Reports

Blood Flow Rates by Nuclear Magnetic Resonance Measurements

Abstract. Blood flow rates may be determined by nuclear magnetic resonance relaxation time measurements. A set of experiments carried out on the flow of blood in mice tails has demonstrated the feasibility of the scheme. Two simple measurement methods are described, and the pertinent equations are given. In addition, some procedures for using nuclear magnetic resonance and electron paramagnetic resonance for tracing the flow of specific materials in the body are outlined.

This paper reports on the application of nuclear magnetic resonance (NMR) techniques to the study of blood flow rates in mice. These animals were chosen to illustrate the method, but there is no limitation implied. We expect soon to measure blood flow rates in the fingers or arms, or both, of human beings. Since the power input involved is less than 0.01 watt of 60 Mcy/sec radio-frequency (R-F) waves, there is no danger to the subject. In this regard, the method is superior to the use of radioactive tracer substances with counters and is clearly preferable to puncturing the veins or arteries as in certain conventional procedures. Nonetheless, it may be well to compare initial measurements with measurements made by other systems, since then the precision may be established.

Several methods have been employed, and all are satisfactory, but some are easier to use and are more precise than others. One procedure is to measure the nuclear relaxation time of the protons in the water of the blood and then note the apparent change in relaxation time after tightening a tourniquet to stop the flow of blood. The means of such relaxation time measurements are known from physical investigations of the nuclei of gases, liquids, and solids (*1–3*).

The simplest method of measurement consists of the following procedure. The NMR R-F absorption curve is determined with no flow—that is, with a tourniquet applied. Next, the tourniquet is removed and another absorption curve observed. Since the flow results in less saturated nuclei entering the observation region, the absorption curve with blood flow is larger in amplitude. The absorption is in fact proportional to the saturation factor,

$$[1 + \tfrac{1}{2}\,\gamma^2 H_1^2 T_1 g(\nu)]^{-1}$$

where γ is the nuclear gyromagnetic ratio, H_1 is the R-F magnetic field intensity, T_1 is the thermal relaxation time, and $g(\nu)$ is the normalized shape function for the resonance line.

Derivations of the formulas will appear in a later, more expanded paper. Only the more important results are quoted below.

The theoretically expected apparent relaxation time $(T_1)'$ of a liquid flowing at a rate less than V_0/T_1 (where V_0 is the sample volume under observation, and T_1 is the static, that is, nonflowing thermal relaxation time of the liquid) is given by:

$$(T_1)' = T_1 V_0/(V_0 + hT_1) \qquad (1)$$

where h is the volume fluid flow per second. Using this relationship and the fact that the signal strength is reduced by a saturation factor, the increment of signal amplitude $(A_f - A)$ due to flow, divided by the amplitude of the signal without flow A, is given by:

$$\frac{A_f - A}{A} = \frac{s\left(1 - \dfrac{V_0}{V_0 + hT_1}\right)}{1 + s\left(\dfrac{V_0}{V_0 + hT_1}\right)} \qquad (2)$$

where

$$s = \tfrac{1}{2}\,\gamma^2 H_1^2 T_1 g(\nu)$$

When notable saturation of the signal occurs, as in our experiments with blood flow, s is much larger than unity, and Eq. 2 may be approximated by

$$\frac{A_f - A}{A} \approx \frac{hT_1}{V_0} \qquad (3)$$

Consequently, this simple formula gives the rate of blood flow after three measurements. Our experiments were as follows. The first measurement consists of determining T_1 for the blood by one of the standard methods (*1–3*) while a tourniquet is applied. We mostly used the saturation method. The determination of T_1 should take a minute or so, and thus presents no difficulty from blood stoppage. Next, the height of the absorption curve is observed by means of an oscilloscope and noted as height A. Finally, the tourniquet is removed, and the absorption curve is again observed on the oscilloscope and noted as height A_f. Equation 3 now gives the average linear flow rate v, in centimeters per second, as

$$v = \frac{(A_f - A)L_0}{AT_1} \qquad (4)$$

where L_0 is the length of tail in the R-F field of measurement.

The major source of error in applying Eq. 4 to blood flow in vivo is that the protons of the water in the tissues also contribute to the amplitudes A and A_f. For this reason, we advocate Eq. 4 only when relative velocities are desired. In that case one takes the ratio of two values of velocity, and the static proton contributions vanish. In this manner we have measured the relative velocity of blood flow in standard white laboratory mice when sedated with ether and sodium pentothal. The tail flow velocity was 4.5 times faster for ether-sedated mice over the pentothal-sedated mice.

Another design, now under construction, will be employed to measure absolute values of blood flow velocities. The method consists of having two sets of coils separated by a distance x. One set of coils is connected with an R-F transmitter and receiver to monitor the R-F resonant absorption. The second coil is associated with an R-F transmitter which is pulsed at a predetermined time. The pulse of R-F can be allowed to cause saturation or inverse polarization of the local nuclei (*1, 3*). At the instant of pulsing, a time base is generated for an oscilloscope sweep. By estimating the time for the perturbed nuclei to reach the monitoring point, the flow velocity is determined. More explicitly, x divided by the time between the pulse and the monitor signal disturbance gives the average velocity. (The term "average

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

velocity" refers to the fact that the flow is laminar, hence there is a velocity distribution of which the average is measured.)

The latter measurement system is applicable to instantaneous velocity measurements; that is, one would be able to measure flow between heart beats as well as during a heart beat, provided that the distance x is chosen small relative to the total distance of fluid travel between heart pumps.

There is no problem of obtaining magnetic field homogeneity over the region of a human finger, but there is some difficulty if measurements are contemplated in a large region such as the human neck. We are planning to measure absolute blood velocities in human fingers as our next project.

It is difficult to attempt a comparison of different flow velocity systems at this stage of development, but in our opinion the nuclear resonance measurement system has potentialities unequaled by any other method. In essence, an NMR measurement implants disturbed nuclei whose path may be traced for any length of time up to the relaxation time T_1. For mouse blood, T_1 is approximately 0.4 sec. Human blood has a relaxation time of the same order of magnitude.

One additional important set of experimental possibilities should be mentioned. One may use nuclear or *electron* magnetic resonance as a tracer detection system. For example, consider the digestion or injection of substances containing nickel, cobalt, iron, or other transition metal salts. These may be detected in the bloodstream in vivo by means of electron paramagnetic resonance.

Detection of as little as one billionth of a gram of paramagnetic material has been achieved; however, it is expected that R-F losses due to surrounding body tissues will reduce the sensitivity somewhat. Nuclear resonance techniques may also be utilized in conjunction with specific tracer substances—almost every chemical has its own specific resonance spectrum and a host of materials would serve as tracers (*4*).

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References and Notes

1. F. Bloch, *Phys. Rev.* **70**, 460 (1946); ———, W. W. Hansen, M. Packard, *ibid.* **70**, 474 (1946).
2. N. Bloembergen, E. M. Purcell, R. V. Pound, *ibid.* **73**, 697 (1948).
3. E. Hahn, *ibid.* **80**, 580 (1950).
4. It is a pleasure to acknowledge numerous stimulating conversations with Professors Enoch Callaway III and Robert E. Harris which initiated my interest in biological measurements.

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Stimulation of Amino Acid Transport in Isolated Diaphragm by Growth Hormone Added in vitro

Abstract. The influence of the pituitary on the transport of α-aminoisobutyric acid-1-C^{14} into the cells of isolated rat diaphragm was investigated. Hypophysectomy results in a lower-than-normal rate of α-aminoisobutyric acid-1-C^{14} penetration into muscle. Adding either simian or bovine growth hormone preparations to the incubation medium in concentrations of 2.5 to 25 μg/ml of medium resulted in a doubling of the α-aminoisobutyric acid-1-C^{14} penetration rate. The stimulatory effect was minimized when the hormone concentration was reduced to 0.25 μg/ml of medium.

The incorporation of leucine-2-C^{14} into the protein of isolated rat diaphragm is markedly reduced by hypophysectomy and stimulated by chronic treatment with bovine growth hormone (*1, 2*) or by the addition of simian growth hormone to the incubation medium in low concentrations (*3*). Although there are many possible explanations for these pituitary effects on amino acid incorporation, it is conceivable that the effects could result, in part, from alterations in the rate of entry of amino acids into the intracellular amino acid pool. The experiment of Noall *et al.* (*4*), in which an acute injection of growth hormone enhanced the cellular concentration of the nonutilizable amino acid, α-aminoisobutyric acid (AIB), suggested that growth hormone might exert an influence on amino acid transport. The present investigation was undertaken to determine the effects of hypophysectomy and growth hormone, added in vitro, on the rate of penetration of AIB-1-C^{14} into the cells of the isolated diaphragm.

Fed female rats of the Charles River strain weighing 60 to 80 gm were used in all experiments. The rats employed in the first series of experiments (Fig. 1) were hypophysectomized 40 days before sacrifice, while those used in the second series (Fig. 2) were hypophysectomized 14 days before the experiment. The method used to measure AIB-1-C^{14} transport was a modification of the procedure described by Kipnis and Noall (*5*). The animals were killed by a blow on the neck, and the diaphragms were rapidly prepared according to the method of Kipnis and Cori (*6*). The "intact" diaphragm preparation was blotted on filter paper and transferred to 125-ml flasks containing 30 ml of Krebs bicarbonate buffer, *p*H 7.4. Glucose was added to the buffer at a concentration of 0.01 mole/lit. and AIB-1-C^{14} at a concentration of 0.05 mmole/lit. (25,000 count/min × ml of medium). The AIB-1-C^{14} was obtained from Isotope Specialties Co., Inc., at a specific activity of 1 mc/mmole. Growth hormone preparations of simian (M425B) and bovine (NIH-BGH-1) origins were dissolved in 0.15 ml of distilled water at *p*H 8 and added to the medium immediately prior to the beginning of an experiment. An equivalent amount of distilled water at *p*H 8 was added to the control flasks. The medium in the flasks was preheated to 37°C, so that the timing of the incubation period commenced with the addition of the diaphragm preparation to the flask. Immediately after the addition of the tissue, the flask was gassed with 95 percent O_2–5 percent CO_2 for 5 minutes and then sealed. Incubation was carried out with shaking (100 cy/min) for various periods up to 2 hours. At the end of the incubation period, the diaphragm muscle was dissected away from ribs and adhering tissue, washed for 10 seconds in two changes of buffer, weighed, and homogenized in 1.0 ml of 0.008*N* acetic acid. The homogenate was centrifuged, and the supernatant was diluted 1:1 with 1 percent sucrose. Aliquots of the diluted supernatant were plated at infinite thinness on aluminum planchets and after drying were counted with a gas-flow, thin end-window counter.

The results are presented as the ratio

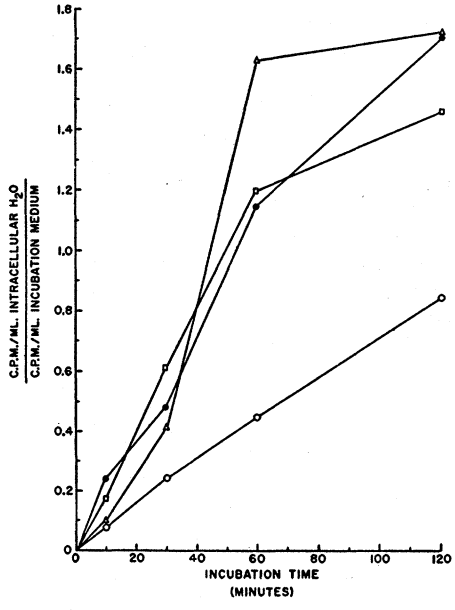

Fig. 1. Penetration of AIB-1-C^{14} into "intact" rat diaphragm preparations. ●, normal; ○, hypophysectomized; △, hypophysectomized + simian growth hormone added to medium (25 μg/ml); □, hypophysectomized + bovine growth hormone added to medium (25 μg/ml). Each point represents one observation, except for the hypophysectomized controls, which represent the mean of three observations per point.