treatment set contained 20 ml Czapek's agar medium, 1 ml extract, 0.8 ml antibiotic solution[6], a fungal disc (5 mm diameter, cut from the periphery of 7-day-old culture) inoculated in the centre. Control set was prepared similarly, except that equal amount of distilled water was added instead of the extract. Fungitoxicity of the volatile vapour of the extract was determined by Latham and Linn's method[11], and the effect of increased inoculum was observed in Czapek's liquid medium. Phytotoxicity and systemic activities of the extract at its lethal (1:40) and hyperlethal (1:20) concentrations were tested following Grewal[12] and Erwin et al.[13].

Observations. As is clear from table 1, the extract retained activity up to 100 °C, beyond which it became gradually ineffective; retained activity on autoclaving; remained completely active up to 15 days when stored at room temperature; killed the test fungi within 5 min; possessed maximum fungitoxicity between pH levels 5.0–9.0. The extract was fungicidal against all the test fungi and its lethal dose was found to be 0.5 ml i.e., 1:40 dilution. Volatile vapours emitted from the extract were also fungicidal (table 1). It possessed a wide range of fungitoxicity, inhibiting all the 26 fungi tested at 1 ml dose, 19 at 0.5 ml dose and 7 at 0.25 ml dose (table 2).

The extract was found to inhibit heavy fungal inoculum (10 discs each of 5 mm diameter). The extract exhibited no phytotoxicity during seed germination, root application and foliar spray studies. However, it did not prove systemic by either of the applications through roots and shoots.

Discussion. Disease resistance in plants has been attributed to the various chemicals present in their tissues. Strong fungicidal action exhibited by *R. sceleratus* in the present investigation may be responsible for its disease-free occurrence. It may be marked that Bhakuni et al.[14] have reported the plant to possess no fungitoxicity, while on the contrary Nene et al.[15] have found it to be active. It may be noted that Bhakuni et al.[14] missed the activity on account of their using dried material, as the plant has been found to loose activity on drying[16]. Loss of fungitoxicity due to dehydration has already been recorded by some workers with several other plants too[4,13]. The extract, on account of its thermostability, quick fungicidal action, activity at broad pH range, tolerance against heavy fungal inoculum, broad fungitoxic spectrum and no phytotoxicity during in vitro trials, proves to be of great significance for in vivo studies. It is hoped that the extract and its active principle(s) will prove to be of great therapeutic value for plants against different diseases.

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2 Acknowledgment: The authors are thankful to Prof. K.S. Bhargava, Head, Department of Botany, Gorakhpur University, Gorakhpur for providing laboratory facilities.
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Water compartments in the myelinated nerve. III. Pulsed NMR results[1]

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Summary. 3 experimentally distinct transverse relaxation components of the water in frog sciatic nerve are obtained by Carr-Purcell-Meiboom-Gill technique. The relative weights of these components: ≈29%; ≈50%; ≈21% fit well with water compartments in this tissue as revealed by previous methods.

The state of water in tissues was, and still continues to be, the aim of many studies, due to its fundamental interest, both theoretical and practical. NMR techniques are widely used and a great deal of experimental data already accumulated, particularly on muscle water and to a somewhat lesser extent on water in nerve and other tissues[2]. However, the problem is far from being solved, the role and properties of tissue water still remaining a matter of considerable dispute[3]. Here we present results on the proton magnetic resonance (PMR) behaviour of the myelinated nerve, revealing the existence of 3 experimentally distinct types of water, with relative slow exchange processes between the various components.

Materials and method. The measurements were made on frog *(Rana temporaria)* sciatic nerves immediately after they were dissected or after keeping them for 30 min in normal Ringer solution. The total water content of the nerves was obtained by heat drying to constant weight at 105 °C. Their 'NMR-visible' water content was determined by calibration of the detector system, using samples of Cu^{2+}-doped water. The transverse relaxation behaviour of water protons in nerve and its variation with temperature and radio-frequency pulse separation, was observed using a Carr-Purcell-Meiboom-Gill sequence. The measurements were made on a Bruker BKR-322s spectrometer operating in this study at 45 MHz. The sequence pulse spacing (τ) was varied between 0.4 and 4 msec and the amplitudes of 256–1024 echoes were recorded. In each experiment, 8–64 transverse relaxation decays were averaged in a BNC-12 minicomputer. The temperature of the samples was gradually lowered from 300 to 268 °K.

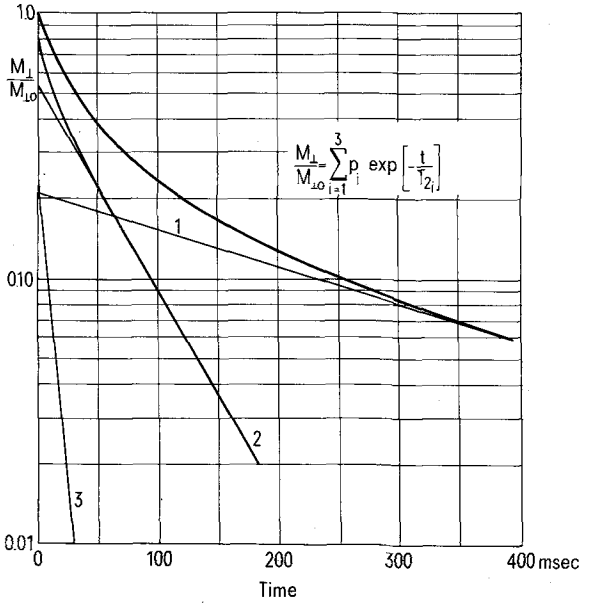
Results. The figure shows a typical multiexponential trans-

Table 1.

p_1(%)	p_2(%)	p_3(%)	T_{2_1}(msec)	T_{2_2}(msec)	T_{2_3}(msec)
20.85±2.71	50.33±1.75	28.72±0.97	310±21	70±14	17±6

Table 2.

T(K)	p_1(%)	p_2(%)	p_3(%)	T_{2_1}(msec)	T_{2_2}(msec)	T_{2_3}(msec)
300	19	49	32	289	67	20
282	20	47	33	374	80	44
273	21	49	30	524	90	35
268	19	51	30	590	85	16



A typical multiexponential transverse relaxation decay of water protons in nerve and its graphical resolution revealing the 3 distinct components.

verse relaxation decay of the water protons in frog myelinated nerve, obtained at room temperature and $\tau = 1$ msec. Its analysis by peeling-off procedure indicates the existence of a multi-exponential decay curve composed of at least 3 exponential terms. This means that the decay of the transverse magnetization may be represented by the equation

$$\frac{M_\perp}{M_{\perp 0}} = \sum_{i=1}^{3} p_i \cdot \exp\left[-\frac{t}{T_{2_i}}\right]$$

where M_1 and M_{10} are the transverse magnetizations observed at times t and $t=0$ respectively; T_{2_i} represent the apparent relaxation times and p_i the populations of the 3 distinct components revealed experimentally. The mean values of these parameters, obtained by computer curve-fitting or by graphical analysis of the multiexponential decays in the case of 7 nerves at 300 °K and $\tau = 1$ msec, are listed in table 1. Table 2 contains the values of the same parameters at different temperatures.

The 'NMR-visible' water-content of the nerves compares very well (SD up to 10%) with their total water content. We failed to observe any differences between the relaxation decays recorded on nerves oriented at 90 °C with respect to the magnetic field and those randomly oriented, as Fung[4] observed to occur in the muscle. The comparison of the transverse relaxation behaviour of nerve water protons at different temperatures and fixed τ leads us to infer the existence of some temperature dependent exchange processes between the various components. When decreasing the temperature, the transverse relaxation first becomes slower as a whole and then the fast component relaxes even faster and the slow one slower (table 2), due to the exchange processes becoming less effective. The presence of some kind of exchange between various sites is also suggested by the τ-dependence of transverse relaxation at fixed temperature: at a lower τ the fast component relaxes faster and the slow component slower. However, so far we cannot prove that these sites are identical with the relaxation components observed experimentally.

Conclusions and discussion. If one accepts that the 3 experimentally distinct relaxation components correspond to physically distinct types of water molecules, one can tentatively ascribe them to different tissue water compartments. The slowly relaxing fraction, amounting to about 21% of the total tissue water, is probably located in the intercellular space, it representing the extracellular water. The physical properties of this fraction are the closest to those of a dilute aqueous solution, however the propinquity to macromolecular surfaces lowers its transverse relaxation time. The fast relaxing component, representing about 29% of the signal, may be ascribed to water closely associated with proteins and phospholipids, having the physical properties strongly affected by this association. The intermediate relaxation time fraction, amounting to about 50% of the whole nerve water, most probably represents the axoplasmic water. Obviously, this interpretation is not the only one, but these NMR results are in very good agreement with others obtained by a quite different method[1], and also consistent with NMR data on the muscle[5,6].

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