the cortisol concentration was varied from 0.25 mM to 1.0 mM and the 5α-androstane-3,17-dione concentration from 0.06 mM to 0.35 mM.

After 10 sec, the incubation was stopped by pouring the suspension into ice-cold stopping solution (2 mM $HgCl_2$, 310 mM NaCl, 1.25 mM KJ). The further experimental procedures have been described before[7,8].

Results and discussion. As has been described, the regions of human erythrocytes, where the glucose transport is occurring, change their physical state at a temperature of about 20 °C[8]; this leads to different apparent activation energies (μ) above and below the transition temperature. Figure 1 shows that a phase transition can be observed as well in the presence of the investigated steroid hormones cortisol and 5α-androstane-3,17-dione. The transition temperature does not change by addition of the steroids into the incubation medium. From the slopes of the Arrhenius relation it follows, however, that the hormones have an influence on the μ-values of the glucose uptake both above and below the transition temperature. The data in the table show that, below the transition temperature, the influence of the two steroids on the increase of the apparent activation energy is small and similar (about 20%). Above the transition temperature, the effect is markedly higher. The increase of the apparent activation energy by 5α-androstane-3,17-dione is about 85%, whereas that of cortisol is about 55%. An increase in μ for reactions catalyzed by membrane-bound enzymes has been attributed to a reduction of membrane fluidity[9].

Parameters for the change of the physical state of the erythrocyte membrane caused by 2 steroid hormones

Steroid in the medium	Apparent activation energy (kcal) of glucose transport	Hill coefficients for the inhibition of glucose transport with hormones
	Above the transition temperature:	
0	7.3	
Cortisol	11.1	−1.0 (at 25 °C)
5α-androstane-3, 17-dione	13.5	−1.2 (at 25 °C)
	Below the transition temperature:	
0	20.0	
Cortisol	24.3	−0.9 (at 10 °C)
5α-androstane-3, 17-dione	23.5	−1.1 (at 10 °C)

It is easy to realize that the influence of the steroids on the decrease of the membrane fluidity is higher above the transition temperature, since at this temperature-range the membrane fluidity is enhanced (in the absence of these compounds). At lower temperature-range, on the other hand, where membrane fluidity is anyway reduced, a further marked reduction cannot be achieved by the hormones. The fact that cortisol is a competitive and 5α-androstane-3,17-dione is a non-competitive inhibitor of glucose transport[7] obviously does not play any role. The Hill coefficients of the inhibition of some membrane enzymes were used as a criterion of the membrane fluidity of rat erythrocytes[10]. From our experiments (figure 2a, b and table), it can be seen that the Hill coefficients of the glucose transport inhibition by the two steroid compounds also show statistically different values (for 10 °C and 25 °C $p < 0.0005$).

Above the transition temperature, the absolute values of the Hill coefficients are higher than below (for cortisol $p < 0.0005$, for 5α-androstane-3,17-dione $p < 0.01$). This points to a higher membrane fluidity above the transition temperature. The interpretation of the relationship between the Hill coefficient and membrane fluidity is still unclear[11].

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Heavy water intake in tissues. II. H_2O-D_2O exchange in the myelinated nerve of the frog[1]

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Summary. The kinetic parameters of D_2O intake in the frog sciatic nerve are obtained by means of infrared spectroscopy. 3 aqueous compartments: a non-exchangeable one (≈29%) and 2 compartments of quasi-free exchangeable water: ≈50% intracellular and ≈21% extracellular, are revealed.

A great deal of experimental studies have been devoted to the effects of heavy water on various biological systems[2], mainly because the use of D_2O is the easiest way to replace a normally occurring isotope (^1H) with its heavy isotope (^2H or D), whose mass ratio is the greatest among those of all stable isotopes. At the same time, this considerable attention is motivated also by the assumption that the replacement of light water with D_2O could reveal the participation of water in various biological processes[3]. However, no molecular understanding of deuterium isotope effects is so far available; large discrepancies and debates still persist with respect to the significance of certain data[4]. In our opinion, this may be partly due to the insufficient knowledge of the time course of D_2O incorporation into each biological object. While not dealing at all with deuterium isotope effects on the nerve, the aim of the present investigation is twofold: a) to obtain the kinetical parameters of heavy water intake in the nerve, and b) to reveal in a proper way the

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distribution of water in this tissue. This latter aspect is essential for the accurate estimation of all intracellular concentrations. In contradistinction to the attempts at measuring the extracellular space by means of large marker molecules that are assumed to remain extracellular[5], the use of D_2O has the essential advantage of avoiding the permanent source of errors entailed by the surface adsorption of these molecules. Similar investigations were reported on the smooth and striated muscles[6], but with the use of a different technique and within a rather different conceptual framework.

Materials and method. Measurements were made on frog, R. temporaria, sciatic nerves, taken from male animals with a weight of about 30 g. Each nerve is weighed (with a 10^{-4} g sensitivity balance) and the parameters of its excitability (rheobase and chronaxie) are determined by standard electrophysiological procedures. At 0 time a bundle of 4 sciatic nerves, with their endings firmly tied, is placed in 99.7% D_2O-Ringer solution.

The substitution of the external D_2O for the tissue water is followed up by means of the optical absorption, E, of the bathing solution at $\lambda = 1470$ nm. At this wavelength (in the near infrared) H_2O has one of its maxima of absorption, while D_2O does not absorb at all, so that the extinction is proportional to the concentration ratio H_2O/D_2O[7]. The optical absorbtion is measured by a Hitachi EPS-2 spectrometer with a PbS photocell as infrared detector. The calibration of the method indicates a resolution of 0.25% in the values of H_2O/D_2O ratio. The temperature around the vessel is regulated by thermostated water circulation and a Cu-Constantan thermocouple allows its monitoring just near the tissue.

After following up (for about 60 min) the H_2O-D_2O exchange, the nerves were again weighed. This control shows a weight increase of less than 5%, which allows us to consider, in the theoretical treatment, that the heavy water intake in the tissue is continuously balanced by an equal water outflow. After this weighing, the excitability of the nerves was once again checked, and in all the cases it remained practically unaltered, inspite of the impossibility of oxygenating the bathing solution during the experiment. Eventually the nerves were dried to constant weight at 105 °C in order to obtain their total water content: V_t.

A complete experiment comprises also the effect of temperature on the rate of D_2O intake in the tissue. Accordingly, in each case the exchange was first followed up at a given temperature T on a bundle of sciatic nerves from the right legs of 4 animals, the corresponding left nerves being kept in the animals until the same exchange was followed up on them at the temperature T′ (usually $T' = T + 10$). The optical absorption of the solution is recorded once per min, except for the first minute which is taken to handle and to adjust the experimental setting.

Computations. The time dependence of the experimental values of the extinction, E(t), is accurately expressed as a biexponential function:

$$E(t) = A[1 - \exp(-\alpha t)] + B[1 - \exp(-\beta t)] \quad (1),$$

whose parameters A, B, α, β are obtained by numerical fitting on an IBM-370 computer. These parameters are indicative of the rate of water exchange for deuterium oxide and of the tissue space accessible to D_2O.

A complete mathematical description of water distribution in a series of tissular compartments was published elsewhere as a theoretical model[8]. In that description, which offers the computational frame for the experimental data presented here, the various water compartments are defined solely on the basis of water accessibility for diffusion exchange, in passing over energy barriers separating the compartments. Obviously, this criterion allows us to consider only a series array of compartments, without any a priori anatomical correspondence. Based on that general description, we restrict ourselves to representing nerve water as distributed in only 2 distinguishable compartments of volumes V_1 and V_2, only the compartment 1 being in direct contact with the external solution of volume V_0. The whole water outflow from the tissue into the external D_2O-solution (linearly related to its optical absorption at $\lambda = 1470$ nm) is given by the biexponential expression (1). Instead of a full derivation which is beyond the scope of this communication[9], we list below the formulas giving the volumes of the 2 compartments, V_1 and V_2, computed within the limits of assuming symmetrical energy barriers separating the compartments; and the apparent free energy changes for heavy water intake in the first tissular compartment from the outer one (ΔG_{1-0}) and into the second compartment from the first one (ΔG_{2-1}). All these are functions of the parameters A, B, α, β (at the temperature T) and A′, B′, α', β' (at the temperature T′), directly obtained by numerical fitting of the experimental points with the equation (1). The formulas are:

$$V_1 = \frac{A\alpha + B\beta}{(9.298-A)\alpha - B\beta} V_0 \quad (2)$$

$$V_2 = \frac{9.298\ B\ (\alpha-\beta)}{[(9.298-A)\alpha - B\beta](9.298 - A - B)} V_0 \quad (3)$$

$$\Delta G_{1-0} = \frac{RT_1T_2}{T_2 - T_1} \ln \frac{(A\alpha + B\beta)[(9.298-A')\alpha' - B'\beta']}{(A'\alpha' + B'\beta')[(9.298-A)\alpha - B\beta]} \quad (4)$$

$$\Delta G_{2-1} = \frac{RT_1T_2}{T_2 - T_1} \ln \frac{B(\alpha-\beta)(A'\alpha' + B'\beta')(9.298-A'-B')}{B'(\alpha'-\beta')(A\alpha + B\beta)(9.298 - A - B)} \quad (5)$$

Results. Owing to the inherent differences among the nerves, it would be misleading to average the experimental values of the extinction or – which is the same – of the parameters A, B, α, β. Accordingly, the fitting procedure was performed for each pair of corresponding curves at the temperatures T and T′ and only the final values of V_1, V_2, ΔG_{1-0} and ΔG_{2-1} were mediated. These values are listed below, together with the errors (mean value ± S.D.) for eight pairs of recordings at temperatures ranging from 20 to 30 °C.

The total water content of the nerves (V_t), obtained by heat drying, as well as the volumes V_1 and V_2, are expressed with respect to the initial fresh weight of the tissue.

The time course of nerve water replacement by heavy water is significantly expressed by the quasi-saturation times of the 2 compartments, τ_1 and τ_2, defined by the conditions: $1 - \exp(-\alpha t) = 0.95$ and $1 - \exp(-\beta t) = 0.95$, so that $\tau_1 = \ln 0.05/\alpha$ and $\tau_2 = \ln 0.05/\beta$. Obviously these kinetical parameters strongly depend not only on the volume of the nerve water, but also on the size of the external compartment. For a ratio $V_t/V_0 = 1:8$ at 22 °C, they are: $\tau_1 = 25$ sec and $\tau_2 = 467$ sec. The quasi-saturation times τ_1 and τ_2 are linearly related to this ratio in an inverse proportionality.

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V_t (ml/kg)	V_1 (ml/kg)	V_2 (ml/kg)	$1 - \frac{V_1 + V_2}{V_t}$ (%)	ΔG_{1-0} (kcal/mole)	ΔG_{2-1} (kcal/mole)
763 ± 27	163 ± 19	382 ± 47	29 ± 7	4.7 ± 0.7	2.1 ± 0.5

Conclusions and discussion. As mentioned before, the 2 aqueous compartments, whose volumes are V_1 and V_2, do not have any unambiguous anatomical correspondents, simply because they are defined only on the basis of the water accessibility for diffusion exchange. Nevertheless, in view of the rapidity with which the exchange between the external solution and the first tissular compartment (as expressed by the quasi-saturation time τ_1) occurs, one can infer that this compartment is extracellular, namely that it represents the intercellular space. This conclusion is further substantiated by the rather low free energy change ΔG_{1-0}, when D_2O passes from the external solution into this compartment. The second aqueous compartment of the nerve, into which the heavy water intake proceeds much more slowly, most probably represents the axoplasmic water. The fact that ΔG_{2-1} is even lower than ΔG_{1-0} can be tentatively accounted for by assuming that water passage through the conjunctive sheath surrounding the nerve is energetically more important than the passage through the axolemma. Anyhow, both ΔG_{1-0} and ΔG_{2-1} show that the intake of heavy water into the nerve is not accompanied by significant energy changes, so that one can conclude that at least 70% of the whole nerve water is almost free for rapid diffusion exchange. The remainder of up to 30% of the nerve water is not revealed by the deuterium oxide replacement, and thus it appears as either 'bound' or somehow 'obstructed' water. Maybe it is more correct to call this compartment 'invisible' from the viewpoint of the present technique. The slowness of the overall process of heavy water permeation into the tissue is to be attributed mainly to the spatial compartmentalization than to differences in the state of water. These conclusions are in very good agreement not only with similar investigations[6], but also with recent NMR measurements on the muscle[10,11].

A practical indication arising from this study is that in the investigations of the physiological effects of deuterium on the myelinated nerve, when deuteriation is obtained by simple immersion in heavy water, one should wait for several tens of minutes until heavy water replaces light water in the deeper tissular compartments.

It is to be observed that our measurements are practically incompatible with stirring, which means that the effect of the unstirred layer at the surface of the tissue is de facto included in the results. Anyhow, this effect leads to an apparent rate of exchange which is lower than the true one, a fact strengthening further the above conclusions.

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Liver regeneration: cultural characteristics of remnant liver cells following a second partial hepatectomy

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Summary. When a 38% hepatic resection (rat) is followed in 24–72 h by a 30% hepatic resection (in the same animal), in vitro cell survival is observed in liver remnant tissue removed at any interval following the second resection.

Cells from the liver remnants of weanling or immature rats survive in culture when removed at any interval following a large hepatic resection[1,2]. In mature rats, however, employing the same primary culture techniques, only cells removed from the hepatic remnant at intervals greater than 46 h following hepatic resection survive in culture. This report describes the influence of a prior hepatic resection on the growth capacity of remnant liver cells (mature rat) removed during this early (< 46 h) interval.

Sixty 200 g male Wistar rats were subjected to a medial lobectomy (38% hepatic resection). At 3 subsequent intervals, i.e., 8 h, 24 h and 72 h, 20 of the same animals were subjected to a second (left) lebectomy. This would constitute a 30% hepatic resection in the intact rat, but more than that in this situation. 40 (control) rats had single 68% or 30% resections. At intervals of 12, 24, 36, and 48 h following the second hepatic resection, animals in the experimental groups were sacrificed and liver tissue removed for culture. At the same intervals following the single (control) resections, tissue was also obtained. Tissue was taken from the caudate lobe, an area not contiguous to prior resection(s).

Liver tissue from 5 rats in each of the 12 experimental and 8 control groups was combined, minced, the cells dispersed with trypsin solution (0.25%), suspended in Eagle's minimal essential medium (MEM) and counted. Each cell pool so formed was inoculated into 20 Petri dishes (1.8×10^6 cells/dish), containing MEM with 20% fetal calf serum (FCS) additive. Cultures were maintained at 37 °C in an incubator flushed with CO_2 and refed with the same media. After 2 days in culture, viable (attached) cell populations are reduced to approx. 5% of the inoculum. After 16 days in culture, cells attached to the plate were removed with trypsin solution, resuspended in MEM and counted with a hemacytometer.

As noted in previous studies[1,2] there was no cell survival in tissue removed 8, 24, and 36 h following a single hepatic resection, either 30% or 68% (table, a and b). A preliminary resection (38%) however, performed 24, 40 or 72 h prior to a 30% resection, resulted in survival

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