Musebeck, was also reported to result from a substance left on its host by earlier females and from the detection of an oviposition deterrent within the parasitized host[13]. The poison gland of this species was considered the source of the deterrent and not the lateral oviducts as was the case for *C. perdistinctus*. The source of the external pheromone was not definitely identified for *O. lepidus*.

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F. S. GUILLOT
S. B. VINSON

Texas A&M University,
Department of Entomology,
College Station, Texas 77843

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Spin Echo Studies on Cellular Water

HIGH resolution nuclear magnetic resonance (NMR) studies on rat skeletal muscle have recently shown that cellular water produces an absorption line almost ten times as broad as the line width of pure water[1]. This suggests that these water molecules are at least partly bounded such that the proton correlation time is increased. Similar observations have also been made for deuterated water in skeletal muscle[2] and for H_2O in other tissues[3–5]. Our results further substantiate the notion that ordered water exists in biological tissue and rules out several criticisms of this interpretation.

To obtain more precise and detailed information about the cellular water, we have complemented the high resolution studies. The spin–lattice relaxation time (T_1), the spin–spin relaxation time (T_2) and diffusion coefficient (D) were measured by procedures given elsewhere[6,7]. All experiments were performed at room temperature and the results are summarized in Table 1. Pure water in identical experimental conditions gave values for T_1, T_2, and D very close to earlier published data[8,9]. We find $T_1/T_2 = 1.85$ for pure water in agreement with the observations of Meiboom *et al.*[8].

These measurements on mammalian muscle water agree with the findings of the previous high resolution[1] and pulsed[3,4] NMR studies. The diffusion coefficient of muscle water was also in qualitative agreement with that determined for a variety of biological tissues[4]. The observations suggest that the change of water properties (in the NMR sense) in the cellular environment is a universal phenomenon which is independent of species and tissue. The interpretation of the

Table 1 Measurements of Relaxation Times and Diffusion Coefficients

	T_1 (s)	T_2 (s)	$D \times 10^{-5}$ (cm²/s)
Pure water	3.09 ± 0.15 (4)	1.52 ± 0.093 (9)	2.78 ± 0.035 (11)
Skeletal muscle	0.72 ± 0.05 (6)	0.045 ± 0.002 (12)	1.43 ± 0.07 (5)

All values are mean ± standard error of the mean. The numbers in parentheses represent the number of samples analysed.

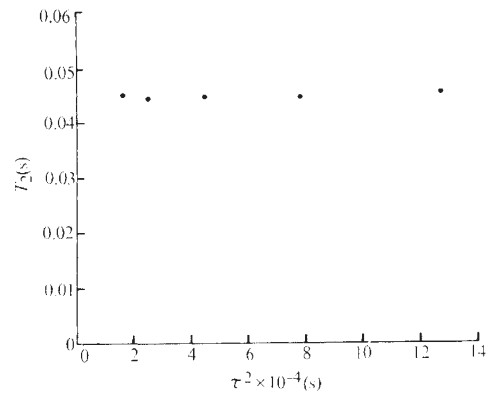

Fig. 1 Spin–spin relaxation time (T_2) of proton in muscle water as a function of observation time (τ).

NMR results, however, is still controversial. Some investigators favour the interpretation that the dramatic shortening of the proton relaxation times implies a structural change of the cellular water; water molecules inside the tissue are more ordered than those of ordinary water[1,2], but others do not agree with this interpretation. Hansen and Lawson[10] argued that the high resolution NMR line broadening might be caused by the diffusion of free water through microscopic magnetic field inhomogeneities, although the pulsed NMR results are not consistent with this broadening mechanism. First, the shortened values of T_1 for cellular water cannot be explained by diffusion in an inhomogeneous field. Second, if this mechanism is to be effective in reducing the apparent spin–spin relaxation time, then an unreasonably large value for the local field inhomogeneity must be assumed. It was shown by Carr and Purcell[6,7] that the measured T_2 in an infinite sample is given by

$$\frac{1}{T_{2(\text{effective})}} = \frac{1}{T_2} + \frac{1}{3}\gamma^2 G^2 \tau^2 D \qquad (1)$$

Here, D is the diffusion coefficient, γ is the gyromagnetic ratio, G is the magnetic field gradient and τ is the time between the 90° pulse and the 180° pulse. In a bounded system the effective D is smaller than the true diffusion coefficient[11–13]. Suppose we assume an upper limit for D and set it equal to the value for pure water. Then for a typical $\tau \sim 0.03$ s and T_2 measured ~ 0.04 s, a value of $G = 2.2$ gauss/cm would be required, which is more than twenty times the natural sample magnetic field inhomogeneity as determined from the width of the echo signal. Furthermore, we have measured the value of T_2 for muscle water for various values of τ from 13 ms to 36 ms. The values of $1/T_{2(\text{effective})}$ were unchanged within experimental error (Fig. 1). If the relaxation is the result of molecular diffusion through a magnetic field gradient, the reciprocal of the measured T_2 would be a linear function of τ^2 (ref. 10 and equation (1)). This dependence is not observed in our experiments.

Other objections to the ordered water interpretation have also been advanced. For example, Glasel has pointed out[14] that the exchange of solvent species between the bulk phase, average local magnetic field, and that which exists near suspended matter is sufficient to cause line broadening, and no ordering or disordering of the solvent must be postulated. To support this argument, Glasel cites experiments on water in systems packed with glass beads to show that the relaxation times of protons and deuterons change as the surface-to-volume ratio varies. We feel, however, that the results of these experiments do not necessarily negate the ordered water interpretation.

Glass is hydrophilic and has a strong Van der Waals interaction with water, which can be easily seen from the capillary effect. Hori's freezing experiments[15] showed that a thin layer (greater than 1 μm) of water held between two glass surfaces

is highly structured. Therefore, it is clear that water molecules must be ordered near the surface of the glass beads used by Glasel[14]. As the radius of the glass bead is reduced, the surface area of the glass–water interface, as well as the percentage of structured water, increases. This increase in water order can cause the relaxation times to be reduced. Glasel's experiments therefore do not disprove the ordered water interpretation, but support it. In addition, evidence for the existence of a highly structured water near interfaces is reported elsewhere for physical[15–20] and biological systems[21–23].

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D. C. CHANG
C. F. HAZLEWOOD
B. L. NICHOLS
H. E. RORSCHACH

Departments of Pediatrics and Physiology,
Baylor College of Medicine,
Texas Children's Hospital, and
Department of Physics,
Rice University,
Houston, Texas 77025

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Trickle Perfusion for Organ Preservation

THE two most successful methods of preserving canine kidneys are those described by Collins *et al.*[1] and Belzer *et al.*[2]. In the Collins system, which is extremely simple, the organ is flushed with a solution ('Collins C4') similar to intracellular fluid with a high potassium, phosphate and magnesium content. This solution is left in the kidney stored at 4° C, which will function well when re-implanted after 24 h. It is essential, however, that there should be no warm ischaemic damage to the kidney[3]. In Collins's experiments, the animals were very carefully maintained so that the kidneys were not maltreated until they were removed and rapidly cooled.

The Belzer system preserves the kidneys at 10° C for longer periods with better initial function on re-transplantation after 24 h[4] and is successful with kidneys that have suffered ischaemic damage[5]. The apparatus, however, is complicated, expensive and difficult to transport. Very careful surveillance and continuous perfusion at relatively high flows with a recirculating perfusate containing specially prepared plasma are needed with this method.

Hypothermia is of value in organ preservation. Oxygen consumption of a kidney at 10° C is approximately 5% of normal[6]. Some of the advantages of the Belzer system can be obtained with a much simpler perfusing apparatus using low flows and single passage of perfusate without recirculation[7]. Flow rates of 100 ml./h of a hypertonic dextran containing buffered salt solution at 4° C gave consistently satisfactory preservation of dog kidneys for 18 h and 50% successful preservation for 30 h when the removal of the second of the kidney pair was delayed for 3 weeks. Lactic acid was continuously released and a progressive rise of lactic dehydrogenase in the venous effluent was detected, indicating active anaerobic metabolism but also continued cell damage.

We felt that this approach deserved further study, with kidney and liver preservation. Livers were preserved for 11–12 h, with 3 l. of perfusate passed through the organ, and kidneys for 24–26 h with a perfusion flow of 30–80 ml./h. Preliminary studies with dyes injected into the perfusate showed that at these very low flows, much of the perfusate did not go through the renal cortical capillaries but came out through capsular arterioles directly. We therefore perfused the kidneys with intermittent pulses of more rapid perfusate flow to improve cortical capillary perfusion.

Johnson *et al.*[8] successfully used plasma protein fractions (PPF)* as a perfusate instead of cryo-precipitated plasma. Our perfusates also contained PPF with additives similar to those used by Belzer and colleagues, or PPF with an equal volume of Collins C4 solution. We have tried to develop a simple perfusing system with better protection of the organ than is possible with unperfused preservation, and eventually to test this system with ischaemically damaged kidneys. It should also be possible to study metabolism of the organ during perfusion, by analysis of the difference between constituents of a perfusate and the effluent, with a view to improving the preservation and developing a better perfusate and perfusion conditions.

Dogs were anaesthetized with 'Nembutal' supplemented with 'Halothane'. Pigs were induced and maintained on 'Halothane' alone. The right kidneys of the dogs were removed without any special preparation; 500–1,000 ml. of 5% dextrose was infused intravenously and the wound was closed. After removal of the kidney, it was flushed with the perfusing solution and this was continued at 4° C. After 24–26 h the kidneys were re-transplanted to the iliac vessels of the original donors[9] and 500 ml. of dextrose was infused before the opposite kidney was removed. In the liver preservation experiments, the pigs were allowed to breathe spontaneously during 'Halothane' anaesthesia. The liver was removed from the donor and the portal vein and hepatic artery were cannulated and infused with 1 l. of Hartmann's solution at 4° C. The perfusion was continued at this temperature with solution containing PPF. The liver was allografted orthotopically to the recipient pig (technical details have been described elsewhere[10]).

Kidneys and livers were placed in sterile polythene bags and the effluent from the renal vein and vena cava collected in the bag and removed by gentle suction. The organs were perfused by a drip with a gravity head of approximately 1 m. The drip line was connected to a simple blood-warming coil placed with

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