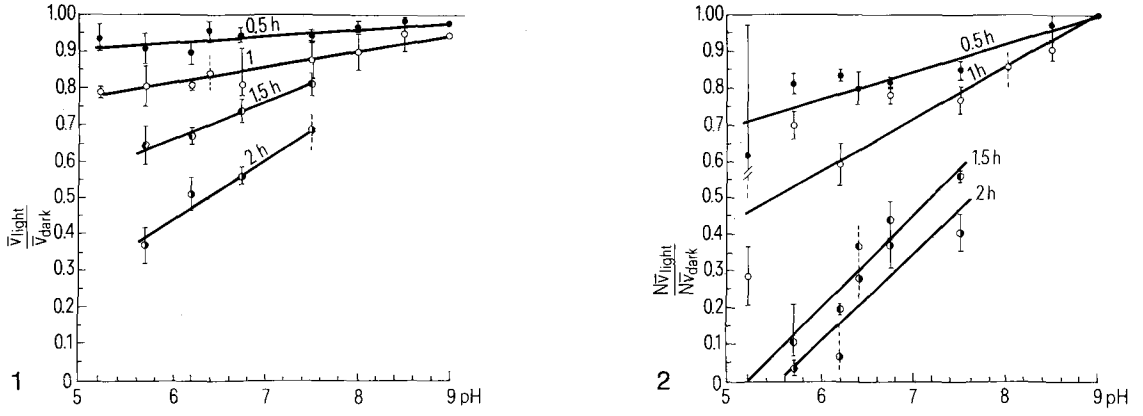

Fig. 1. Ratios of the mean velocities $\bar{v}$ under illumination and in darkness in relation to pH. The lower this ratio, the higher the photodynamic effect.

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.

The extracellular space (ECS) is calculated from the formula:

$$ECS = \frac{\text{Dilution ratio} \times \text{dilution factor} \times 100}{\text{Wet wt of auricle}}.$$

The values presented in figure 2 are means±SD of means. n is the number of measurements.

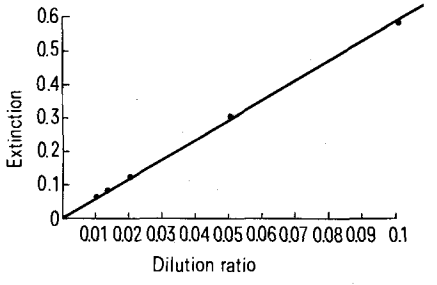

Fig. 1. Extinction of hemiglobincyanide as a function of dilution ratio c/c_0; $c_0 = 1.6$ g/100 ml. SD is smaller than the diameter of the symbols.

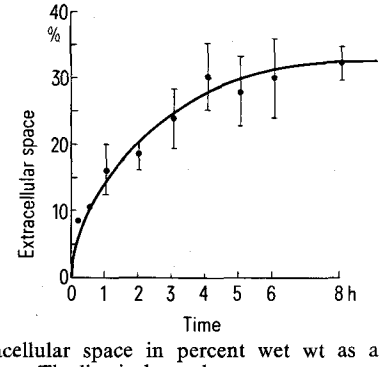

Fig. 2. Extracellular space in percent wet wt as a function of incubation time. The line is drawn by eye.

Results and discussion. Figure 2 shows the time-dependent uptake of hemoglobin in guinea-pig auricles. A steady state is attained after 6 h of incubation. Note that the values are not significantly different for incubation times between 4 and 8 h. It can be concluded therefore that the determination of extracellular space of guinea-pig auricles using hemoglobin requires incubation times of about 6 h. The values then obtained for the ECS in percent wet wt is (32.2±2.6)% (n=9), a result which is in good agreement with data from the literature[10,11]. Some experiments on frog sartorii, where the steady state of hemoglobin uptake is obtained after an incubation time of 2 h, yield a value of (12.4±1.0)% (n=5). This result corresponds with data from the literature[12,13].

Hemoglobin fulfills 2 preconditions necessary for the determination of ECS: a) It does not penetrate into the cells, and b) does not impair electrical and mechanical properties of heart muscle cells. The advantage of the method described lies mainly in its technical simplicity.

1 This work was supported by the Deutsche Forschungsgemeinschaft SFB 114 'Bionach'.
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