the alkaline extraction, only two washes with 0·1 volume distilled water were necessary to obtain neutral washings. Addition of this procedure to urinary neutral steroid methods with less efficient removal of acids and phenols[4,5] abolished the interference associated with the presence of β-naphthol in the urine.

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Nuclear Magnetic Resonance Spectrum Changes accompanying Rigor Mortis

INFORMATION on the behaviour of water in biological cells is essential to developing an understanding of the roles of that ubiquitous molecule in biological systems. This communication reports observations of nuclear magnetic resonance spectra of cod muscle over a period of time from shortly after death to the cessation of rigor mortis, which suggests that cellular water suffers a loss in structure and an increase in mobility during this time.

Two codfish (*Gadus morhua*), each weighing approximately 4 lb., were filleted within 10 min of death. Three core samples of the dorsal muscle fillet were obtained using a modified core cutting nuclear magnetic resonance sample tube. Nuclear magnetic resonance spectra were run in a 'Varian *A*-60' high resolution nuclear magnetic resonance spectrometer (14·092 kG field) adjusted for optimum field homogeneity, and 'ringing' with distilled water. The probe temperature was +15° C. Spectra of the three samples were taken at approximately 45, 280 and 380 min after death.

At 150 min after death the fillets from which samples were removed were stiffened and had a rough, hardened texture. Rigor mortis subsided 5·5 h after death, at which time the fillet tissue had softened and was exuding clear liquid.

The muscle spectra all consist of a single symmetrical water peak which is four to eight times broader than that produced by pure water. The peak width, taken at 0·6 of the peak maximum, decreases by 14–22 per cent in approximately 6 h, as shown in Fig. 1. Peak area does not change appreciably.

Broadening of the nuclear magnetic resonance absorption peak of water in the presence of proteins, cellular material, starches and other materials has been previously reported[1–5]. Broadening is evidence of a decrease in transverse relaxation time ($T_2 = f$[1/peak width]), and reduction in proton mobility such as might result from adsorption or bonding of water by cell protein[1,6]. Bratton *et al.*[4] have reported that a reversible narrowing of water peak width (and increase in T_2) accompanies the contraction of living frog muscle, and suggest an explanation in terms of change in extent of the 'bound water' phase. Reduction in the water-holding capacity of muscle in rigor mortis is well established[7].

The core-cutting sample tube is essential for producing geometrically uniform samples and reproducible spectra, and may be useful to other nuclear magnetic resonance investigations of semi-solid materials. The tubes are made from 'Varian' 5-mm nuclear magnetic resonance sample tubes, by cutting off the sealed end and grinding a scalloped cutting edge in its place. The tube cuts a

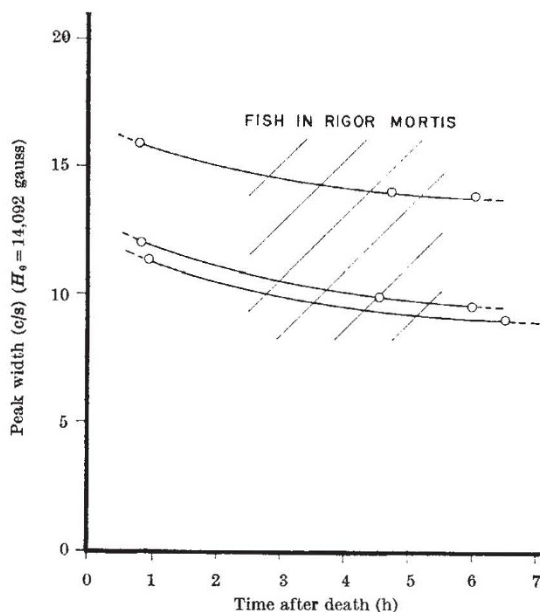

Fig. 1. Nuclear magnetic resonance peak width plotted against time after death

uniform cylindrical core when screwed into fish muscle. The core remains in the tube, the lower end of which is then sealed with a 5-mm plug of putty. The water contents of sample cores have been estimated at 81 ± 2 per cent by comparing their absorption peak areas to samples of known water content.

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Two-dimensional Thin-layer Chromatography of the Major Mononucleotides of Ribonucleic Acid

THIN-LAYER chromatography, by virtue of its convenience, sensitivity, and short developing time, is useful for the separation and quantitation of many types of nucleic acid derivatives[1–3]. This communication describes the method used by this laboratory for the separation of the products of alkaline hydrolysis of ribonucleic acid.

Preparation of thin-layer plates: 15 g of '*MN*-cellulose powder 300*G*' (Macherey and Nagel, Düren, Germany) is mixed with 90 ml. of distilled water by shaking for 1 min. Clean 20 × 20 cm glass plates are coated with the use of a Desaga-Brinkmann 'Fixed-Thickness Applicator'. The plates are dried in air for 30 min and the cellulose is activated by heating for 10 min at 110° C. The plates are washed with water and air-dried before they are used.

Chromatography procedures: RNA is hydrolysed by incubation either for 18 h at 37° C in 0·3 N potassium hydroxide, or for 48 h at 60° C in 10 per cent piperidine. The potassium is removed by precipitation with perchloric

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