperatures. The high prevalence of mobility of wounds in snakes held at 13.5°C probably reflects a slower rate of fibrin exudation and scab formation.

Voluntary and optimal temperature ranges vary among different species of reptiles (12). It is not known whether the effect of temperature on rate of healing is proportional to the absolute difference in temperature, or to the difference relative to the voluntary range for the species. The rate of wound healing in two species of fish with different environmental temperature requirements appeared to be more highly correlated with absolute temperature, than with the relative position of the temperature within the range for either species (17). Reepithelialization of cutaneous wounds which we observed in snakes was slower than that in fish (17) and lizards (18) held at similar temperatures, though the rate of fibroplasia was similar in snakes and lizards. The magnitude of the effect of differences in temperature on reepithelialization and induction and maturation of fibrous tissue appears to be greater in garter snakes than in fish, although the effect of temperature was more evident on the rate of reepithelialization of large defects (of unspecified area) in fish (19). In lizards, no difference in rate of epithelialization of wounds was noted between 24°C and 32°C, though at 4°C there was no cellular response observed during the first four days after wounding (17).

Skin wounds on mammals also heal more rapidly in warm environments than in cool ones. This is probably due to reflex local vasoconstriction or sludging of blood at lower ambient temperatures (6). In snakes held at 30°C, the experimental temperature closest to mammalian body temperature, reepithelialization of simple linear incisions required five to ten days as compared to 24 to 48 hours in mammals (6). Fibroblasts appeared in the dermis of snakes held at this temperature by five days after surgery, only slightly later than the three day average in mammals (6). Subsequent fibrosis proceeds rapidly in snakes at

TABLE VII. The Effect of Temperature, Duration and Type of Wound on Mean Dermal Score (see Table I for Explanation; Range = 1-3, n = 6)

	Unsutured Incision			Sutured Incision			Round Excision			Square Excision		
	C			C			C			C		
Day	13.5	21	30	13.5	21	30	13.5	21	30	13.5	21	30
2	1.0	1.0	1.0	—[a]	—	—	1.0	1.0	1.0	—	—	—
5	1.0	1.0	1.2	—	—	—	1.0	1.0	1.0	—	—	—
10	1.2	1.3	2.2	—	—	—	1.0	1.0	2.0	—	—	—
21	1.2	2.2	3.0	1.2	2.0	2.8	1.0	1.3	2.7	1.0	1.2	2.8
42	1.8	2.7	3.0	1.0	2.5	2.7	1.2	3.0	3.0	1.0	2.7	3.0

[a]Not examined

Fig. 1-4. The effect of temperature on the inflammatory response in skin wounds of various duration. Bar height = mean value. Open bar — 13.5° C; hatched bar — 21° C; solid bar — 30° C. Subdivided where necessary due to interactions ($P < 0.1$) between temperature and duration or type of wound. Within each subgroup differing letters (eg. a,b) indicate a statistically significant difference ($P < 0.05$). Comparisons between subgroups may not be statistically valid. Distance along Y axis from X axis to S = standard error of the difference between two means.

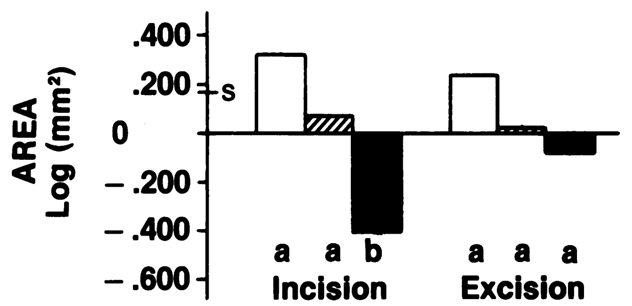

Fig. 1. The effect of temperature on the area of dermal inflammation in wounds of two, five and ten days duration.

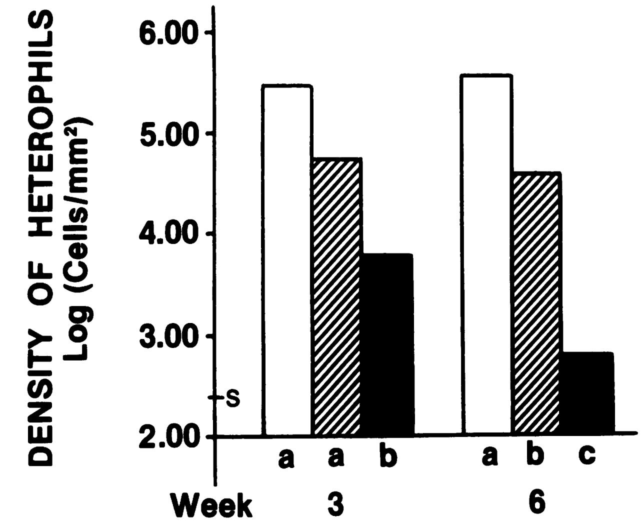

Fig. 2. The effect of temperature on the density of heterophils in the dermis in wounds of three and six weeks duration.

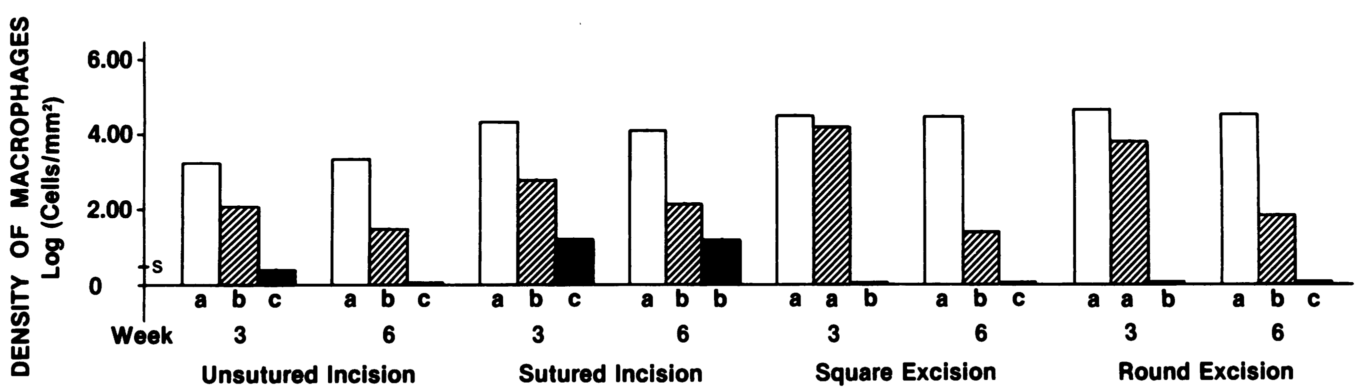

Fig. 3. The effect of temperature on the density of macrophages in the dermis in wounds of three and six weeks duration.

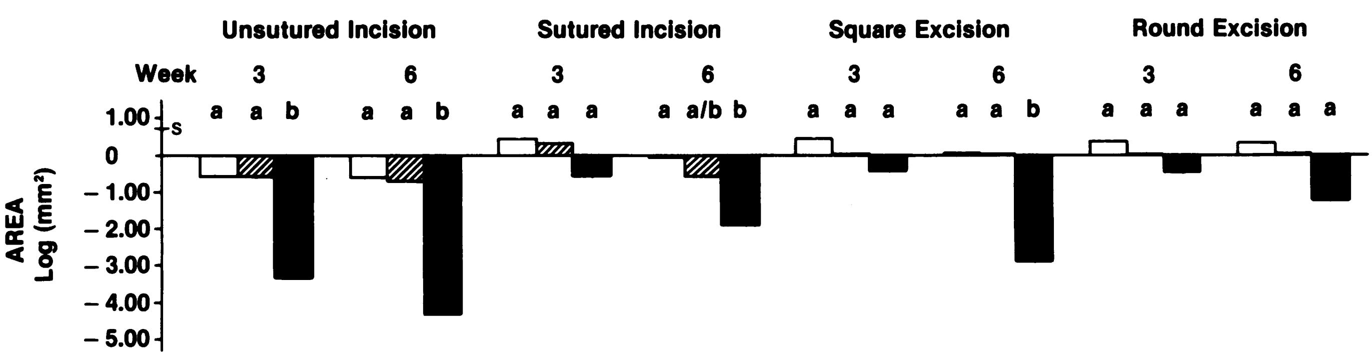

Fig. 4. The effect of temperature on the area of dermal inflammation in wounds of three and six weeks duration.

TABLE VIII. Changes in the Area of Inflamed Dermis and Density of Heterophils and Macrophages in the Area of Inflammation in Wounds of Three to Six Weeks Duration[a]

		Week		
	Subgroup[b]	3	6	SE[c]
Area containing inflammatory cells — log (mm^2)	13.5°C	0.182*	-0.048*	0.345
	21°C	-0.032*	-0.310*	0.345
	30°C	-1.185*	-2.577†	0.345
Density of heterophils — log (cells/mm^2)	13.5°C	5.466*	5.545*	0.270
	21°C	4.738*	4.559*	0.270
	30°C	3.777*	2.794†	0.270
Density of macrophages — log (cells/mm^2)	13.5°C	4.171*	4.078*	0.255
	21°C	3.183*	1.647†	0.255
	30°C	0.387*	0.279*	0.255

[a]Comparisons to be made horizontally within each subgroup. Means with differing superscripts are statistically different from each other ($P < 0.05$). n = 24
[b]Based on the presence of interactions ($P < 0.1$) between time and temperature
[c]Standard error of the difference between two means

this temperature as it does in mammals.

Snakes held at all three environmental temperatures were capable of mounting an inflammatory reaction to wounding within two days. The reaction was more widespread but of the same character and of equal intensity at lower temperatures. Inflammatory cells disappeared from wounds more rapidly at higher temperatures, heterophils persisting longer than macrophages. The character of the piscine inflammatory reaction was similar in fish held at different temperatures (20,21), but the rate of development of the reaction was temperature dependent. This effect was apparent in fish two days after surgery, in contrast to the observations in snakes at this time. The temperature range at which snakes were held was higher than that to which the fish were exposed; therefore earlier examination might have revealed differences in the time of onset of inflammation in relation to temperature.

Increasing ambient temperature may have beneficial effects on wound healing other than those evaluated in these experiments. The frequency of ecdysis, during which the rapid proliferation of the epithelial basal cell layer seemed to accelerate reepithelialization (10), may be increased at higher temperatures (22). In garter snakes, transfer from 21°C to 28°C constant temperature has been shown to induce ecdysis (23).

Wounds often became grossly contaminated with fecal material. However there was no apparent bacterial infection in snakes held at any temperature, though low ambient temperature is reported to decrease the survival rate of reptiles with bacterial infections (24).

There was no apparent advantage in suturing small linear incisions. Wound edges were difficult to appose because of the thin dermis and the stiff superficial keratin layers. There were no differences in the gross appearance of unsutured or sutured incisions, but microscopically there was greater overlap of wound edges and disruption of the scale pattern, and slightly slower epithelial maturation in sutured wounds. Incisional wounds were created parallel to the lines of skin tension; had distractive forces been present, suturing of wounds might have been advantageous. Suturing increased the intensity of the inflammatory response in the dermis, as has been reported in mammals (5).

The eventual shape of excisional wounds appeared to be influenced by the anteroposteriorly oriented lines of skin tension, and fixation of wound margins by scab formation. Wounds rapidly decreased in dorsoventral dimension before contraction associated with fibroplasia might be expected to occur. Even so, round wounds resulted in larger, more irregular defects than did square wounds. In mammals, the rate of contraction is a function of the area of the wound and is independent of wound shape (8).

Several recommendations to optimize surgical care of reptiles, particularly snakes, can be drawn from these experiments. 1) Ambient temperature should be held in the upper portion of the voluntary range for the species to accelerate healing. The frequency of

TABLE IX. Comparison of Area of Inflammation and Density of Heterophils and Macrophages in the Inflamed Dermis in Unsutured and Sutured Incisional Wounds and Between Square and Round Excisional Wounds on Snakes[a]

	Subgroup[b]		Incisional Wounds		Excisional Wounds		
	Week	C	Unsutured	Sutured	Square	Round	SE[c]
Area containing inflammatory cells — log (mm^2)[d]	3	13.5	-0.586*	0.495*	0.432*	0.387-	0.690
		21	-0.567*	0.337*	0.047*	0.055*	0.690
		30	-3.341*	-0.558†	-0.381*	-0.460*	0.690
	6	13.5	-0.590*	-0.032*	0.082*	0.346*	0.690
		21	-0.706*	-0.539*	0.001*	0.005*	0.690
		30	-4.321*	-1.848†	-2.915*	-1.221†	0.690
Density of heterophils — log (cells/mm^2)[e]	—	—	3.962*	4.758†	4.494*	4.706*	0.220
Density of macrophages — log (cells/mm^2)[f]		13.5	3.297*	4.207†	4.427*	4.568*	0.361
		21	1.723*	2.448*	2.713*	2.776*	0.361
		30	0.192*	1.140†	0*	0*	0.361

[a]Comparisons to be made horizontally, within each subgroup. Means with differing superscripts are statistically different from each other ($P < 0.05$). Comparisons between incisional and excisional wounds are not valid
[b]Based on the presence of interactions ($P < 0.1$) between wound type and time or temperature
[c]Standard error of the difference between two means in the same row
[d]n = 6
[e]n = 36
[f]n = 12

feeding must be increased to compensate for the increase in general metabolic activity. 2) Incisional wounds should be created parallel to the anteroposteriorly oriented lines of skin tension, if possible. This will diminish the effect of distractive forces on wound margins and reduce the number of sutures necessary. It may not be necessary to suture small wounds oriented in this direction. 3) When debriding open wounds or excising large amounts of skin, the long axis of the defect should be oriented anteroposteriorly if possible. This will take maximum advantage of contraction resulting from mechanical forces and minimize the area which must be reepithelialized. There may be a slight advantage in creating square or rectangular rather than round wounds. Elliptical defects, not examined here, might be the optimum wound shape to promote contraction.

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REFERENCES

1. **McDONALD HS.** Methods for the physiologic study of reptiles. In: Gans C, ed. Biology of the Reptilia, Vol. 5. London: Academic Press, 1976: 28-34.
2. **HOSKINS MAH, ALEKSIUK M.** Effects of temperature, photoperiod and season on *in vitro* metabolic rates of tissues from *Thamnophis sirtalis parietalis*, a cold climate reptile. Comp Biochem Physiol 1973; 45A: 737-756.
3. **REGAL PJ.** Thermophilic response following feeding in certain reptiles. Copeia 1966: 588-590.
4. **BUSTARD HR.** Activity cycle and thermoregulation in the Australian gecko *Gehydra variegata*. Copeia 1967: 753-758.
5. **PEACOCK EE, VAN WINKLE W.** Wound Repair, 2nd ed. Toronto: WB Saunders, 1976: 54-80.
6. **SWAIM SF.** Surgery of Traumatized Skin: Management and Reconstruction in the Dog and Cat. Toronto: WB Saunders, 1980: 70-115.
7. **MONTGOMERY TC, SHARP JB, BELLINA JH, ROSS LF.** Comparative gross and histological study of the effects of scalpel, electric knife, and carbon dioxide laser on skin and uterine incisions in dogs. Lasers Surg Med 1983; 3: 9-22.
8. **McGRATH MH, SIMON RH.** Wound geometry and the kinetics of wound contraction. Plast Reconstr Surg 1983; 72: 66-72.
9. **POSTLETHWAIT RW, WILLIGAN DA, ULIN AW.** Human tissue reaction to sutures. Ann Surg 1975; 181: 144-150.
10. **SMITH, DA, BARKER IK.** Healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). Can J Vet Res 1988; 52: 111-119.
11. **SMITH DA, BARKER IK, ALLEN OB.** The effect of certain topical medications on healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). Can J Vet Res 1988: 52: 129-133.
12. **BRATTSTROM BH.** Body temperatures of reptiles. Am Midl Nat 1965; 73: 376-422.
13. **DIXON WJ, BROWN MB, ENGELMAN L, FRANE JW, HILL MA, JENNRICH RI, TOPOREK JD.** BMPD Statistical Software. Berkeley: University of California Press, 1981: 330-344.
14. **FLEISS JL.** Statistical Methods for Rates and Proportions. 2nd ed. New York: John Wiley and Sons, 1981: 24-26.
15. **SAS INSTITUTE INCORPORATED.** SAS Users Guide: Statistics. Cary, North Carolina: SAS Institute Inc., 1982.
16. **MADERSON PFA, BARANOWITZ S, ROTH SI.** A histological study of the long-term response to trauma of squamate integument. J Morphol 1978; 157: 121-136.
17. **ANDERSON CD, ROBERTS RJ.** A comparison of the effects of temperature on wound healing in a tropical and a temperate teleost. J Fish Biol 1975; 7: 173-182.
18. **MADERSON PFA, ROTH SI.** A histological study of the early stages of cutaneous wound healing in lizards *in vivo* and *in vitro*. J Exp Zool 1972; 180: 175-186.
19. **ROBERTS RJ, BALL HJ, MUNRO ALS, SHEARER WM.** Studies on ulcerative dermal necrosis of salmonids. III. The healing process in fish maintained under experimental conditions. J Fish Biol 1971; 3: 221-224.
20. **FINN JP, NIELSEN NO.** The effect of temperature variation on the inflammatory response of rainbow trout. J Pathol 1971; 105: 257-268.
21. **ROBERTS RJ, MACQUEEN A, SHEARER WM, YOUNG H.** The histopathology of salmon tagging. I. The tagging lesion in newly tagged parr. J Fish Biol 1973; 5: 497-503.
22. **CHIU KW, MADERSON PFA.** Observations on the interactions between thermal conditions and skin shedding frequency in the Tokay (*Gekko gecko*). J Herpetol 1980; 14: 245-254.
23. **MADERSON P, CHIU KW, CONTARD P, BANK S.** Temperature and shedding in snakes. J Am Soc Zool 1982; 22: 886.
24. **MONAGAS WR, GATTEN RE Jr.** Behavioural fever in the turtles *Terrapene carolina* and *Chrysemys picta*. J Therm Biol 1983; 8: 285-288.