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Whole-Body NMR Spectrometer

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by

Jasper A. Jackson
Wright H. Langham

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WHOLE-BODY NMR SPECTROMETER

Jasper A. Jackson and Wright H. Langham

ABSTRACT

A low-frequency NMR spectrometer has been built that will accommodate sample volumes of 0.2 to 1.25 liters. A shielded solenoid provides a uniform field of about 10 G. Signals are detected by a symmetrical rf bridge whose coils are bank wound of litz wire with Q ∿ 300 to 400 at 40 kHz. In the smaller coil homogeneity is sufficient to distinguish signals of protons in protein from those in fat and water. Proteins show a broad resonance underlying the fat and water resonance which is not clearly resolved. The broad resonance is identified with protein by spectra of pure H_2O (sharp peak only) and gelatin made with D_2O (broad peak only). Protein hydration water is believed to be included in the main water peak. Lard is used to identify the signal from fat. Some unidentified lines are occasionally seen. Spectra have been obtained from gelatin, whole eggs, egg whites, egg yolks, and dead mice. In addition, what is believed to be the first NMR signal ever obtained from a whole living animal has been taken on an anesthetized rat. Potential application to analysis of living animals into their whole-body composition is discussed as is detection of signals from other nuclei.

1. INTRODUCTION

Since the original low-frequency nuclear magnetic resonance (NMR) experiment of Brown and Purcell,[1] relatively little work has been done in this field. There has been some work by the Swiss group,[2] by geophysicists interested in magnetometer applications,[2,3] and by a few others.[4] There have been a number of applications of standard NMR techniques to biology,[5] but apparently there has been no application of low-frequency NMR to biology. Therefore, it is appropriate to determine if a low-frequency NMR spectrometer capable of taking large samples can obtain signals of biological interest, particularly from living animals.

2. LOW-FREQUENCY NMR SPECTROMETER

Frequency Generator

Figure 1 shows a block diagram of the low-frequency NMR spectrometer. A General Radio Company Model 1163-A synthesizer locked to a General Radio Model 1115-B frequency standard provides the rf drive for the bridge. The synthesizer is powered by 24 V dc from two 12-V storage batteries to eliminate the 60-cycle modulation present on the output of the synthesizer at low

output levels.

rf Bridge

Figure 2 shows a circuit diagram of the rf bridge and the field effect transistor preamplifier. The symmetrical (Wheatstone) configuration was chosen because circuit analysis using the Los Alamos Scientific Laboratory NET II code for ac circuits indicated a greater fractional transfer of the NMR voltage in the sample coil to the symmetrical bridge output than for other configurations. Also, in theory, a perfectly symmetric bridge should be in balance at all frequencies and, therefore, less sensitive to frequency drift. The input is through a General Radio Model 941-A toroidal transformer. The coarse amplitude balance is controlled by R_2 and R_2', and the fine amplitude balance is controlled by R_3. C_1 through C_6 and C_1' through C_6' are 0.002-μF high Q ARCO Type PJ capacitors with polystyrene dielectric. Coarse phase balance is provided by air tuning capacitors C_7, C_7', C_8, and C_8'; and fine phase balance is maintained by the ceramic trimmers C_9 and C_9'. Monitoring of the coil rf level is done through the high-impedance 100:1 voltage divider formed by C_{13} and C_{14}. Level is read on a Hewlett-Packard Model 400-D ac voltmeter. The bridge configuration shown in Fig. 2 is for balance at 30 kHz (for operation at 40 kHz the balance capacitance is reduced as in Fig. 2).

The preamplifier consists of a high-input impedance field effect transistor. The circuit was designed to give an amplification factor of 10. This preamplifier is reliable and it has low noise characteristics. The 24-V dc supply is that described above.

rf Coils

The sample and reference coils were bank wound (400 turns on small coils and 1,000 turns on large coils) of 50/38 nylon-covered litz wire to give Q's in the range from 300 to 400 at 40 kHz, which is more than 60% of the theoretical Q ($\omega L/R_s$) at this frequency. The coil Q is reduced not more than about 25% by insertion of the sample into the coil (estimated from the sharpness of the bridge balance). The smaller coils gave superior NMR signals because of better field homogeneity over the smaller volume. All the coils were wound to give nearly the same inductance (13.7 mH); the members of each set (small and large) were closely matched.

Receiver

A National Radio Model HRO-500 with an LF-10 preselector is used to amplify the signal from the preamplifier. The receiver S-meter is used to monitor the balance (or degree of unbalance) of the bridge. Balance is also monitored by a Lissajous pattern on an oscilloscope as shown in Fig. 1; the signal at the rf level monitor is used to drive the horizontal deflection plates, and the preamplifier output the vertical deflection plates.

Lock-in Amplifier and Recorder

The output of the receiver detector is fed to the input of a Princeton Applied Research Corporation Model HR-8 lock-in amplifier. The modulation level is read by an H-P 400-D ac voltmeter. The signal from the HR-8 is then displayed on a Brown chart recorder. Modulation of the magnetic field is provided by the internal reference of the HR-8, which drives a Helmholtz pair mounted on Lucite rings which are slipped over the solenoid.

Solenoid Power Supply

The stable current for the solenoid is provided by a PAR TC-602CR current source. The field is scanned by injecting a small control voltage into the current source. The swept voltage is provided by a Varian Model V4280-A motor-driven helipot.

Solenoid

An air core solenoid, 30.48 cm (12 in) in diameter and 91.44 cm (36 in) long (corrected to fourth order in H_z), was constructed according to the design of Hanson and Pipkin[6] (except for some details of the magnetic shielding). Figure 3 shows the solenoid with two sizes of sample coil. Each of the four solenoid windings (main, second-order correction, fourth-order correction, and linear correction) consists of two layers: a right-hand winding over a left-hand winding. Each layer is laid in a threaded groove cut on a lathe according to the procedure described in detail by Hanson and Pipkin (except that No. 17 wire was used instead of No. 18). Homogeneity was maximized by adjusting the current through the second- and fourth-order correction windings until the best NMR signal was obtained. Hanson and Pipkin achieved a homogeneity of one part in 10^5 over a 300 cm^3 volume at the center of the solenoid as measured by the line width in their optical pumping experiment at 29.3 MHz. The low frequency in the present experiment reduced the signal strength making comparable measurement impossible. However, it seems reasonable to assume that inhomogeneity is not likely to be more than an order of magnitude worse than theirs. This would mean $\Delta H/H_o \leq 10^{-4}$ or $\Delta H \leq 1$ mG for H_o=10 G for protons at 40 kHz.

Magnetic Shield

A soft iron shield, 0.3175-cm (1/8 in) thick, was rolled, welded, and hydrogen annealed by the James Millen Manufacturing Company of Massachusetts. Two grooved, 2.54-cm (1-in) thick, iron end plates were machined at LASL and attached to sliding mounts for access to the solenoid interior. Center holes, 2.54 cm (1 in) in diameter, were drilled in the end plates for cable connections when the ends were closed. Figure 4 shows an end view of the solenoid and shield after assembly. Figure 5 shows the complete bridge with the magnetically shielded solenoid and sample coil above and the electrically shielded reference coil below the table, together with the heavy aluminum box in which were mounted the other bridge components. In operation the box was completely surrounded by a 7.62-cm (3-in) thick Styrofoam container to reduce the effect of room temperature fluctuations. The electronics were in standard racks and are not shown. Figure 6 shows an anesthetized rat being inserted into the sample coil.

3. RESULTS

The spectrometer is extremely sensitive. Thus, proton resonance signals from water to which had been added 0.0005 M $MnSO_4$ for proton spin relaxation were easily obtained with a signal-to-noise ratio of 100:1. Unfortunately, the bridge balance was also extremely sensitive to the temperature change and motion of the sample. The temperature sensitivity limited lock-in response time to a maximum of 10 sec. In many cases, since the experiments were done in an uncontrolled temperature environment, response times were 3 sec. Nevertheless, with sufficient patience, useful traces can

be obtained.

Figure 7 shows comparison traces of protons in water and water to which had been added 0.0005 M $MnSO_4$ for proton spin relaxation (all traces are derivatives of absorption). The settings are those which optimize the biological traces (discussed later). They were $H_1 \cong 2\text{-}3$ mG; $H_{mod} \cong 2\text{-}3$ mG at 14 cps. Scan rate was 3.75 mG/min for the 3-sec response and 1.88 mG/min for the 10-sec response traces. It is clear that at these settings proton resonances in water with no Mn^{++} ions present are partially saturated. Figure 8 shows comparison traces of various nonbiological substances. Figure 8 (a) shows again the partially saturated signal from tap water. The resonance of protons in lard is a representative signal from fat, shown in Fig. 8 (b). Figure 8 (c) is the signal from protons in gelatin made with H_2O. Dry gelatin gave no signal, presumably due to unfavorable relaxation times. Figure 8 (d) is another trace from H_2O gelatin at slightly different settings. In both gelatin traces two signals are detectable: a broad signal superimposed on a sharper signal. The broad signal is assigned to the gelatin protons and the narrow signal to the water protons. To confirm this assignment, gelatin was prepared using D_2O. This trace is seen in Fig. 8 (e) to show only the broad gelatin signal. Figure 8 (f) shows the signal from the empty sample coil.

Figure 9 shows the signal obtained from a mixture of lard and dry lactalbumin. The lard was melted and the mixture stirred thoroughly to wet the lactalbumin. The mixture was then allowed to cool until solid. The trace shows the broad resonance associated with protein and the sharper resonance from fat.

Figure 10 shows comparison traces of several biological samples. Figure 10 (a) is the trace from egg whites, which contain only protein and water, but no fat. The broad signal and the sharp leading edge and trailing peak of the partially saturated water signal are clearly seen. Figure 10 (b) shows the trace from egg yolk with the composition as shown. The broad protein signal is still seen, but the sharp component is less pronounced since it now has contributions from both fat and water. Figure 10 (c) is the trace from whole eggs. The characteristic sharp leading edge and trailing peak are more pronounced now due to the large percentage of water, but less so than in Fig. 10 (a). The bumps near the center of the resonance may be real since they seem to reproduce, but a higher signal-to-noise level will be necessary before this can be decided.

Figure 10 (d) shows the NMR trace of an anesthized rat. This is believed to be the first NMR signal ever obtained from a whole living animal. The bridge balance was extremely sensitive to the respiratory motion of the animal, the balance oscillating violently at the breathing frequency of about 1 cps.

Nevertheless, a 10-sec response time averaged the oscillations sufficiently to obtain fair traces. A hint of the broad protein peak can still be seen in Fig. 10 (d). To demonstrate the response of the bridge to the respiratory motion, the trace in Fig. 10 (e) was taken at a 1-sec response time. It shows the respiratory motion superimposed on the NMR signal. At the time this trace was taken, the rat had been under anesthesia for 3 hours and the

spike is where it twitched when starting to awaken.

A signal from 7 dead mice showed the same general characteristics (broad protein resonance, unresolved fat and water signals). However, this could be obtained only after allowing the mice to remain in the spectrometer overnight, since the body temperature change after death caused a steady drift of the bridge balance.

4. DISCUSSION

It appears that the whole-body NMR spectrometer would be a useful tool in studies of gross body composition if the signal-to-noise ratio could be increased. The primary limitation at present is the extreme sensitivity of the bridge balance to the temperature change and motion of the sample. The drift with temperature makes it impractical to use response times longer than 10 sec.

One obvious remedy is to use the newly developed "signal averaging" techniques in which the resonance is scanned rapidly many times at fast response time and the information on each scan is stored in a multi-channel analyzer, the noise being proportional to $\sqrt{N}$ and the signal proportional to N, where N is the number of scans. If the bridge balance began to drift due to temperature change, the scan could be stopped, the accumulated information left stored in the analyzer, the bridge rebalanced, and the scan restarted. In this manner the total time spent scanning can be extended until the desired signal-to-noise ratio is obtained. The effect of respiratory motion could be reduced by making the time for any given scan short compared with the 1-sec respiratory period so that the bridge balance did not change during the scan.

A possible remedy for drift of the bridge phase (frequency) balance due to temperature change is to use an automatic bridge balance. This, however, only rebalances the bridge in phase and not in amplitude. Therefore, since temperature change can also cause an amplitude unbalance, compensation would be incomplete. However, in this connection, it may be worth noting that the respiratory motion seemed to affect the phase balance to a much larger degree than the amplitude balance. The dispersion (phase) balance control could be adjusted to a point where the bridge balance was nearly independent of the respiratory motion. The bridge balance would remain nearly stationary for several seconds before starting to drift off. In this condition the respiratory motion (as measured by bridge balance) seemed to consist of a single periodic motion. However, when the bridge balance was not so adjusted and was oscillating with large amplitude, there appeared a second component so that a recording of the output might appear as shown in Fig. 11. The response of the Brown recorder was not fast enough to actually obtain such a trace.

5. OTHER NUCLEI

Additional signal to noise should allow detection of signals from other nuclei of biological interest. A list of the more abundant stable isotopes with nonzero magnetic moments, together with their relative signal strengths, was combined with figures on the relative abundance of elements in the body. The combination of these two lists eliminated or made unlikely many nuclei. However, there is a strong possibility that at least two

isotopes, ^{31}P and ^{14}N are very likely candidates for measurement. They are both nearly 100% abundant, are fairly prominent in body composition, and knowledge of their concentration in the body is of interest biologically, phosphorous being associated largely with bone and nitrogen with muscle.

At 40 kHz, ^{31}P requires a field of about 23.2 G. The solenoid produces about 18.6 G/amp; therefore, a field of 23.2 G requires a current of 1.25 amp, which is within the 2-amp capability of the power supply. ^{31}P signals were detected in phosphoric acid and in calcium phosphate to simulate bone. The latter, however, were of very poor quality because of unfavorable relaxation time of ^{31}P in solid phosphate. If it were possible to gain a factor of 10 in S/N with signal averaging, it might be possible to make useful ^{31}P measurements.

At 40 kHz, ^{14}N requires a field of about 130 G. This would require about 7 amp and is beyond the capability of the present power supply. An attempt was made to detect ^{14}N at 10 kHz, but this is far from the design frequency of the bridge and was not successful. With a supply capable of delivering 7 amp it should be possible to detect ^{14}N; however, the additional S/N gained by signal averaging may be required to make useful measurements. The field homogeneity would probably be worse at 130 G, but ^{14}N resonances are characteristically much broader than proton signals and may not suffer. ^{23}Na and ^{39}K are of biological interest. They are both more than 90% abundant, but their occurrence in the body is rather low and would require even larger signal to noise than ^{14}N and ^{31}P.

It is hoped that results with the present instrument might give design data for constructing a spectrometer capable of accepting larger animals and/or humans. Calculations show that the conductivity of the human body ("normal saline solution") should allow electromagnetic waves in the 20-kHz range to penetrate to a depth of the order of 1 meter.

6. ACKNOWLEDGMENTS

The authors thank Bob E. Watt for his help in the early design stages of the spectrometer; John Buchen for his aid and advice on the electronics; J.A. Spalding and L.M. Holland for supplying mice and rats; Ray Squires for his aid in construction of the solenoid; Richard Heibert for the preamplifier design. Finally, one of us (J.A.J.) wishes to thank his wife for her aid in preparing the samples.

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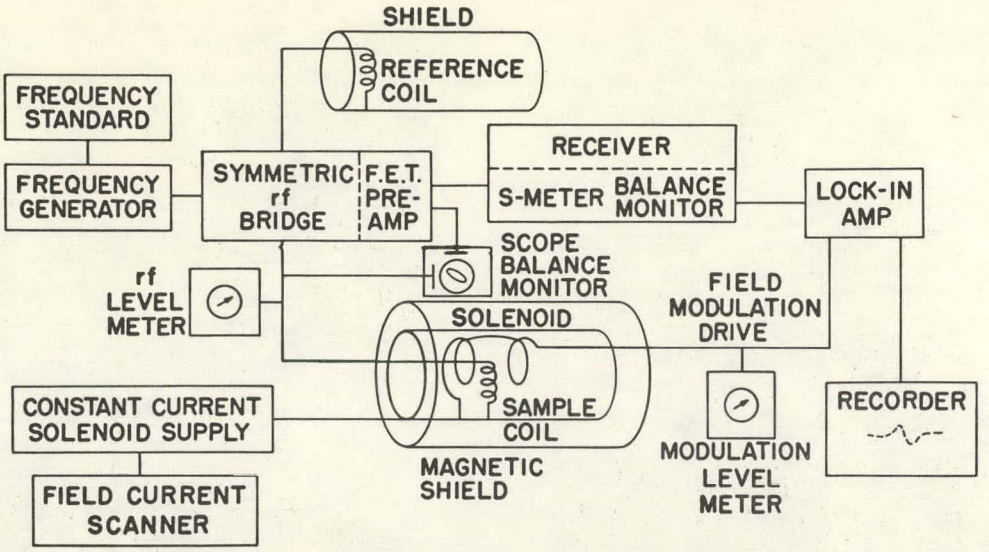

Fig. 1. The low-frequency NMR spectrometer.

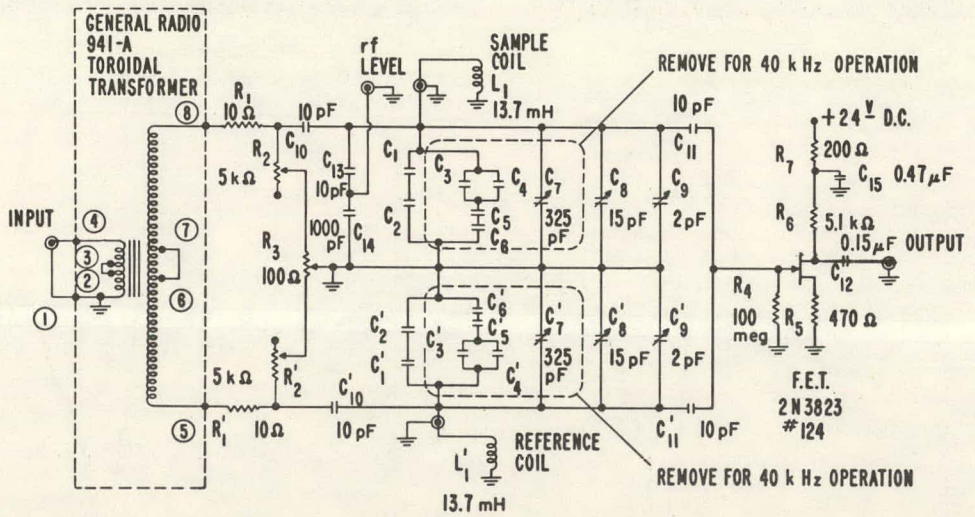

Fig. 2. Low-frequency bridge (30 and 40 kHz).

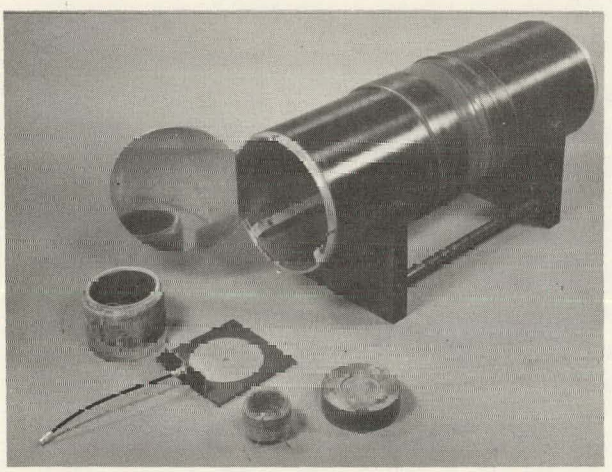

Fig. 3. Solenoid with 0.2- and 1.25-liter sample coils and holders.

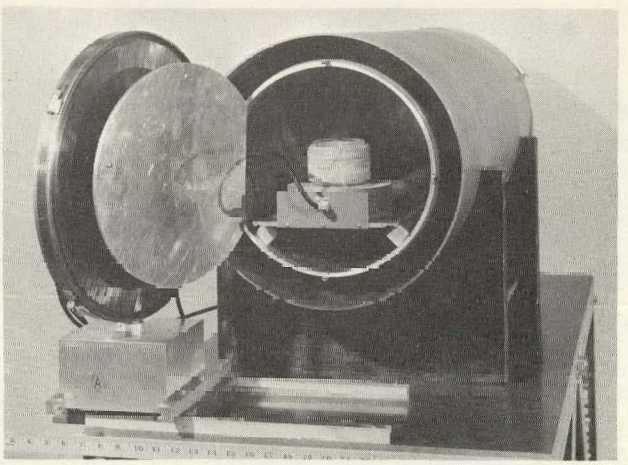

Fig. 4. End view of the solenoid and magnetic shield after assembly with 0.2-liter sample coil.

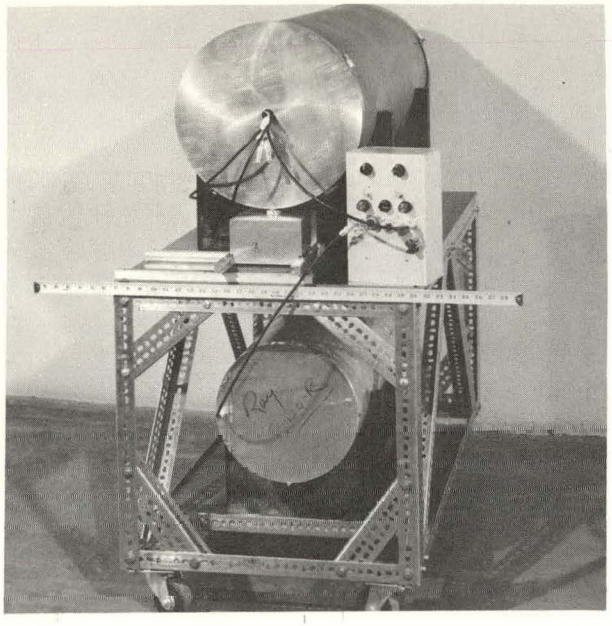

Fig. 5. Complete low-frequency bridge.

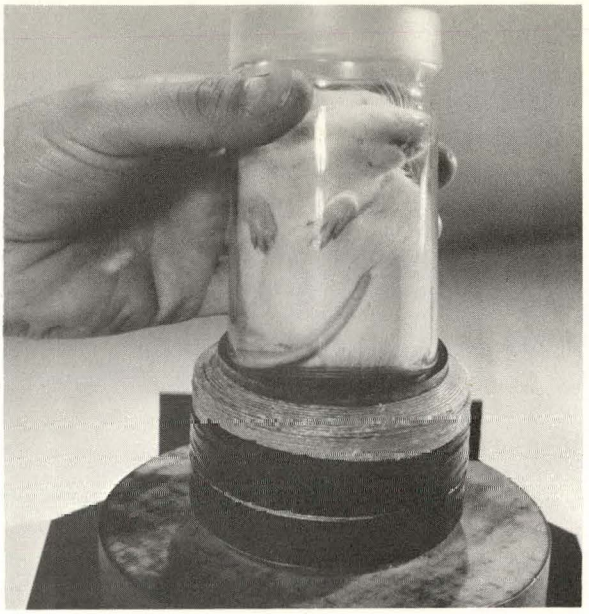

Fig. 6. One-hundred-fifty-gram rat being inserted into the sample coil.

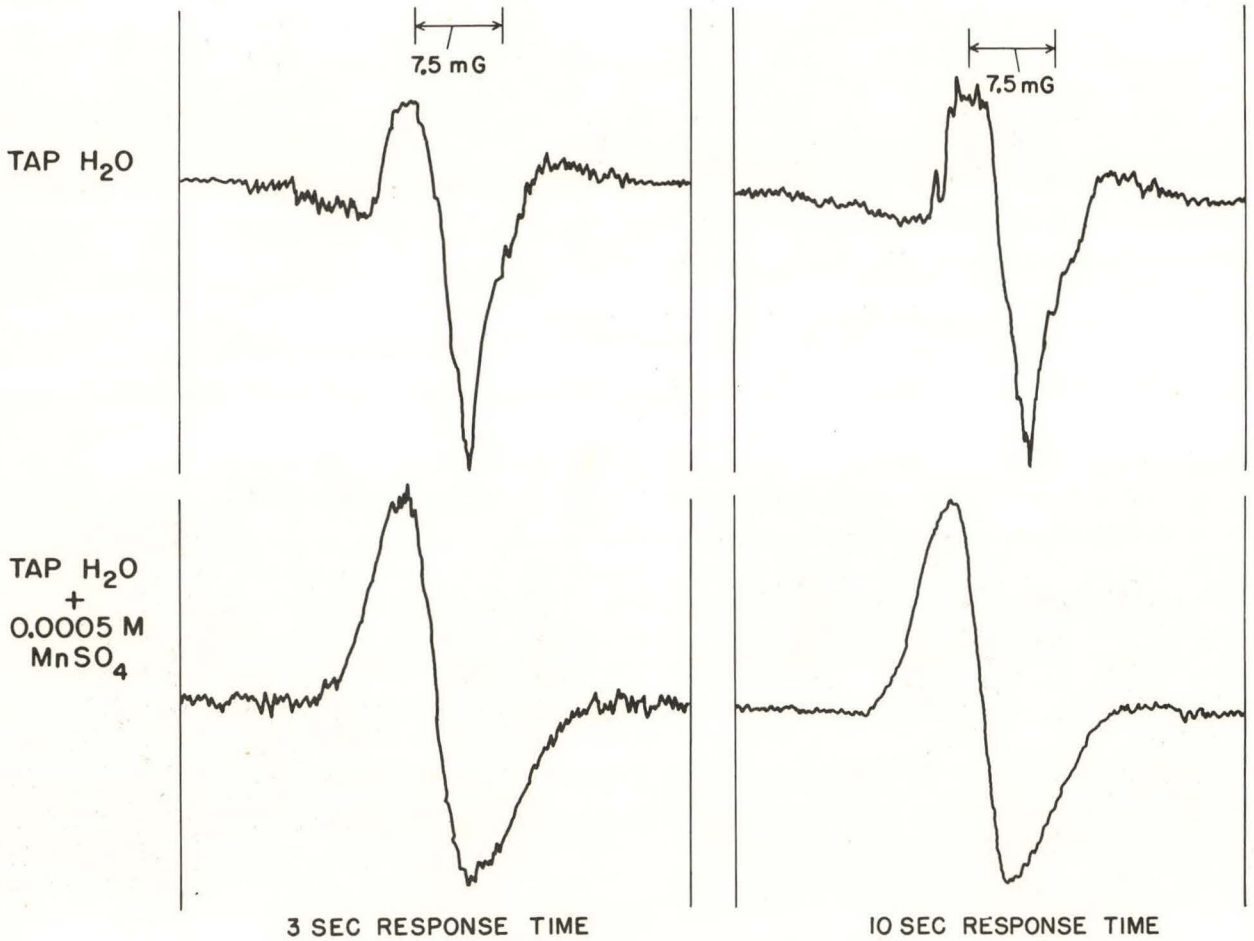

Fig. 7. Comparison traces of proton resonances in tap water and tap water plus 5 x 10^{-4} M $MnSO_4$.

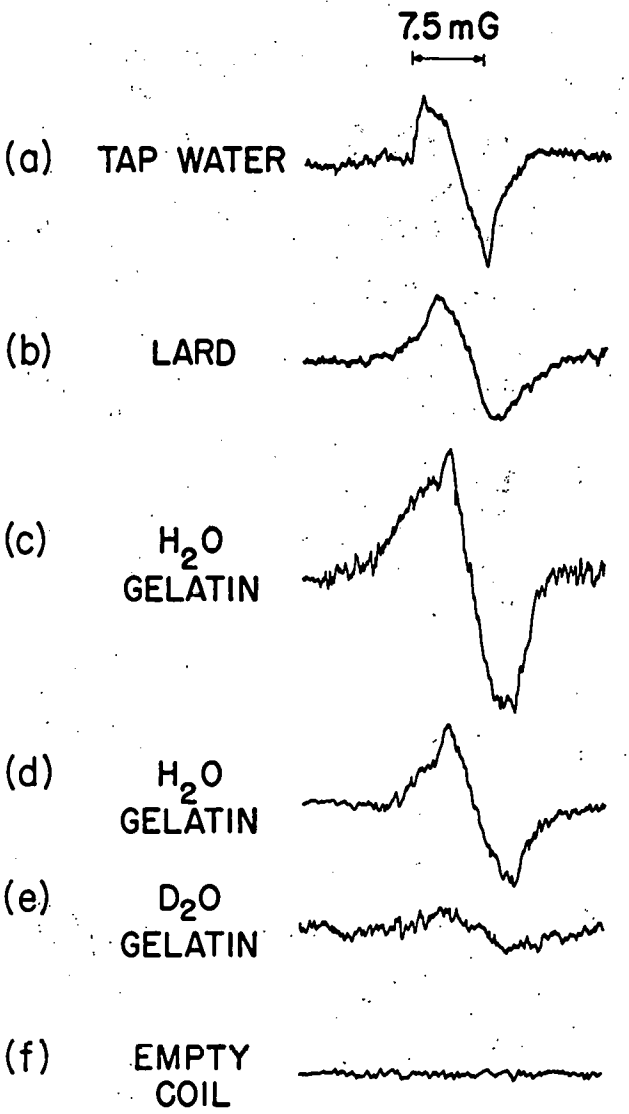

Fig. 8. Comparison traces of proton signals from nonbiological samples.

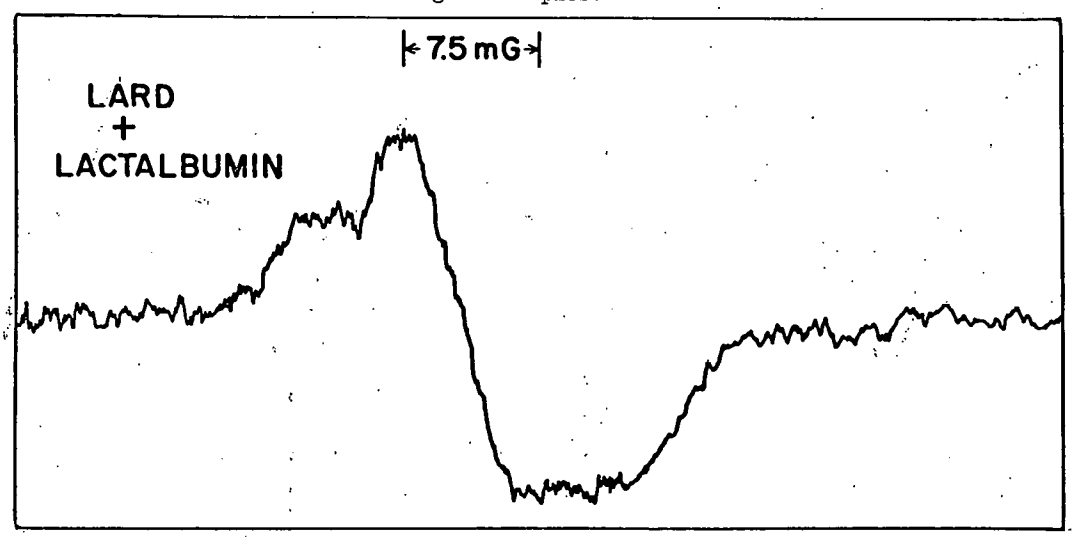

Fig. 9. Proton resonance in a mixture of lard plus lactalbumin (no water present).

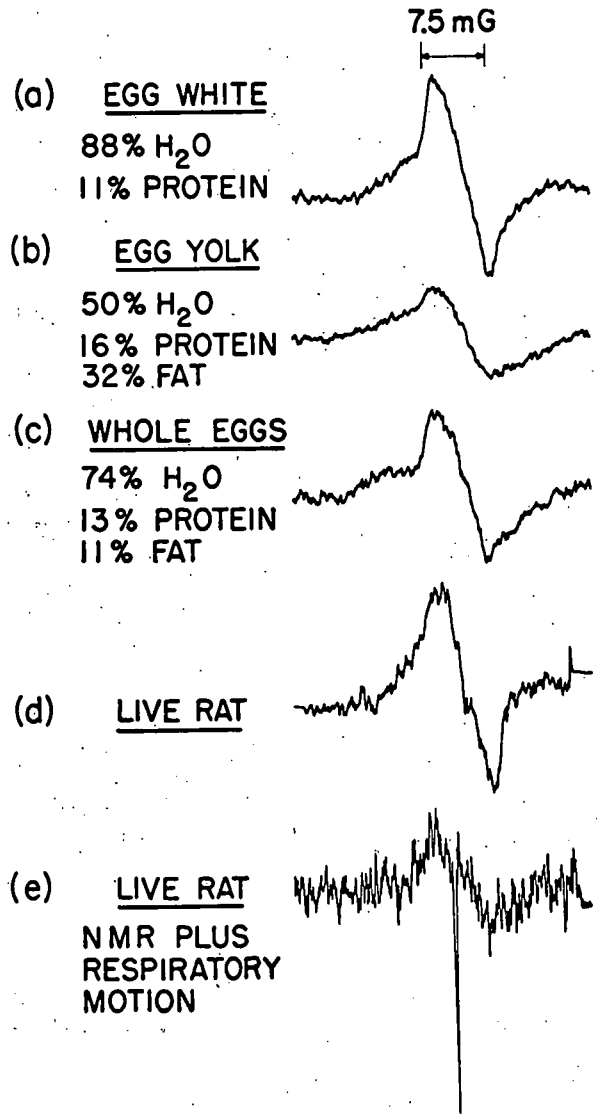

Fig. 10. Comparison traces of proton signals from several biological samples.

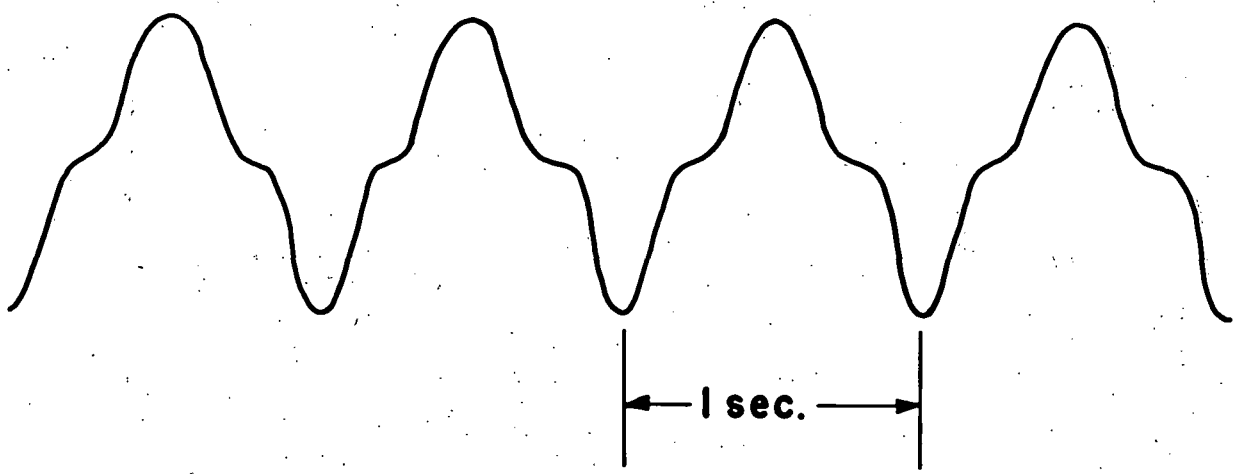

Fig. 11. Response of bridge balance to respiratory motion.