PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

A Survey of Some Applications of NMR Chemical Microscopy [and Discussion]

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A survey of some applications of NMR chemical microscopy

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We address two related aspects of NMR chemical microscopy of intact mammalian tissues: attainment of high spatial resolution images; and incorporation of a level of chemical discrimination by sensitizing the image intensity of either chemical shift or nuclear relaxation. These concepts are illustrated with images of human finger joints and of the head or heart of rat.

1. Introduction

During the past decade, nuclear magnetic resonance imaging (MRI) has been developed largely as a method for clinical diagnosis in man, such that two-dimensional slice images can now be obtained with slice thicknesses of 2 mm, and in plane resolution of less than 1 mm. During the same period, nuclear magnetic resonance spectroscopy (MRS) has also advanced rapidly so that localized spectra can now be obtained from suitably large regions of the human body. The area of 'nuclear magnetic resonance chemical microscopy' addresses that broad range of measurements which attempt to combine the spatial localization of MRI with the chemical selectivity of MRS. Inevitably, given the generally poor signal-to-noise sensitivity of the NMR method, optimization of any measurement in favour of one experimental parameter imposes limitations on some others; in particular, high spatial resolution images cannot be obtained from the NMR responses of any species which is present in high dilution.

The focus of this article is *in vivo* proton NMR of human extremities and small laboratory animals, with particular emphasis on optimization of the resolution attainable both spatially and chemically. Following a brief review of high spatial resolution imaging, the traditional perception of 'NMR chemical microscopy' is extended to question what chemical data can be obtained without sacrificing spatial resolution; this can be achieved not only by using chemical-shift resolution, but also nuclear spin relaxation times. Finally, the results obtained are discussed with a view to identifying possible applications, limitations and future prospects.

2. Experimental methods

All measurements were made using a 2 T, 31 cm bore Oxford Instruments magnet together with an Oxford Research Systems Biospec I console. Because high-resolution imaging requires the highest attainable signal-to-noise ratio (SNR) the

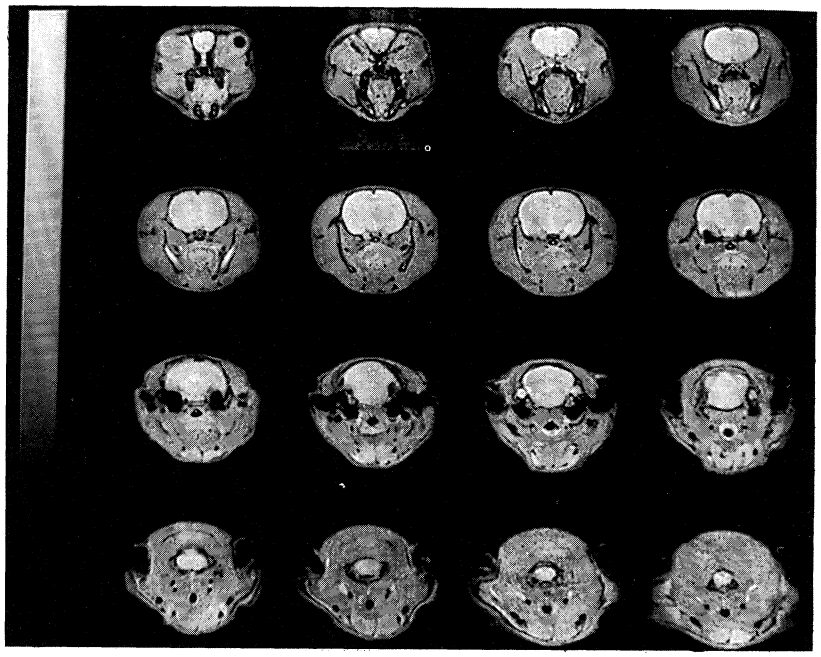

Figure 1. Sixteen adjacent transverse spin-echo images of the head of a 200 g Wistar rat. Slice thickness is 2 mm and in-plane resolution is 0.16 mm.

entire magnetic assembly was surrounded by a radiofrequency screen. Furthermore, a closely fitting radiofrequency probe was constructed for each object, based on either the sine-spaced birdcage (Bolinger *et al.* 1988) or a solenoid design. Gradients were designed of a sufficient strength to obtain the resolution needed (40 mT m^{-1}) together with fast enough switching times to allow minimization of unnecessary T_2 decay (Carpenter *et al.* 1989).

Most MRI data-sets were obtained using the spin-warp method (Edelstein *et al.* 1980) because it is relatively robust to a small amount of motion of the object, and also to variation in the homogeneity of the static magnetic field.

All animals were anaesthetized before and during data acquisition, following approved practice. Cardiac gating was triggered from the electrocardiograph. As the human volunteers were neither anaesthetized nor sedated, care was necessary to ensure that they were sufficiently comfortable to remain still during the entire experiment.

Image processing and relaxation time calculations were performed on SUN 4/150 and DEC 3100 workstations using software designed in house.

3. Results

Providing that care is taken to optimize the SNR, *in vivo* MRI now allows the routine acquisition of images from small laboratory animals with SNR and spatial resolution equivalent to that demanded for clinical studies in man. For example, figure 1 shows a set of adjacent 2 mm thick slice images taken through the head of a rat, with in-plane resolution of *ca.* 0.160 mm; by using interleaved multislice imaging the

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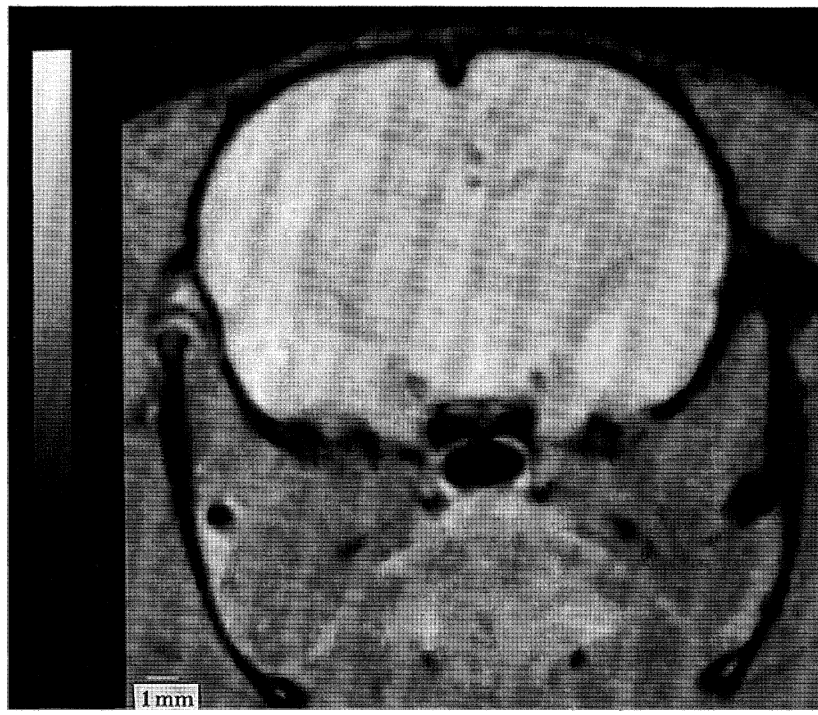

Figure 2. Single slice from figure 1.

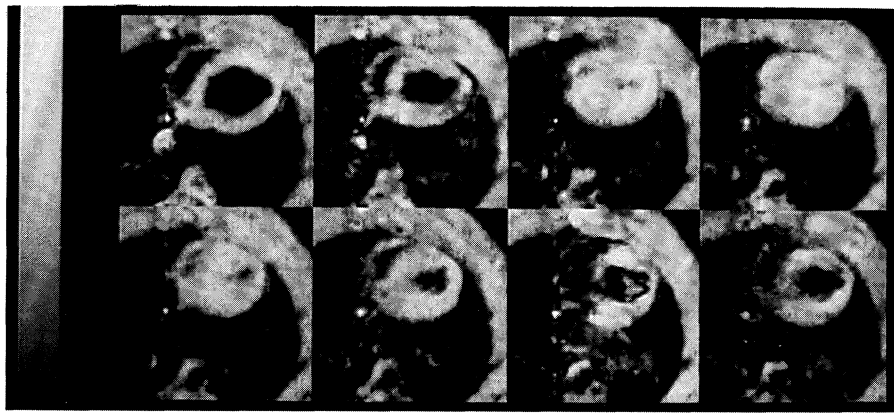

Figure 3. Single slice spin-echo image through a rat heart (300 beats per minute). Images were gated to different points in the cardiac cycle using a ECG trigger developed in-house. Slice thickness is 2 mm and in-plane resolution is 0.18 mm.

complete set was acquired in a total of 20 min. Figure 2, which is a software expansion of a single slice, shows that the relative resolution is similar to that obtained clinically in man. It is also possible to image more difficult subjects within reasonable time periods. Thus figure 3 shows a set of 2 mm thick single slice images of a rat heart, gated to eight points in the cardiac cycle with 0.18 mm in-plane resolution; data acquisition took a total of 40 min. Although images such as these yield anatomical information at relatively high spatial resolution, as can be seen in

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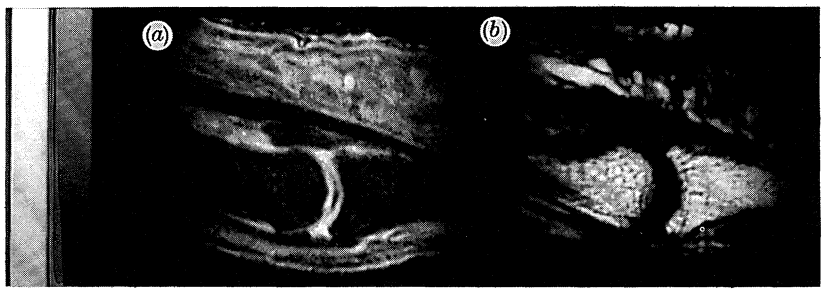

Figure 4. Chemical shift selective saggital images of the distal interphalangeal joint of the first finger of a healthy adult volunteer. (*a*) Water only, (*b*) fat only. The images were obtained using the spin-echo protocol together with a 90° 1331 frequency-selective binomial pulse. Slice thickness is 1.7 mm and in-plane resolution is 0.13 mm.

figure 2, they contain relatively little contrast, and essentially no chemical information.

The application of localization techniques to NMR spectroscopy giving high quality chemical information cannot approach the spatial resolution of an image. Not only is spatially localized spectroscopy vastly more demanding technically, being very sensitive to eddy currents generated by the localizing gradients, necessitating excellent main field homogeneity and requiring good suppression of the water signal, but a far higher overall SNR is required from each volume element to show separate peaks from compounds present in much lower concentration than water. Thus, although useful spectra have been obtained from voxels of sizes down to $5 \times 5 \times 5$ mm (Frahm *et al.* 1987), chemical information of this accuracy cannot be obtained at the spatial resolution of images.

Nevertheless a much lower degree of chemical shift specificity may be obtained at high image resolution; this is routinely performed in clinical examinations to remove the 'chemical shift artefact' which otherwise degrades spatial resolution. Since both fat and water give an intense NMR signal from many tissues, it is possible to obtain separate images from each, either consecutively or concurrently. Figure 4 shows water and fat images of a normal finger. The cartilage, clearly visible in the water image, would be obscured by the fat signal from the fatty bone marrow in a normal spin-echo image. The simultaneous requirement for fat/water distinction, together with high spatial resolution is particularly apparent in the skin, where thin layers of different chemical composition can be clearly identified; the structure of the fatty bone marrow within the trabecular bone is also well visualized. Images such as these may be obtained without the difficulties associated with localized spectroscopy. There are two methods for achieving chemical shift resolved images. In this case, subtraction techniques such as the Dixon method (Dixon 1984) were not used, because the short T_2 value of the protons of the water in the cartilage would result in a noticeable T_2 decay during the chemical shift evolution period. Rather, selective excitation using a binomial pulse proved satisfactory, a 1331 pulse being chosen as having sufficiently sharp frequency selectivity while being as short as possible to minimize unnecessary loss of the rapidly relaxing magnetization.

Powerful as it is, the chemical shift is not the only way of obtaining chemical information about a system. A large part of image contrast between different regions of an object is caused by neither proton density nor chemical shift effects but, rather, by the variations in spin–spin and spin lattice relaxation times. Figure 5, a series of

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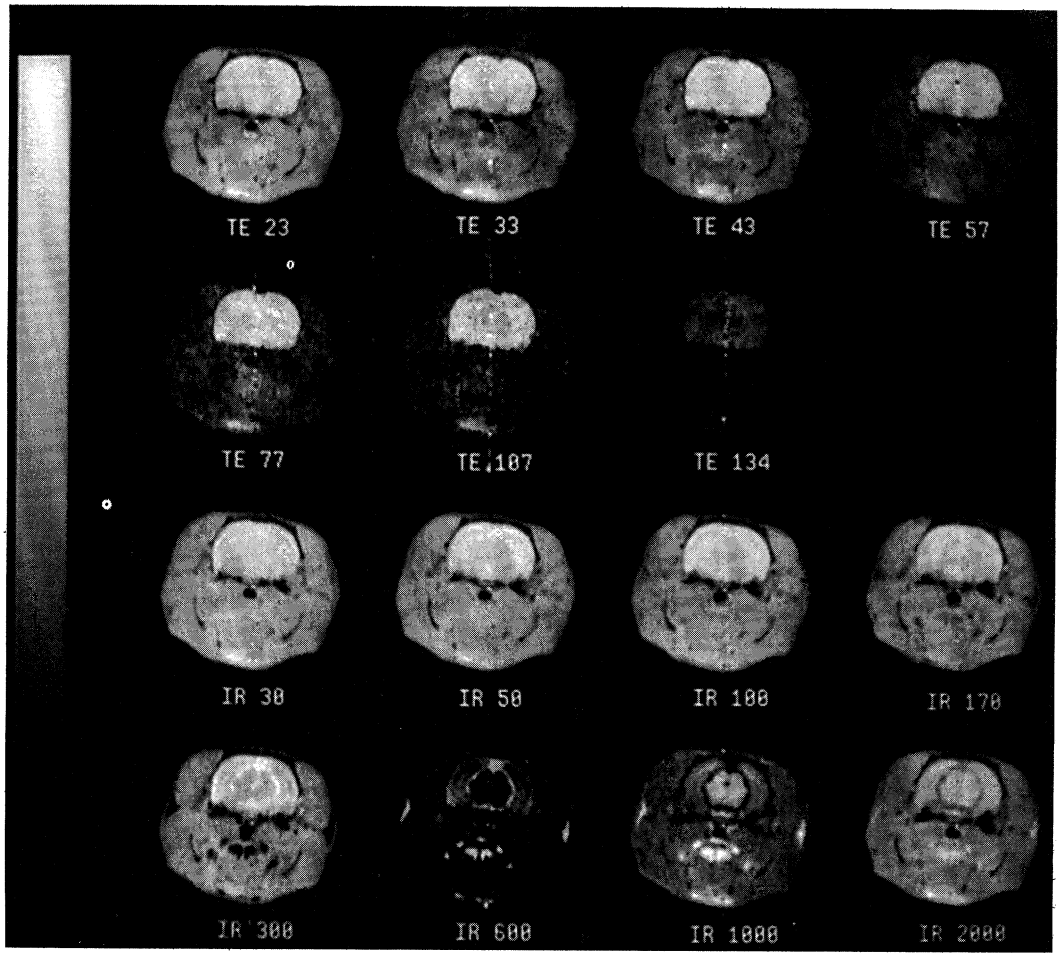

Figure 5. Images of the same 2 mm slice of a rat brain with in-plane resolution 0.16 mm. The first seven images were obtained using the spin-echo experiment with increased echo time to introduce T_2 contrast. The last eight were obtained by inversion recovery with increasing recovery times to highlight T_1 contrast.

images of a single slice of a rat brain illustrate this point; the first seven slices are variable spin-echo time (T_E) images demonstrating T_2 contrast, while the last eight are inversion-recovery images which show T_1 contrast. The latter images clearly delineate different brain tissues. This T_1 and T_2 information, although invariably used when attempting to delineate anatomy and pathology, can also yield information about the chemical environment of the water. Figure 6 illustrates this phenomena in a courgette; although the water content in the skin and cortex is similar, the T_2 in the cortex is considerably depressed. One reason for this lowering of the T_2 in comparison with that of pure water is that the cortex water undergoes proton exchange with the cellular components; there are other reasons, such as diffusion in field gradients, but these are beyond the scope of this article (Hills & Duce 1990). Thus, whereas at this spatial resolution it would be impossible to see a proton spectrum from any cellular components, their presence can still be detected via their influence on the T_2 values of the water.

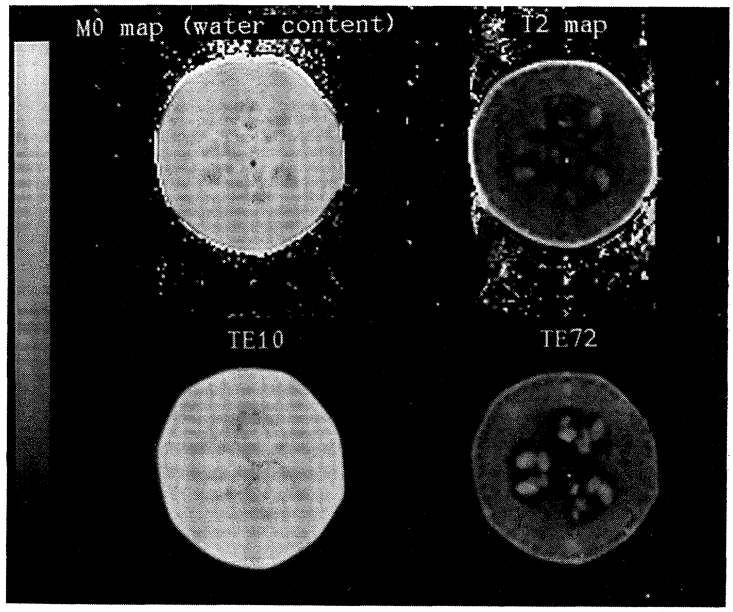

Figure 6. Images of the same slice through an intact courgette. The upper two images show water content and the T_2 maps of the courgette, derived from a set of eight spin-echo experiments at different T_2, two of which are shown.

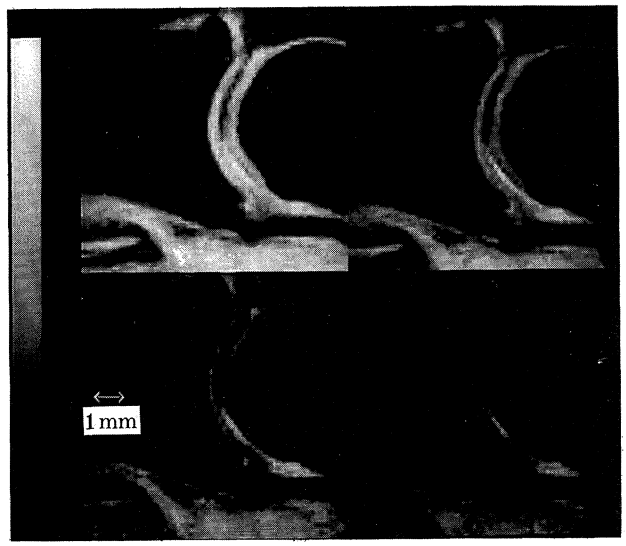

Figure 7. Single slice spin-echo fat-suppressed images of the distal interphalangeal joint of the first finger of a healthy volunteer, with T_Es of 22, 32, 42 and 52 ms showing the long T_2 of the synovial fluid with respect to the cartilage. Slice thickness is 1.7 mm and in-plane resolution is 0.13 mm.

Chemical shift information provides a measure of the local electronic distribution around the nucleus, whereas relaxation times sample a much greater region about it. Chemical changes may effect the relaxation times via other routes. In protein aggregation, for example, during gelatinization the T_2 of the water drops as its

mobility is restricted by exchange with the polymer (Hills *et al.* 1989). In large chain polymers, such as cartilage, the overall structure of the matrix is often altered by small biochemical changes; these morphological changes may effect the T_2 of water within the matrix because of diffusive exchange through field gradients as well as changed mobility. Figure 7 shows a water-only chemical shift selected image of the distal interphalangeal joint of the first finger of a healthy volunteer. The joint consists of two layers of cartilage with synovial fluid between them; the fluid in the cartilage matrix is essentially the same as the synovial fluid and it is possible to differentiate between the two only because of interactions of the water with its environment. High resolution is needed in this case because the physical structures to be distinguished are of the order of 0.2 mm. The concentration of water is very similar in both, as can be seen in the first image (short T_E value), but the subsequent images clearly demonstrate the longer T_2 of the synovial fluid outside the cartilage. The dark line in the middle is believed to be cartilage water at a lower concentration.

4. Discussion

In vivo imaging down to 0.13 mm pixel size has been demonstrated at 2 T, but the resolution obtainable is not necessarily limited by the SNR. As has been pointed out, the fat/water chemical shift artefact may degrade resolution by a factor of 10 in one direction, but this can easily be removed with chemical shift suppression; motion may also be a limiting factor, although this too can sometimes be accounted for (for example, cardiac gating).

Although the use of relaxation times for attaining images with contrast in them is well known, their causes are not well understood and are often not even considered. Relaxation time data can be acquired for voxels of $1.7 \times 0.13 \times 0.13$ mm in *in vivo* mammalian tissue is less than 10 min with few difficulties. Although a high quality NMR spectrum can undoubtedly give more information than a relaxation time, as well as being easier to understand, it is important to realize that the 'relaxation times' and 'chemical shifts' reflect interactions in different regions around the nucleus. Thus while 'chemical shifts' reflect purely chemical properties, relaxation times represent the physiochemical environment through features such as exchange and morphology; hence these two phenomena cannot be directly compared, and indeed they often offer complementary insight. Nevertheless, in many circumstances chemical questions may be answered by relaxation time measurement, and in some cases only by relaxation time measurement. This leads to the concept of measuring the properties of a substance which is NMR insensitive (perhaps because of low concentration as in a tissue metabolite, or of a very short spin–spin relaxation time due to slow molecular tumbling, as for a cell wall matrix) by measuring the NMR properties of water with which it interacts dynamically.

Despite the widespread use of relaxation time measurements in NMR spectroscopy and of both T_1 and T_2 tissue contrast in clinical MRI, there is clearly much work to be done in understanding the basic processes that contribute to transverse and longitudinal relaxation mechanisms. The possibility of extracting chemical and other information from high-resolution studies of laboratory animals is even now apparent, information that would certainly be of value in studying disease processes and pharmaceutical intervention.

This paper illustrates that chemical shift selective imaging and relaxation times are useful probes into chemistry at the microscopic level. Future development in the

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technology for localized spectroscopy and an increased understanding of the relaxation processes will ensure a bright future for NMR in the high-resolution study of chemical interactions.

It is a pleasure to thank Dr Herchel Smith for his munificent endowment which made this work possible.

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Discussion

D. K. Menon (*Hammersmith Hospital, London, U.K.*). Is it perhaps unimportant that spatial resolution in NMR microscopy is far less than that in other forms of microscopy? Perhaps the true potential of MR microscopy will not be in providing superior resolution in space, but in distinguishing between normal and abnormal tissue at a stage when this is otherwise impossible.

L. D. Hall. I agree. Clearly one would prefer to have the highest possible spatial resolution, but even if this is achieved at the cellular level, there will still be 'volume averaging' of the NMR responses. In practical terms it is important only to ensure that the NMR responses from the abnormal tissue are not 'diluted' by signals from the adjacent normal tissues. Thereafter, one can use all of the time available for data acquisition, to characterize that defined region. It is for this reason then, that we have chosen to develop methods such as 'zoom-imaging', in which the highest possible spatial resolution is achieved from a small volume within a larger object.

M. S. Silver (*Philips Medical Systems, Best, The Netherlands*). These presentations on NMR microscopy covered well the technical developments Professor Hall has achieved; however, clinical applications were not discussed. What clinical applications is he planning? Has he begun work on the application of MR microscopy to the food industry or the agricultural industry?

L. D. Hall. In response to the first question I make two points. First, the existing apparatus based on 31 cm bore magnets is being used to study human cartilage in the finger joints of healthy volunteers and patient studies will follow. Furthermore, the experience gained in that way will be applied to other studies of man, using the 1.5 T whole-body instrument in Addenbrooke's Hospital.

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With regard to other application areas, we already have extensive experience of food, of polymers, of fibre-reinforced composites, of petrogeological samples and to a lesser extent of various agriculture-related problems. To our pleasant surprise we find that our apparatus is well suited to all of the above areas and we have already obtained data from each area which, we are informed by others who are experts in these areas, have provided them with novel insights not attainable by other methods. For that reason we intend to sustain a major programme in 'non-medical' applications.

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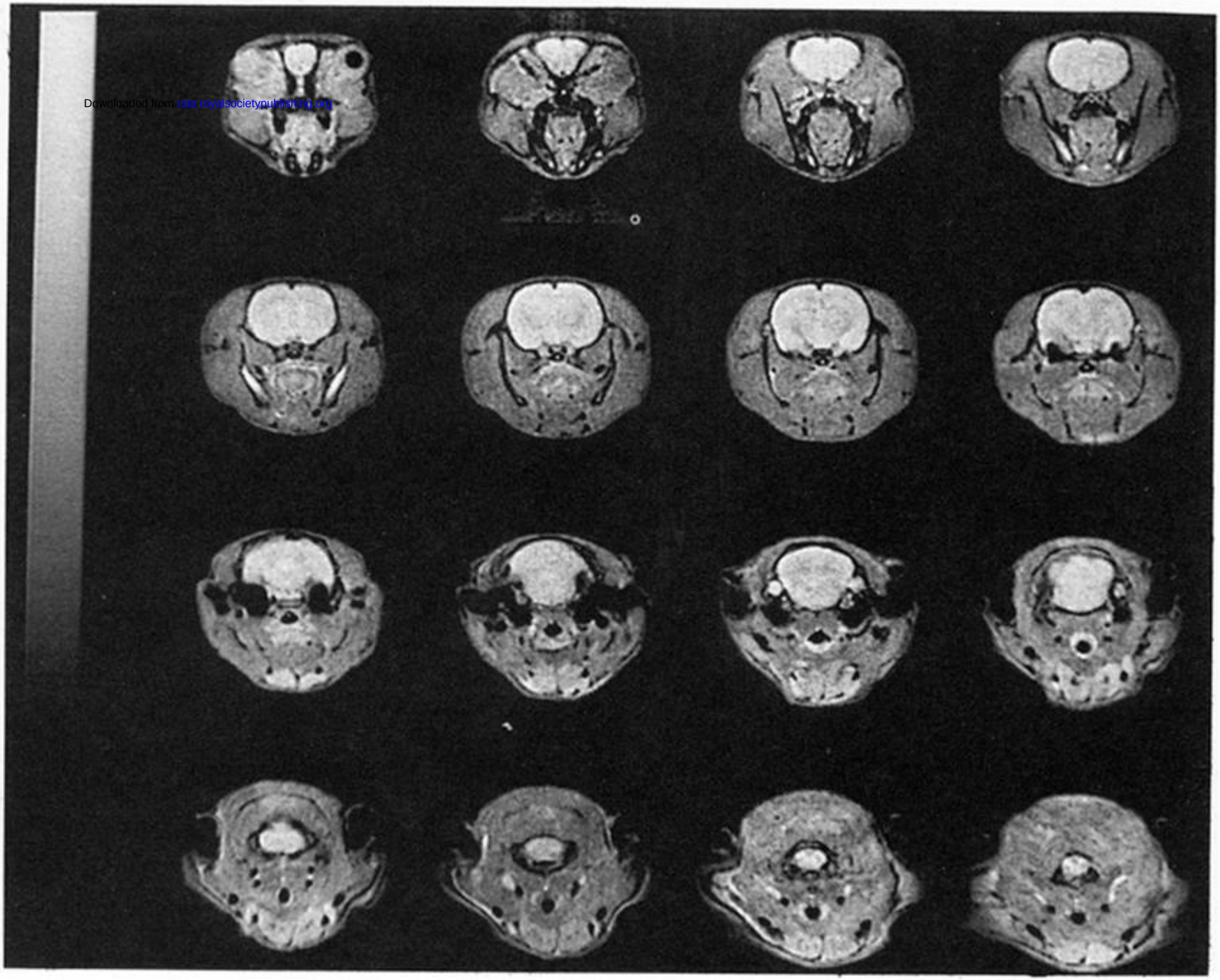

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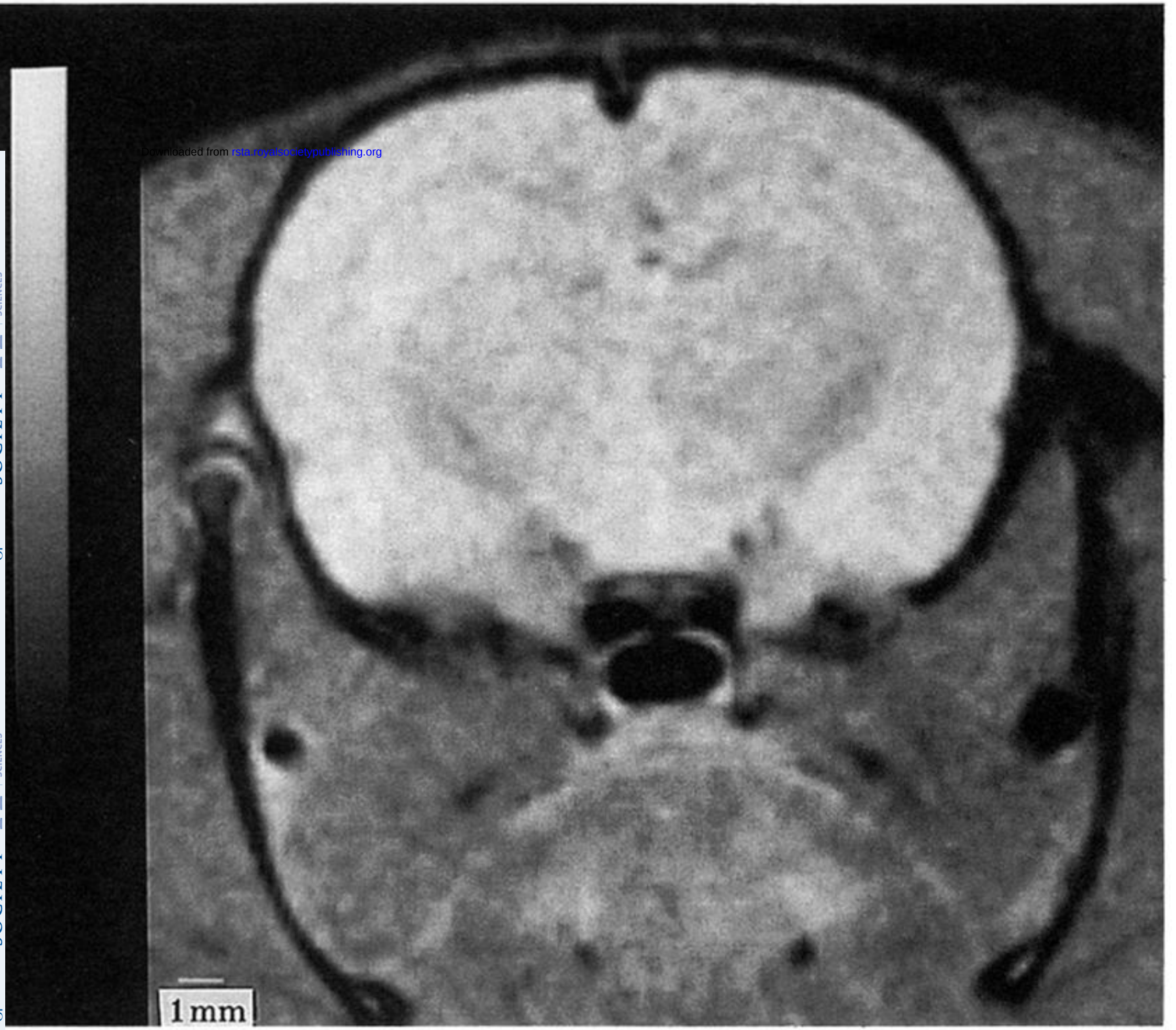

Figure 2. Single slice from figure 1.

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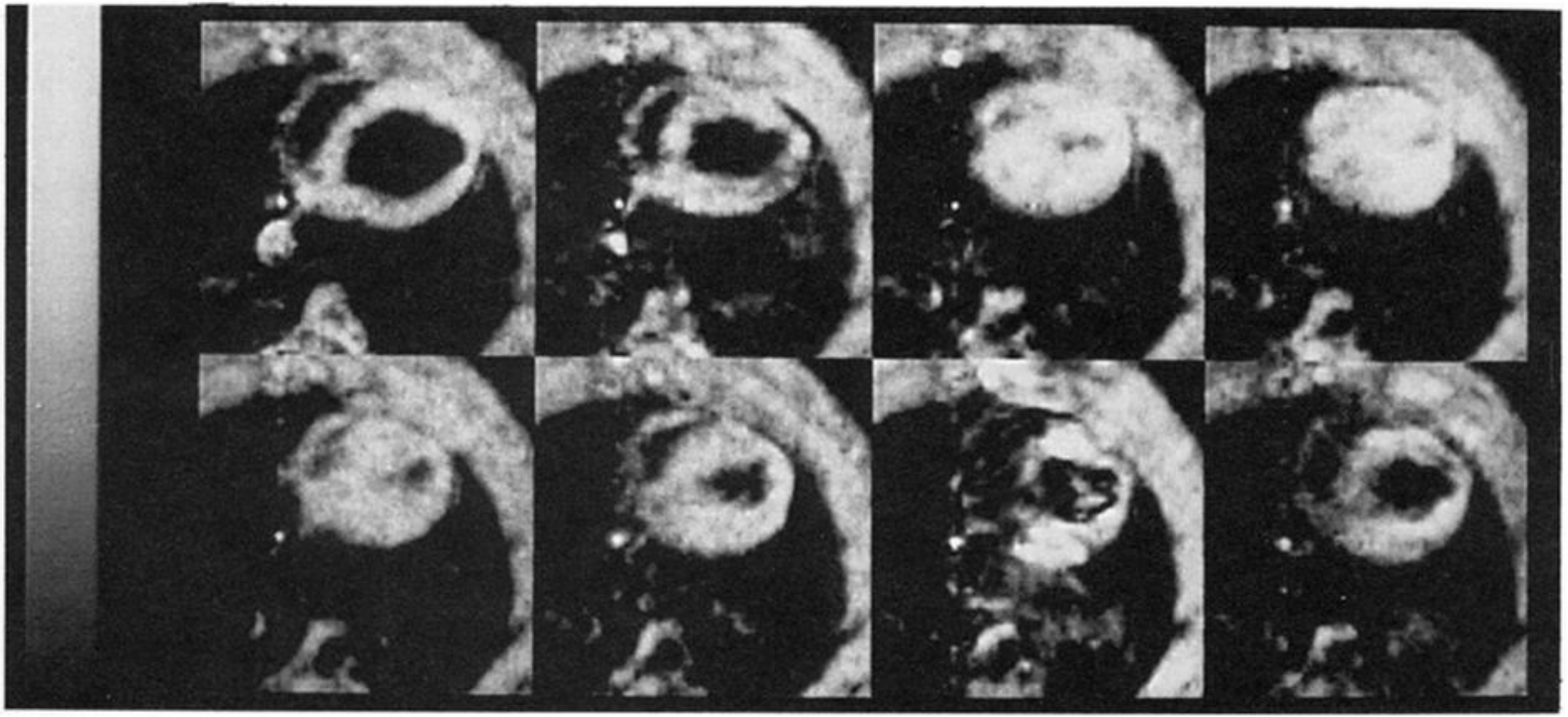

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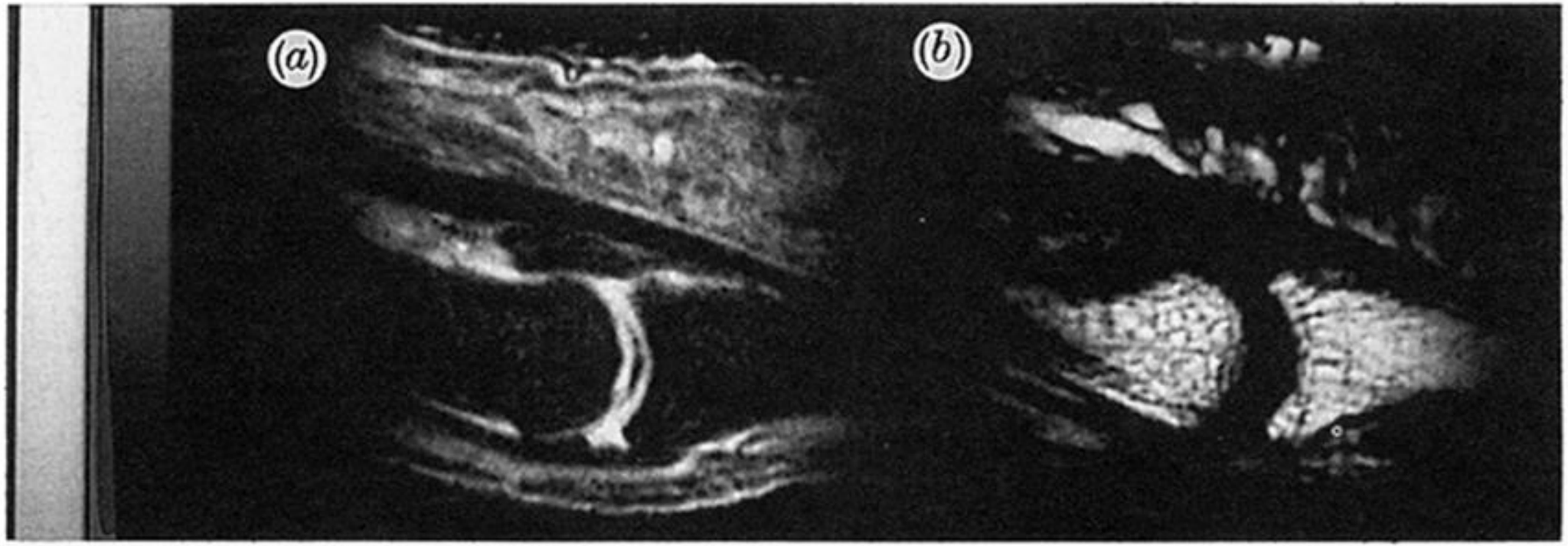

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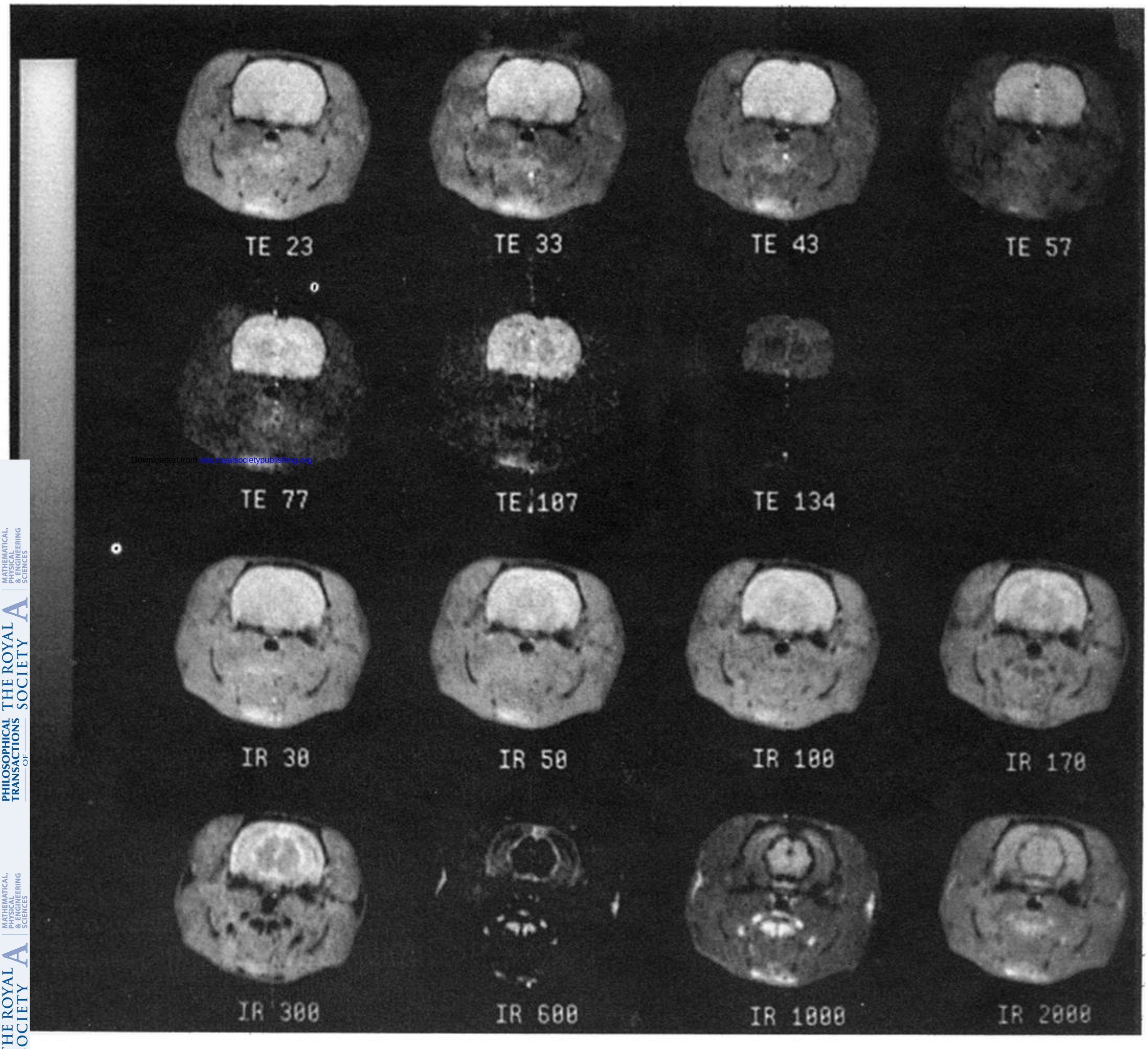

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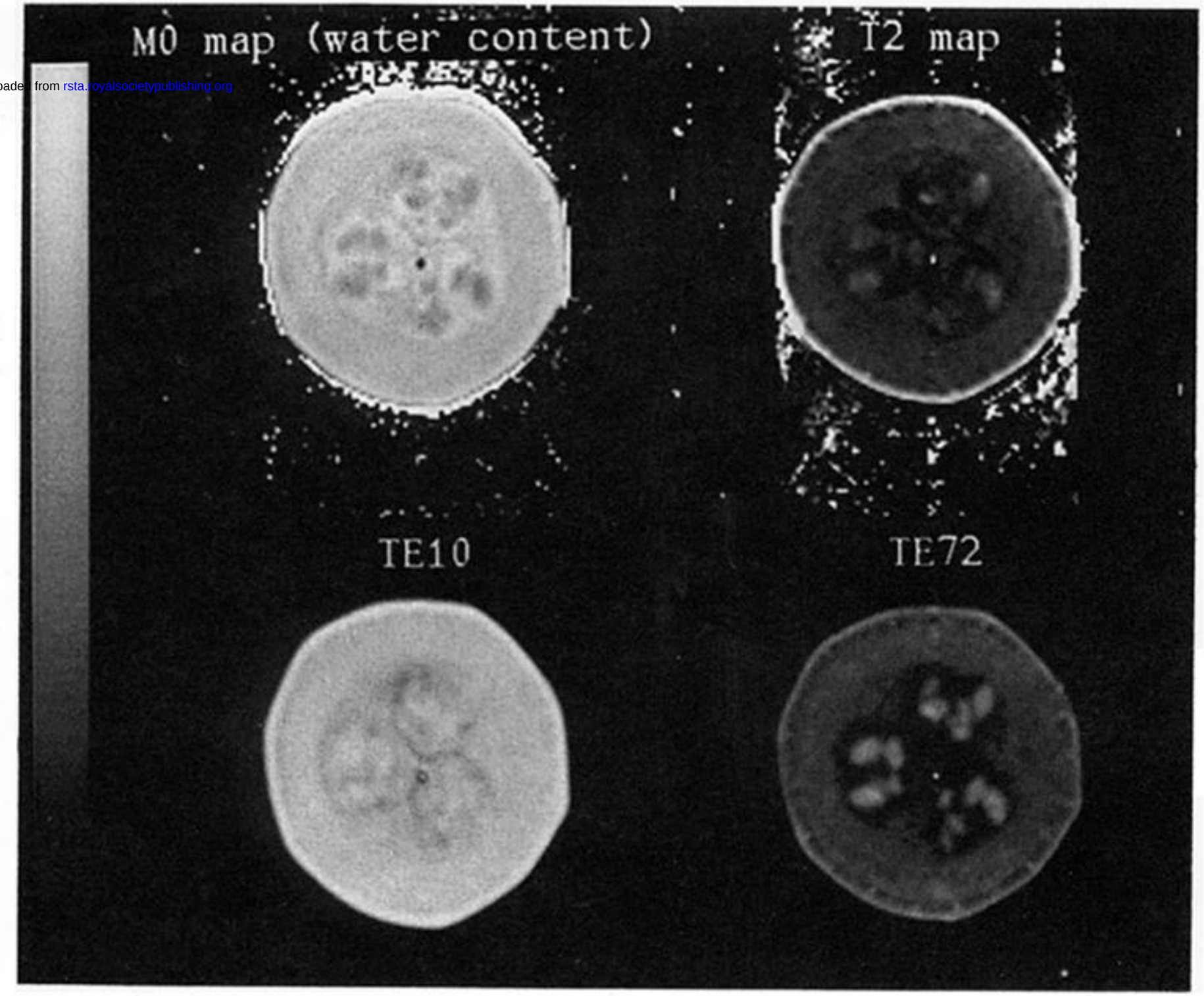

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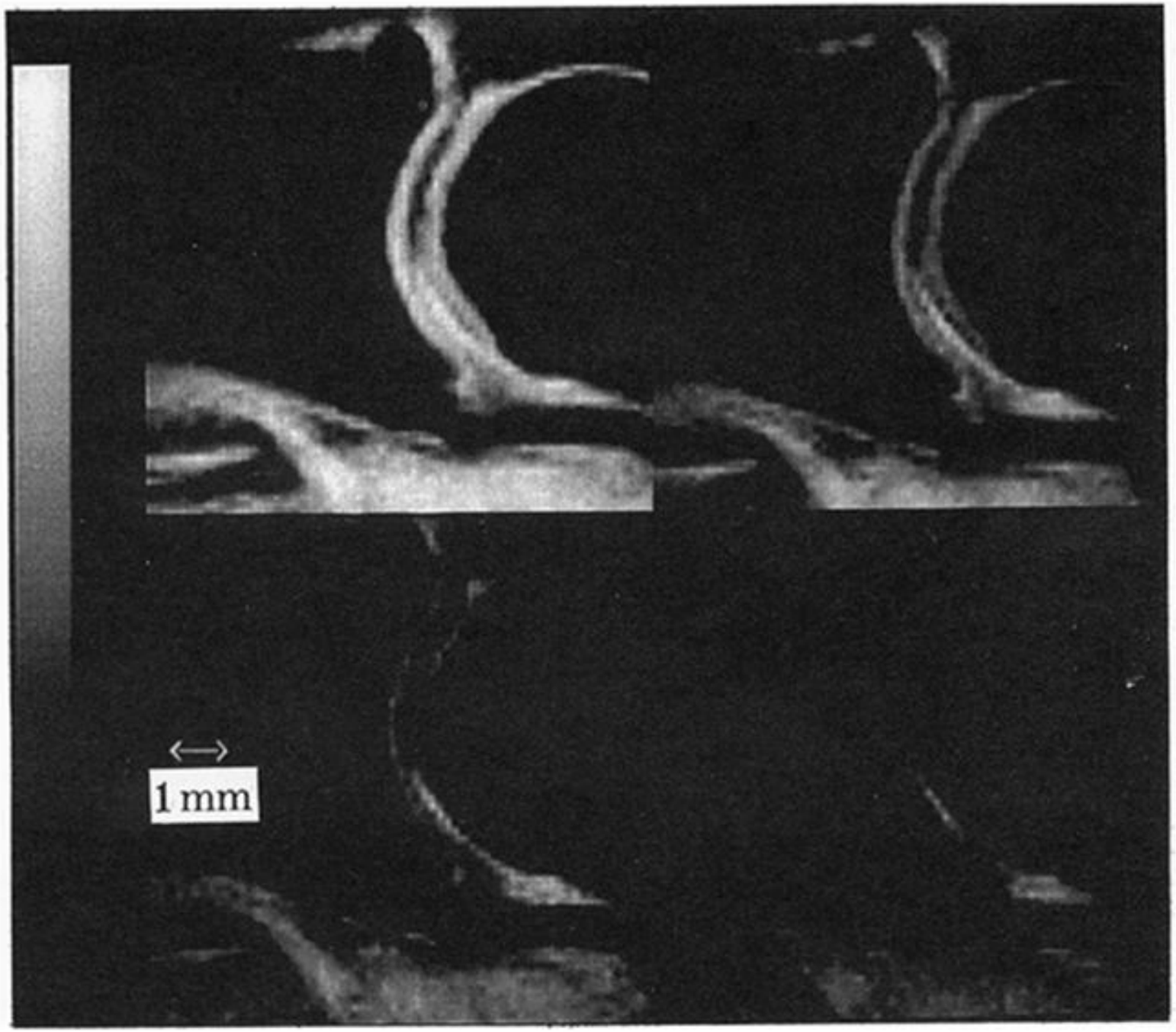

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