Blood Velocity Measurements in Intact Subjects

Abstract. *Venous blood velocities in intact human forearms can be measured by the use of nuclear magnetic resonance techniques. In essence, two separated coils are placed over the vein, and the arm is held in a magnetic field. Radio-frequency energy in one coil "flips" over the protons in the blood stream, and the second coil detects the arrival of the "flipped" protons. Human blood in vivo and in vitro has a nominal nuclear magnetic resonance relaxation time of 0.4 second.*

The use of nuclear magnetic resonance (NMR) for the measurement of blood flow was initiated on mice (*1*, *2*) in 1959. We have recently developed the NMR blood flow measurement technique to the point where we were able to measure venous flow in the forearms of humans. A major difficulty was the substantially larger radio-frequency noise picked up from human bodies relative to mice bodies. The measurements reported here were carried out with the use of significantly improved electronic circuitry, and the system was redesigned to maximize the signal-to-noise ratios of the NMR signals. The background experiments in NMR and measurements of relaxation time have been discussed by Bloch (*3*) and Bloembergen *et al.* (*4*).

Our measurements were carried out on median veins in the forearms of human subjects. Figure 1 shows the placement of the coils over the skin surface of a subject. The coils used were 0.6 cm in diameter and were separated various distances along the vein, about 1 to 3 cm in the course of the experiments. Most of the measurements were carried out in a field of about 3600 gauss with a radio frequency of 15.4 megahertz.

A block diagram of the measurement system is shown in Fig. 2. The principle of the measurement is as follows: The water molecules in the blood in the arm and hand are polarized by the main magnetic field. The coil on the left is energized by a pulse of radio-frequency energy of sufficiently long period to cause a reversal of polarization of the hydrogen nuclei (the adiabatic fast passage reversal technique) in the close vicinity of that coil. Our system swept the field at the rate of 10 gauss per millisecond to accomplish the adiabatic fast passage. The portion of the blood stream reversed in polarization by that radio-frequency pulse is called the "bolus." Because the hydrogen nuclei within the bolus are reversed in orientation in the magnetic field, the bolus contains the "tagged" hydrogen molecules. The bolus remains changed in polarization for a time defined as T_1, a characteristic time to return to within 1/2.718 of the equilibrium polarization.

Fig. 1. Subject and magnet for venous blood flow measurement.

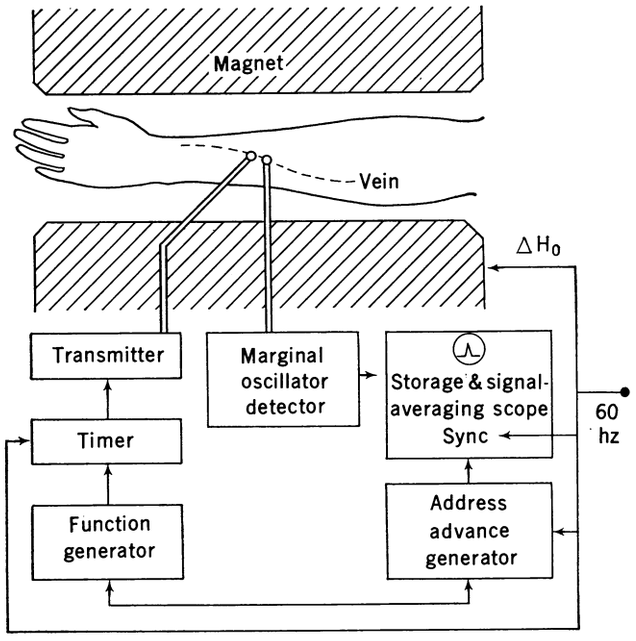

Fig. 2. Block diagram of the measurement system.

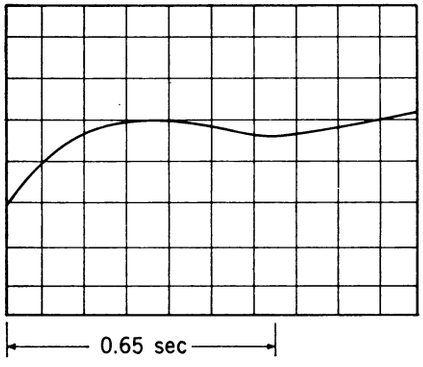

Fig. 3. Envelope of the NMR signals detected in the second coil with blood flow measurement. The dip indicates the passage of "tagged" bolus. Each division represents 0.1 second. The coil separation is 2.5 cm, and the elapsed time is 0.65 second; this gives a flow velocity in the median forearm vein of 3.85 cm/sec.

(Blood has a T_1 of 0.4 second in vivo or in vitro with an accuracy of measurement of approximately 0.03 second.) When the bolus arrives at the second coil a dip in the NMR resonance curve is observed in the oscilloscope. By use of a storage oscilloscope or a signal averaging system, the time scale for observation of the polarization reversal and the arrival of the bolus is defined exactly in time.

The arrival of the bolus is reproduced in Fig. 3 as the oscilloscope pattern. The dip in the signal shows when the bolus of fluid arrives at the detection coil. In order to obtain better signal-to-noise ratios, the detected signal is observed every 1/120 of a second and averaged by a signal-averaging system to nullify the random noise. In Fig. 3 we see the envelope of the NMR signals detected by the marginal oscillator detector and averaged over 256 sweeps (each sweep taking 1 second). Measurements of 120 NMR signals per second averaged for 4.25 minutes give the envelope shown in Fig. 3, which shows a venous blood flow velocity of 3.85 cm/sec.

The flow velocity is determined as follows: The inverting pulse is recorded as the start of the scope sweep. The scope face is calibrated at 0.1 sec/cm, and the center of the NMR signal dip is taken as the time (t) required for the bolus to reach the center of the NMR receiving coil. Since the distance (L) between coils is known, the mean flow velocity is simply L/t. The measurement shown in Fig. 3 indicates a velocity of blood flow in the median forearm vein of one of us (O.C.M.) of 3.85 cm/sec. We could observe flow measurement times fractionally longer than T_1 because more than one-third of the bolus remains "tagged" for a time longer than T_1.

As a comparison, flow measurements were also carried out in water and in water solutions of paramagnetic salts. The solutions could be adjusted to have a relaxation time of 0.4 second so as to simulate blood. Using the same instrumental setup as that for the blood flow measurements, we carried out measurements of water flow in a plastic tube. One of the authors previously used this same type of system to measure fuel flow (*5*).

In the course of these experiments, measurements of the relaxation time (T_1) of human blood in vivo as well as in vitro were carried out. Fresh blood was drawn from one of us (O.C.M.) for the in vitro measurement and an anticoagulating agent was added. The effect of the anticoagulant on relaxation measurements was measured separately, and the anticoagulant was found to have no effect on T_1. The blood sample in vitro had a T_1 of 0.4 ± 0.03 second. The in vivo measurement gave the same T_1, in agreement with our earlier measurement on mice (*1*), and experimenters in the U.S.S.R. (*6*) have found that dog blood also has a T_1 of 0.4 second.

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References and Notes

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5. Nuclear magnetic resonance measurements of jet fuel were successfully carried out by J.R.S. on NASA contract No. NAS8-1581.
6. A. Zhernovoi and G. Latyshev, *Nuclear Magnetic Resonance in a Flowing Liquid* (Consultants Bureau, New York, 1965), p. 71.
7. We thank Prof. I. Fatt for the use of part of the equipment, T. Grover for suggestions, and J. Zeevi, Martha Singer, and others who volunteered to be subjects for blood flow measurements.

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Bimodal Sedimenting Zones Due to Ligand-Mediated Interactions

Abstract. *Ligand-mediated association-dissociation reactions can give rise to band sedimentation patterns showing bimodal bands despite instantaneous establishment of equilibrium. Weaker interactions result in unimodal bands whose sedimentation coefficients decrease with time of sedimentation and in characteristic patterns of total ligand. The implications of these results for fundamental investigations of protein interactions and for conventional analytical applications of zone sedimentation and molecular sieve chromatography are considered.*

Macromolecular interactions mediated by small molecules are important in current thought concerning biological control mechanisms. Binding of ligand molecules to sites on a protein may affect a change in macromolecular conformation or state of aggregation, thereby modulating the biological activity of the large molecule. Of particular interest in the present context are ligand-mediated association-dissociation reactions, examples of which include the facilitated association of carbamyl phosphate synthetase by positive allosteric effectors such as inosine monophosphate or ornithine (*1*) and the dissociation of lamprey hemoglobin into subunits upon binding oxygen (*2*). Both of these reversible reactions were detected and studied by the method of sedimentation velocity—zone sedimentation through a preformed density gradient in the first instance and moving-boundary analytical sedimentation in the second. For some time we have been developing the theory of sedimentation of ligand-mediated interactions of this sort (*3*; *4*, chapters 4 and 5), since such calculations provide one with the understanding required for quantitative, indeed, sometimes even qualitative, interpretation of their sedimentation patterns.

Consider, for example, the reversible reaction

$$m\mathrm{M} + n\mathrm{X} \rightleftharpoons \mathrm{M}_m\mathrm{X}_n$$

in which a macromolecule M associates into a polymer containing m monomeric units (m-mer), with the mediation of a small molecule or ion X, of which a fixed number n are bound into the complex. The set of conservation equations for sedimentation in a sector-shaped ultracentrifuge cell takes the form

$$\frac{\partial(C_1 + mC_2)}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[(D_1\frac{\partial C_1}{\partial r} - C_1 s_1 w^2 r)r + m(D_2\frac{\partial C_2}{\partial r} - C_2 s_2 w^2 r)r\right] \quad (1)$$

$$\frac{\partial(nC_2 + C_3)}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[n(D_2\frac{\partial C_2}{\partial r} - C_2 s_2 w^2 r)r + (D_3\frac{\partial C_3}{\partial r} - C_3 s_3 w^2 r)r\right]$$

In these equations C designates molar concentration; D, diffusion coefficient; s, sedimentation coefficient; w, angular rotation; r, radial distance; and t, time. The subscripts 1, 2, and 3 designate M, $\mathrm{M}_m\mathrm{X}_n$, and X, respectively. The calculations have been made for rates of reaction very much larger than the rates of diffusion and sedimentation so that local equilibrium attains at every instant. In this limit

$$C_2 = KC_1^m C_3^n$$

where K is the equilibrium constant of the reaction. A rapid and accurate calculation has been formulated for the numerical solution of these equations (*4*, chapter 5). The computer code (*5*) is written so as to be applicable not only