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METHOD FOR INVESTIGATION OF EXPERIMENTAL FRACTURES BY MEANS OF FLUORESCENT SUBSTANCES

By

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The desire to accelerate the healing of fractures has given rise to many experimental investigations. Despite this fairly wide interest, it appears that no reliable repair-promoting method has become available. Of the difficulties encountered in such an experimental investigation, some are unsurmountable such as the production of identical fractures in a series of animals and sufficiently accurate assessment of the rate of healing in the individual animals. The rate of repair is believed to depend to some extent on the circulating conditions at the site of the fracture (see *Block* 1940). However, owing to lack of suitable physiological methods it has not been possible to study the circulation in and around the callus. Therefore, the availability of such a method would promote the investigation of problems bearing on fracture healing. It was with this in mind that the following method was devised for the investigation of the blood flow to the metaphyseal region of the tibia of the rabbit (*Brodin* 1955). The method consists of the intravenous injection of a fluorescent substance, after which the animal is immediately killed and the fractured bone removed as well as the corresponding bone of the other side, which is used as a control. The preparations are deep-frozen, tissue is planed off, and the planed surface photographed in ultraviolet light. In the above-mentioned investigation a tibia fracture occurred in one of the animals. The bone was examined, and the results are given in order to exemplify the applicability of the method. It appears that a detailed investigation of fractures by this method is not yet advisable (see Discussion of the method).

MATERIAL

Sodium-3-oxypyrene-5,8,10-trisulphonate (Bayer, Leverkusen, Germany), 5 % solution in 0.9 % sodium chloride, pH 5.3.

Ultraviolet light source.

Carbon dioxide ice.

Alcohol 96 %.

Cold room with a temperature of —10° C to —15° C.

METHOD

An anaesthetized rabbit of about 1 kg. weight with a tibial fracture receives 2 ml. of the oxypyrene in an ear vein. About 20 sec. later the

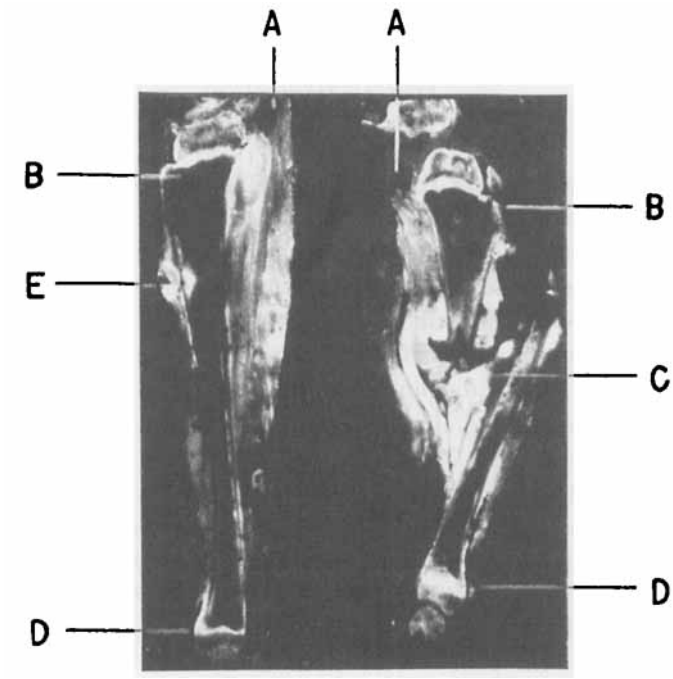


Fig. 1.

Two tibiae of young rabbit that had received 100 mg. oxypyrene intravenously 20 seconds before death. The left leg was fractured 1 week before. The fluorescence appears white in the reproduction.—*A.* No fluorescence in the vein in the fold of knee on the intact right side; slight fluorescence is seen in the corresponding area of the other side.—*B.* The intensity and spread of the fluorescence in the proximal metaphysis is, roughly speaking, equal on both sides.—*C.* The fluorescence in the callus and in the marrow cavity near the fracture is intense.—*D.* In the distal metaphysis the intensity and spread of the fluorescence is greater in the fractured bone than in the intact one.—*E.* Note the intense fluorescence round the previous site of an indicator in the cortex.

hind quarters are cut off and immediately placed into a mixture of carbon dioxide ice and alcohol. In the cold room the legs are wiped and planed until the actual structures appear in the experimental bone and in the control bone. The surfaces are then smooth and shiny. The ends of the bones are fixed, with the planed surfaces uppermost, against the lowermost surfaces of two horizontal parallel bars. The planed surfaces thus lie on one and the same horizontal level (Fig. 1). The ultraviolet light is placed so that it illuminates corresponding parts of both bones with the same intensity. The bones are photographed, using a yellow-green filter. The blackening of the film can be evaluated by means of a photometer in some cases, e.g. in the metaphyses, or with the naked eye, if the difference is marked.

RESULTS

Fig. 1 shows the tibiae of a young rabbit. One week before the investigation the animal had fractured its left tibia proximally to the foramen nutritium. The fluorescent parts are white in the picture. The fluorescence appears in the vicinity of the epiphyseal cartilages, chiefly in the metaphyses. It is much stronger in the distal left metaphysis (fractured bone) than in the right metaphysis. Proximally, no difference in fluorescence intensity can be demonstrated between the metaphyses. The fluorescence of the callus including adjacent parts of the marrow, is strong, indicating a quick blood supply. The periosteum and endosteum show some fluorescence as do some small arteries in the muscles. No fluorescence can be seen in the large veins in the posterior of the right (control) knee joint. A faint fluorescence can, however, be distinguished in the corresponding veins on the left side.

DISCUSSION OF THE METHOD

The interval between the injection of the fluorescent substance and the death of the animal was not long enough to permit the substance passing the capillaries of the lower leg of an intact rabbit and reaching the large veins (control side, Fig. 1 A). If the rate of the circulation increases, some of the fluorescent substance will pass out into the above mentioned veins (fracture side, Fig. 1 A). The circulation on the left fractured side thus appears to be faster than on the control side. Despite the possibility that some of the fluorescent substance reaching the fractured bone passed the capillaries of the bone, some regions of the bone were more intensely fluorescent than corresponding regions in the control bone, (distal metaphysis and the marrow nearest the fracture).

The fractured bone had thus received more fluorescent substance, which implies a larger flow of blood to the fractured bone than to the control bone.

Various objections can be raised against the fluorescent substance used. It is acidic and, possibly because of this, shows an affinity for bone tissue (*Brodin* 1955). It diffuses readily through the vessel walls. Owing to these less desirable qualities of the properties of the fluorescent substance, it was decided not to use the method until some suitable fluorescent substances is available. For such a substance to be satisfactory, it should preferably be stable in different concentrations, non-toxic, non-coagulant and should not permeate the vessel wall. (The high molecular Vasoflavine (*Moses et al.* (3) 1951) changes its degree of polymerisation and its light absorption when diluted).

When a suitable fluorescent substance becomes available, the method will probably prove valuable in the investigation of fractures.

SUMMARY

Fluorescence studies with 3-oxypyrene-5,8,10-trisulphonate, deep-freezing, planing and photography of a one week old, proximal diaphyseal tibial fracture of a rabbit revealed increased blood supply to the distal metaphysis of the fractured bone and to the marrow cavity close to the fracture. The callus had a rich blood supply.

RESUME

Des études sur le procédé de fluorescence avec 3-oxypyren-5,8,10-trisulphonate, congélation, examen et photographie d'une fracture diaphysale proximale du tibia, vieille d'une semaine, chez un lapin, ont montré qu'il y avait un afflux plus abondant de sang dans la métaphyse distale de l'os fracturé que dans la cavité de la moëlle toute proche de la fracture. Le callus avait un très riche afflux sanguin.

ZUSAMMENFASSUNG

Fluoreszenz-Untersuchungen mit 3-Oxypyren-5,8,10-Trisulphonat, Tieffrierung, und Fotographierung an einem eine Woche alten, proximalen Diaphysenbruch der Tibia eines Kaninchens, zeigten eine Zunahme der Blutversorgung in der distalen Metaphyse des gebrochenen Knochens und in der Markhöhle dicht an der Bruchstelle. Der Kallus wies reichliche Blutversorgung auf.

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