Stress and wound healing

by

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Abstract

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Groups of six week old male and female CF1 white mice were exposed to cold, heat or noise stressors either before or during skin wound healing. Healing progress was measured daily and the rate and variability of the entire process was calculated for each subject, after wound closure had been achieved. All stressors, in one or both of the methods of presentation, were found to increase the mean total time to heal. A significant correlation was found between pre-exposure, reduced wound contractibility and faster rate of healing, and, conversely, between during-exposure, normal contractibility and slower rate of healing. The physiological meaning of these correlations will be explored in a future study of stress and hormonal control of skin thickness with respect to amount of initial wound contraction and rate of healing.

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History

In order to explain man's unique ability to survive in a variety of ecological settings, it is necessary to examine the organic mechanisms evolved by the species to deal with the wide range of stressors encountered. The organic foundation for most physiological and behavioral adaptations lies in the endocrine system and more specifically in the hypothalamic-pituitary-adrenal axis.

Stimuli which provoke accelerated activity of the hypothalamic-pituitary-adrenal axis are diverse. Such stimuli are responsible for the psychological and physical changes that we equate with the experience of stress. Selye (1955; 1973) calls this experience the General Adaptation Syndrome (G.A.S.) and divides it into three phases: alarm, resistance and exhaustion. The alarm phase is characterized by a series of reactions in which the organism seeks homeostatic function, and the exhaustion phase by a decline of acquired adaptation and reduced ability to react when in homeostatic jeopardy.

Although it may not always be possible to judge <u>a prior</u> which stimulus is stressful to which organism,

operational criteria for the experience of stress, based on physiological measurements, have been partially established. In 1936 Selye (1955) described the typical profile of responses elicited by different stressors (e.g., infection, intoxification, trauma, nervous stress, heat, cold, muscular fatigue). Among the most important responses to stressful stimulation are the following:

1) a shift in pituitary activity toward increased adrenocorticotropic hormone output
2) adrenal hypertrophy
3) a shift in protein, glucose and lipid metabolism
4) thymico-lymphatic involution and gastrointestinal ulcers.

Through an as yet ill-defined, but presumably central nervous system (CNS) pathway of mediation, the stressor induces the secretion of corticotropin releasing factor, from the arcuate and pre-optic areas of the hypothalamus, which travels in the blood, via the hypothalamic-hypophysial portal system, to the anterior pituitary which responds with a release of adrenocorticotropic hormone (ACTH).

ACTH then is carried by the bloodstream to the adrenals

where it exerts its characteristic inductive effects on the various regions of the adrenal glands. The fasciculate and reticular portions of the adrenal cortex are permitted by the presence of ACTH to utilize cholesterol* in the production of a variety of steroid hormones called glucocorticoids (GC's). These hormones are responsible for a chain of internal reactions that are adaptive in situations of acute stress but which may become destructive in the course of chronic stress exposure or in cases of pathological responses of adrenals and/or target tissues, producing what Selye (1952) called "the diseases of adaptation."

The chain of internal reactions catalyzed by the GC's includes increased protein catabolism, increased glucose and glycogen synthesis, decreased fibrin-collagen formation, decreased inflammatory-immune responses, and simultaneously the potentiation of catecholamine reactions.

Certain GC's can cause an increase in mRNA and rRNA in target cells of the liver. These nucleic acids then direct the production of enzymes (tryptophan pyrolase, alanine-, tyrosine- and tryptophan-α-ketoglutarate transaminases)

* Yoffey and Robinson (1953) noticed depletion of cholesterol from the zona reticularis of the adrenal cortex in as little as 5 minutes after exposure to cold or injection of adrenaline.

that will increase amino acid metabolism and the eventual breakdown of protein tissues (Nichol and Rosen, 1965).

Amino acids which are made available through protein catabolism can be employed in gluconeogenesis, which is the focus of GC activity, as carbohydrate reserves become depleted during prolonged stress (Nichol and Rosen, 1965). GC's also have been shown to selectively increase the production of other liver enzymes (glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphohexose isomerase) specifically needed for gluconeogenesis.

The formation of sugar by the liver from noncarbohydrate molecules and the production of glycogen will provide the body with rapidly mobilized sources of energy (Gray, 1971). The net result of these interrelated processes of deamination, glycogenesis and gluconeogenesis is a greater capacity for metabolism necessary to maintain homeostasis (Nichol and Rosen, 1965) under stress.

However, the longterm effects of high circulating GC's, with respect to deamination/energy molecule formation, will be a negative nitrogen balance (Ruch and Patton, 1965) and consequent impairment of tissue maintenance and growth (Rehder, 1967).

Chronic stress, prolonged corticosteroid treatment,

congenital adrenal hyperplasia and Cushing's disease, with their characteristically high GC levels, all may result in muscular weakness due to continuous deamination. In such cases GC's may also interfere with growth by blocking new cartilage and bone formation and may even cause osteoporosis. In the course of adaptation to prolonged stress, decreased thyroid activity, probably arising from a decrease in thyroid stimulating hormone (TSH) secretion (Mason, 1968), and decreased somatotropin (GH) secretion also will contribute to depression of growth and reduced tissue maintenance. These effects are the result of generally depressed anterior pituitary activity due to the negative feedback of high circulating concentrations of GC's during continued exposure to stress or with pathologic overactivity of the adrenal cortices.

In the short run, connective tissue changes due to GC effects on protein are not marked enough to be considered maladaptive concomitants to GC mobilization. These changes are probably not realized under normal conditions when stress is transitory and GC levels quickly return to normal physiological limits. However, in the long run, continued stimulation of adrenal cortical tissues or administration of cortical hormones, coupled with depressed activity of the anterior pituitary that reduces TSH and GH output, will result in

destructive connective tissue changes. These include fibroblast reduction and consequent collagen atrophy (Iverson, 1954), retardation of granuloma formation (Funk and Jensen, 1967), and loss of bone matrix (Forsham, 1963).

These connective tissue responses are related to corticoid depression of the immune system, which reduces granulation tissue and histamine secretion, causing decreased capillary permeability (Mason, 1968; McMinn, 1969; Jensen and Rasmussen, 1970), as well as to the accentuation of glucose and glycogen formation at the expense of protein. The general adaptive advantage of suppression of the immune system is unclear. Involution of the thymus begins rapidly, as noted by Selye in experiments with rats subject to the stress of immobilization for 48 hours (Mason, 1968). Resistance to viral and bacterial infection (Forsham, 1963) and tumorigenesis (polyoma) (Jensen and Rasmussen, 1970) is reduced. Antibody, lymphocyte and eosinophil numbers decline, and it would seem that these are maladaptive but inescapable consequences of adjustment to stress.

On the positive side, this GC suppression of the immune system will protect target tissues from destructive allergic

and immune responses (Forsham, 1963). This fact has proved useful in many clinical applications, but can be helpful in stress situations only when the source of stress is itself a deleterious inflammatory or antigen-antibody reaction.

GC potentiation of catecholamine responses galvanizes another set of interrelated events that takes place concurrently with those already discussed. GC's sensitize blood vessel walls to adrenaline and noradrenaline. Vasodilation in the gut, mucous membrane, skeletal muscle, and viscera; vasoconstriction in the extremities and skin and increased blood pressure all result. These changes plus increased heart rate and force of contraction, increased activity of the sweat glands, contraction of pilomotor muscles, and alertness seem to work reciprocally upon the adrenal cortices, via CNS and pituitary, because the state of excitement, as perceived from within, further depletes the adrenal cortical stores of cholesterol, presumably for use in more steroid hormone production (Yoffey, 1953).

Thus the organism under stress responds with a multifaceted pattern of arousal. The organism is poised for physical confrontation with or escape from the stressful situation. If the physical employment of specifically elaborated energy is obstructed, a continuous physiological

call to arms ensues and prevails so long as the hypothalamic-pituitary-adrenal axis is activated. The result is a state of readiness that obliterates the normal rhythm of activity and rest, and the organism experiences a sleep that brings no relaxation or renewal. If energies provided by the G.A.S. can not be fully dissipated in activity, tension remains. The destructive extensions of the initially adaptive, GC-stimulated responses appear and are joined by other maladaptive, longterm changes. The organism experiences longer periods of wakefulness (Bullough and Laurence, 1961; Forsham, 1963), increased susceptibility to infection (Jensen and Rasmussen, 1970) and consequent delay of growth and reparative processes (Toivanen et al., 1960; Ruch and Patton, 1965; Goss, 1970).

In this connection, it has been noted by many investigators that all the GC-mediated events may directly or indirectly retard the process of skin wound healing (Perasalo et al., 1953; Duran-Reynals, 1954; Iverson, 1954; Toivanen, 1960; Reddan and Rothstein, 1965; Funk and Jensen, 1967; Goss, 1970) in both warm- and cold-blooded species.

By the thesis of non-specific actions of stress (Selye, 1973), it is inferred that any stressors which are strong enough to affect the hypothalamic-pituitary-adrenal axis to

sufficient and similar intensity will affect skin wound healing in a similar and negative way.

With respect to this hypothesis, some of the influences of the G.A.S. specific to skin, its component substances, and its behavior when challenged by foreign bodies or injury will now be reviewed.

The rate of healing is generally found to be impeded by the physiological reactions of the G.A.S., but there may also be aspects or phases of the G.A.S. that enhance the healing process. The positive effects of G.A.S. on healing are at present speculative and rightfully overshadowed by the substantial proofs of the negative effects of stress on wound healing.

Protein catabolism and related enzymatic activity may depress healing in the following ways. Since decreased alanine transaminase activity and increased nitrogen retention are characteristic of protein anabolism in the liver and in fetal development (Nichol and Rosen, 1965), it would seem, conversely, that stress, which increases alanine transaminase activity via GC induction and decreases nitrogen retention, could retard the growth of new tissue in a wound.

In addition to these possible systemic effects, there is a demonstrated local effect of cortisone on epidermis,

causing reduced fibroblast formation, vascularization and deposition of extracellular ground substance (Aldrich etal., 1951; Iverson, 1954). Since collagen formation starts in the endoplasmic reticulum of fibroblasts, with later transfer to Golgi vesicles or extracellular space (McMinn, 1969), it is logical that reduction of fibroblasts will inhibit collagen formation which is essential to the growth of new skin. Normally, collagen is extruded from the fibroblasts and becomes arranged in a cross-striated pattern, forming collagen fibrils (McMinn, 1969). But with local application of cortisone to rat skin, the collagen fibrils become arranged in a compact mass (Iverson, 1954). Asboe-Hansen (1954) observed a decrease in the number and presence of granulations in the residual mast cells of the skin with ACTH and cortisone injections in man, rabbits, guinea-pigs and mice. Consequent to these changes the dermis is thinned and the ground substance depleted.

Overall, stress or stress plus glucocorticoid administration has been shown repeatedly to limit connective tissue proliferation. However, there may be some positive aspects to GC responses for the healing process. If injury to the skin is experienced apart from other stressors and the wound is permitted to heal under conditions that would

not further activate the adrenals, then the fact that GC's increase glycogen production may assist the healing process. Although the glycogen increases due to GC action have been measured in liver tissue, it is possible, bearing in mind other local actions of GC's in connective tissue, that glycogen formation may be induced in epidermis also. This may have a positive value in that glycogen accumulation, noted in the hypertrophic and migrating cells of skin wounds in many species including man, is thought to precede and supply the energy for a burst of mitotic activity in the basal cells. This conjecture finds support in the facts that glycogen accumulation is not measurable until about 8 hours after injury, but glycogen has disappeared completely after about 48 hours, at which time mitotic activity in the healing wound reaches a peak (Lobitz and Holyoke, 1954; McMinn, 1969).

There is still some doubt, however, as to the source of the accumulation. Is the glycogen synthesis of these cells increased or is it synthesized at a normal rate but blocked for utilization? If increased glycogen is called for in the mitotic processes of the cells of a healing wound, then it is conceivable that GC's aid in its synthesis, although the target cells usually specified for this corticoid action

are hepatocytes.

Another positive aspect of GC activity for skin healing is noted in consideration of the depressed immune system reactivity. Although GC's increase the possibility of infection in wounds, they may assist healing when new tissues must be transplanted. Wistar and Hildemann (1960) showed that stress will significantly depress skin homograft rejection in mice. The fact that the same sort of glycogen accumulation observed in normally healing wounds also occurs in skin homografts and autografts (McMinn, 1969), may provide additional proof that GC's are indirect aids in connective tissue repair--through their suppression of immune reaction to transplanted skin, and their possible role in the pre-mitotic elaboration of glycogen.

There is further evidence that depression of the immune response may bear favorable results for healing. Cavallero (1954) notes the anti-metachromatic effect of cortisone on myxomatosis viral infection in rabbits. He points out that the pathologic responses caused by different sorts of pathogens must be considered before the nature of cortisone's affect on connective tissue can be assessed. Robinson (1953) also says that "the effects of cortisone in infectious diseases will vary with the animal species, the invading micro-

organism, the pathology of the infectious lesion and the amount of cortisone administered." In general, large doses of cortisone or ACTH have an infection-permissive effect, but small doses of cortisone may even have an infection-suppressive effect, as found in rabbits receiving less than 0.5mg of cortisone daily for 5 days prior to pneumococcal injection.

It is important to note that one of the many inflammatory responses at the wound site, release of histamine, which is responsible for fluid exudation, will be suppressed by cortisone (Cameron, 1953). This may be due to the fact that cortisone reduces capillary permeability. Apparently some species, like mice and rabbits (Rehder, 1967), are more cortisone-sensitive and manifest this anti-histamine action of the hormone more readily than man (Robinson, 1953). This may have something to do with GC interaction with differential catecholamine secretion (noradrenaline:adrenaline= fearful-anxious responses:angry-aggressive responses), according to species and possibly according to individual (Gray, 1971).

Like depression of the immune response, the GC-potentiated effects of catecholamines, secreted by the adrenal medulla during stress (Forsham, 1963; Mason, 1968), also

may have positive and negative effects on healing. For instance, the single response of vasoconstriction can have two opposing effects for wounds. On the positive side, it prevents hemorrhage, and on the negative side it cuts down nutritive blood supply (creates eschemia) which has been shown to retard skin healing in rabbits (McMinn, 1969). This is corroborated by the fact that the skin of sympathectomized rabbits (McMinn, 1969) and denervated skin of rats (Elfving, 1959) will heal faster than normal skin.

The adrenal medullary involvement in mitosis in skin wound healing is important to assess here. Bullough and Laurence, quoted by McMinn (1969), suggest that normal epidermal cells of the active organism are mitotically inhibited. Bullough and Laurence (1961) succeeded in creating almost complete cessation of epidermal mitotic activity in mice through starvation. They speculate that the normal inhibitor of skin mitosis is adrenaline and that its increased secretion during the stress of starvation led to the increased inhibition of mitosis. Their view is supported by the facts that 1) skin mitoses increase during sleep, when adrenaline is low and decrease during activity, when adrenaline is high; 2) this diurnal pattern is eliminated (i.e., mitoses remain high at all times of day) by

adrenalectomy, whereby most adrenaline is obviated, but it is restored by injection of 10μg of adrenaline.

It was observed (Bullough and Laurence, 1961; Kreyberg et al., 1965) that stress, particularly that associated with fear and anxiety (Gray, 1971), would, by increasing adrenaline, depress the number of skin mitoses. It is inferred, therefore, that sleeplessness, experienced as part of prolonged stress, will depress skin mitoses. It is noted, however, by Bullough and Laurence (1961) that wounded epidermis seems to be exempt from the diurnal pattern, the epidermal mitotic activity at the wound site apparently being less sensitive to adrenaline inhibition. While in normal skin 5μg of adrenaline are sufficient to depress mitotic activity, this dose and even up to 50μg will not appreciably suppress mitosis in wounds.

It is conceivable, however, that wound epidermis, in the presence of additional systemic stress or in conjunction with GC administration, would show wound site mitotic depression as a result of the potentiating effect of GC's on adrenaline. Also, the GC, cortisone, and its tropic hormone, ACTH, can independently exert an anti-mitotic effect systemically (Bullough, 1952; Bullough and Laurence, 1961; Green and Ghadially, 1951) and cortisone locally

(Baker and Whitaker, 1950; Green and Ghadially, 1951; Cameron, 1953) on the skin.

The following chart is offered in summary of the general pattern of stress-provoked arousal.

Chart 1.

CNS input (stress stimuli)
↓
hypothalamic release of CRF
↓
pituitary release of ACTH
↓
adrenal release of corticoids and catecholamines

Targets of glucocorticoids

Liver	Thymus and Lymphatics
protein catabolism ↑	viral & bacterial susceptibility ↑
gluconeogenesis ↑	antibodies, lymphocytes, eosinophils ↓
Anterior pituitary	**Skin**
TSH ↓	fibroblasts ↓
GH ↓	collagen ↓
ACTH ↓	granuloma formation ↓
	bone matrix ↓
	histamine & capillary permeability ↓

Targets of GC-potentiated catecholamines

Skin	Blood vessels
histamine ↓	vasoconstriction ↑ in skin and extremities
sweat gland activity ↑	
piloerection ↑	vasodilation ↑ in gut, mucous membrane & skeletal muscle

N.B. Some of these reactions take longer than others to develop. Some are of shorter duration than others.

The following chart is offered in summary of the pattern of arousal with respect to skin and wound healing.

Chart 2.

Systemic GC effects

fibroblast ↓ ⟶ collagen ↓

granulations and cellular migration and infiltration ↓

glucose uptake* ↑

homograft and autograft rejection ↓ (via ↓ immune response)*

mitosis ↓

formation of new capillaries and lymphatics ↓ (Cameron, 1953)

Local GC effects

compacting collagen fibrils ⟶ thinning of skin

phagocytic activity ↓ ⟶ delaying removal of byproducts of tissue damage (Cameron, 1953)

glucose uptake* ↑

vascular hyperemia ↓

mitosis ↓

permeability of capillaries ↓ (via ↓ histamine)

GC-potentiated catecholamine effects

hemorrhage ↓

vascular hyperemia ↓

mitotic inhibition ↑

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* May assist healing

In view of the foregoing evidence of the involvement of stress-initiated changes, mediated mainly via adrenal cortical hormones, in connective tissue maintenance and wound repair, the modest intention of restoring a simple "macro" approach to the study of skin wound healing, in mice exposed to environmental stressors, is proposed. With the method of exposure and the stressors used, it is hoped that comparisons will be drawn between the different phases of the adaptation syndrome and, by extension, between the relative intensities of impact on the hypothalamic-pituitary-adrenal axis of the different stressors.

It remains only to point out that the GC effects that alter the course of wound healing have been presented here as potential aspects of a stress situation. However, the experimental evidence is often derived from effects that have not been produced by GC's generated in response to environmental stimuli. That courses of injections or local applications of GC's or tropins will most likely not mimic the native G.A.S. needs hardly be mentioned. Furthermore, these entail the additional stress factor of handling. Although vital for detailed histochemical analysis of hormonal involvements at the wound site, these studies can not reveal the sensitivity of the normal response to stress as can a basic study of the rate of healing under strictly delimited conditions of single-type environmental stress.

Experimental Procedure

Materials

140 CF1 one month old mice (Carworth)

Hotpack environmental room

Mettler semi-micro analytic balance (#H20T d ≙ 0.1mg)

Olivetti desk computers Programma 101 and P652

Concord Automatic 350 tape recorder

one hour recording tape (Coach)

Oster small animal clipper (model #A2)

Nair depilatory cream; ether

"Shred-a-bed" 7" x 9" nesting mats

0.5cm x 0.5cm rubber stamp (Krengel); blue food color

Avcom acetate roll (catalog #950)

Sharpie indelible pen (Sanford); micrometer (Central)

Method

Let: S=subject E=experimenter

Controls

21 mice (10 ♂ and 11 ♀), 6 weeks old, were wounded in the following way:

1) S was removed from a large cage in which all other males or all other females were housed for two

weeks after delivery to the premises.

2) S was placed on top of a small, empty cage with a wire mesh top. As S gripped the mesh, E shaved an area of approximately 8.5cm in circumference on the caudal half of S's back.

3) Nair was applied to the area and allowed to dry for 5 minutes.

4) Nair was washed from the area.

5) S was anesthetized, and the rubber stamp outline was applied on the approximate center of the naked area.

6) Using small surgical tongs and scissors the outlined square of skin was excised down to the deep fascia covering the muscles of the lower back.

7) S was placed in a small cage in which a similarly wounded member of the opposite sex was placed.

8) All Ss remained untouched, under ordinary laboratory conditions, for the next 24 hours.

9) On each day subsequent to the 24 hour rest period, until complete healing* was achieved, the wound was measured between the hours of 8 and 8:30 P.M., in the following way:

* Complete healing was judged by inspection to have been achieved when the scab was gone and the wound completely closed.

10) S was removed from its cage and placed on a wire mesh cage top. As S gripped the mesh, E placed a clean piece of acetate (about 2cm x 2cm) over the wound and drew an accurate outline of the wound on the acetate. This procedure took about 60 seconds.

11) Later, the outline of the wound was cut out of the acetate square with surgical scissors and weighed.
Steps 1-11 were followed for all control and experimental Ss.

Pre-cold

21 mice (10♂ and 11♀) were kept in a cold room (4°C ± 0.5°; 50% humidity) for 5 hours on day 1, for 6 hours on day 2, for 7 hours on day 3 and for 8 hours per day for the next 16 days before undergoing steps 1-11. Two Ss and one half of a "Shred-a-bed" nesting mat were kept in each cage.

Cold-during

20 mice (10♂ and 10♀) underwent steps 1-11 and were placed in a cold room at the same temperature and humidity and according to the same schedule of acclimatization used for pre-cold Ss. Each S was kept in the cold room until its wound had healed completely. Two Ss and one half of a

"Shred-a-bed" nesting mat were kept in each cage.

Pre-heat

17 mice (9♂ and 8♀) were kept in a warm room (33°C ± 0.5°; 28% humidity) for 5 hours on day 1, for 8 hours on day 2, for 10 hours on day 3 and for 24 hours per day for the next 17 days before undergoing steps 1-11. Two Ss were kept in each cage, and no bedding material was provided.

Heat-during

20 mice (10♂ and 10♀) underwent steps 1-11 and were placed in a warm room at the same temperature and humidity and according to the same schedule of acclimatization used for pre-heat Ss. Each S was kept in the warm room until its wound had healed completely. Two Ss were kept in each cage, and no bedding material was provided.

Pre-noise

14 mice (7♂ and 7♀), kept 2 in a cage, were exposed to loud noise (99dB on the C scale overall, with the following pitch breakdown: 9600-4800Hz - 65dB; 4800-2400Hz - 83dB; 2400-1200Hz - 94dB; 1200-600Hz - 96.5dB; 600-300Hz

- 89dB; 300-150Hz - 80dB; 150-75Hz - 46dB; i.e., almost "white noise" in which there is almost equal volume in each pitch range)* during a 4 hour period daily, between the hours of 7 and 11 P.M., for 15 consecutive days, according to the following schedule (Siegel and Smookler, 1973) before undergoing steps 1-11.

Day 1	½	½	1	1	1		
Day 2	1	½	½	½	½	½	½
Day 3	½	½	½	½	1	½	½
Day 4	1	½	1	1	½		
Day 5	½	½	1	½	½	½	½
Day 6	1	½	½	1	1		

Repeat sequence _____ = noise on

Each 4 hour session consisted of 2.5 hours of loud noise and 1.5 hours of silence. The recorded noise was produced by editing and retaping the roar of an express subway train.

Noise-during

20 mice (10♂and 10♀) underwent steps 1-11 and were exposed to loud noise, as described for pre-noise Ss.

* Courtesy of Dr. M. Margulies, Institute of Health Sciences, 105 E. 106th Street, New York City, N.Y.

Each S was exposed to noise daily until its wound had healed completely. These Ss were sacrificed on the day of healing, and their adrenals were weighed wet to obtain data for a future comparative study.

Statistical Methods

Total time to heal

1) The number of days to heal was counted for each S.

2) These total-days-to-heal figures were added and the mean and standard deviation (S.D.) found for each group and for males and females within groups.

3) t tests were performed on the means of each group compared to control, and between pre and during groups for each experimental condition, and between males and females of each group.

Rate of healing

1) The rate of healing was calculated for each S using the following formula: $y = a + bx$, where y is the dependent variable of cut-out weight; a is the y-intercept; b is the slope constant, and x is the independent variable of day-since-wounding.

2) The b figures were added and the mean and S.D. were obtained for each group and for males and females within groups.

3) Using t scores, the b figures for all Ss of a group were compared simultaneously to all the b figures of the control. For each experimental condition, pre and during groups were compared in the same way. Males and females within groups were compared also.

Comparison of initial wound contraction

1) 20 acetate cut-outs of the standard stamp (i.e., size of the original skin wound) were weighed, and the mean and S.D. of these weights was obtained.

2) The mean and S.D. of the first-day-wound-outline acetate cut-outs were obtained for each group and for males and females within the groups.

3) t scores were obtained for:

each group against the control

pre against during groups for each condition

males against females within groups.

Graphic Methods

1) Weights of cut-outs for each S in a group were

plotted against the day-since-wounding.

2) The line which connected the centers of all daily point distributions was drawn.

3) The line of each group was transferred from graph to acetate paper.

4) The lines were superimposed on each other to facilitate visual comparison of the total-time-to-heal and the rate of healing.

Test of acetate uniformity

1) 20 clean squares of equal size (0.5cm x 0.5cm) were cut out of acetate and weighed. The mean and S.D. of these weights were found.

2) These parameters were compared to similar parameters obtained for 20 finger-smudged squares of equal size cut from the same roll of acetate.

3) 32 micrometer measurements were taken of the width of the acetate sheet at different points along the roll. The mean thickness and the percentage of variation in thickness were obtained.

Results

The results of testing the acetate used for wound-outline weights showed an acceptable consistency of weight and thickness. The weight of a sample of 20 clean, hand-cut acetate squares (0.5cm x 0.5cm) varied only ±0.027mg against a mean weight of 4.82mg. Finger-smudged acetate squares of the same size varied ±0.047mg against a mean weight of 4.96mg. The mean weight of smudged acetate was significantly greater than that of the clean acetate ($t=2.28$ $P < .05$). Therefore, care was taken to use only clean acetate for wound outlines and not to have finger-prints or dust on the outlines before weighing. The thickness of the acetate was 0.11mm ± 0.003.

Initial wound contraction

In order to establish the extent of the influence of stress exposure on skin, it was decided to compare all pre groups as a unit to all during groups and controls as a unit for amount of wound contraction after the first 24 hours. All pre group Ss had been exposed to their respective stressors for 15 to 17 days before receiving a wound. All during group Ss and controls had resided under normal

laboratory conditions with no excessive noise, temperature change or handling before receiving a wound.

Comparing group means, the pre unit shows less contraction of the wound after 24 hours than does the during unit (see Table 1). However, before drawing any association between pre treatment and reduced initial wound contraction, it must be noted that one of the groups, noise-during, showing low initial contraction was in the unit considered normal before wounding.

The difference between the initial size of the wound and the size after 24 hours is indicated by $\triangle$. The smaller $\triangle$, the less the wound contracted.

Considering all Ss in each unit, the pre groups show significantly less contraction than the during groups ($t=2.43$ $P < .02$). There seems to be an inverse relationship between stress exposure and the ability of the skin to contract initially.

There were no significant differences for amount of initial wound contraction between males and females of any group (Appendix I).

It was observed that some Ss have thick skin that retains the square outline of the carefully excised wound, while others have thin skin that "spreads" after cutting,

so that the sharp square outline is not retained. The consistency and distinctness of this individual variation did not become clear until several groups had been wounded. Therefore, the early quality of the skin of each S was not noted for thickness, and it is not known whether or not the thin, "spreading" type of skin was present more often after exposure to stress or whether these skin differences were randomly distributed through the groups, on the basis of individual genetic makeup and/or differences in food intake.

Another way in which the pre unit differed from the during and control unit was in the total number of fatalities. Five deaths occurred, by the end of healing, in the pre unit, while no deaths occurred in the during and control unit.

Total time to heal

Number-of-days-to-heal is used as one index of the rate of healing. Referring to Figures 1-7 and the raw data in Appendix III, it can be seen that there is a wide range in the total time to heal, but that the extremes of this range, 11 days and 25 days, are provided by only a few Ss in a total last day count of 133 Ss. Most Ss from

all groups healed within the 14 to 17 day range. Fifteen days is the mode for all groups combined, and individual group modes fall on days 14, 15, 16 or 17, except in the noise-during and pre-noise groups where "modes" occur on several days.

Table 2 shows that the mean total number of days to heal is significantly greater than that of the control in every treatment group except pre-cold. Both simultaneous and pre treatment with stress can result in a greater number of days to heal when compared to the mean number of days to heal required by controls. All pre Ss, as a group, do not differ significantly from the control group in mean total days to heal. All during Ss, as a group, however, take significantly longer to heal than controls ($P < .02$) (see Appendix I).

Within each experimental condition there were no significant differences between pre and during groups in mean number of days to heal.

There were no significant differences between males and females in any group (Appendix I).

"b" rate of healing

The slope (b) of the line that best fits the

distribution of points, when weight of wound-outline is plotted against the day-since-wounding, is the second index of the rate of wound healing.

The b coefficients of each group are shown and compared in Table 3. In every experimental condition there is a significant difference between the pre and during groups in rate of healing. In both cold and heat it is the pre group that is healing at a faster rate than the during group. In the noise stress situation, the pre group is healing more slowly than the during group. Within each experimental condition, whichever group is healing at the slower rate (i.e., where b is smaller) also shows the greater mean total time to heal (see Table 2). All pre Ss, considered as a group, do not differ significantly from the control in rate of healing, but all during Ss, as a group, do differ significantly from the control ($P < .05$). As a group, all during Ss are healing significantly slower than the controls (Appendix I).

Differences in rate of healing may be evaluated in connection with initial wound contraction. Three of the treatment groups (pre-cold, pre-heat, noise-during) healed at a significantly faster rate than the other three

treatment groups (cold-during, heat-during, pre-noise), $t=5.83$ $P < .01$.

As a group, the fast healers always show less initial wound contraction than slow healers. The fact that reduced initial wound contraction is so perfectly correlated ($X^2=15$ $P < .001$) with increased rate of healing and that both are significantly correlated with pre exposure ($X^2=4.33$ $P < .05$) is treated in the discussion.

The fast healers with less initial wound contraction were all pre-exposed to stress, except in the case of noise, where faster healers are found in the during group. The slow healers with more initial wound contraction were all exposed to stress during healing, except in the case of noise, where slower healers are found in the during group. With respect to these findings, it is important to observe that the difference between the rates of healing of the pre-noise and noise-during groups is of a much lower order of significance ($P < .01$) than between the pre and during groups of the other two experimental conditions ($P < .001$).

There is a similar consistency of effect seen when treatment groups are compared to the control. Only two groups differ significantly from control in the initial

size of the wound. Pre-heat shows significantly less initial wound contraction compared to control ($P < .01$), and cold-during shows significantly more initial wound contraction compared to control ($P < .001$). In terms of treatment, this is consistent with the finding that pre-exposed Ss show significantly less initial wound contraction than during-exposed Ss ($P < .001$) (Appendix I). Thus, the pre-heat group, showing less initial wound contraction with respect to control, is also the group healing significantly faster than the control. And the cold-during group, showing more initial wound contraction with respect to control, is also the group healing significantly slower than the control (Appendix I).

Graphic comparisons

Pre-cold, cold-during, control. (Figs. 1,2,3 and Superimposition I)

The variance, as indicated by the vertical lines joining the daily point distributions, appears to be much greater in the pre group than in the during group, when both are compared to the control.

In both pre and during groups there is peaking on the third day. Peaking indicates that the size of the wound

became larger.

The graphs of all three groups are jagged; that is, they describe a course of alternating accelerated and decelerated healing.

Pre-heat, heat-during, control. (Figs. 1,4,5 and Superimposition II)

In contrast, the heat-exposed Ss show smoother progress. Again the variance of the pre group is greater than that of the during group, when both are compared to the control.

Pre-noise, noise-during, control. (Figs. 1,6,7 and Superimposition III)

The variance of both noise groups is low compared to the control, as the healing progress of both groups is less sporadic than in other experimental groups. However, due to circumstances beyond the control of E, the lack of measurements for four consecutive days in the pre-noise group makes the variance picture more uniform than it actually may be.

Other observations

Looking at Figures 1-7, it is clear that every group, except pre-heat, had at least two Ss with incompletely closed wounds on day 16, which is the last day on which there were still two control Ss with incompletely closed wounds. This means that all stressors, except pre-heat, were effective in extending the total healing time beyond that of the control.

It is also apparent from these graphs that the median size of wounds at the end of day 1 is greater than control's in every group, except cold-during and heat-during, which are both very close to the control.

It was noted, by gross inspection, that regrowth of hair occurred most rapidly in the heat-during group. One week after wounding, the Ss in the heat-during group showed almost complete regrowth of hair in the shaved area. In all other groups, the shaved area remained hairless or nearly so, even when healing was complete at 2 to 2½ weeks after wounding.

It was observed also that heat-during Ss showed a thinner-looking, more translucent skin over the closed wound than other Ss.

Table 1.

Comparison of initial wound contraction and total number of deaths between exposed and unexposed Ss, as of 24 hours after wounding.

Unexposed group	Day 1 N	$\bar{X}$ weight of 0.5x0.5cm acetate cut-out	Grp. $\bar{X}$ wt. mg. day 1 outline	Δ	All Ss $\bar{X}$ and S.D. acetate cut-out	expsd/ unexpsd t	deaths
Control	21	4.82±0.03	2.98	1.84			0
Cold-d.	22		2.12	2.70			0
Heat-d.	20		2.54	2.28	2.72±0.75		0
Noise-d.	20		3.31	1.51			0
	83						0
						2.43 P < .02	
Exposed group							
Pre-cold	21		2.72	2.10			0
Pre-heat	18		3.85	0.97	3.05±0.84		2
Pre-noise	18		2.64	2.18			3
	57						5

Table 2.

Comparisons of mean number of days to heal.

Group	$\bar{X}$ # of days to heal	t with control	P values	t between pre & during groups	P values
Control	14.86				
Pre-cold	14.81	0.06	n.s.	1.60	n.s.
Cold-during	16.30	2.83	<.01		
Pre-heat	16.12	2.86	<.01	0.33	n.s.
Heat-during	16.30	2.62	<.02		
Pre-noise	16.36	2.07	<.05	1.12	n.s.
Noise-during	15.50	1.27	n.s.		

Table 3.

Comparisons of rate of healing (b).

Group	b	t with control	P values	t between pre & during groups	P values
Control	-.24				
Pre-cold	-.24	0.03	n.s.	4.17	<.001
Cold-during	-.15	4.63	<.001		
Pre-heat	-.32	4.07	<.001	8.40	<.001
Heat-during	-.18	3.32	<.01		
Pre-noise	-.22	1.12	n.s.	2.93	<.01
Noise-during	-.28	1.68	n.s.		

Group	N	$\bar{X}$ b	t	P value
All pre Ss	52	-.26	4.01	<.001
All during Ss	60	-.20		

Table 4.

Comparisons of rate of healing and initial wound contraction.

Fast healers	Group $\bar{X}$ Rate (b)	Fast v. slow t	Day 1 $\bar{X}$ wt. of wound outline mg.	Large v. small t
Pre-cold	-.28		2.72	
Pre-heat	-.32		3.85	
Noise-during	-.28		3.31	
		5.83 P <.01		6.46 P <.001
Slow healers				
Cold-during	-.15		2.12	
Heat-during	-.18		2.54	
Pre-noise	-.22		2.18	

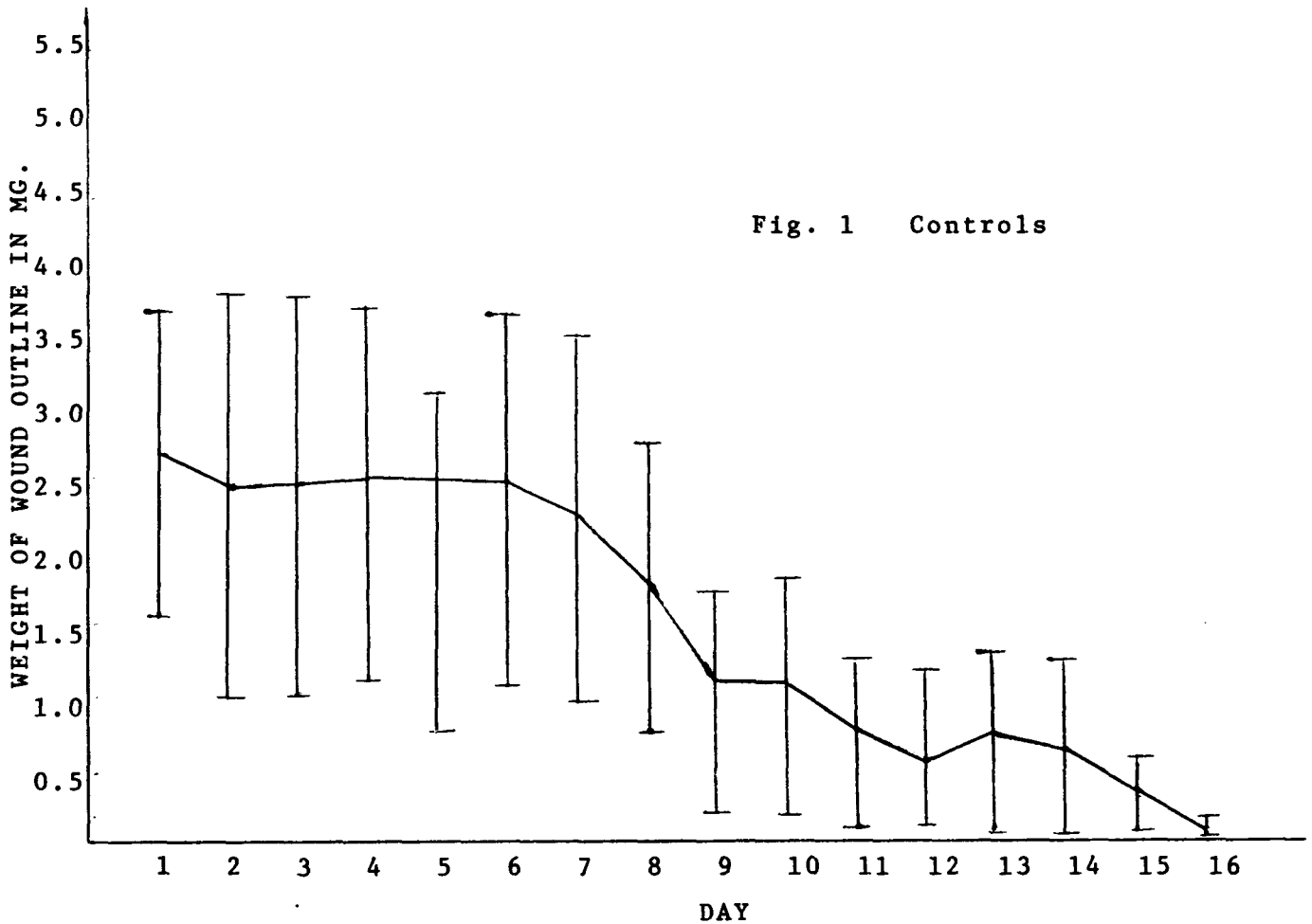


Fig. 1 Controls

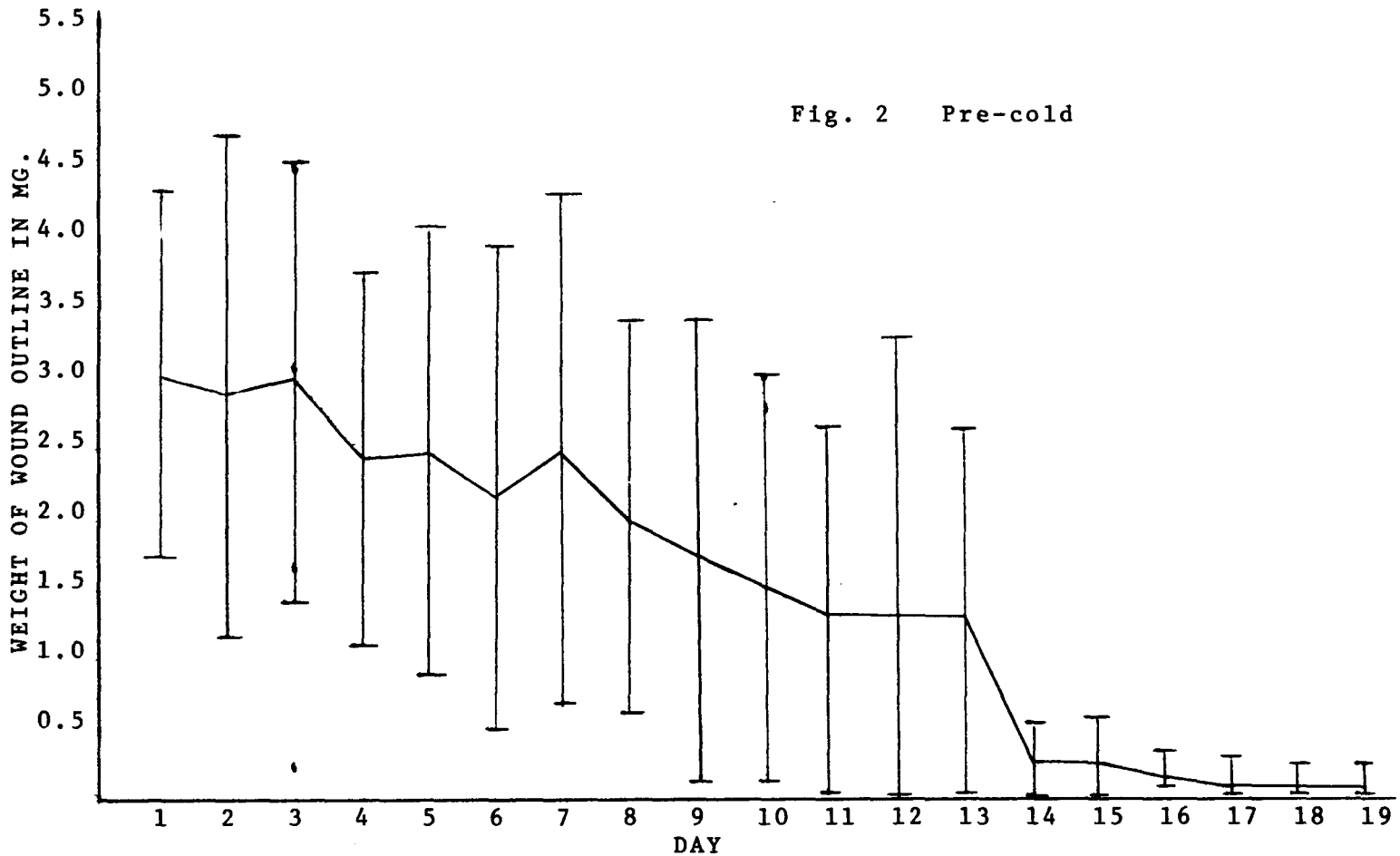


Fig. 2 Pre-cold

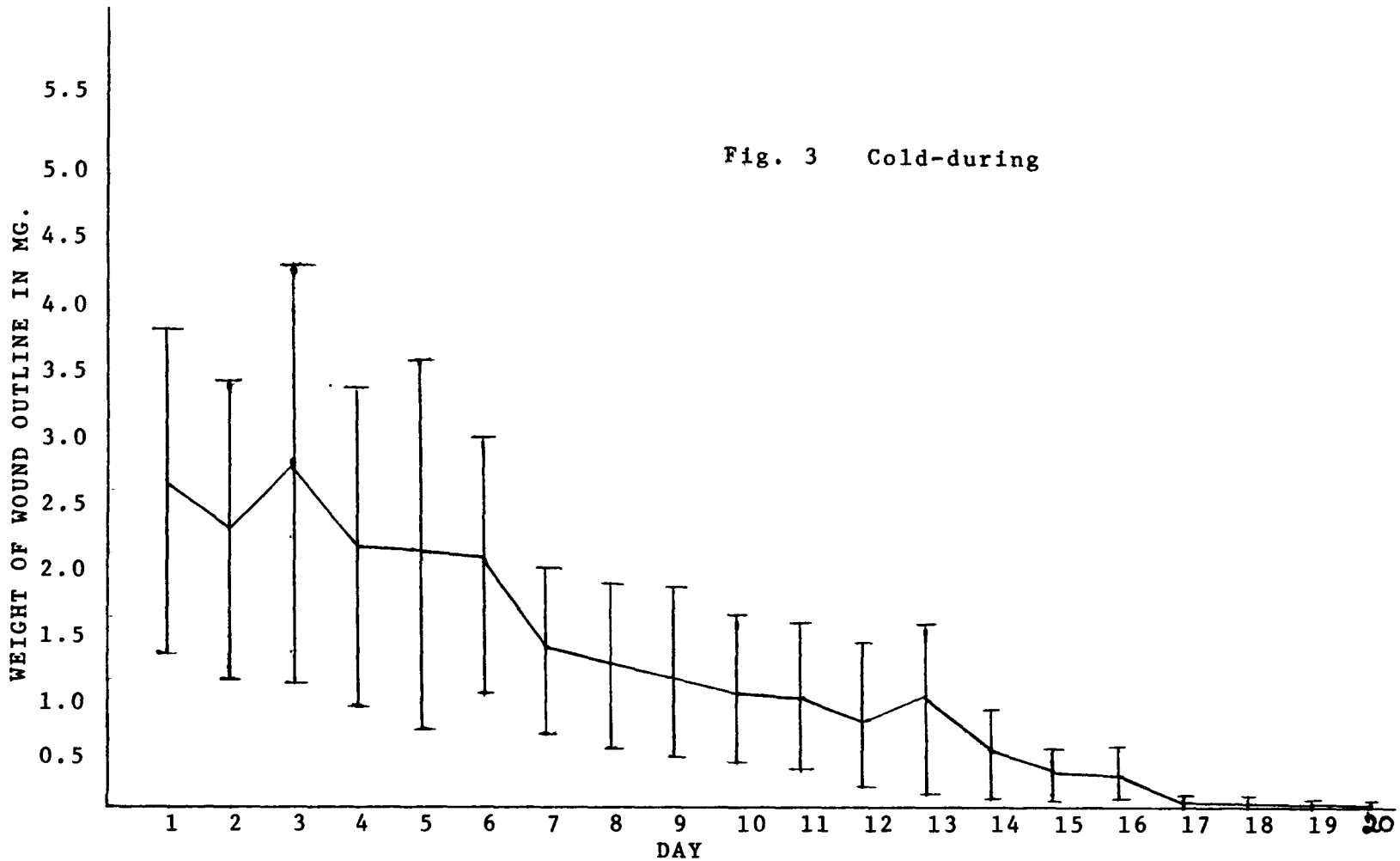


Fig. 3 Cold-during

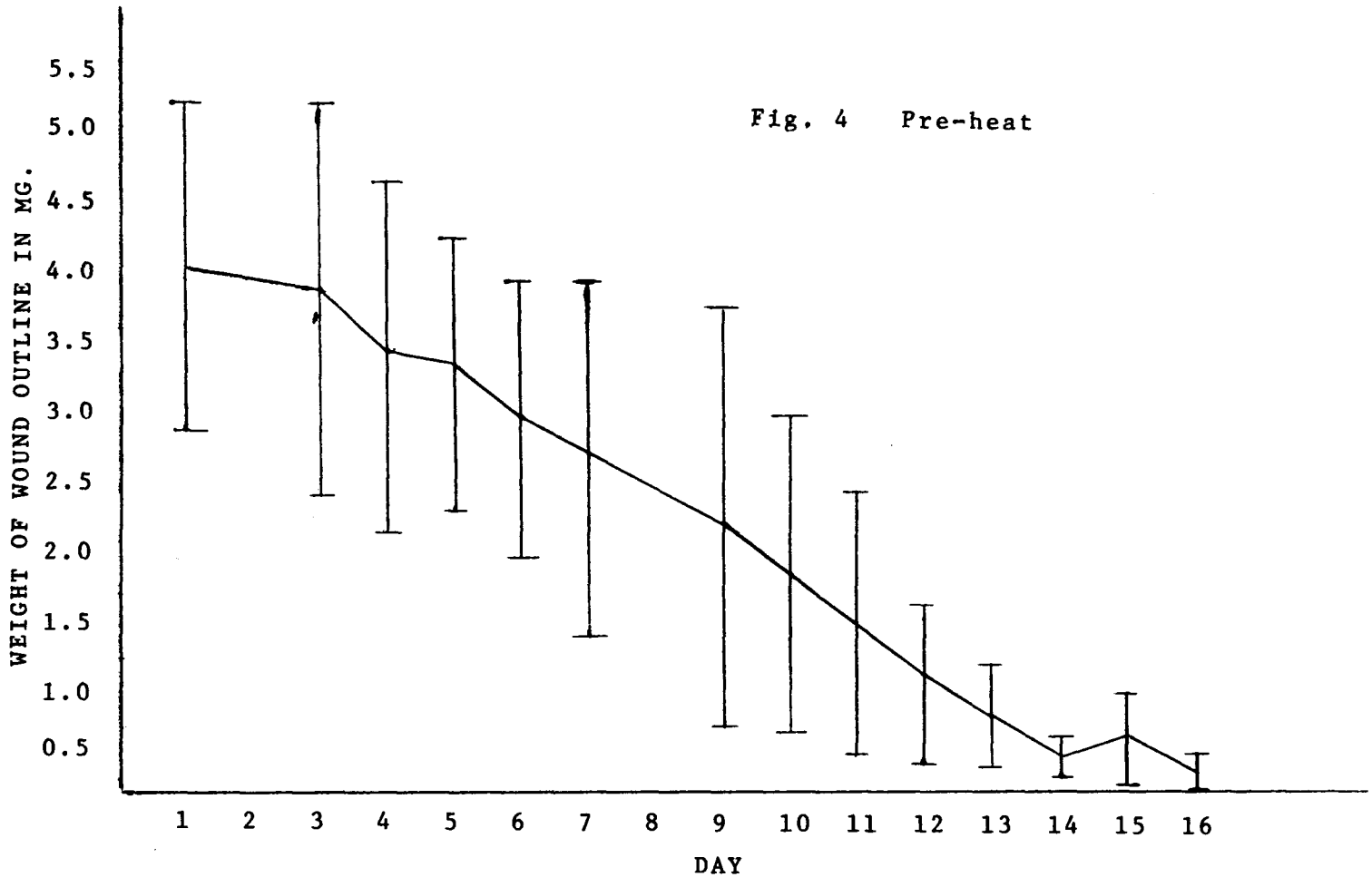


Fig. 4 Pre-heat

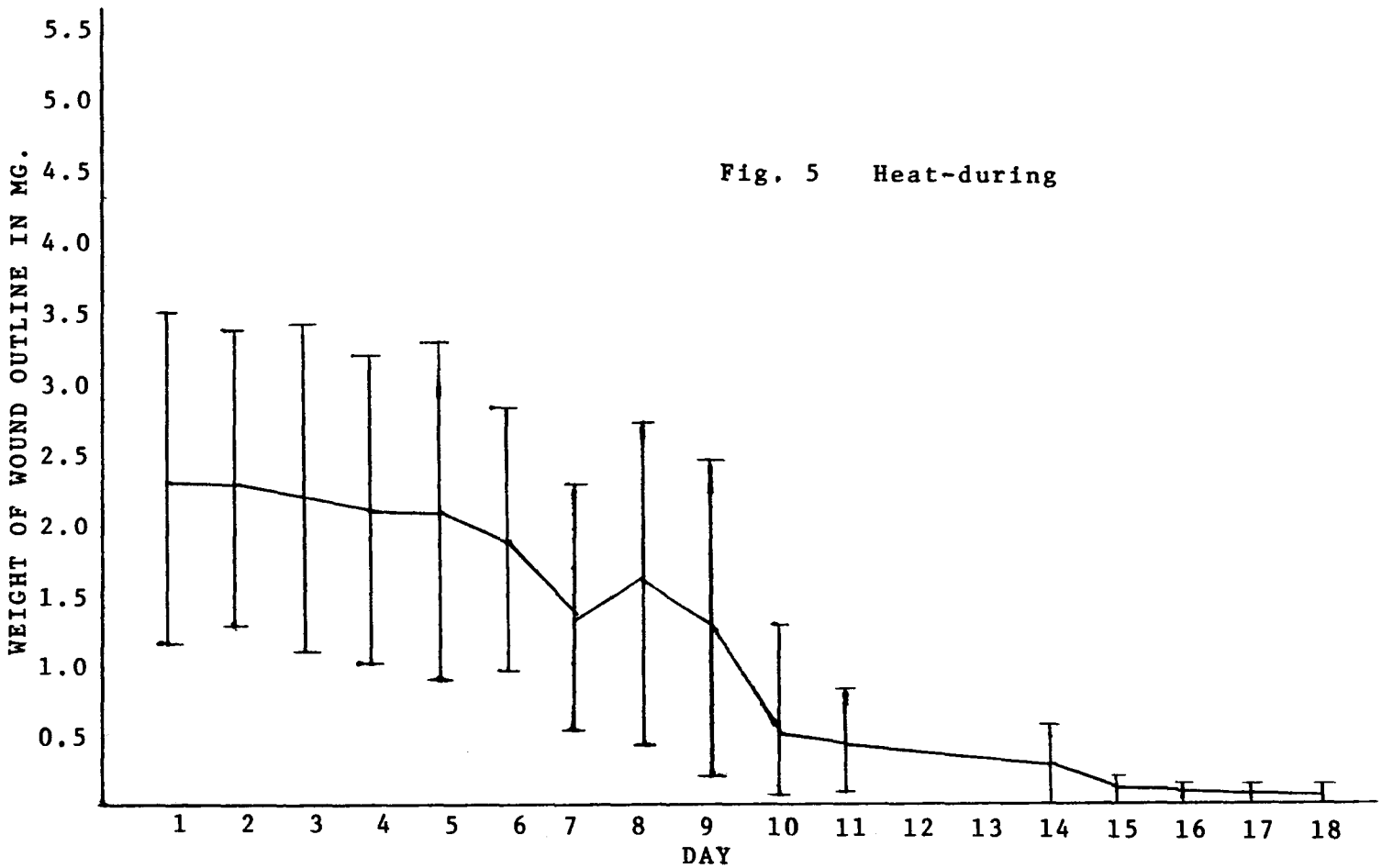


Fig. 5 Heat-during

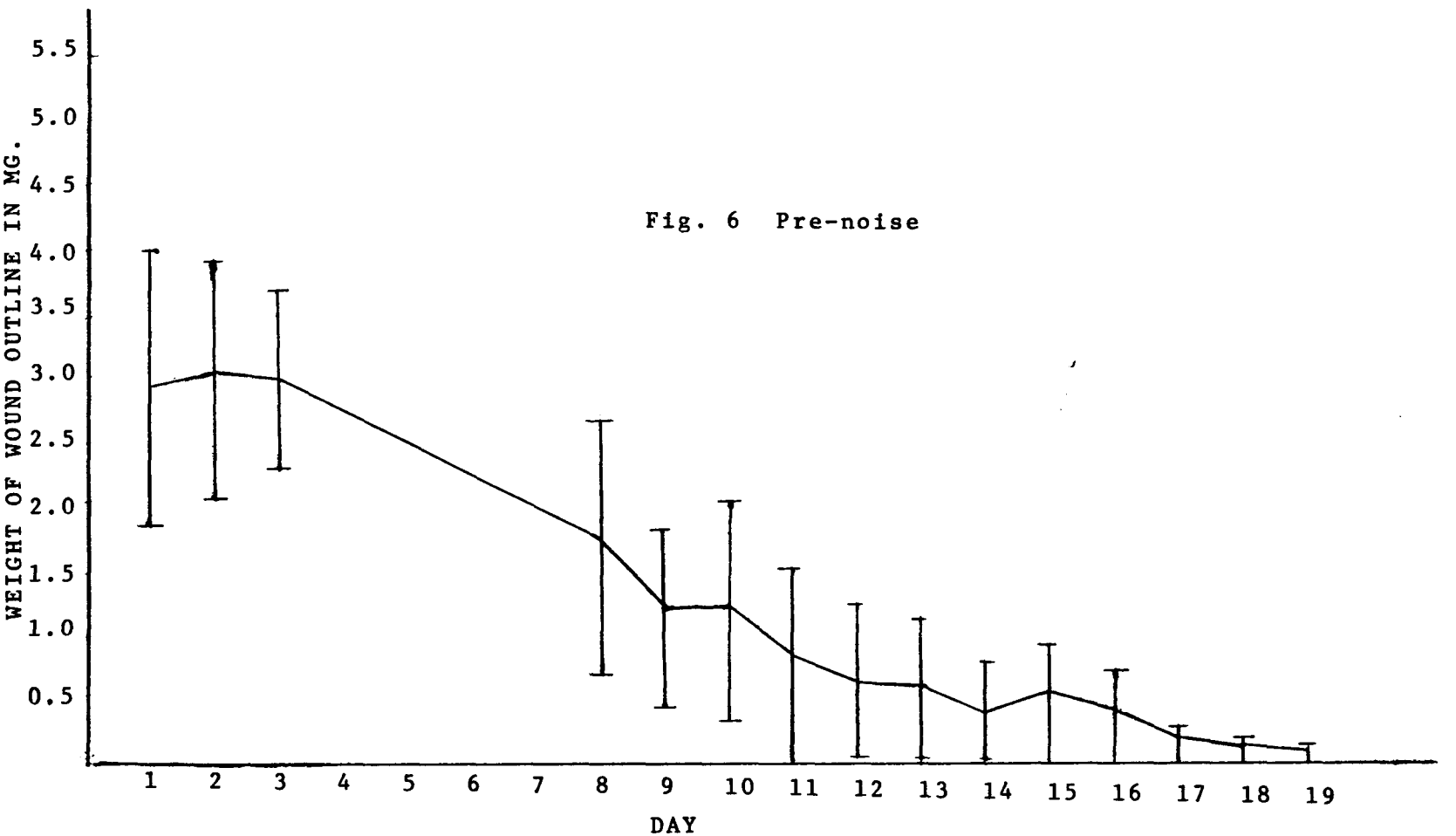
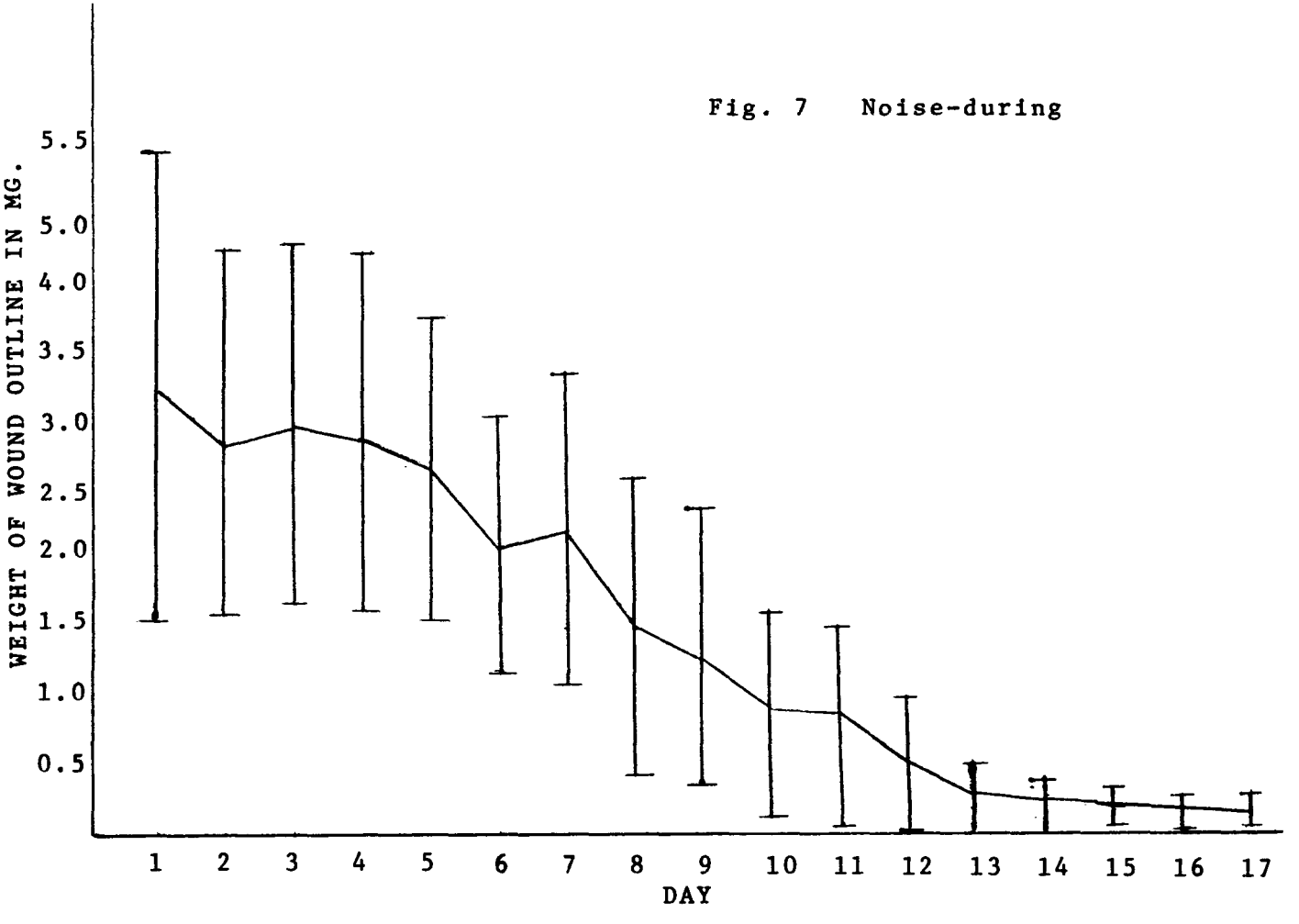
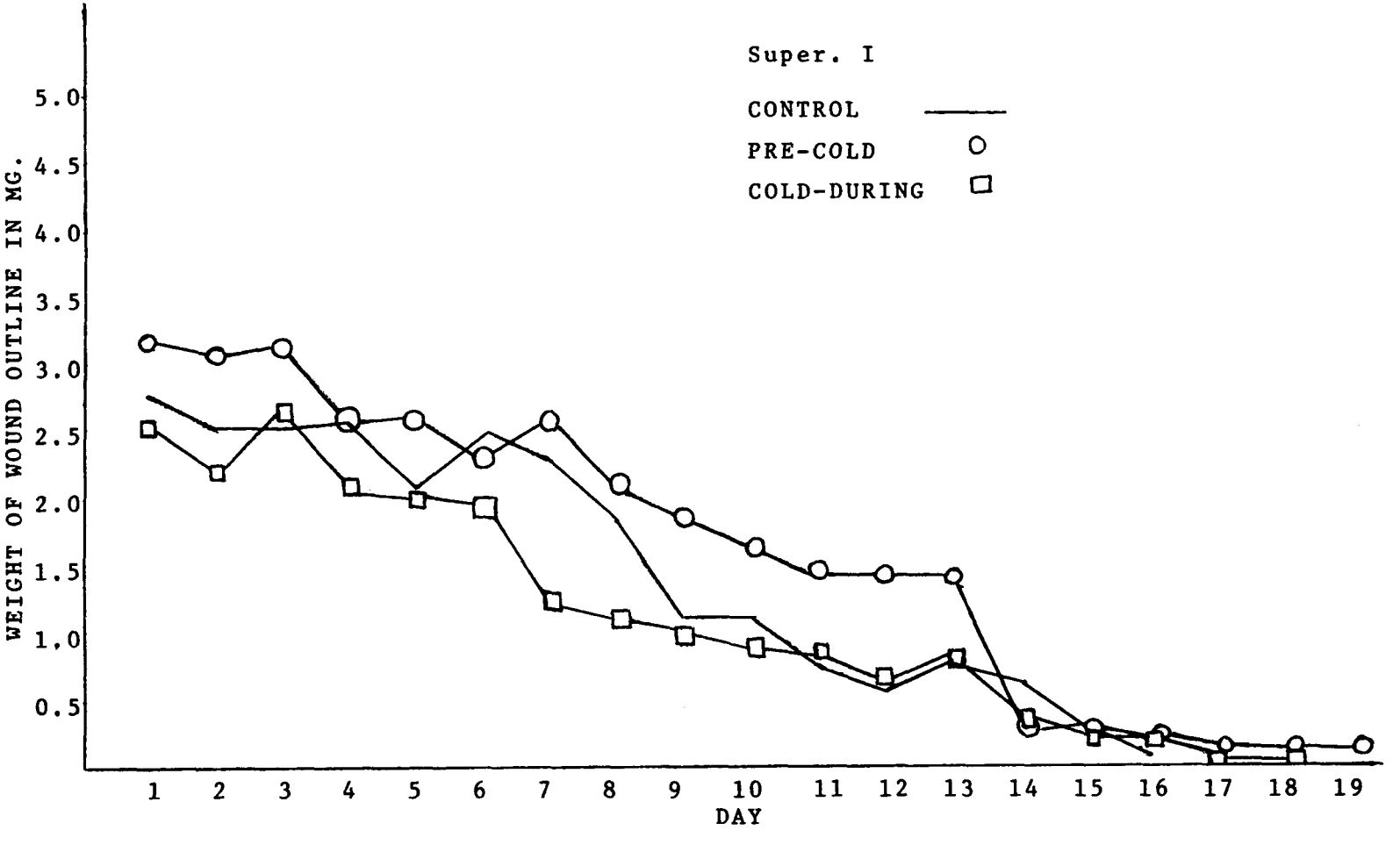
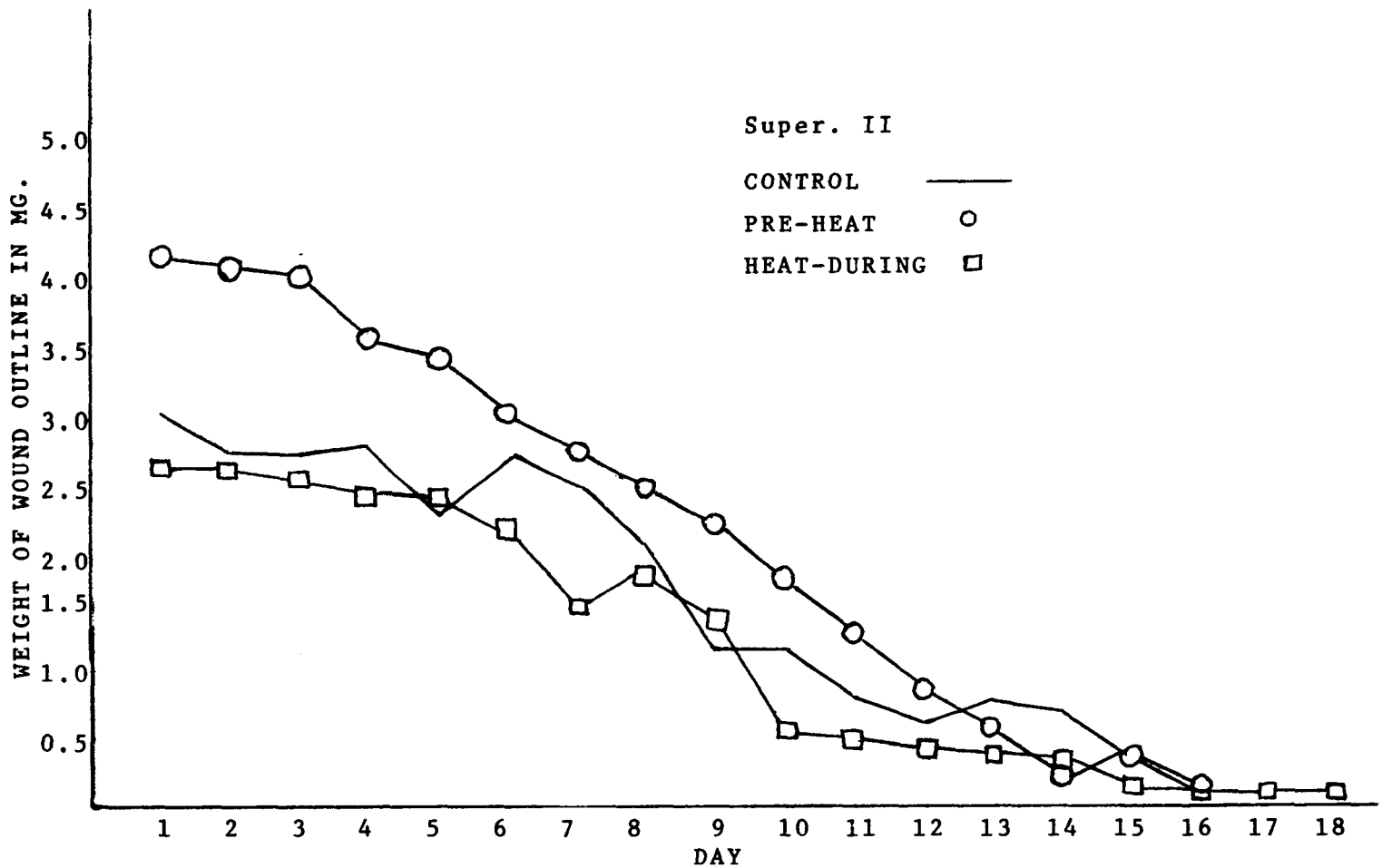


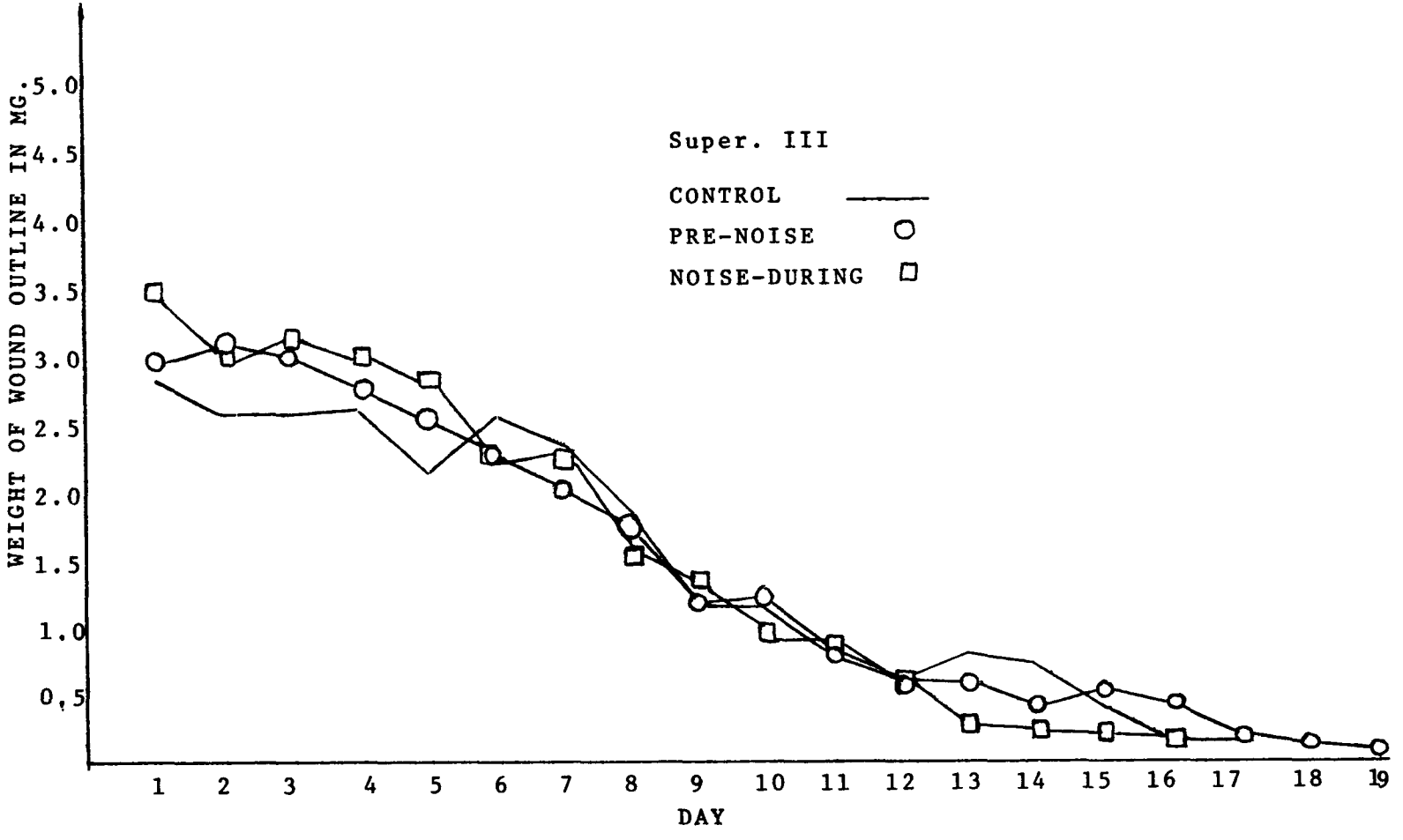
Fig. 6 Pre-noise

Fig. 7 Noise-during




Super. I
CONTROL
PRE-COLD
COLD-DURING
WEIGHT OF WOUND OUTLINE IN MG.
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
DAY


Super. II
CONTROL
PRE-HEAT
HEAT-DURING
WEIGHT OF WOUND OUTLINE IN MG.
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
DAY


WEIGHT OF WOUND OUTLINE IN MG.
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Super. III
CONTROL
PRE-NOISE
NOISE-DURING
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
DAY

Discussion

Blood vessels, fibrous tissue and epithelium all must regenerate in order to achieve wound closure. Microscopic sections of wounds treated locally with cortisone show less than the normal amount of endothelial proliferation, fibroplasia and epithelialization (Howes, 1954).

The stressors used in this experiment did not affect wound healing in the same way or to the same degree. Relative to controls, the stressors sometimes did not affect the initial contraction of the wound, the rate of healing, or the total-days-to-heal at all (pre-cold and noise-during; see Appendix I). It must be presumed, therefore, that these conditions were not sufficiently stressful to provoke the adrenocortical activity that retards skin healing (Spain and Molomut, 1952; Perasalo et al., 1953; Toivanen, 1960).

However, even in the two experimental conditions that did not differ significantly from controls there were indications of rate of healing differences that may depend on exposure conditions. Comparing Figures 2 and 7, it is possible to see that Ss healing after the end of stress exposure (pre-cold) and Ss healing during stress exposure (noise-during) follow a similar course for the first week.

Then, the effects of simultaneous exposure seem to depress the rate of noise-during Ss, and the effects of pre exposure, having dissipated, seem to allow the rate of pre-cold Ss to increase. The healing curves diverge, although their paths remain almost identically parallel from day 6 to day 11. It is important to note that there are internal rate differences during the course of healing which may be opposite to the direction of overall mean rate comparisons. For example, in the final analysis, pre-cold Ss have a slower mean rate of healing than noise-during Ss because of greater daily deviations, although noise-during Ss actually are healing at a slower rate than pre-cold Ss during half of the total healing time (day 6 to 14).

Noise

It seems clear (see Super. III) that both noise conditions were not stressful enough to retard the sprouting of blood vessels, the deposition of reticulin or the proliferation of fibroblasts. In both of these experimental conditions there is the least graphic divergence from the control.

Although Smookler et al. (1973) found 100±2.3dB to provide sufficient stress to induce hypertension and

molaral asymmetry in rats, and Jurtshuk et al. (1959) found 100±5dB sufficient to cause an increase in adrenal weight and a decrease in blood glutathione in female rats, this level of noise apparently was not adequate to retard healing of skin wounds in the male or female CF1 mice used in the present experiment.

This does not mean that 100dB of noise produced no effect on healing. The fact that pre-exposure to noise caused the wounds of pre-noise Ss to take significantly ($P < .05$) more days to heal and those of noise-during Ss to take somewhat longer than controls (P .20) indicates the direction of the inhibitive force of stress on vascular, connective and epithelial tissue growth.

Possibly, volume of noise similar to that used by Jensen and Rasmussen (1970) and Funk and Jensen (1967), 120-123dB, would produce a more pronounced effect. The fact that Funk and Jensen (1967) found decreased granuloma formation in mice, and Toivanen et al. (1960) found wound healing retardation in male rats, using noise (unmeasured) combined with other stimuli, shows that sound can affect connective tissue. Volume, pitch, schedule of presentation and vibrational components of noise may all contribute to this effect. Arguelles (1967) points

out that he and Blivaiss find that adrenocortical stimulation by high intensity sound can be independent of acoustical transmission. Normal men, wearing ear protectors to white noise of 140dB for 30 minutes, showed increases in blood 17-hydroxycorticosteroid (17-OHCS) and urinary 17-ketogenic steroids and 17-OHCS that persisted for two to three hours after the end of exposure (Arguelles, 1967).

An experiment in which mice are deafened by brief, painless exposure to high frequency and intensity noise and are later exposed to loud noise could reveal the extent to which the vibrational component of noise may be an adrenocortical activator. Congenitally deaf strains of mice could be used alternatively, but there may be other genetic differences, like predisposition to middle ear infections, that might affect the pituitary-adrenocortical system.

It is possible that rodents react to the vibration of the cages or directly to vibrations of noise that are not necessarily perceived by either tactile or auditory modalities but still create what Toivanen et al. (1960) call "psychic stress." At first, this may appear unlikely, but consideration of radiant energy, forces recognition of

the fact that light can burn and destroy skin cells without being seen or felt. Perhaps intense sound energy can induce endocrine activity without being heard. This possibility becomes more plausible when the ill effects, such as reduced fertility (Arguelles, 1967; Jurtshuk et al., 1959) and aggravation of gastrointestinal ulcer (Arguelles, 1967), of noise, to which people may feel they have accomodated, are taken into consideration (Sackler et al., 1959). This problem is especially pertinent to urban living, jet travel, and certain types of industrial and office work. Even exposure to the ignorable buzz of flourescent lighting may be sufficiently stressful to elicit a low-grade arousal state, which might predispose some individuals to diseases of adaptation when new stressors are introduced.

The factors of volume and vibration having been reviewed, there remain the pitch and presentation aspects of noise to consider. White noise was used in this experiment. This means that there were about equal amounts of all pitch ranges in the sound produced by the recording. Therefore, the effects of extreme pitch were controlled for. Previously, the schedule of presentation used here had been found effective in producing stress in rodents (Smookler et al.,

1973), but it is not known whether the significant results obtained by these experimenters and the trends noted in their study were the consequence of the shock-effect of intermittent presentation, of loud volume, or of vibration. Random negative reinforcement schedules consistently have been found to be most behavior-disintegrative and anxiety-provoking of all conditioning protocols (Gray, 1971). Therefore, it would be advantageous to explore the possibility that Ss may undergo stress reactions by virtue of programmed inconsistency of arousal, even when the stimulus used is not noxious *per se*.

Heat

Both heat groups differ significantly from the controls for all parameters except day 1 size of wound in heat-during Ss. Heat seems to be able to slow the rate of healing, as indicated by the significantly increased mean number of days to heal for pre-heat and heat-during Ss ($P < .01$ and $P < .02$, respectively). This situation may be due to specific effects of heat on skin, as well as to the general systemic response elicited by any stressor.

Riesenfeld (1973) has concluded that skin weight loss in heat-exposed rats "seems to be merely a nutrition-

specific response." However, his data would seem to support better the conclusion that inanition is only a contributory factor to skin weight loss in heat-exposure. There is total body weight loss in both starvation and heat-exposure, but compared to control, the relative skin weight loss in starved animals is less than that in heat-exposed animals. Therefore, heat itself must be partly responsible for the significant losses in absolute skin weight observed.

Iverson (1954) also has pointed out that inanition enhances the depressive effect of adrenal cortical hormones on wound healing through protein depletion beyond that caused by protein breakdown under stress.

Hence, one of the routes of heat stress influence may be starvation, which can effectively reduce the thickness of the skin (Riesenfeld, 1973), possibly resulting in a depressed capacity to contract upon wounding and to regenerate.

It is noteworthy that the least initial wound contraction of all groups occurred in pre-heat. It seems that the combined stress induced by heat and the resultant body weight loss had already established connective tissue

changes, before infliction of the wound, that would reduce the contractibility of the skin.

Therefore, the very significantly ($P < .001$) larger wounds of the pre-heat Ss compared to heat-during Ss and to controls ($P < .01$) must be a function of conditions that prevailed before healing started. The apparently contradictory finding that pre-heat Ss healed at a much faster rate and in slightly less absolute time than heat-during Ss may also be due to pre-treatment and to a possible direct relationship between increased size of wound and increased rate of healing, in the following way.

Heat-during Ss showed mean initial wound contraction sufficient to reduce the size of the original wound (i.e., 4.82±0.03mg) by almost half, after only 24 hours. Pre-heat Ss, on the other hand, showed mean initial contraction sufficient only to reduce the size of the original wound (also 4.82mg) by slightly more than one fifth. It is possible that the broader wound perimeters of pre-heat Ss triggered the increased mitotic rate which permitted the wounds of these Ss to heal in slightly less mean-total-days than those of heat-during Ss, in spite of the fact that there was more surface area to be closed in pre-heat wounds. This possibility is supported by the fact that

regardless of experimental conditions and consequent reasons for decreased initial wound contraction, the rate of healing that accompanies a large perimeter day 1 wound is always significantly more rapid than the rate that accompanies a small perimeter day 1 wound (see Appendix I). As Bullough (1960, quoted in McMinn, 1969) has shown, it is the injured cells, within 1mm of the cut edge of the wound, that become mitotically disinhibited for healing. This may be due either to the loss of a mitotic inhibitor by diffusion out of the injured cells or to production of a specific inhibitor of adrenaline, a mitotic suppressor, by these cells (McMinn, 1969). In either case, it would seem that the more cells in the perimeter of the wounds, the more chances for mitosis to ensue, and the more rapid will be the rate of healing.

There are other explanations for the repeated significant correlation between large wounds and rapid healing that will be addressed later. Here it must be observed only that stress during healing would seem, according to this interpretation, not to be able to suppress mitosis (i.e., increase mitotic duration) in <u>wound</u> tissue. This conclusion is well-supported by the research of Bullough and Laurence (1961). Therefore, stress does not appear to

be able to retard wound healing during the major portion of its duration, i.e., when wound closure is being completed by mitotic proliferation. This finding stands in contrast, but not in contradiction, to the fact that stress produced by noise (Kreyberg et al., 1965), sleep deprivation (Bullough and Laurence, 1961) or overcrowding (Bullough, 1952) does effectively suppress mitosis by increasing its duration in normal epidermis of mice.

A possible explanation of the rapid growth of hair in heat-during Ss may lie in a reduction of mitotic inhibition, caused by adrenaline or its oxidation product, adrenochrome (Bullough and Laurence, 1961), in hair follicle cells, as in wound tissue cells. The assumption here is that adrenaline inhibits hair growth as well as mitosis of normal skin cells. This assumption is indirectly substantiated by the converse finding (Baker, 1951) that adrenalectomy in rats causes a state of accelerated hair growth that lasts for up to 20 weeks after ablation. The rapid hair growth in heat-during Ss must have something to do with a heat-specific desensitization to adrenaline or other hair growth inhibitor, rather than with a generalization of this response from the wound cells to the hair follicle cells in the shaved region. If generalization were

possible, then it ought to occur also in the other groups where Ss have the same sort of wounds. In any case, the observation of rapid growth of hair in heat-during Ss has not been quantified here, and since it is contrary to the effect of heat observed by other investigators (Riesenfeld, 1973) and is apparently counteradaptive, it would not be appropriate to speculate further about its cause, without a controlled follow-up study.

Cold

Both the greatest and the least divergence from controls occurs under the cold experimental condition. Pre-cold Ss are more like control Ss than those in any other group, and cold-during Ss are more unlike control Ss than those in any other group. In spite of these respective differences from the controls, pre and during cold groups are not significantly different from each other for mean-days-to-heal. This is because of large internal variance for mean-days-to-heal in the pre-cold group and should not be construed to mean that the Ss of these two groups took the same amount of time to heal. In fact, in absolute mean number of days to heal, without consideration of internal variance, pre-cold and during-cold are more different

from each other in this parameter than are any other two groups (see Appendix I).

As in heat-exposure, the possibility of specific, as well as systemic, effects of cold in relation to wound healing retardation must be taken into account. Again there is the observation that cold-exposure, like heat-exposure, can cause weight loss (Barnett, 1959; Riesenfeld, 1973), reduce the absolute weight of the skin (Riesenfeld, 1973), and thereby impede healing.

One factor that unavoidably may have been accentuating the affect of the cold stressor was the shaving of a large area of the back, preparatory to excision. In heat, this may have reduced or, more likely, not affected the experience of stress; in noise, shaving most likely did not change the experience of stress at all. But in cold-during, the removal of a large portion of the coat may well have contributed to the significantly retarded healing of Ss in this group. This is probably why the cold stressor could only be tolerated for about 8 hours, after which time many of the shaved and wounded pilot Ss died.

Mice of the cold-during group were as normal as controls upon wounding. Their initial wound contraction was as great or greater than controls', which means there was

a relatively small perimeter of injured cells to become disinhibited to the mitotic suppression of adrenaline, followed, according to this theory, by the observed reduced rate of healing. Uninjured skin cells of Ss in cold-during must have been particularly mitotically inactive, since low skin temperatures cause sympathetic stimulation, which leads to adrenal medullary secretion possibly in excess of that produced by any acute, nonspecific reaction to a stressor.

Pre-cold Ss, in contrast, had been exposed to cold before wounding; their skin already had undergone the reductive changes of cold, documented by Barnett (1959), and, therefore, the initial wound contraction was significantly less than in cold-during Ss, leaving a large wound on day 1, a long perimeter of many disinhibited cells and the associated increased rate of healing.

Cold seems to have a more transient affect on skin than heat. This can be seen when the time-to-heal for pre groups of both conditions is observed. The pre-cold Ss are healing like normal controls, while pre-heat Ss are slowed significantly ($P < .01$). Both pre groups show a similar pre treatment trend of large day 1 wound size, but the depressive effect of cold on fibroblasts, collagen,

reticulin and ground substance apparently was not as pronounced as that of heat. This was confirmed by the gross observation of the skin while performing the excisions. Pre-heat Ss appeared to have the thinnest skin, which tended to lose the shape of the square cut-out immediately. The sharp corners of the wound became round, and the edges sagged outward.

The skin of mice seems to be able to recover from pre-exposure to cold and heal normally. However, simultaneous cold-exposure can retard wound-healing, as shown strikingly in cold-during Ss, who, in spite of having the smallest day 1 wounds, relative to controls, took significantly more days to heal than controls. Not only must systemic stress effects induced by cold participate in retarding healing via connective tissue depletion, but cold-induced peripheral vasoconstriction and reduced vascular proliferation also must contribute to the retardation. Solomon et al. (1964) found peripheral vasoconstriction in rats exposed to cold. Lundgren et al. (1959 quoted in McMinn, 1969) found delayed healing in incised wounds of rabbits subjected to cold. It was judged that this delay was due to local vasoconstriction because this delay disappeared almost completely after

denervation. Slow epithelial migration and contraction have been attributed to cold-exposure (McMinn, 1969). It is evident that vasoconstriction in the skin surrounding a wound would reduce circulation and cause oxygen deprivation in the area. Oxygen is of vital importance to cell division in tissues of mammalian epidermis (Bullough, 1955).

Since this discussion primarily has focused on the effects of adrenal cortical and medullary hormones, it is necessary to correct the impression that these are the only endocrine secretions that will influence skin and healing. Thyroid hormones also play an important role in adjustment to stress, especially in cold-exposure. Ducommen et al. (1967 quoted in Masón, 1968) found that the thyroid activity in rats decreases during the first 10 days of emotional stress caused by handling, sham injection and brief ether anesthesia, but that if general stressful stimulation continues, thyroid activity gradually increases over the next 10 days, until thyroid stimulating hormone in the blood exceeds normal levels. The time element in thyroid response to stress is important from the point of view of skin wound healing because most closure occurs during the first 10 days.

Since thyroid hormones act antagonistically to those of the adrenal cortex in relation to skin (i.e., thyroid hormones promote proliferation in stratum compactum), a depression of thyroid activity during the healing process would permit even greater impact of glucocorticoids on the wound target. For all stress-during Ss this would mean accentuation of any tendency to slow down healing. For all pre-stress Ss this could result in a boost for skin healing, since the thyroid would be in its recovery phase. The possible role of thyroid in healing of pre-stress Ss will be considered again in the discussion of increased or normal rates of healing observed in pre groups.

The case of cold presents an interesting contradictory situation. In so far as cold activates the adrenals, as a systemic stressor, it may inhibit the thyroid, but as cold is also a CNS-activator it stimulates the thyroid via the hypothalamus (Turner and Bagnara, 1971). Thyroid activity rises rapidly in rats exposed to cold and results in increased metabolic rates (Turner and Bagnara, 1971). It may be inferred, therefore, from the decreased rate of healing and increased number of days to heal in cold-during Ss, that systemic stress, caused by cold and handling of cold Ss, when they were placed into and removed from the temperature-

controlled room at the beginning and end of each 8 hour session, was sufficient to produce adrenal activity that superseded the effects of cold-induced thyroid activity.

At the beginning of this discussion, the two groups, pre-cold and noise-during, which did not differ significantly from controls were considered. Now the two groups, pre-heat and cold-during, which differ most significantly from controls will be discussed, particularly in connection with initial wound contraction and rate of healing.

In the pre-heat group, initial wound contraction is small; therefore, day 1 wounds are large. In the cold-during group, initial wound contraction is great; therefore, day 1 wounds are small. It is also observed that pre-heat and cold-during are not significantly different from each other in mean days to heal. Therefore, it would seem that rate of healing (b) is strictly a function of day 1 wound size. In fact, it may be true that rate, as indicated by the slope (b) of the healing curve, is a Cartesian artefact*, in so far as the graphs are linear. If this is true, then much of the course of healing depends on what happens at the wound site in the first 24

* Observation courtesy of Dr. C. Ember, Hunter College, N.Y.C. and Dr. D. Mitra, Bell Telephone Laboratories.

hours after wounding.

Wound closure in healthy, unstressed animals has been shown to consist of two phases: contraction and cicatrization (McMinn, 1969). As already stated, stress does not affect mitotic proliferation in wound tissue (Bullough and Laurence, 1961). Therefore, it may be that stress has its most crucial effect on the contraction phase of healing. Once connective tissues have undergone some degree of involution, as in pre-stress Ss, the starting point of the graph (i.e., day 1 wound size) is fixed high, while that of during Ss is set as in normal controls or lower.

This seems to be the essential difference between the pre and during groups, which pre-heat and cold-during illustrate most clearly. In pre-stress Ss, stress reactions change the skin before wounding, causing marked depression of initial wound contraction (i.e., contraction which occurs in the first 24 hours). In during-stress Ss, stress acts to change the skin while healing is in progress, causing retardation of cicatrization (i.e., closure that occurs in the 14 to 16 days subsequent to the rest day after wounding). Although there is a great time disparity between the contraction phase and the cicatrization phase, this should not lead to the judgment that cicatrization provides

the greater contribution to closure of wounds. As mentioned earlier, in the case of heat-during, contraction can account for one half or more of the total closure. Mitotic proliferation, which is the foundation for filling in the wound with new tissue, is not the source of this early wound size reduction. In fact, mitosis does not maximize in mouse skin until two to three days after wounding (Bullough and Laurence, 1961).

Wound contraction was significantly ($P < .01$) greater for the during groups, taken as a unit, than for the pre groups, taken as a unit, which would support this new theory that time of application of stress with respect to wound infliction is critical to the initial stages of healing.

The primary foci of stress on skin, with regard to its ability to contract, are fibroblasts and histamine production. Fibroblasts are necessary for collagen production; histamine is necessary for granulation. Both collagen and granulation are needed to maintain the tensile strength and thickness of the skin (McMinn, 1969), which enables the skin to hold a cut outline and contract readily. Church and Warren (1968 quoted in McMinn, 1969) found fibroblasts on the edge of the wound to be of prime

importance in contraction of wounds in the web membrane of fruit bats. Higton and James (1964 quoted in McMinn, 1969) found wound contraction in rabbits to be proportional to granulation.

A simple way to corroborate the finding that pre-exposure to stress impedes the "snapping back" phase of wound closure would be to measure the skin weight of equal size skin cut-outs at the time of excision in normal Ss and pre-stress Ss. If the normal Ss have heavier cut-out weights and these correlate with smaller mean day 1 wound size, then it is true that healthy skin is thicker and initially contracts faster than skin of pre-stress Ss, who, conversely, would show lighter cut-out weights correlated with larger mean day 1 wound size. A study, such as this, in which skin thickness and its relation to contractibility are examined after various stressor and hormone pre-treatments is in preparation.

Another explanation of the very different healing trends in pre and during units is that of recoup effect in pre-treated Ss. Recoup is the concept that second stressors are responded to with less intensity than first stressors. Although this theory is not solid, there is some evidence to support it. Sandblom (1944 quoted in

Chassin et al., 1953) found that second wounds healed faster than first wounds in rabbits. Frenkl (1971) found that rats pre-conditioned with forced swimming showed reduced response to histamine and surgical stressors. A similar type of recovery coupled with the increasing thyroid activity, mentioned earlier, may have been working to allow pre-stress Ss to heal in the same time as or faster than controls, in spite of day 1 wounds that were equal to or greater than those of controls, and in spite of exposure to stressful stimuli. This recovery or apparent immunity to stress typifies the adaptive phase, which Selye has described for the G.A.S. (Selye, 1955, 1973). It is believed that pre Ss in these experiments were not only entering a phase of accomodation but were better equipped, through pre-exposure, to cope with the stress of wound healing.

In contrast, during groups, as a unit, heal at a significantly ($P < .05$) slower rate and over a significantly ($P < .02$) longer period of time than controls, in spite of day 1 wounds that are equal to or smaller than those of controls. This must be attributed to the various consequences of the acute phase of stress brought on by the double effect of noise, heat or cold superimposed on wounding.

Comparability of stress effects on rate of healing in mice ought to be good with respect to man. Howes (1954) found the same rate of healing in skin wounds of mice and men, in spite of the higher metabolic rate of smaller mammals. However, it is important to remember that wounds that take approximately 2 weeks to heal in mice are comparable in size to wounds that take about 11 months to heal in man. The point is that the wounds inflicted in this experiment were large and of long duration relative to the body size and lifespan of the Ss. Therefore, it would seem that the responsiveness of the pituitary-adrenal and CNS-thyroid systems in mice must be rapid, and Ss must pass through the three phases of G.A.S. quickly, in terms of absolute time, when compared to larger mammals like man. This is borne out by the fact that initial mitotic bursts in wounds of mice are seen after 12 to 24 hours, but not until 42 to 60 hours in man (McMinn, 1969).

Summary

The results of this experiment lead to the conclusion that certain stressors can affect wound healing by changing the quality and thickness of the skin and thereby its ability to contract. Possible specific effects of various

stressors, as well as their general non-specific consequences via adrenal cortex and medulla and thyroid glands, have been considered.

The importance of time of exposure to initial contraction and resultant wound perimeter with regard to rate of healing, via disinhibition of cells for mitosis, and to relative amount of closure was emphasized.

Pre and during exposure conditions were compared. Pre Ss were found to have significantly larger wounds that healed at significantly faster rates than those of during Ss. Pre-exposed groups, as a unit, were not significantly different from controls for mean-days-to-heal or for rate of healing. The recoup effect and adaptive phase were discussed in this connection. Simultaneously exposed groups, as a unit, had greater initial contraction and smaller wounds but took significantly more days to heal and had significantly slower rates of healing than controls. The double effect of during exposure and acute phase were discussed in this connection.

The need for further study of the specific component of stressors and the endocrine control of skin thickness and contractibility was mentioned.

APPENDIX I: COMPILED RESULTS

GROUP	N	$\bar{x}$ DAYS TO HEAL	t with CONTROL	t PRE vs. DURING	t ♂ vs. ♀	b	t with CONTROL	t PRE vs. DURING	t ♂ vs. ♀	$\bar{x}$ DAY 1 WT. of outline in mg.	t with CONTROL	t PRE vs. DURING	t ♂ vs. ♀
CONTROL	21	14.86			n.s.	-0.24				2.98			n.s.
PRE-COLD	21	14.81	n.s.	n.s.	n.s.	-0.24	n.s.	P<.001 4.17	n.s.	2.72	n.s.	2.43	n.s.
COLD-DURING	20	16.30	2.83 P<.01	n.s.	n.s.	-0.15	P<.001 4.63		n.s.	2.12	3.57 P<.001	P<.02	n.s.
PRE-HEAT	17	16.12	2.86 P<.01	n.s.	n.s.	-0.32	4.07 P<.001	8.39	n.s.	3.85	3.44 P<.01	5.16	n.s.
HEAT-DURING	20	16.30	2.62 P<.02	n.s.	n.s.	-0.18	3.32 P<.01	P<.001	n.s.	2.54	n.s.	P<.001	n.s.
PRE-NOISE	14	16.36	2.07 P<.05	n.s.	n.s.	-0.22	n.s.	2.93	n.s.	2.64	n.s.	2.77	n.s.
NOISE-DURING	20	15.50	n.s.	n.s.	n.s.	-0.28	n.s.	P<.01	n.s.	3.31	n.s.	P<.01	n.s.
	LAST DAY N								LAST DAY N				FIRST DAY N
ALL PRE Ss	52	15.65	n.s.	n.s.	n.s.	-0.26	n.s.	4.01	52	3.05	n.s.	2.81	57
ALL DURING Ss	60	16.03	2.59 P<.02	n.s.	n.s.	-0.20	2.02 P<.05	P<.001	60	2.63	n.s.	P<.01	62

Appendix II: Chi-square tests

Fast b	Slow b	
3	0	Large wound
0	3	Small wound

$X^2=15.00$

$P < .001$

Fast b	Slow b	
2	1	Pre-exposed
1	2	During-exposed

$X^2=4.33$

$P < .001$

Large	Small	
2	1	Pre-exposed
1	2	During-exposed

$X^2=4.33$

$P < .05$

Appendix III

RAW DATA -- CONTROL

DAY	1	2	3	4	5	6	7
S							
1	3.39	2.49	2.61	2.65	2.58	2.77	2.25
2	2.74	1.73	2.14	1.77	1.35	1.76	1.22
3	3.68	3.18	3.18	2.89	2.82	3.86	3.36
4	3.08	3.04	2.61	2.68	2.04	1.91	1.59
5	2.34	2.02	2.04	2.17	1.80	2.22	2.23
6	3.45	2.25	1.97	2.13	2.00	2.02	1.66
7	3.64	3.27	3.42	2.80	2.77	3.04	1.80
8	3.69	3.28	2.74	2.70	2.84	2.50	2.72
9	2.40	1.00	1.09	1.14	.99	1.21	1.21
10	3.27	4.03	2.89	2.61	2.66	2.24	1.86
11	2.24	2.11	1.93	2.58	1.97	2.62	1.96
12	3.25	2.91	3.68	3.32	2.50	2.62	2.14
13	2.37	2.15	2.22	2.32	1.85	2.19	1.80
14	2.86	2.63	2.31	2.39	2.40	1.91	1.99
15	2.88	3.72	2.82	2.32	2.03	2.62	1.23
16	2.64	2.06	2.62	2.57	2.55	1.59	1.63
17	2.79	3.15	3.00	3.37	2.80	3.09	2.06
18	3.00	3.28	3.03	3.20	2.39	1.72	1.06
19	1.60	1.31	1.43	1.33	.78	1.18	1.00
20	3.30	2.80	1.67	1.91	1.69	1.38	1.25
21	3.87	3.80	3.98	3.95	3.28	3.37	3.65

Appendix III

RAW DATA -- CONTROL (CONT.)

DAY	8	9	10	11	12	13	14
S							
1	1.40	1.27	0.79	0.70	0.25	0.17	healed
2	0.80	0.56	0.56	0.67	0.01	0.16	healed
3	2.81	1.85	1.96	0.85	0.77	0.53	0.01
4	1.78	0.89	1.00	0.16	healed		
5	2.61	1.37	0.88	0.25	0.05	0.10	0.10
6	0.95	0.55	0.59	0.45	0.02	healed	
7	1.80	1.33	0.99	0.95	0.78	0.27	0.24
8	2.00	1.21	0.84	0.39	0.07	0.09	0.01
9	1.11	0.97	0.82	1.04	0.74	0.17	healed
10	1.18	0.85	0.25	0.12	0.18	healed	
11	2.37	1.55	1.45	0.74	0.14	0.07	0.06
12	1.47	1.03	0.65	0.46	0.15	0.22	0.05
13	1.39	1.21	1.04	1.21	0.22	0.10	0.02
14	1.91	1.75	1.75	1.39	1.13	1.33	1.31
15	1.60	1.02	0.38	0.37	0.22	0.15	0.01
16	0.87	0.77	0.55	0.60	0.10	healed	
17	1.68	1.37	0.48	0.41	0.25	0.14	0.09
18	1.16	1.01	0.90	0.84	0.23	0.16	0.14
19	0.82	0.28	0.20	0.20	0.04	0.18	0.14
20	1.30	0.91	0.90	0.94	0.81	0.36	0.24
21	2.74	1.79	1.65	0.52	0.39	0.41	0.30

Appendix III

RAW DATA -- CONTROL (CONT.)

DAY	15	16	17	18
S				
1				
2				
3	healed			
4				
5	.10	.14	.04	healed
6				
7	.15	healed		
8	healed			
9				
10				
11	healed			
12	healed			
13	.04	healed		
14	.61	.08	healed	
15	healed			
16				
17	died			
18	healed			
19	healed			
20	.17	.13	healed	
21	healed			

APPENDIX III

RAW DATA COLD-DURING

DAY	1	2	3	4	5	6	7
S							
1	1.96	1.69	1.74	2.07	1.49	0.96	0.83
2	2.10	1.81	1.01	0.98	0.94	1.03	1.10
3	1.47	1.75	1.61	1.92	1.62	1.91	1.42
4	2.28	1.80	1.83	2.60	2.01	1.40	1.27
5	2.05	2.26	2.01	1.85	1.75	1.26	1.38
6	2.06	2.36	1.92	1.97	2.14	2.03	1.28
7	2.88	3.39	3.34	3.34	2.12	1.87	1.58
8	2.83	2.38	1.93	1.20	2.14	1.45	1.23
9	2.38	2.85	3.12	1.27	1.58	1.14	1.05
10	3.79	3.37	4.33	3.21	3.56	2.95	1.91
11	1.22	1.04	0.96	0.90	1.07	0.88	0.80
12	1.24	1.33	1.08	1.28	1.28	1.43	1.25
13	2.28	2.48	2.55	1.92	2.02	1.80	1.39
14	1.97	1.89	1.13	1.27	0.64	1.16	0.54
15	2.53	2.54	died				
16	1.47	1.32	1.16	0.79	0.94	0.87	0.67
17	2.14	2.35	2.46	2.00	1.81	1.62	1.53
18	1.49	1.50	1.38	0.95	1.01	0.99	died
19	2.08	2.13	2.70	2.87	1.00	1.33	1.15
20	1.29	1.19	1.63	1.52	1.60	1.26	0.86
21	2.06	1.78	1.58	1.41	1.12	1.65	1.30
22	2.96	2.85	3.26	2.94	1.37	1.88	1.14

Appendix III

RAW DATA COLD -- DURING (CONT.)

DAYS	8	9	10	11	12	13	14
1	.68	.61	.62	.75	.50	.53	.22
2	1.12	.39	.38	.60	.34	.19	.14
3	.46	.61	.40	.32	.13	.04	
4	.83	.45	.45	.63	.34	.28	.07
5	1.26	.82	.83	.61	.78	.21	.05
6	.99	1.03	1.00	1.00	.46	.21	.08
7	1.23	.90	.66	.93	.39	.17	.07
8	.86	1.00	1.12	.78	.46	.45	.30
9	.73	.90	.65	.30	.57	.27	.08
10	1.81	1.71	1.50	1.46	1.27	1.48	.67
11	.68	.81	.54	.54	.47	.16	healed
12	1.38	1.13	1.36	1.31	.93	.63	.27
13	1.31	1.00	.99	1.12	1.24	.84	.72
14	.78	.55	.26	.26	.39	.17	.38
15							
16	.59	.48	.36	.37	.23	.24	.06
17	1.64	1.63	1.32	1.25	1.14	.68	.51
18							
19	1.05	.84	.57	.75	.47	.48	.17
20	1.17	.82	.80	.87	.72	.44	.36
21	1.06	.93	.91	.76	.68	.18	.12
22	1.14	.98	.93	.52	.51	.38	.54

Appendix III

RAW DATA COLD -- DURING (CONT.)

DAY	15	16	17	18	19	20
S						
1	.21	.13	.09			
2	healed					
3						
4	healed					
5	.08	.02	healed			
6	healed					
7	.07	healed				
8	.09	.08	.06	1.05	healed	
9	.14	.13	.01			
10	.24	.48	.04	.03	.02	healed
11						
12	.13	healed				
13	.43	.09	healed			
14	healed					
15						
16	healed					
17	.11	.02	.16			
18						
19	.07	.23	healed			
20	.02	healed				
21	healed					
22	healed					

Appendix III

RAW DATA PRE-COLD

DAY	1	2	3	4	5	6	7
S							
1		1.80	2.07	2.01	1.86	.90	1.27
2		2.54	2.48	2.92	2.47	1.93	1.67
3		3.27	2.93	2.54	2.75	2.31	1.79
4		2.00	1.21	1.72	1.13	1.22	.49
5		4.52	4.97	4.73	3.91	3.47	3.21
6		3.00	3.02	3.36	3.89	3.80	3.49
7		3.33	2.76	2.86	2.47	2.51	1.74
8		2.83	2.92	2.64	2.72	2.31	1.59
9		2.53	2.07	2.69	2.42	2.65	2.31
10		2.55	3.19	2.72	2.01	2.02	1.55
11		2.18	1.68	1.81	1.13	1.30	.81
12		3.41	2.64	2.70	2.95	2.21	1.93
13		2.90	2.23	2.17	2.03	1.50	1.75
14		2.69	3.13	2.37	2.25	1.70	1.34
15		1.80	1.71	1.48	1.60	1.82	1.65
16		2.62	2.30	1.90	2.14	2.01	2.26
17		2.06	2.05	2.26	2.10	1.56	1.18
18		2.52	2.68	3.22	3.16	3.88	3.51
19		2.15	2.56	2.04	1.86	1.76	2.08
20		3.03	2.87	3.55	3.68	4.27	4.11
21		3.30	3.62	2.81	1.87	1.43	1.12

Appendix III

RAW DATA PRE-COLD (CONT.)

DAY	8	9	10	11	12	13	14
S							
1	.98	1.54	.73	.74	.74	.28	.03
2	1.27	1.46	.93	.49	.48	.27	healed
3	1.87	.82	.18	.48	healed		
4	.77	.77	.66	.10	.14	.04	healed
5	2.36	1.74	3.53	.47	.41	.03	.08
6	3.34	3.22	.80	2.72	2.72	3.43	2.70
7	1.14	1.29	.55	.41	.15	.06	.02
8	1.20	1.30	2.33	.59	.38	.04	.03
9	2.34	2.16	.31	1.87	2.13	1.98	.50
10	.69	.62	.10	.30	.25	.13	healed
11	.93	.67	1.71	.11	.01		
12	2.02	2.03	1.31	.57	.31	.27	.07
13	2.04	2.02	1.13	1.32	.89	.63	.73
14	.74	.94	.54	.32	.42	.02	.16
15	1.28	.84	1.59	.39	.13	.14	healed
16	1.74	1.44	.75	1.00	1.23		
17	1.18	.76	.99	.57	.05		
18	2.47	2.40	1.35	1.17	.95	.46	.29
19	.85	1.30	3.51	1.41	1.05	1.00	.49
20	4.49	3.53	.53	3.14	1.04	.65	.49
21	.76	.70		.47	.13	.12	healed

Appendix III

RAW DATA PRE-COLD (CONT.)

DAY	15	16	17	18	19	20	21
S							
1	.02	.01	.01	healed			
2							
3							
4							
5							
6	.19	.10	.01	.02	.02	.01	healed
7							
8							
9	.30	.61	.34	.31	.24	.25	.24
10							
11							
12	healed						
13	.23	.03	.06	.01	healed		
14	healed						
15							
16							
17							
18	.19	.05					
19	.20	healed					
20	.58	.10	.07	.04	healed		
21							

Appendix III
RAW DATA PRE-COLD (CONT.)

DAY	22	23	24	25	26
S					
1					
2					
3					
4					
5					
6					
7					
8					
9	.22	.25	.09	.03	healed
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					

APPENDIX III

RAW DATA HEAT-DURING

DAYS	1	2	3	4	5	6
1	2.83	2.26	1.94	1.82	1.28	2.00
2	3.13	3.00	2.42	1.79	2.02	1.42
3	2.03	1.89	1.38	1.30	0.90	1.42
4	2.67	2.84	1.85	1.55	1.31	1.44
5	2.68	2.70	2.18	1.45	1.59	1.28
6	3.03	2.71	2.29	1.82	1.53	1.66
7	3.67	3.50	3.53	3.29	3.47	2.93
8	2.24	1.67	1.36	1.37	1.30	1.65
9	2.37	1.66	1.22	1.05	1.08	1.00
10	1.74	1.32	1.13	1.06	1.16	0.99
11	2.26	1.93	1.65	1.36	1.45	1.16
12	2.96	3.46	2.33	2.16	1.72	2.03
13	1.67	1.30	1.56	1.12	1.67	1.13
14	2.41	2.03	1.70	1.48	1.54	1.41
15	2.82	2.25	1.98	1.56	1.02	1.28
16	1.83	2.02	2.14	1.86	1.73	1.02
17	2.83	2.44	2.42	2.35	1.65	1.72
18	3.07	2.60	2.82	2.81	2.74	1.72
19	2.73	1.60	1.10	1.01	1.32	1.49
20	1.80	1.61	1.26	1.07	1.26	1.18

Appendix III

RAW DATA HEAT -- DURING (CONT.)

DAY	7	8	9	10	11	12	13
S							
1	.91	.44	.34	.43	.27		
2	1.05	.86	.95	.85	.26		
3	.94	.77	.87	.65	.69		
4	1.02	.87	.63	.35	.28		
5	.91	1.04	.85	.94	.20		
6	1.68	1.30	1.32	.63	.62		
7	2.38	2.84	2.54	1.03	.44		
8	1.15	.65	.55	.77	.71		
9	.52	.74	.42	.31	healed		
10	1.10	.40	.12	.15	.18		
11	1.61	1.25	1.37	1.32	.83		
12	1.59	.76	.80	.55	.80		
13	1.05	1.04	.70	.55	.20		
14	1.30	1.73	1.09	.71	.12		
15	.95	.96	1.37	.35	.27		
16	.54	.59	.27	.14	.12		
17	.87	.88	.85	.67	.50		
18	2.05	1.62	1.52	.83	.39		
19	1.00	.99	1.00	.99	.68		
20	1.09	1.35	.58	.05	.07		

Appendix III

RAW DATA HEAT -- DURING (CONT.)

DAY	14	15	16	17	18	19	20
S							
1	.20	healed					
2	.18	.01	healed				
3	.12	.09	healed				
4	.07	.06	healed				
5	.02	.10	healed				
6	.01	.08	.07	healed			
7	healed						
8	.14	.07	.07	.06	.04		healed
9							
10	.12	.06	.03	healed			
11	.15	.08	.13	.15	.10	healed	
12	healed						
13	.03	.01	.01	healed			
14	.01	.01	healed				
15	.20	.04	.05	.11	healed		
16	.10	healed					
17	.11	.06	.06	.02	.03	healed	
18	.57	.16	.11	healed			
19	.36	.16	.13	.03	healed		
20	.13	healed					

Appendix III

RAW DATA PRE-HEAT

DAY	1	2	3	4	5	6	7
S							
1	4.80		5.30	4.67	4.20	3.87	2.76
2	3.13		4.00	3.08	3.79	3.24	3.04
3	4.04		3.80	3.61	3.94	3.52	3.05
4	3.93		3.98	3.76	3.66	2.77	2.25
5	3.23		3.85	3.87	3.40	2.97	2.39
6	3.64		3.78	3.90	3.94	3.16	1.53
7	4.01		4.63	4.23	4.25	3.78	3.41
8	3.05		2.99	2.63	2.25	1.99	1.16
9	died						
10	3.61		4.20	3.74	3.68	2.33	1.88
11	3.70		2.24	1.88	2.17	2.30	2.07
12	4.31		4.87	4.17	3.79	3.09	2.75
13	2.73		2.38	2.52	2.72	1.75	1.28
14	3.83		3.91	3.89	2.30	2.10	1.24
15	5.12		died				
16	4.05		3.84	4.14	3.97	3.52	3.21
17	4.12		3.86	3.68	3.91	3.65	3.86
18	3.39		3.49	3.65	3.45	2.93	2.46
19	4.67		4.47	4.16	4.16	2.99	2.62

Appendix III

RAW DATA PRE-HEAT (CONT.)

DAY	8	9	10	11	12	13	14
S							
1		2.93	2.66	1.32	1.00	.93	.16
2		1.17	.79	.65	.54	.13	.12
3		1.21	.95	.50	.33	.12	.28
4		1.55	.83	.76	.35	.13	.19
5		1.42	1.25	.87	.88	.36	.19
6		2.39	2.54	2.25	.66	.26	.29
7		1.92	1.99	1.69	1.41	.64	.27
8		.60	.43	.27	.27	.14	.14
9							
10		.92	.60	.24	.33	.35	.39
11		.80	.79	.51	.31	.20	.07
12		2.60	2.86	1.00	.84	.38	.26
13		1.00	.99	.66	.19	.25	.22
14		.43	.56	.67	.30	healed	
15							
16		1.94	1.08	1.02	.43	.21	.24
17		3.62	2.53	1.31	.90	.39	.38
18		1.72	2.27	.96	.54	.35	.22
19		.78	.99	.99	.72	.13	.21

Appendix III

RAW DATA PRE-HEAT (CONT.)

DAY	15	16	17	18
S				
1	.16	.26	healed	
2	.13	.08	healed	
3	.18	.13	healed	
4	.07	.02	healed	
5	healed			
6	healed			
7	.15	.14	healed	
8	.01	.10	healed	
9				
10	.42	.12	healed	
11	healed			
12	.05	healed		
13	.18	healed		
14				
15				
16	.29	healed		
17	.73	.21	.04	healed
18	healed			
19				

Appendix III
RAW DATA NOISE -- DURING

DAY	1	2	3	4	5	6	7
S							
1	2.65	1.59	1.65	1.45	1.69	1.26	1.61
2	3.74	3.96	4.38	3.86	3.26	2.07	1.95
3	3.96	1.97	2.29	1.90	1.60	1.76	1.47
4	4.20	4.09	4.09	4.30	2.67	1.83	1.97
5	3.39	3.12	2.08	2.26	1.56	1.17	1.15
6	3.31	1.75	1.77	1.61	1.66	1.25	.99
7	2.89	2.94	2.90	2.25	2.14	1.94	2.09
8	3.59	2.63	2.39	2.31	2.57	1.83	2.45
9	1.54	1.77	2.04	1.85	1.88	1.27	1.19
10	5.05	3.73		3.71	3.34	2.98	2.81
11	3.63	4.06	2.71	2.13	1.79	1.62	1.46
12	3.02	3.82	3.83	3.70	3.33	3.09	3.43
13	2.90	3.15	2.73	2.19	2.33	1.33	1.62
14	3.21	4.36	3.98	3.73	3.27	2.76	2.35
15	3.07	3.63	4.05	3.50	3.86	2.94	3.30
16	2.72	2.32	2.40	2.30	2.29	2.30	1.44
17	3.97	3.29	3.79	4.06	3.47	3.14	2.25
18	2.96	3.41	3.87	3.59	2.71	2.78	2.67
19	2.91	3.17	3.08	2.59	1.98	1.37	1.83
20	3.45	4.25	3.64	3.82	2.53	2.70	2.72

APPENDIX III

RAW DATA NOISE-DURING

DAY	8	9	10	11	12	13	14
S							
1	1.49	1.23	1.30	1.20	0.20	0.20	0.09
2	2.23	1.42	0.88	0.30	0.16	0.05	0.01
3	1.31	1.26	0.47	0.31	0.18	0.07	0.02
4	1.84	0.82	0.47	0.15	0.06	0.27	healed
5	0.64	0.56	0.42	0.15	0.12	0.01	0.09
6	0.41	0.36	0.09	0.01	0.02	healed	
7	1.67	1.13	0.69	0.15	0.02	healed	
8	1.75	1.22	0.64	0.56	0.50	healed	
9	1.12	0.96	0.40	0.40	0.45	0.22	0.12
10	2.64	2.31	0.87	0.41	0.40	0.52	0.19
11	0.84	0.77	0.51	0.31	0.05	0.03	healed
12	2.61	1.17	0.79	0.54	0.64	0.11	0.39
13	1.25	1.00	0.82	0.45	0.10	0.02	0.24
14	2.15	2.04	0.79	0.18	0.25	healed	
15	2.18	1.67	1.05	0.80	0.72	0.33	0.40
16	1.14	1.07	1.04	0.37	0.46	0.17	healed
17	1.67	1.79	1.67	1.56	1.00	0.30	0.40
18	2.39	1.84	1.42	1.15	0.98	0.39	0.40
19	1.05	1.00	1.04	1.02	0.61	0.08	0.36
20	1.96	1.10	0.72	0.61	0.30	0.01	0.21

Appendix III

RAW DATA NOISE -- DURING (CONT.)

DAY	15	16	17	18
S				
1	.02	.15	.05	healed
2	healed			
3	.03	healed		
4				
5	healed			
6				
7				
8				
9	.05	.01	healed	
10	.03	healed		
11				
12	.32	.04	healed	
13	.20	.13	.20	healed
14				
15	.04	.01	healed	
16				
17	.27	.21	healed	
18	.24	.25	.24	healed
19	.28	healed		
20	.04	healed		

Appendix III

RAW DATA PRE-NOISE

DAY	1	2	3	4	5	6	7
S							
1	2.33	2.56	2.79				
2	2.99	3.42	2.72		2.29	1.49	1.18
3	2.98	died					
4	2.18	2.79	2.86		1.10	.70	.77
5	2.12	2.67	2.74		.77	.43	.51
6	2.75	2.91	2.32		1.63	1.00	.87
7	2.00	3.00	2.82		1.31	1.36	1.42
8	4.01	3.39	3.69		2.71	1.58	1.47
9	2.47	2.01	2.22		1.09	1.08	.89
10	2.66	3.23	2.68		.65	.56	.31
11	2.25	2.38	2.40		1.50	1.47	1.35
12	2.10	2.54	2.43		1.29	1.49	1.56
13	2.63	died					
14	1.81	2.65	2.71		.80	.89	.90
15	2.45	2.77	2.74		.69	.42	.40
16	3.43	3.92	3.47		2.67	1.80	1.65
17	3.44	3.37	3.54		2.24	1.20	.89
18	3.00	3.25	3.06		1.70	1.54	2.02

Appendix III

RAW DATA PRE-NOISE (CONT.)

DAY	8	9	10	11	12	13	14
S							
1							
2	1.00	.68	.50	.26	.24	.21	.02
3							
4	.10	.24	.17	healed			
5	.02	.06	.03	.01	healed		
6	.66	.66	.46	.20	.06	.07	.16
7	.99	.73	.43	.43	.10	healed	
8	1.19	1.00	1.13	.80	.91	.76	.31
9	.53	.14	.07	.01	.04	healed	
10	.34	.28	.17	healed			
11	1.14	.67	.20	.17	.14	.22	.08
12	1.10	1.28	.72	.22	.19	.16	healed
13							
14	.44	died					
15	.19	.28	.36	.24	healed		
16	1.51	.29	.25	healed			
17	.85	.35	.20	healed			
18	.93	.09	.21	.06	healed		

Appendix III

RAW DATA PRE-NOISE (CONT.)

DAY	15	16	17	18
S				
1				
2	.01	.01	healed	
3				
4				
5				
6	.01	healed		
7				
8	.20	.10	.11	healed
9				
10				
11	.11	healed		
12				
13				
14				
15				
16				
17				
18				

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