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

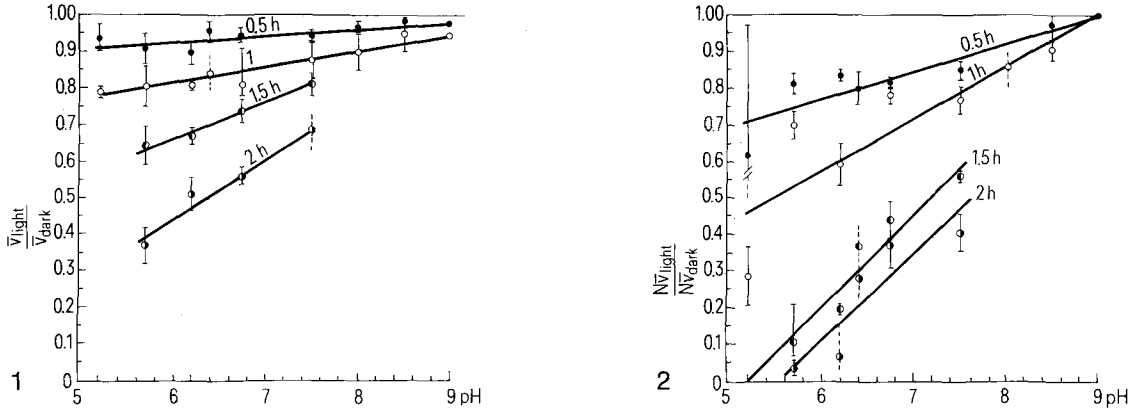

Fig. 1. Ratios of the mean velocities $\bar{v}$ under illumination and in darkness in relation to pH. The lower this ratio, the higher the photodynamic effect.

Fig. 2. pH-dependence of ratios of the average numbers of spermatozoa passing by a fixed spot in unit time (migration rate, $N\bar{v}$) under illumination and in darkness. The photodynamic effect increases with decreasing pH.

(equivalent to the number of sperms passing by a fixed spot in the field of the microscope of the measuring system) were calculated. Then the correlations of these ratios and pH were checked after each time interval. The ratios were taken to account for the dependence of the separate parameter values on pH also when in darkness[8], as well as for eliminating scatter due to natural variation between samples.

Over the ranges as investigated, an apparently linear relationship between both ratios and pH could be established (figures 1 and 2). The numerical results are represented in the table.

From these data and the graphical presentations, it is clear that there is a pronounced effect of pH on photosensitivity of spermatozoa at all pH values lower than 9, increasing with decreasing pH.

Since increasing time of illumination is equivalent to increasing dosage, the effect increases with time, as demonstrated by the significance of the correlations between the slopes of the regression curves with time.

For $d[\bar{v}(\text{light})/\bar{v}(\text{dark})]/dpH = f(t)$ the relationship is not straight, but it becomes straight if the square root of the slope is taken; the corresponding straight-line correlation is $r = 0.995$, $p < 0.001$. For $d[N\bar{v}(\text{light})/N\bar{v}(\text{dark})]/dpH = f(t)$ the best possible fit was obtained by the first order approximation, with $r = 0.948$, $p < 0.01$.

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The determination of extracellular space using hemoglobin[1]

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Summary. The ECS of guinea-pig atria and frog sartorii can be determined using hemoglobin. For guinea-pig atria an ECS of (32.2±2.6)% wet wt for frog sartorii an ECS of (12.4±1.0)% wet wt can be measured.

Extracellular space (ECS) is often determined using inulin[2-5], mannitol[6] or ^{51}Cr EDTA[7,8]. These methods are relatively complicated. The method described here is very simple and yields equally good results. The experiments described below are carried out mainly on guinea-pig right and left auricles. Some experiments, however, are also performed on frog sartorii.

Method. The isolated atria of guinea-pig are equilibrated in conventional Tyrode's solution, which is continuously bubbled with a CO_2/O_2 mixture for 30 min at 35 °C. The auricles are then transferred in a Tyrode's solution containing 1.6 g/100 ml hemoglobin (Merck).

After an appropriate incubation time, the auricles are blotted gently on ash-free filter paper, weighed and washed for 2 h in 2 ml Tyrode's solution; this solution containing the hemoglobin of the extracellular space is centrifuged for 10 min to eliminate suspended matter. The hemoglobin is determined by the cyanmethemoglobin method[9], oxidizing hemoglobin by ferricyanide to methemoglobin, which forms the stable hemiglobincyanide complex with cyanide ions. The complex absorbs light at 540 nm and can be analyzed by photometry. Standards are obtained from the hemoglobin Tyrode's solution in which the atria are incubated.

In figure 1 the extinction ($\lambda = 540$ nm) of the hemiglobincyanide complex is plotted against the dilution ratio of the hemoglobin Tyrode's solution. In order to oxidize and complex hemoglobin, a hemoglobin reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate (ASID Diagnostika) was used.