Cryolesions of Bone

An Experimental Study. Part I: Examinations in Technique of Controlled Cryolesion on Bone*

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Summary. Three different approaches of freezing rabbit tibia bones are demonstrated. Intramedullary freezing seems to be the safest method to avoid soft tissue necrosis. Bone specimens removed immediately after the freezing-thawing process showed electron optically evidence of irreversible damage of bone cells in all cases.


The aim of cryosurgery is a controlled tissue destruction by freezing. Cryolesions can be caused by either contact (tube, spray) or by penetration (tube, inhibition). As the cooling agent, liquid nitrogen (LN₂), nitrous oxide, carbondioxide, or freon is used. The working temperature of the different cryosurgery systems is determined by physical phenomena, such as phase-transformation (steam—fluid), Joule-Thomson effect (lowering of temperature by flow of a fluid through a valve), or Peltier effect (lowering of temperature by an electric current passed through two dissimilar semi-conductors.)

The development of cryobiology in recent years has contributed considerably to the understanding of the influence of low temperatures on living tissue of which the main component is water. By removal of heat, water crystallizes and ceases to function as either a solvent or as a structural molecular element. By slow cooling, ice formation occurs after fall below the freezing point firstly in the intercellular space, whilst the cell loses water and is first of all supercooled. The temperature of complete freezing is indicated as the eutectic point and lies, in biological tissue, at about 21 deg (physiological salt solution). By rapid cooling of tissue (more than 100 deg per min) intra- and extracellular ice crystal formation occurs simultaneously. These observations were carried out on single cells (Mazur, 1965) and also on tissue cultures (Smith, 1961).

The following evidence was obtained:
1. Freezing followed by thawing destroys a large portion of the treated cells.
2. The combination of rapid cooling (temperature gradient greater than 100 deg per min) and slow thawing (1–10 deg per min) shows an especially high cell death rate.

Point of the Experiment

The main point of this examination was to test by which technique a controlled cryodestruction on extremity bones would be possible (i.e., a selective devitalizing of a chosen portion of bone without causing lesions in the neighboring soft tissue). Furthermore, a temperature profile should be determined with the help of thermo-measuring elements, which is essential for the reproduction of an experiment of this type. Finally, it should be tested whether the cells of bone tissue were reliably destroyed by the effect of low temperatures.

Order of Experiment

The experiments were carried out on 24 hybrid rabbits with a weight of 4–5.5 kg and an age of 8 months to 1.5 years. A cryosurgical system (ERBE, Tübingen)
with temperature pre-selection and an automatic probe thawing device was chosen. As the cooling agent, liquid nitrogen was used.

There were 3 groups each of eight animals each:
- Group 1: Freezing in vitro;
- Group 2: External freezing (E.F.);

These divisions were made according to coldness application. The length of the tibial bones was radiologically measured pre-operatively (Fig. 1) as we wished to devitalize 1/5 (20%) of the tibial diaphysis.

The animals were premedicated with 50 mg/kg Ketalar i.m. After 15 min they were anaesthetized with 15 mg Nembutal per kilo body weight (i.v., ear vein). After cleaning of the operation area, and under sterile conditions, a 5 cm long skin incision was made on the front of the tibia and the diaphysis was exposed extra-periosteally. Before and after the coldness application, small bone splinters were removed for histological examination.

Results

Group 1 (Freezing in vitro)

Freezing in vitro is a certain type of controlled cryo-destruction because the selected tissue (bone) is removed, frozen outside of the body, and then reimplanted. Thus, an unwanted lesion of the neighboring soft tissue could certainly be avoided.

Using the oscillating saw one fifth of the total bone length was removed from the mid-shaft and super-cooled for 5 min in a container of liquid nitrogen. After thawing, the bone segment was reimplanted using an osteosynthesis plate. However, this order did not stand the test as the frozen bone, due to its brittleness, fractured after the boring of the screw holes so, that a stable osteosynthesis was not possible. For this reason, fractures and sometimes complete disbandment of the implants were the rule during the first postoperative days.

Group 2 (External freezing)

This form of coldness application is called external freezing because after circular exposure from the outside, the bone is wound several times with a plastic tube (Fig. 2).

This tube (a 14 Ch. Nelaton-catheter) was connected to the cooling tube, and filled with liquid nitrogen.

A disadvantage of this technique is the necessity for good isolation from the surrounding soft tissue. Despite the use of polystyrene sponge, extensive muscle, and skin-rim necroses sometimes occurred. For this reason, Teflon troughs with a rim thickness of 3 mm were used later, and permitted a better isolation (Fig. 3).

Fig. 1. Area of frozen bone between arrows (20% of bone length)

Fig. 2. Device for external freezing

Fig. 3. Soft tissue retractor (Teflon)

Despite generous use of isolation material, a reliable protection of the neighbouring soft tissue was not possible by the external method.

Group 3 (Intramedullary freezing)

After osteotomy (V-shaped osteotomy) in the mid-shaft, a cooling tube of 3 mm diameter was pushed 2 cm