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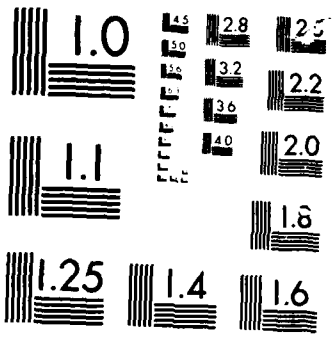

MICROCOPY CHART

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The use of vascular microcorrosion casts has made it possible to demonstrate the degree of damage to the microcirculation in experimentally induced frostbite. This approach provides a direct method for demonstrating vascular patency. Four groups of animals were used in this investigation. The left hind limbs of anesthetized rats were cooled to -10°C in groups one and three and -20°C in groups two and four, as measured by needle thermocouples placed under the gastrocnemius muscles. Thermocouples were also placed in the left hind footpads of groups three and four. The sheathed limbs were cooled in an

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alcohol bath at approximately 1.1°C per minute. All cooled limbs were rewarmed to 37°C in a 40°C water bath. The right hind limbs served as uninjured controls. The footpad temperatures recorded in groups three and four were used to characterize the footpad temperatures in groups one and two. Vascular microcorrosion casts were made from the left and right hind paws of groups one and two using Batson's modified methyl methacrylate. Scanning electron microscopic examination of the casts demonstrated dramatic differences between the vascular integrity of control paws and that of cooled paws. Exposure to the cold temperatures destroyed most of the microcirculation. In addition, the weights of the casts from the control paws were significantly different from the weights of the casts from the cooled paws. It was concluded that this model for evaluating frostbite injury accurately demonstrates the extent of microvascular damage and has significant potential as a method for evaluating therapeutic drug regimens.

VASCULAR CASTS DEMONSTRATE MICROCIRCULATORY INSUFFICIENCY IN ACUTE FROSTBITE

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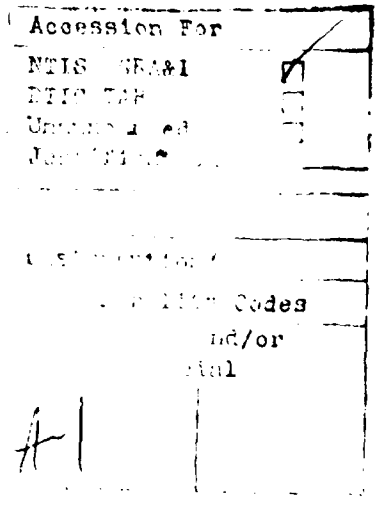

ABSTRACT

The use of vascular microcorrosion casts has made it possible to demonstrate the degree of damage to the microcirculation in experimentally induced frostbite. This approach provides a direct method for demonstrating vascular patency. Four groups of animals were used in this investigation. The left hind limbs of anesthetized rats were cooled to -10°C in groups one and three and -20°C in groups two and four, as measured by needle thermocouples placed under the gastrocnemius muscles. Thermocouples were also placed in the left hind footpads of groups three and four. The sheathed limbs were cooled in an alcohol bath at approximately 1.1°C per minute. All cooled limbs were rewarmed to 37°C in a 40°C water bath. The right hind limbs served as uninjured controls. The footpad temperatures recorded in groups three and four were used to characterize the footpad temperatures in groups one and two. Vascular microcorrosion casts were made from the left and right hind paws of groups one and two using Batson's modified methyl methacrylate. Scanning electron microscopic examination of the casts demonstrated dramatic differences between the vascular integrity of control paws and that of cooled paws. Exposure to the cold temperatures destroyed most of the microcirculation. In addition, the weights of the casts from the control paws were significantly different from the weights of the casts from the

cooled paws. It was concluded that this model for evaluating frostbite injury accurately demonstrates the extent of microvascular damage and has significant potential as a method for evaluating therapeutic drug regimens.

Progress in frostbite research has been severely hampered by the lack of suitable animal models. Earlier studies with rabbit skin grafts (18), hamster cheek pouches (15, 19), and mouse hindlimbs (2) indicated that the extent of tissue loss was primarily a function of the degree of microvascular damage. However, most of the existing animal models for human frostbite suffer from the variability inherent in the process of freeze injury in vivo. Variability in neurological responses, blood levels of vasoactive substances, and the lack of adequate techniques for evaluating vascular integrity are contributing factors. Until recently, rapid rewarming (3, 5) was the only major advance in the treatment of frostbite. The present approach to therapy involves treatment of symptoms; however, recent investigations suggest that complex therapeutic regimens (which might include vasodilators, dextran, heparin, membrane stabilizers or sympathetic neural blockade) improve tissue retention (1, 4, 8, 10, 11). Although Xenon-133 (16, 17) and infrared thermography (6) improve the early predictions of tissue loss, both are indirect measures of blood flow which are affected by underlying vessels. In general, clinical evaluation of both frostbite and potential therapeutic drugs remains subjective.

Since it is universally accepted that the major factor in tissue loss from frostbite is a vascular injury, procedures employed in studies of normal vascular integrity can and should be applied to the study of vascular integrity in an animal model for frostbite injury. The application of vascular microcorrosion casting

techniques with subsequent examination by scanning electron microscopy (SEM) has proven to be an excellent method for investigating microvascular patency (7, 9, 12, 13, 14). The purpose of this investigation was to utilize vascular microcorrosion casts to evaluate the extent of vascular occlusion in experimentally induced frostbite and to establish a model for evaluating therapies.

MATERIALS AND METHODS

Male CD strain rats (Charles River Laboratories, Wilmington, MA) were housed in wire bottom rat cages with food and water available ad libitum. Forty-three animals, weighing 285-367 grams were used in this experiment. All animals were randomly placed in four groups and anesthetized prior to experimentation with an I.P. injection of Nembutal (5mg/100 g body weight); if necessary, an additional dose of one third the initial dose was administered. Group one contained 12 animals, group two contained 11 animals and groups three and four contained ten animals each. A 23-gauge copper-constantan needle thermocouple was inserted one centimeter below the knee under the gastrocnemius muscles of the right and left hind limbs of all animals. An additional 29-gauge copper-constantan needle thermocouple was inserted in the left hind footpads of groups three and four. The thermocouples were connected to a Leeds and Northrup Speedomax Recorder. All groups were characterized by the temperature recorded under the gastrocnemius muscle, although

footpad temperatures were obtained in groups three and four. The left hind limb of each animal was enclosed in a finger cot and immersed in an alcohol bath to the distal end of the femur. It was cooled at an average rate of 1.1°C per minute until the temperature under the gastrocnemius reached -10°C in groups one and three and -20°C in groups two and four. This procedure allowed a comparison of leg and footpad temperatures without a needle in paws used for casting (Table 1). The cooling bath temperature was controlled by regulating the flow of alcohol from a reservoir of alcohol and dry ice into a jacket surrounding the bath chamber. A rectal probe was used to monitor core temperature, which was maintained by circulating 37°C water through tubing coiled around the animal. When the limb reached the desired temperature, it was withdrawn from the cooling bath and rewarmed in a 40°C water bath until the temperature under the gastrocnemius muscle reached 37°C. In all groups the right hind limb served as an uninjured control.

Following rewarming, the left hind limb was removed from the 40°C bath. A midline incision followed by careful dissection exposed the abdominal aorta. The aorta was cannulated below the left renal artery and vein and flushed, first with 1% heparin in saline and then with 2.0% glutaraldehyde in cacodylate sucrose. Immediately after this perfusion, Batson's anatomical casting compound, modified with methyl methacrylate (13), was injected through the aorta at a flow rate of approximately 3.5ml per minute until the vena cava filled with the casting compound. To prevent premature hardening of

the casting material during injection, the hindlimbs of the rat were covered with ice (14). After an initial polymerization time of 30 minutes, the tissues containing the casts were dissected from the animal and placed in a 60°C water bath for further polymerization overnight. Then the tissues containing the casts were placed in a solution of concentrated potassium hydroxide in a 50°C oven. The casts were periodically rinsed in distilled water and placed in fresh maceration solution until all tissue was removed. The casts were then placed in a buffered formic acid solution for decalcification. When this process was complete, the casts were rinsed repeatedly in distilled water and air dried. The specimens were mounted on stubs and sputter coated with gold-palladium prior to examination in the SEM.

RESULTS

Visual observation of the casts indicated substantial loss of vascular network in those from the cooled hind paws (Fig. 1), while casts from the control paws (Fig. 2) revealed the huge volume normally occupied by the minute blood vessels. SEM examination of the vascular microcorrosion casts prepared from the uninjured control hind paws of rats revealed the intricate details of vascular pathways through the microcirculatory beds (Figs. 3, 4). On the other hand, the cooled paws retained little or no continuity in the microvasculature (Figs. 5, 6). This change was confirmed by a

quantitative comparison of the weights of the casts from the cooled paws with those of the control paws (Dunn's Multiple Comparison Procedure, $p = 0.05$). There was a significant difference between the two (Fig. 7). There was no difference between the casts from the cooled paws of groups one and two. This can be explained by the small difference (5.5°C) in footpad temperatures in groups three (-30.9°C) and four (-36.3°C) for corresponding leg muscle temperatures of -10°C and -20°C. There was also no difference between the casts from the control paws of groups one and two.

DISCUSSION

The amount of tissue lost as a result of frostbite injury can be affected by a number of variables, including the rate and depth of freeze as well as the rate and temperature of thaw. In our experiments, these variables were controlled in order to establish an accurate model of clinical frostbite injury. The rate of cooling was slow and constant in an attempt to mimic conditions under which frostbite occurs in humans. The temperatures to which the gastrocnemius muscles were cooled were predetermined at -10°C and -20°C. When those temperatures were reached, rewarming was initiated. Since it is generally accepted that rapid rewarming after frostbite is more beneficial than slow rewarming, the former was used (3, 5).

Previous methods for determining damage to the microvasculature from frostbite have utilized techniques which by themselves could cause alterations to the tissue. Rabb et al. (15) everted the hamster cheek pouch and pinned it over a specially designed cooling platform. Zacarian et al. (19) used a copper disc cooled in liquid nitrogen to locally freeze the hamster cheek pouch. In both investigations, the circulation was observed in vivo through the light microscope. These methods have the potential for causing mechanical damage and dehydration of the tissue.

The indirect method employed by Sumner et al. (16, 17) dramatically improved the prediction of tissue loss in frostbite; however, this approach has not been widely used. Overlapping blood vessels and the use of radioactive isotopes are potential problems. Infrared thermography techniques have been used to evaluate experimentally produced frostbite of rabbit feet (6). In their study of fourth-degree frostbite, Hamlet et al. (6) demonstrated that thermographic patterns allowed early prediction of the line of demarcation and sloughing. However, the surface heat-loss pattern seen with this technique is a combination of the deep temperature and the skin temperature which is subject to overlapping vessels. Although both of these methods improve prediction of tissue loss, neither accurately reflects the actual capillary density as well as the direct method.

The vascular replicas studied in this investigation indicate that vascular microcorrosion casts accurately represent the loss of

microvasculature as a result of exposure to freezing temperatures. SEM examination of casts from all hind paws allowed visualization of patent vessels. Damage to the vasculature ranged from the loss of a moderate amount of the microcirculation to the loss of all vessels, except major blood vessels. In all cases, the casts from cooled paws were dramatically different from the casts from control paws. It should be possible to correlate infrared thermography or Xenon clearance with direct replicas to improve interpretation. In the present study, the injury was acute and extreme. Less severe injuries should be evaluated by correlation of casts with tissue loss. It is our intent to apply this direct method for quantitative evaluation of the microvascular network to the evaluation of potential treatments or new therapeutic regimens for frostbite.

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The views, opinions, and/or findings contained in this report are those of the author and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

The conduct of the research described in this report adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

TABLE 1

Comparison of the temperature in ^{o}C under the cooled gastrocnemius muscles with the temperature under the control gastrocnemius muscles and the temperature in the cooled footpads (groups three and four), and the core temperature. Mean $\pm$ SD are given in each case.

	Gastrocnemius Cooled (Left)	Gastrocnemius Control (Right)	Footpad Cooled (Left)	Core
Group 1	-10.0	34.4 $\pm$ 1.8		36.0 $\pm$ 1.3
Group 2	-20.0	33.4 $\pm$ 2.1		35.7 $\pm$ 1.2
Group 3	-10.0	34.5 $\pm$ 1.7	-30.9 $\pm$ 4.4	35.8 $\pm$ 0.9
Group 4	-20.0	34.9 $\pm$ 2.4	-36.3 $\pm$ 5.2	36.4 $\pm$ 1.2

FIGURE LEGEND

FIG. 1. Photograph of entire vascular microcorrosion cast from a cooled hind paw from group one (-30.9°C ± 4.4).

FIG. 2. Photograph of entire vascular microcorrosion cast from a control (uninjured) hind paw.

FIG. 3. Scanning electron micrograph of a portion of a vascular microcorrosion cast of the nail bed from a control (uninjured) hind paw. Bar = 50um.

FIG. 4. A high magnification view of the cast shown in fig. 3. Bar = 10um.

FIG. 5. Scanning electron micrograph of a portion of a vascular microcorrosion cast from a cooled hind paw from group one (-30.9°C ± 4.4). Bar = 100um.

FIG. 6. A high magnification view of a scanning electron micrograph of a portion of a vascular microcorrosion cast from a cooled hind paw from group one (-30.9°C ± 4.4). Bar = 10um.

FIG. 7. Histograms of the weights in milligrams of vascular microcorrosion casts from the control and cooled hind paws of groups one and two. Mean ± SEM are given in each case. The letter in parentheses indicates mean which differs from all other means with the same letter.

REFERENCES

1. Anderson, R.A., and Hardenbergh, E. Frostbite treatment in the mouse with low molecular weight dextran. J. Surg. Res. 5, 256-261 (1965).

2. Bowers, W.D., Hubbard, R.W., Daum, R.C., Ashbaugh, P., and Nilson, E. Ultrastructural studies of muscle cells and vascular endothelium immediately after freeze-thaw injury. Cryobiology 10, 9-21 (1973).

3. Crismon, J.M. and Fuhrman, F.A. Studies on gangrene following cold injury: VI. Capillary blood flow after cold injury, the effects of rapid warming and sympathetic block. J. Clin. Invest. 26, 468-475 (1947).

4. Foray, J., Baisse, P.E., Mont, J.P., Cahen, Cl. Le traitement des gelures de montagne. Sem. Hop. Paris 56, 490-497 (1980).

5. Fuhrman, F.A. and Crismon, J.M. Studies on gangrene following cold injury. VII. Treatment of cold injury by means of immediate rapid warming. J. Clin. Invest. 26, 476-485 (1947).

6. Hamlet, M.P., Veghte, J., Bowers, W.D., and Boyce, J. Thermographic evaluation of experimentally produced frostbite of rabbit feet. Cryobiology 14, 197-204 (1977).

7. Imayama, S. Scanning and transmission electron microscope study on the terminal blood vessels of the rat skin. J. Invest. Dermatol. 76, 151-157 (1981).

8. Kaplan, R., Thomas, P., Tepper, H. Strauch, B. Treatment of frostbite with guanethidine. Lancet, 940-941 (1981).

9. Lametschwandtner, A., Lametschwandtner, U. and Weiger, T. Scanning electron microscopy of vascular corrosion casts-technique and applications. Scanning Electron Microsc. II, 663-695 (1984).

10. McCauley, R.I., Hing, D.N., Robson, M.C., and Heggers, J.P. Frostbite injuries: A rational approach based on the pathophysiology. J. Trauma 23, 143-147 (1983).

11. Mundth, E.D., Long, D.M., and Brown, R.B. Treatment of experimental frostbite with low molecular weight dextran. J. Trauma 4, 246-257 (1964).

12. Murakami, T. Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch. Histol. Jpn. 32, 445-454 (1971).

13. Nopanitaya, W., Aghajanian, J.G., and Gray, L.D. An improved plastic mixture for corrosion casting of the gastrointestinal microvascular system. Scanning Electron Microsc. III, 751- 756 (1979).

14. Potter, R.F., and Groom, A.C. Capillary diameter and geometry in cardiac and skeletal muscle studied by means of corrosion casts. Microvasc. Res. 25, 68-84 (1983).

15. Rabb, J.M., Renaud, M.L., Brandt, P.A., and Witt, C.W. Effect of freezing and thawing on the microcirculation and capillary endothelium of the hamster cheek pouch. Cryobiology 11, 508-518 (1974).

16. Sumner, D.S., Boswick, J.A., Criblez, T.L. and Doolittle, W.H. Prediction of tissue loss in human frostbite with xenon-133. Surgery 69, 899-903 (1971).

17. Sumner, D.S., Criblez, T.L., Novak, J.K., and Doolittle, W.H. Prediction of tissue loss in experimental frostbite with radioactive xenon. Surg. Gynecol. Obstet. 131, 417-423 (1970).

18. Weatherley-White, R.C.A., Sjostrom, B., and Paton, B.C. Experimental studies in cold injury. II. The pathogenesis of frostbite. J. Surg. Res. 4, 17-22 (1964).

19. Zacarian, S.A., Stone, D., and Clater, M. Effects of cryogenic temperatures on microcirculation in the golden hamster cheek pouch. Cryobiology 7, 27-39 (1970).

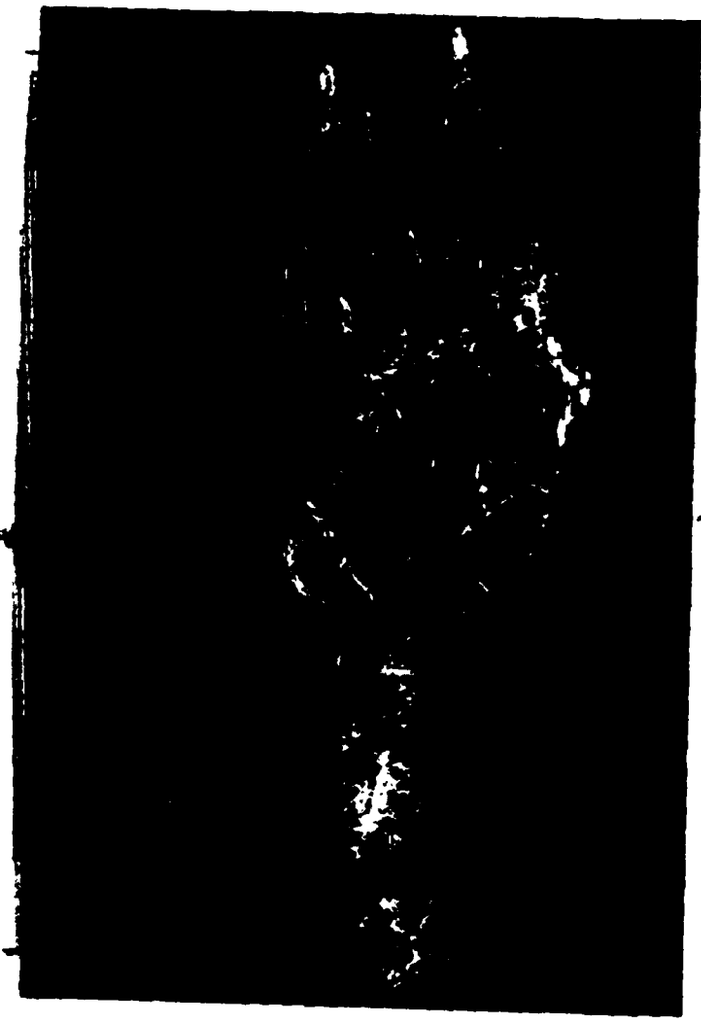


FIG. 1

FIG. 2

FIG. 3

FIG. 4

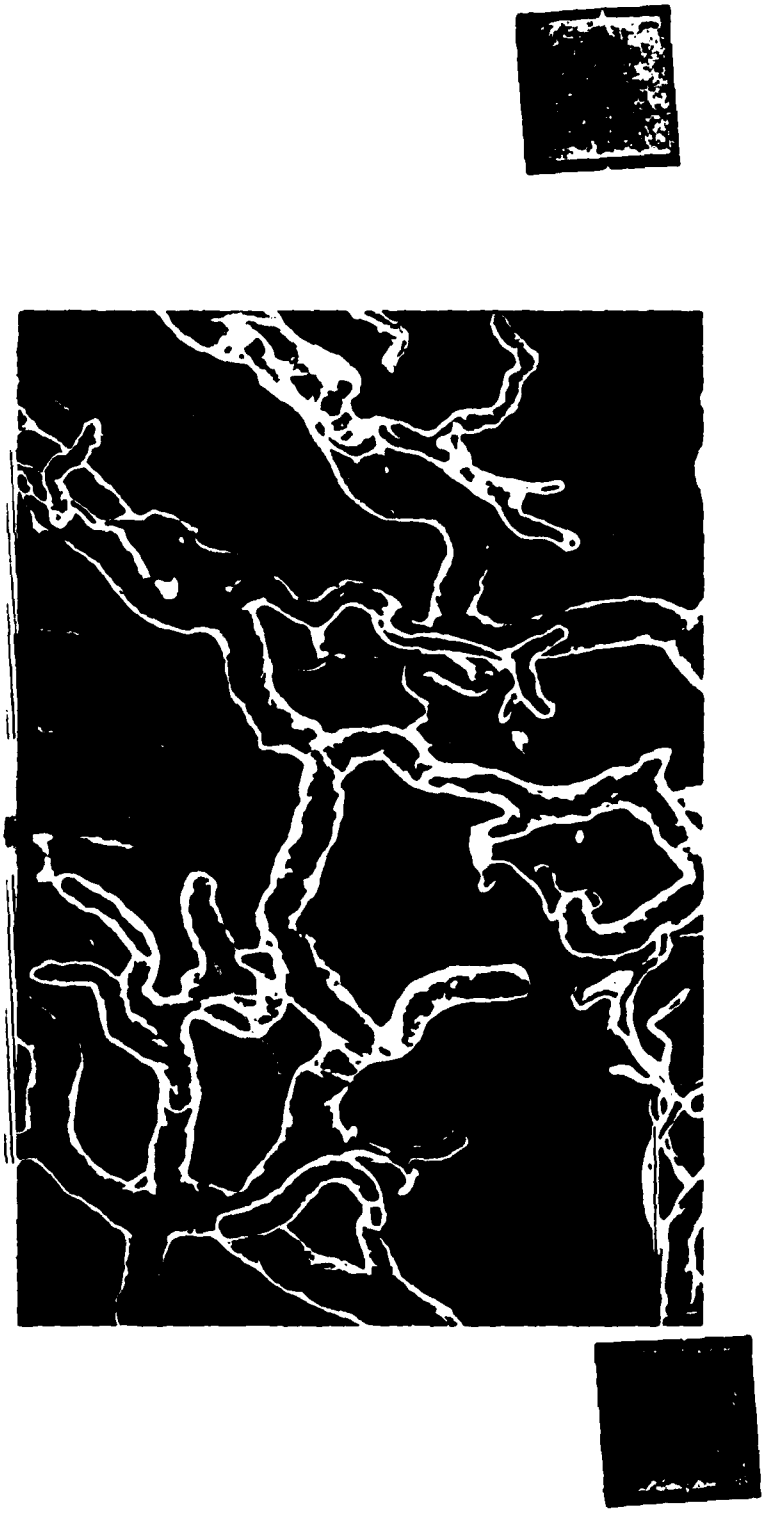


FIG. 5

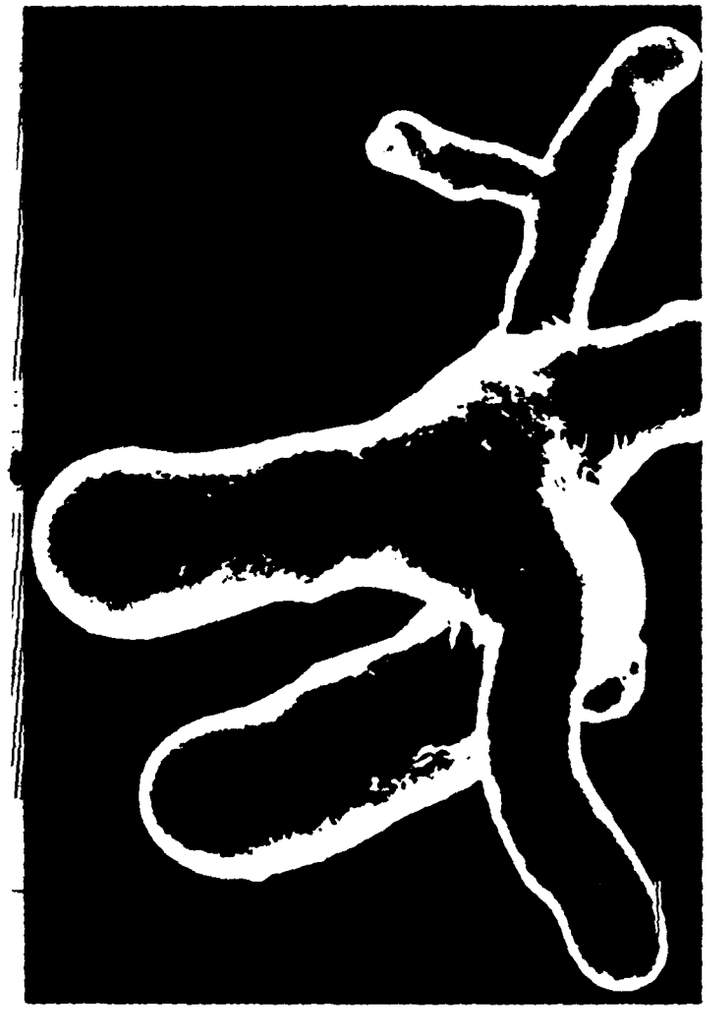
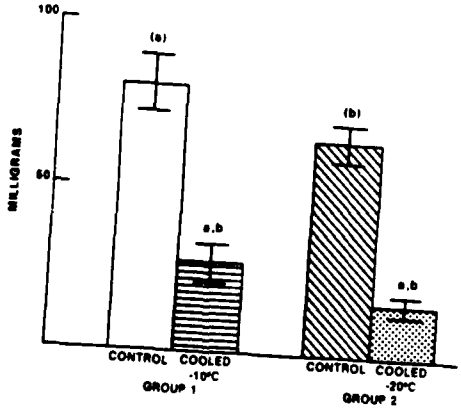


FIG. 6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MILLIGRAMS
(a)
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CONTROL
COOLED
-10°C
GROUP 1
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-20°C
GROUP 2

FIG. 7

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