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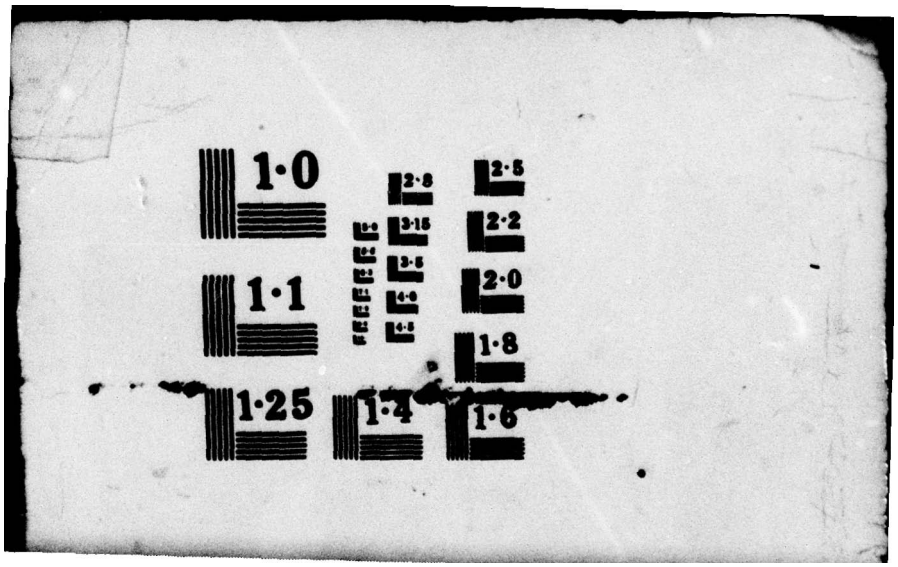
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REPORT

MRL-R-741

A FLUORESCENCE HISTOLOGICAL TECHNIQUE FOR THE INVESTIGATION OF CAPILLARY DAMAGE IN EXPERIMENTAL MUSCLE TRAUMA

Brian M. Paddle and Shirley E. Freeman

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FEBRUARY, 1979

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(6) # A FLUORESCENCE HISTOLOGICAL TECHNIQUE FOR THE INVESTIGATION OF CAPILLARY DAMAGE IN EXPERIMENTAL MUSCLE TRAUMA

(10) Brian M. Paddle and Shirley E. Freeman

(12) 16 p.

(11) Feb 78

ABSTRACT

A fluorescein-labelled dextran of high molecular weight has been used to visualize the capillary bed in muscle tissue adjacent to the permanent wound cavity created by a high-velocity missile.

The fluorescent dye is confined to the vascular system prior to wounding. The distribution of the dye as seen in frozen sections by fluorescence microscopy reveals where damage has occurred.

In undamaged tissue the capillaries appear as small discrete yellow-green discs in transverse section. In tissue taken from near the wound track the radial spread of dye about the capillaries indicates damage. Where many capillaries are damaged muscle cells appear completely surrounded by the dye.

Close to the wound track the dye may be taken up by damaged muscle cells, which are swollen and separated.

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A fluorescein-labelled dextran of high molecular weight has been used to visualize the capillary bed in muscle tissue adjacent to the permanent wound cavity created by a high-velocity missile.

The fluorescent dye is confined to the vascular system prior to wounding. The distribution of the dye as seen in frozen sections by fluorescence microscopy reveals where damage has occurred.

In undamaged tissue the capillaries appear as small discrete yellow-green discs in transverse section. In tissue taken from near the wound track the radial spread of dye about the capillaries indicates damage. Where many capillaries are damaged muscle cells appear completely surrounded by the dye.

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CONTENTS

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A FLUORESCENCE HISTOLOGICAL TECHNIQUE FOR THE INVESTIGATION OF CAPILLARY DAMAGE IN EXPERIMENTAL MUSCLE TRAUMA

INTRODUCTION

The development of rifles that fire high-velocity bullets led early to the observation that the resulting wounds appeared as though they had been caused by an explosion within the body. Entrance and exit wounds were frequently small but an unexpectedly large amount of damage occurred within. This effect was noted from the middle of the last century by Horsley (5) and is particularly pertinent with present-day armaments (3). Wounds from a spear or a nearly spent revolver bullet correspond to a cylinder of disintegrated tissue approximately the size of the missile. However high-velocity missiles leave behind a pulsating temporary cavity which disrupts tissue at a distance from the missile track. The cavity collapses, but tissue is widely devitalized around the smaller permanent cavity, and must be removed by the surgeon. The extent of debridement depends on the surgeon's understanding of the wounding process, and the macroscopic appearance of the wound. In a soft tissue such as skeletal muscle the damaged area will extend radially in an irregular manner for some centimetres from the missile track. Adjacent to the track tissue appears darkened and oedematous, with a general extravasation of blood. It will fail to respond to contractile stimuli. Focal bruising is usual, and the greater part of the damaged limb of an experimental animal may show a reactive hyperaemia. Splitting along the fascial planes will extend into the muscle mass of the limb.

The microcirculation of the muscle is disrupted around the missile track, and the sequestration of bacteria and dead tissue within the wound readily leads to infection. Hopkinson and Watts (4) discussed the histopathological damage inflicted by bullets in anaesthetized experimental animals. These authors were able to show by Indian-ink perfusion of the injured limb that there was a large volume of muscle around the bullet track where the capillaries were disrupted. It could be seen both macroscopically and microscopically that blood vessels in this area did not fill with ink. This volume of hypoxic muscle is at risk in the development of infections such as gas gangrene.

Because of the importance of this phenomenon we have developed an alternative method to study damage to the microcirculation following high-velocity missile wounding. This has enabled us to delineate the radial

extent of the microscopic effects of the wounding process. It is hoped that it will yield information relevant to the treatment of human gunshot casualties. The method depends on the localization of a fluorescein-labelled dextran of high molecular weight in the muscle. Animals are given an intravenous injection of the dextran prior to the wounding of a limb. Samples of muscle are taken during wound excision, rapidly frozen and sectioned in a freezing microtome. Extravasation of fluorescent plasma is visualized in sections with a fluorescence microscope.

MATERIALS AND METHODS

Five rabbits were used in the development of our current procedures. The average weight was 3 kg. The animals were anaesthetized with urethane (25% w/v in 0.9% saline, approximately 2 g/kg) administered via an ear vein, and fitted with a tracheal cannula. The hind legs were shorn of fur and the animal tied to a soft particle board supported at approximately 60° to the horizontal plane, so that the medial aspect of one upper hind leg was vertical and facing the weapon. The target area was selected so that there would be no direct hit on bone or large blood vessels. Thus the muscles penetrated by the projectile were largely the semimembranosus, the adductor group, the gracilis and the biceps femoris.

The weapon was an Australian self-loading rifle (S.L.R.) firing standard 7.62-mm ammunition. The range was 9.5 m and the average muzzle velocity was 800 m/s.

Just prior to the wounding 5% w/v fluorescein isothiocarbamoyldextran of mean average MW 154,000 (FITC-Dextran 150, Pharmacia), in 0.9% saline was injected via an ear vein. The dose was 250 mg/kg.

Investigation and measurement of the wound cavity followed by tissue sampling was begun 15 to 20 min after wounding. In the initial experiments sampling proceeded after opening up the cavity completely from entrance to exit. Later it was found more useful to remove tissue samples from muscles as they were successively exposed along the missile track. In order that a comparison of transverse with longitudinal sections could be made, the tissue samples were of sufficient size to enable two adjacent blocks to be cut from them. Each of these was a little over 3 cm long in a direction parallel to the muscle fibres and was approximately 25 mm^2 in cross section. The boundary of the wound cavity was at one end. They were sandwiched between 2-mm-thick strips of 2% Agar gel and the exposed areas covered with O.C.T. compound (Ames) before being frozen in *iso*pentane cooled in liquid nitrogen. The overall procedure from excision to freezing took less than 3 min. Frozen blocks were wrapped in aluminium foil and stored in liquid nitrogen until required for sectioning. Control samples were taken from the contralateral unwounded leg.

The anaesthetized animals remained in good condition throughout the experiments (up to 2 h) and were killed afterwards by an overdose of urethane.

Subsequently each of the frozen blocks was cut into three 1-cm lengths. From these either longitudinal or transverse sections were obtained at -20°C in an Ames cryostat II. The 10-μm thick sections were freeze-dried at the same temperature at an ultimate vacuum of <0.1 mmHg for about 10 min. They were then brought to room temperature under vacuum, flooded with xylene and mounted in a medium consisting of polystyrene and dibutyl phthalate in xylene. Immediately after mounting the sections were viewed under a Leitz Ortholux microscope for :

(a) General structural characteristics, for which transmitted-light dark-ground or phase-contrast optics and tungsten illumination were used.

or (b) The distribution of fluorescence, for which transmitted-light dark-ground optics and mercury-arc illumination (HBO 50W/AC, Wotan) with a Leitz excitation filter (KP500) and a Leitz suppression filter (K510) were used.

The microscope magnification was either X80 or X50. Kodak Tri-X film was used for black and white photography and Kodak high speed Ektachrome daylight film for colour photography. In both cases the exposure rating was 320 ASA on the Wild photoautomat camera attachment (35 mm format).

RESULTS

Externally the missile appeared to inflict a minimal amount of damage. The entrance and exit wounds were 5-7 mm in diameter (Figs 1 and 2); the missile appeared to traverse the leg with little alteration to its angle of yaw. The length of the wound track ranges from 2.5 to 4.0 cm. There was generally very little bleeding from the wound. Upon opening up the wound cavity the extent of the internal damage became apparent (Fig. 6). There was extensive bruising of muscle around the wound track and at some distance from it in adjacent muscles. There was marked splitting along fascial planes perpendicular to the track. This not only increased the extent of the permanent wound cavity but also made it difficult to measure. Histological sections were prepared from blocks taken within 2 mm of the permanent cavity, and at distances of 1, 2 and 3 cm from it. The distribution of fluorescence was studied, and compared with that in control samples taken from the uninjured leg.

In both transverse and longitudinal sections from control samples the FITC-dextran 150 was confined to the capillaries (Fig. 7) and small intramuscular blood vessels. Sections cut from tissue approximately 3 cm from the wound track also showed fluorescence localized in the region of capillaries. However some leakiness of capillaries was evident, which appeared as focal spreading of the fluorochrome outwards from the capillary boundary (Fig. 3b). Examination of the architecture of muscle cells with dark-ground illumination did not show any abnormality at 3 cm, in any sections that were examined (Fig. 3a). Sections taken closer to the wound cavity (i.e. at 1 or 2 cm) showed much more leakage of the fluorochrome from blood vessels (Fig. 8). In many instances the muscle cells were completely outlined by fluorescence due to leakage of plasma from disrupted

capillaries (Figs 4b and 9). It should be emphasized however that most areas viewed under dark-ground illumination appeared normal (Fig. 4a), and extravasation of red blood cells was focal, rather than distributed over all sections that were examined. Areas with minimal spreading of fluorochrome from the capillaries were seen in a number of sections as close as 1 cm from the wound track. These were often adjacent to strands of perimyseal connective tissue, indicating that it offered some protection from the disruption caused by the temporary cavity. This effect was also noted by Hopkinson and Watts (1963). Sections taken at less than 1 cm from the permanent cavity rarely showed intact capillaries, and gross damage to muscle fibres was frequently evident. The fluorescent marker had spread throughout the extracellular space and had been taken up by some of the muscle cells (Fig. 5b), which were swollen and separated (Fig. 5a). Estimates of cell size obtained from planimeter measurements indicated that cells 3 cm from the wound track had a mean diameter of 73 μm, whereas adjacent to the wound track they had swollen to 102 μm. This difference was significant ($P < 0.001$, $n = 256$). In some sections the connective tissue bundles of the perimysium were filled with the fluorochrome (Fig. 8). This appeared to be due to the rupture of venules and/or arterioles, since diffusion of spilt blood from the surface of the muscle block could not have accounted for its presence. Care was always taken during muscle excision to avoid contamination with spilt blood. Further, blocks were trimmed so that any trauma due to excision would not affect the results.

DISCUSSION

The disruption of the microcirculation of skeletal muscle by expansion of the temporary cavity during the passage of the bullet through the tissue leads to sequestration of bacteria and dead tissue within the wound. This can be a determining factor in wound infection. It is consequently important to know the extent and nature of the capillary damage. This communication describes one method for the study of this effect. Other authors (4) have studied the distribution of carbon black perfused into the wounded area *post mortem*, or have used the technique of angiography (6). Fluorescein-labelled dextran is well tolerated by the animal (7), is present at the time of wounding, and demonstrates clearly the damage to the microcirculation.

The nature and extent of the wound in the rabbit thigh cannot be compared with a wound in man, since the size of the target and the range are much less than would be expected in a military operation. In fact the thigh of a rabbit is so small that it was not possible to follow the extent of damage to a distance of more than approximately 4 cm from the wound track. The bullet did not tumble in the wound, as would be expected from the short track length (2.5 - 4.0 cm) and the fact that this bullet is stable at as short a range as 10 m. Eason *et al* (2) noticed petechial haemorrhages as far as 10 cm from the track of a sphere travelling at a similar velocity to our bullets. Any description of damage to the microcirculation which is relevant to gunshot wounds in man will require the use of larger animals and a range close to that expected in battle.

Dubin (1) has measured the maximum extent of the temporary cavity in gelatine blocks as a function of the velocity of the penetrating missile.

In conditions similar to those of our experiments the maximum diameter would be approximately 8 cm. Further experiments will be required to determine the coincidence or otherwise in the size of the temporary cavity and the extent of capillary damage.

ACKNOWLEDGEMENTS

The work described in this report was done with the technical assistance of Mr. B. Leake and Ms. Dale Leonard. The woundings were carried out by the Ballistics Research Group.

REFERENCES

1. Dubin, H.C. (December 1974). A cavitation model for kinetic energy projectiles penetrating gelatin. USA BRL Memorandum Report No. 2423.

2. Eason, R.L., Pryor, W.H. and Adams, J.F. (1975). Wound studies in porcine skin, muscle and liver as related to variation of velocity of spherical missiles. USA BRL Report AD/A - 004636, pp. 1-13.

3. Harvey, E.N., McMillen, J.H., Butler, E.G. *et al.* (1962). In *Wound ballistics* (eds J.B. Coates and J.C. Beyer), pp. 143-235. Washington, D.C.: Office of the Surgeon General, Department of the Army.

4. Hopkinson, D.A.W. and Watts, J.C. (1963). Studies in experimental missile injuries of skeletal muscle. *Proc. R. Soc. Med.*, 56, 461-468.

5. Horsley, V. (1894). The destructive effect of small projectiles. *Nature*, 50, 104-108.

6. Lewis, D.H., Rybeck, B., Sandergård, J. *et al.* (1974). Early circulatory changes following missile wounding of soft tissue. *Acta. Chir. Scand.*, Supp. 450.

7. Schröder, U., Arfors, K.E. and Tangen, O. (1976). Stability of fluorescein labelled dextrans *in vivo* and *in vitro*. *Microvas. Res.*, 11, 33-39.

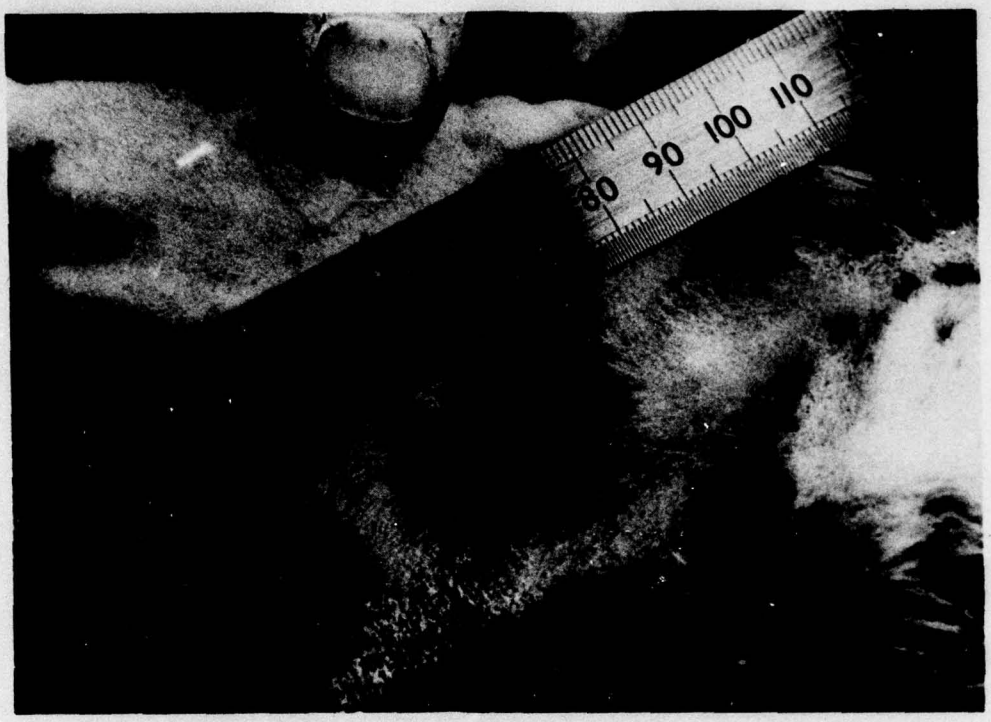


FIG. 1 - Entrance wound: Rabbit hind leg.
Weapon: Australian self-loading rifle firing standard 7.62 mm ammunition.
Range: 9.5 m.

FIG. 2 - Exit wound: Same animal as in Fig. 1.

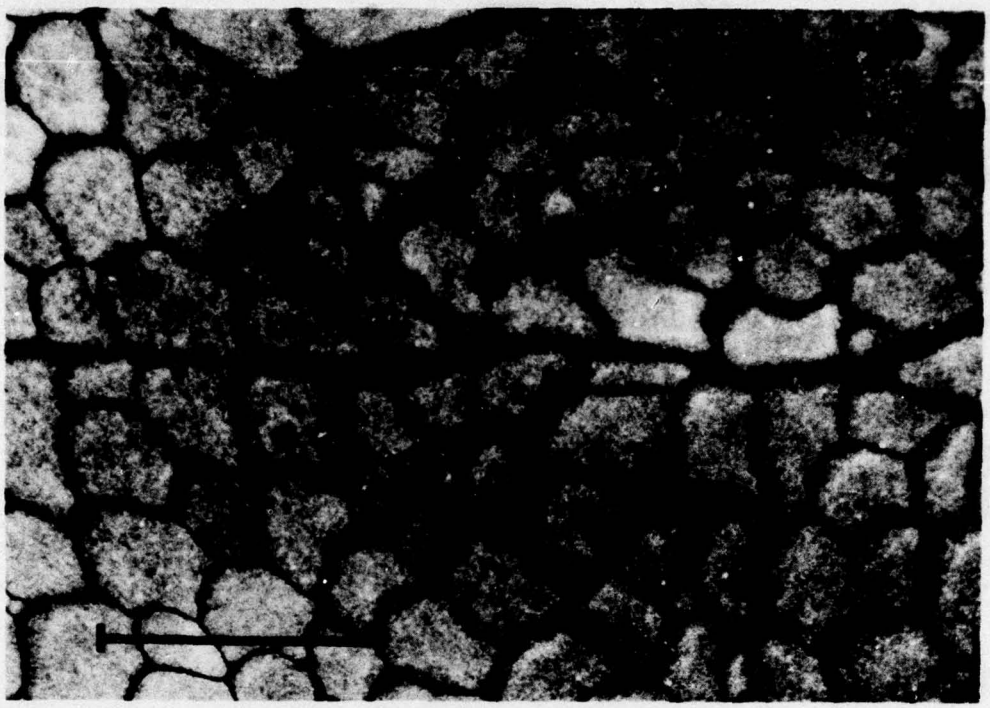


FIG. 3a - Transverse section from superficial muscle at wound entrance. Distance from the permanent wound cavity was 3 cm. Transmitted dark-ground tungsten illumination.

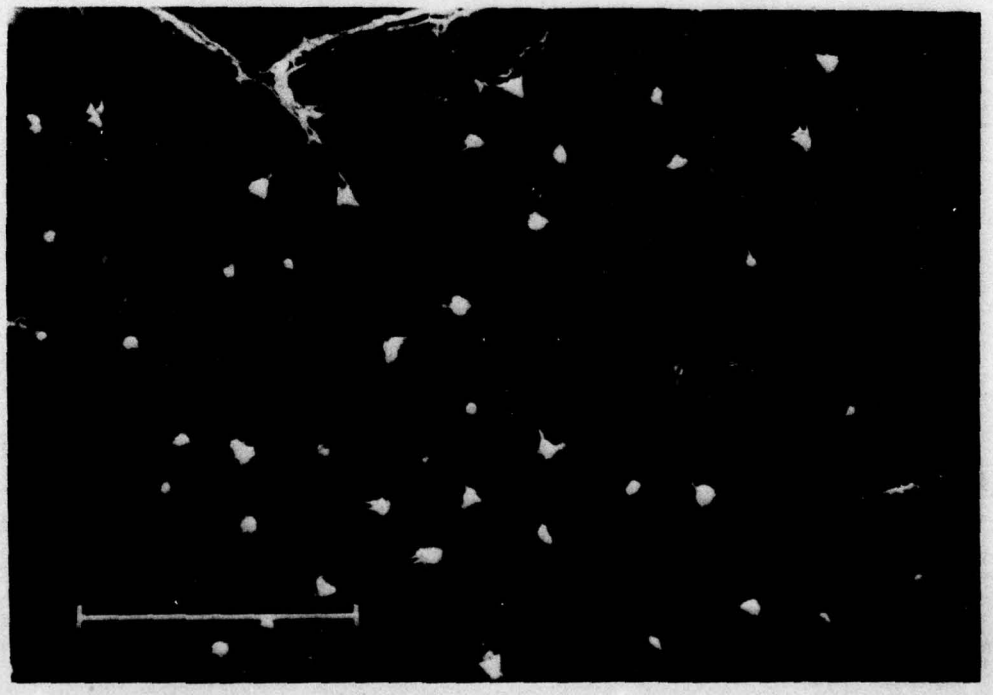
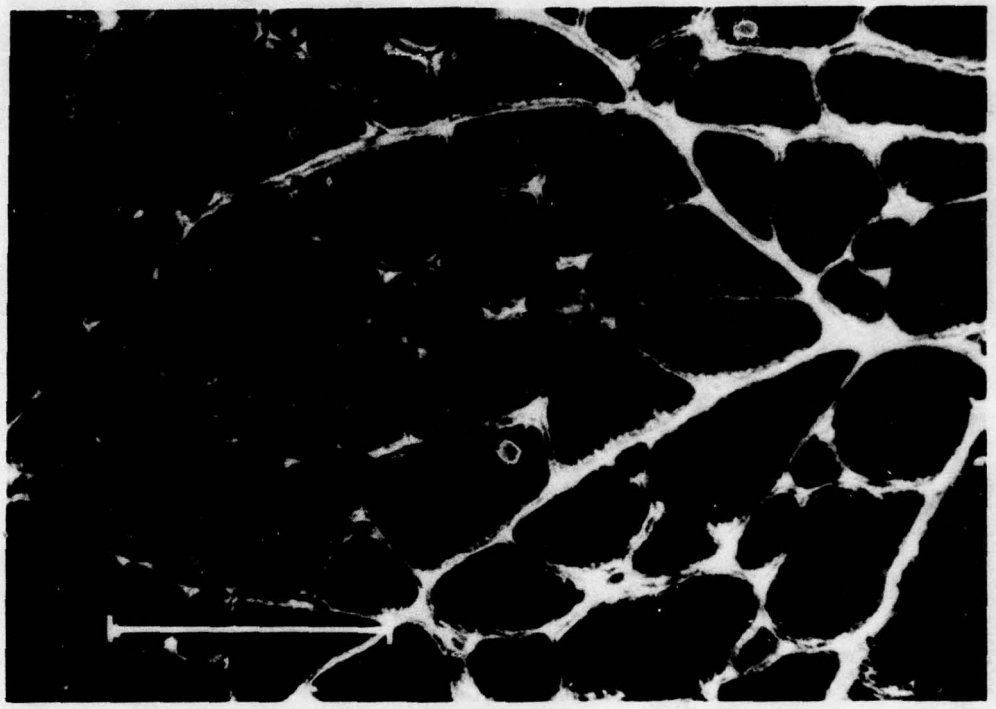
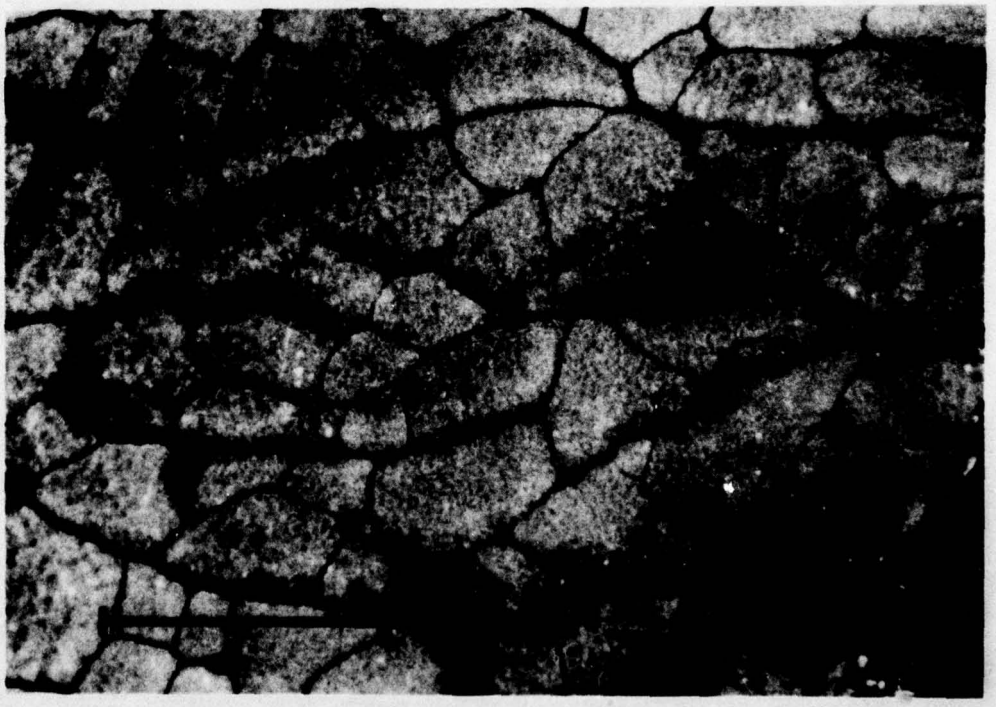


FIG. 3b - Same section as in Fig. 3a, photographed with dark-ground fluorescence excitation for FITC-dextran.

Bars represent 200 μm.



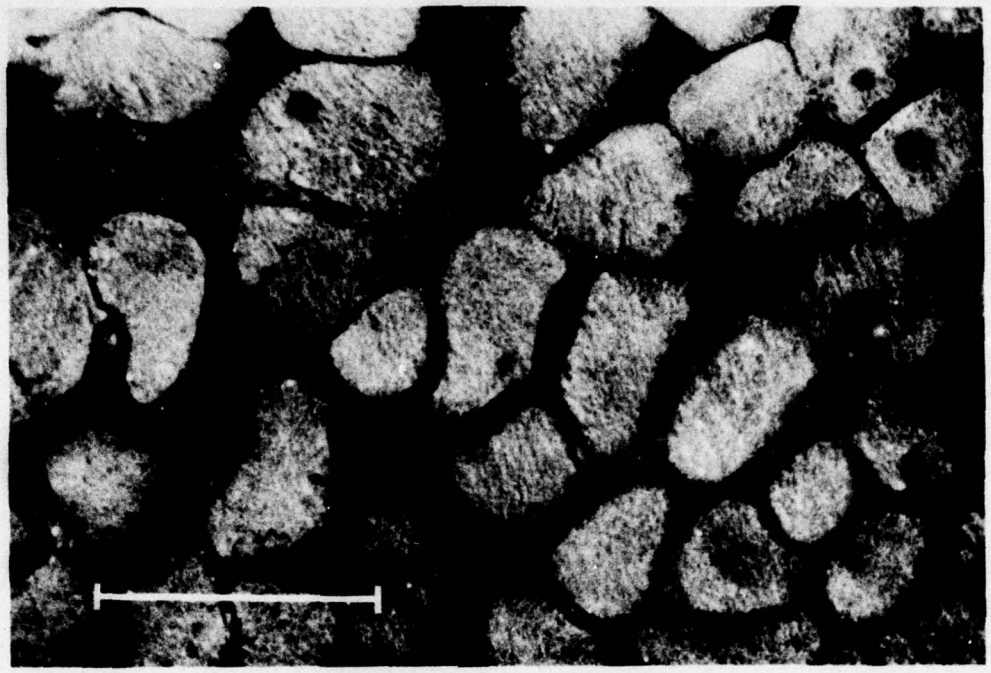
4a



4b

FIGS 4a and 4b - As for Figs 3a and 3b; section was located at about 2 cm from permanent wound cavity.

Bars represent 200 μm.



5a

5b

FIGS 5a and 5b - As for Figs 3a and 3b; section was located at about 2 mm from permanent wound cavity.

Bars represent 200 μm.

FIG. 6 — Wound cavity after removal of superficial muscles and some deeper muscle. Conditions as for Fig. 1.

FIG. 7 — Transverse section from contralateral superficial muscle of the unwounded hind leg; FITC-dextran fluorescence. Bar represents 200 μm.

FIG.8 — Transverse section from superficial muscle at wound entrance. Distance from the permanent wound cavity was 1.5 cm: FITC-dextran fluorescence. Bar represents 200 μm.

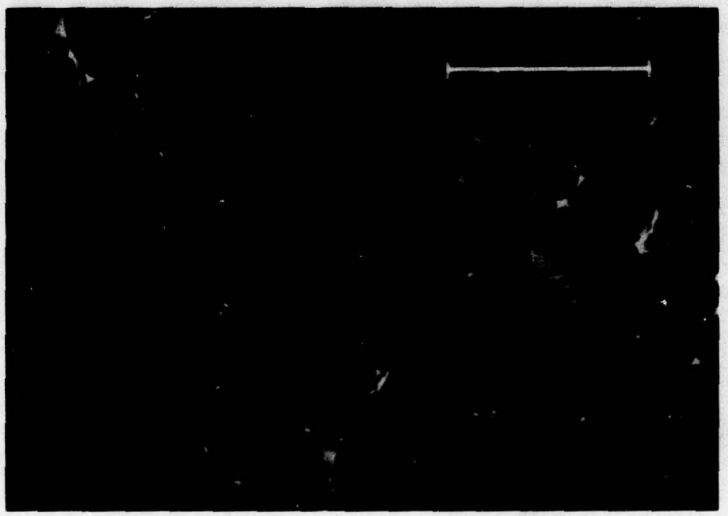


FIG. 9 — Same muscle as in Fig. 8; section was located at 2 cm from permanent wound cavity. FITC-dextran fluorescence. Bar represents 200 μm.

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