PRO EXPERIMENTIS

An anatomical study of the microcirculation in the rabbit femur

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Summary. A technique is described which will allow a study of the microcirculation to a long bone such as the femur. It involves use of a silicone rubber compound (Microfil) in combination with the Spalteholz clearing technique. Preliminary results in the normal rabbit skeleton suggest that this technique can be used to study the changes in vascularity which occur under various pathological conditions.

Silicone rubber compounds (Microfil) can be used to fill and opacify microvascular and other tissue spaces if carefully administered under physiological injection pressure. The material usually polymerizes after injection to form a rubbery cast of the selected vascular area. Since its introduction it has been used with success to study the microcirculation to many types of organs and tissues e.g. the lung[1], kidney[2], intestine[3], liver metastases[4], cerebral infarcts[5], and connective tissue[6].

Microfil compounds have hydrophobic properties, and although several colours are available, the white variety permits a high degree of contrast so that the injected vessels can clearly stand out to provide a three dimensional view of their distribution within the tissue.

In this work a pilot experiment was carried out using Microfil to study the microcirculation of the rabbit femur, particularly the distribution of the nutrient artery to both cortical bone and marrow. The Spalteholz technique[7] was used to produce a clear specimen for microscopic examination and photographic illustration.

Materials and methods. Experiments were carried out using 2.5-3.5-kg New Zealand albino rabbits. Each animal was anaesthetized with Nembutal (30 mg/kg b.wt) by i.v. injection into an ear vein. A tracheotomy was performed, and oxygen supplied continuously through a fine manometer tubing into 1 limb of the tracheotomy tube. This served to keep the airway clear and to maintain normal respiration.

An incision was made in the femoral region to expose the femoral artery which was then cannulated retrogradely using fine polyethylene tubing (1.02 mm outer diameter). The tip of the catheter was positioned close to the origin of the nutrient artery supplying the femur. The rabbit was given an anticoagulant (sodium heparin) to ensure effective removal of its blood volume during perfusion.

The injected material was prepared by mixing equal parts of the Microfil compound (MV-112) and MV-diluent in a disposable plastic container, followed by 3% MV curing agent to achieve the required viscosity. A working time of 15 min was recommended by the manufacturer (Canton Biomedical Products), and this began with addition of the curing agent.

The catheter was flushed with heparinized saline, and the preparation introduced into it slowly under physiological injection pressure. The injection was terminated when there was an increase in resistance, and the vasculature appeared to be filled. At this stage the animal was killed by an intracardiac overdose of Nembutal, and it was left overnight with the catheter in position in a refrigerator at 2-4 °C. During this time curing of the silicone rubber took place. The following day the perfused femur was carefully dissected and cleaned of soft tissue.

The Spalteholz technique was modified slightly to clear the bone and to produce a transparent specimen. The following protocol was employed:

a) Bone fixed in 10% formal saline for 5 days to preserve, harden, and to aid visual differentiation of the structures.

b) Decalcified in a buffered solution of formic acid (15 ml formic acid + 5 g trisodium citrate + 100 ml distilled water). The buffer was added to counteract the injurious effects of the acid, but at the same time it increased the time required for complete decalcification. The bone took approximately 2 weeks to decalcify, and this was controlled radiographically. The acid solutions were changed 4 times during this period.

c) Bleached in 10% hydrogen peroxide for 24 h.

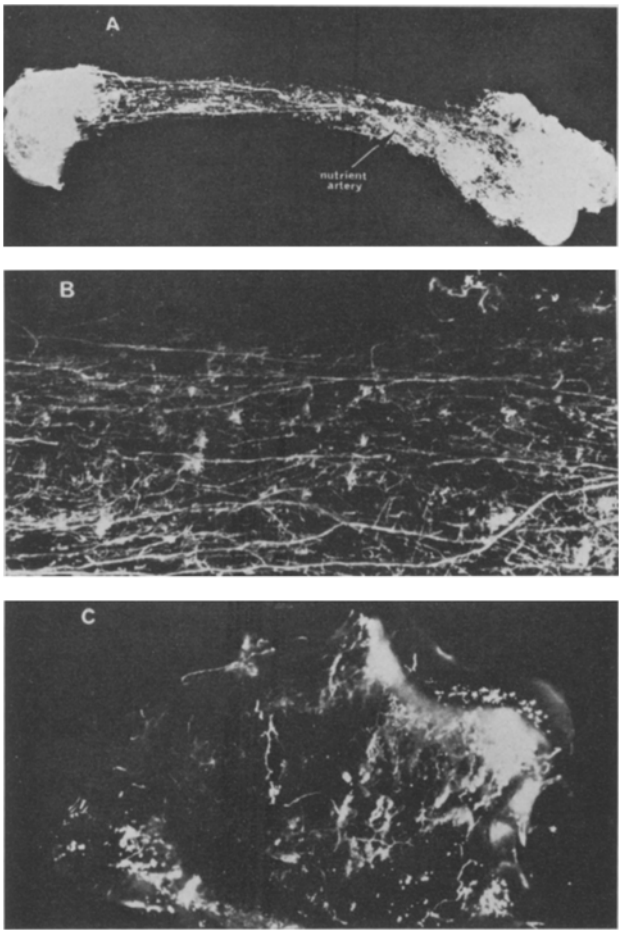
d) Transferred to 70% alcohol for 2 days.

e) Transferred to 100% alcohol, 3 changes over 6 days.

f) Transferred to benzene, 2 changes over 2 days.

g) Transferred to benzyl benzoate, 2 changes over 3 days.

h) Mounted in a mixture of equal parts benzyl benzoate and methyl salicylate (oil of wintergreen).



Photomicrographs of the rabbit femur following Microfil perfusion and clearing by the Spalteholz technique to show *A* the distribution of the nutrient artery, *B* cortical and medullary branches and *C* arborization in the epiphyseal region

Results and discussion. An attempt was made to demonstrate the internal vascularization of a long bone by Microfil angiography. Figure A shows the nutrient artery entering the femur antero-medially at about the junction of the upper and middle thirds of the bone. It then divides into ascending and descending branches which run longitudinally in the periphery of the medullary canal. These vessels not only give off radially oriented branches to the cortex but they also appear to provide a large proportion of the blood supply to the ends of the bone.

The branching network in the endosteal region gives off twigs which appear to extend outwards into the cortex (Figure B). This photomicrograph does not show whether some capillaries swing back from the cortex into the marrow to enter marrow sinusoids. There seems to be a continuity of the vessels in the cortex, and this may indicate that the arterial and venous systems are united in this region, and may therefore be in series with each other. Figure C shows the terminal arborization of the medullary vessels in the epiphyseal region of the femur.

Although a number of workers, particularly M. Brookes[8-10], have carried out extensive studies on the microcirculation to a long bone, the relative supply of nutrient, epiphyseal-metaphyseal, and periosteal vessels is still controversial. In view of this it was decided to undertake a study of the vascularisation of the rabbit femur using a combination of Microfil compound and the Spalteholz clearing technique to render the bone transparent so that it can be viewed under the dissecting microscope.

This work has undoubtedly confirmed the usefulness of the technique in studying the blood supply to an organ such as bone. This technique is currently being used to examine the change in vascularity which occurs in various pathological conditions such as a fracture, to study the regional distribution of the nutrient artery, epiphyseal-metaphyseal, and periosteal arteries in a long bone since the relative role of these vessels is not fully understood, and to determine whether the blood supply of cortical bone is in series or in parallel with that of the medullary system.

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Effect of pH on photosensitivity of bull spermatozoa

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Summary. Photosensitivity of bull spermatozoa increases with decreasing pH in the investigated range of pH 5.2–9.0.

In continuation of systematic studies of photodynamic effects on spermatozoa[1-4], the effect of pH on photosensitivity was investigated. Semen from Dutch-Friesian (FH) and Meuse-Rhine-Yssel (MRIJ) bulls was diluted split-sample in a 2-step procedure with optically clear egg-yolk – citrate diluents[5], adjusted at pH values ranging from 5.2 to 9.0 (included). At each pH one half of the subsample was continuously exposed to light, whereas the other half was kept in a small tube of the same size but made of brown glass, painted black, wrapped in aluminium foil and pressed into a white tube of soft plastic to avoid rise of temperature due to the heating effect of absorbed light radiation. The control tubes were placed alongside the unprotected glass tubes in a water-bath kept at a constant temperature of 5 °C. All tubes were agitated by a single agitator to prevent sedimentation of the spermatozoa.

A constant illumination of 983 ± 3 lx was maintained with a battery of incandescent bulbs (220 V, 150 W) with internal mirrors. A cooling tank, operated with running cold water, was placed between the water-bath and the lamps.

The low temperature was chosen for the experiments to improve the sensitivity for detection of any effect, because it had already been shown that photosensitivity of bull spermatozoa is highest at low temperature[3,4].

Series of photo-electric recordings of moving spermatozoa[1,6,7] in sample drops were made at 38 °C (body temperature of cattle) at intervals of 0.5 h up to 2 h. However, measurements over the whole range of pH 5.2–9.0 had to be restricted to the series after 0.5 h and 1 h, because the average life-span of the spermatozoa at the more extreme values of $pH < 5.7$ and $pH > 7.5$ was too short. The pH values as given are those determined at 22–23 °C; since the pH change with temperature of the egg-yolk – citrate diluents was 0.03% per °C this range corresponds to pH 5.17–8.95 at 5 °C and to pH 5.22–9.04 at 38 °C. The absolute accuracy of the pH values was ± 0.05 pH unit in all samples.

For determining any effect of pH on photosensitivity the ratios of the mean swimming velocities $\bar{v}$ under illumination and in darkness, and those of the migration rates $N\bar{v}$

Effect of pH on photosensitivity of bull spermatozoa

Correlation	Interval	Correlation coefficient (r)	Significance	Regression coefficient
$\frac{\bar{v}(\text{light})}{\bar{v}(\text{dark})} \times \text{pH}$	0.5 h	0.74	$p < 0.05$	0.016
	1 h	0.958	$p < 0.001$	0.052
	1.5 h	0.988	$0.02 > p > 0.01$	0.093
	2 h	0.976	$p < 0.05$	0.167
$\frac{N\bar{v}(\text{light})}{N\bar{v}(\text{dark})} \times \text{pH}$	0.5 h	0.914	$0.002 > p > 0.001$	0.076
	1 h	0.873	$0.02 > p > 0.01$	0.147
	1.5 h	0.968	$p < 0.01$	0.264
	2 h	0.901	$p < 0.05$	0.234