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Attenuation and speed of 10 MHz ultrasound in canine blood of various packed cell volumes at 37 °C

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Abstract

The attenuation coefficient and the speed of 10 MHz ultrasound were determined in canine blood at 37 °C by a differential path length technique. Blood specimens with packed cell volumes (PCV) ranging from 0 to 53% were prepared by separating the cells from the plasma and mixing the two components. The mean attenuation coefficient increased linearly with packed cell volume, the least squares regression function being α (dB/cm) = 0.992 + 0.039 PCV with a standard error of the estimate = 0.255. The speed of 10 MHz ultrasound, c , in millimetres per second, increased with packed cell volume, the regression function for a wave equation model being $1/C^2 = 0.418 + 2.09 \times 10^{-4} (\text{PCV}) - 1.75 \times 10^{-5} (\text{PCV})^2$ with a standard error of the estimate = 0.0049 (mm/s)⁻². Both the attention coefficient and speed of 10 MHz ultrasound were greater in blood than in plasma to a degree dependent on the packed cell volume.

Key words: Attenuation coefficient, Blood, Packed cell volume, Ultrasound

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1 Introduction

Accurate knowledge of the attenuation coefficient and speed of ultrasound in blood is required for ultrasonic ranging of soft-tissue/blood interfaces, such as blood vessel walls, and is important clinically for M-mode echocardiography and Doppler blood flow measurements. The speed of ultrasound in plasma is well known and depends on the concentration of plasma proteins (CARSTENSEN et al., 1953; ANGELSEN, 1975; AUBERT et al., 1978). Several studies of the speed of ultrasound in whole canine blood have been reported (CARSTENSEN et al., 1953; KIKUCHI et al., 1972; AUBERT et al., 1978), but none has systematically evaluated the effect of the formed elements and their intracellular proteins that constitute the most abundant protein fraction in whole blood. The simplest measure of the concentration of formed elements (red cells, white cells and platelets) in whole blood is the packed cell volume (PCV). The present study was undertaken to determine the dependence of the attenuation coefficient and speed of 10 MHz ultrasound in dog blood on the concentration of formed elements or PCV.

2 Materials and methods

To measure the propagation characteristics of 10 MHz ultrasound in blood, a differential path length apparatus was employed (Fig. 1). Pulsed ultrasound was transmitted through a column of blood that was varied in height while ultrasonic time of flight and echo amplitudes were recorded on a high frequency oscilloscope. The blood specimen was contained in a transparent plastic chamber of 7.07 cm^2 cross-sectional area. The bottom of the chamber was formed by a parafilm membrane $100 \mu\text{m}$ thick. This specimen chamber was immersed in a 37°C water bath. The ultrasonic beam entering the bottom of the chamber encountered three reflecting surfaces, two of which (A and B) provided echoes used to calculate ultrasonic time of flight through the blood column. The A and B echoes were displayed on a Tektronix 7403N high frequency oscilloscope (Fig. 1). Echo A represents the parafilm membrane/blood interface, which remains fixed with respect to the transducer beneath it. Normalisation of the incident beam on the interface A was assured by adjusting the beam direction for maximal echo amplitude on the oscilloscope. Echo B represents the blood/air interface.

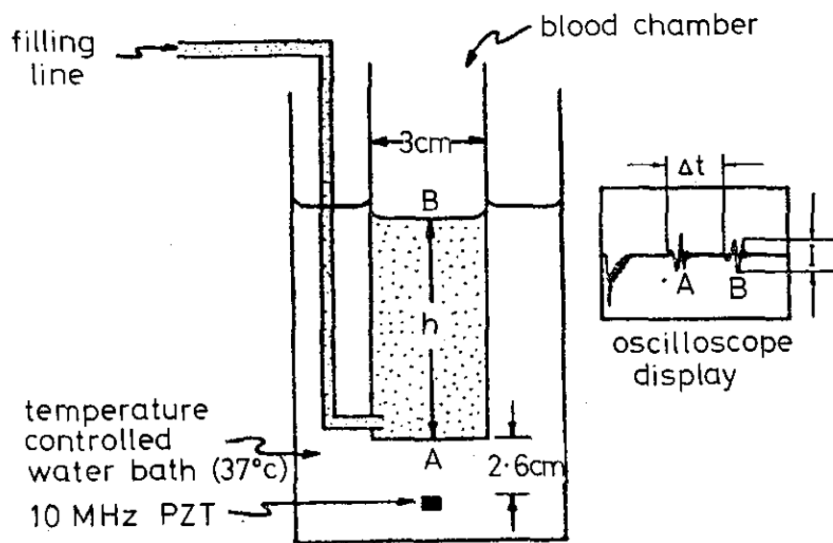


Fig. 1 *Differential path length apparatus for measurement of attenuation coefficient and speed of 10 MHz ultrasound in blood sample*

The blood-column height, h , was increased in 5 mm increments by carefully injecting volumes of 3.5 cm^3 ($0.5 \text{ cm} \times 7.07 \text{ cm}^2$) via the filling line using a 5 cm^3 glass syringe. A magnifying gradicule provided a visual double check on the calculated increase in ultrasonic path length. The time of flight was measured as the difference, Δt , between the initial deflections of the A and B echoes for each height. Echo amplitude was recorded as the maximal peak-to-peak amplitude of the echo B envelope. In all cases, the level of the water bath was matched to the level of the

blood column to minimise distortion of the parafilm membrane by differences in hydrostatic pressure. Sample inhomogeneities due to sedimentation of blood cells were prevented by frequent stirring.

Blood samples of different PCVs were prepared from fresh canine blood stored at 4 °C with acid citrate-dextrose anticoagulant. The cells and plasma were separated by centrifugation, and plasma-cell mixtures were prepared to the desired PCV, values. Each sample was warmed to 37 °C and placed in the sample chamber. The PCV was measured by withdrawing duplicate specimens into capillary tubes and centrifuging them in a Readacrit® apparatus.

Haemolysed samples were identified and discarded. The ultrasonic path length, $2h$, was varied by injecting known volumes of blood into the chamber. The times of flight and echo amplitudes were plotted against path length. Linear least-square regression functions were computed for the delay times, and an exponential least-square fit was computed for the echo amplitudes as functions of total path length.

Since the blood/air interface was incrementally heightened, a correction was necessary for the effect of ultrasonic beam divergence in order to estimate attenuation coefficients with accuracy. Beam divergence, as illustrated in Fig. 2, causes a smaller and smaller fraction of the reflected energy from the blood/air interface to be received by the transducer as sample height, h , is increased. In Fig. 2 the effect is illustrated as a decrease in the fraction of the reflected beam area (A_1 to A_2) received at the transducer. Some correction for this effect of beam divergence must be applied to any pulse-echo technique in which the total path length for propagation is varied.

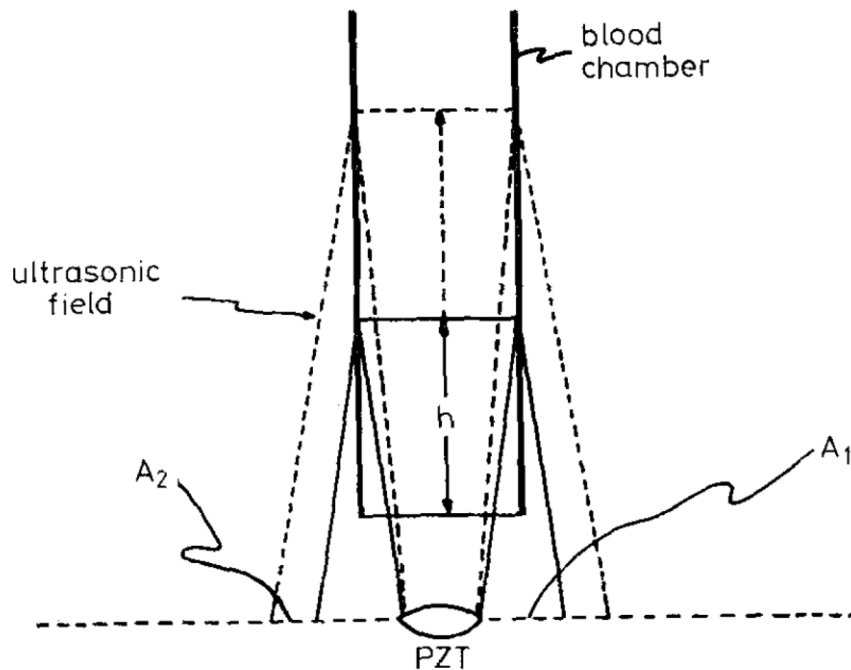


Fig. 2 Simple geometric model of ultrasonic beam divergence

Rather than assuming a particular beam energy distribution and calculating the correction factor analytically, an experimental correction was made. The attenuation coefficient of degassed, distilled water was measured in the experimental apparatus and compared to that previously reported (MARKHAM et al., 1951). The difference between this well known value of attenuation in degassed water and the value measured in the apparatus was interpreted as geometric attenuation. This geometric attenuation was 0.56 dB/cm of path length. Unbiased estimates of the attenuation coefficient in blood were then calculated as the measured attenuation coefficients minus 0.56 dB/cm. This correction typically amounted to 15-20% of the measured values for blood specimens.

3 Results

Fig. 3 shows typical data attained with a packed cell volume of 41%. The straight line represents the least-squares regression for path length against time-of-flight data. The speed is the slope of this line, which is 1.589 mm/ μ s in this example. The curved line is an exponential least-squares fit for echo B amplitude against path length. The attenuation coefficient in this example is 0.031 Np/mm.

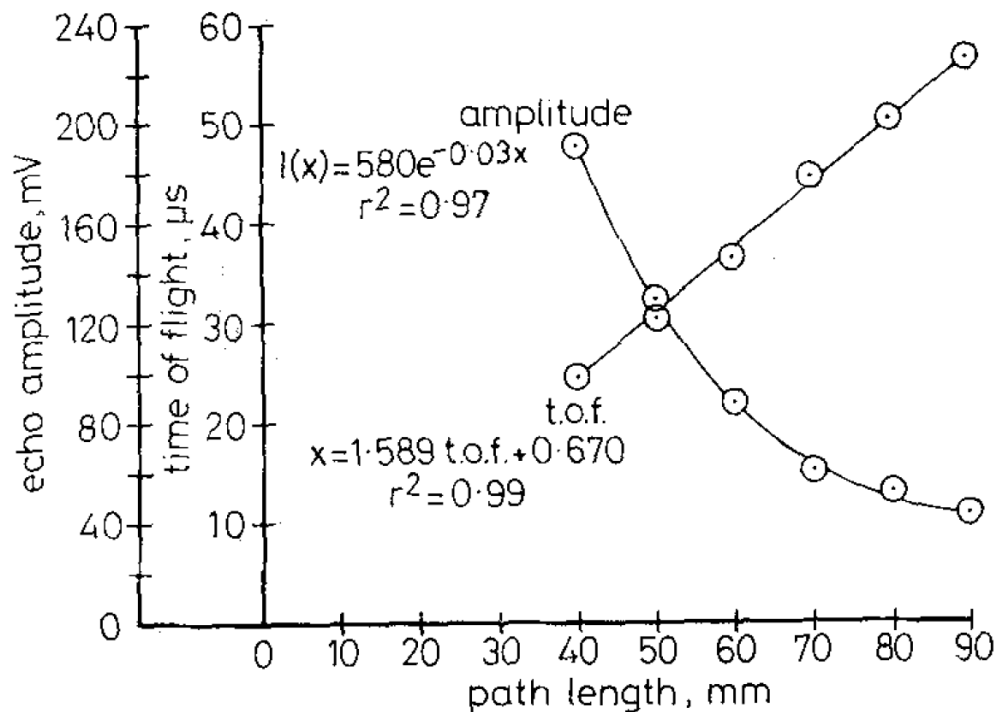


Fig. 3 Typical B-echo amplitude (I) and time-of-flight (t.o.f) data for a packed cell volume of 41%

Fig. 4 shows the corrected data for the attenuation coefficient of 10 MHz ultrasound in canine blood at 37 °C. Note the suppressed zero and vertical bars indicating ± 1 standard deviation. The attenuation coefficient, α , is an increasing function of PCV. A linear regression function for the data is $\alpha = 0.992 + 0.039 \text{ PCV}$, in dB/cm ($r^2 = 0.86$), where $0 < \text{PCV} < 100$ is the packed cell volume in percent. The standard error of the estimate is 0.255.

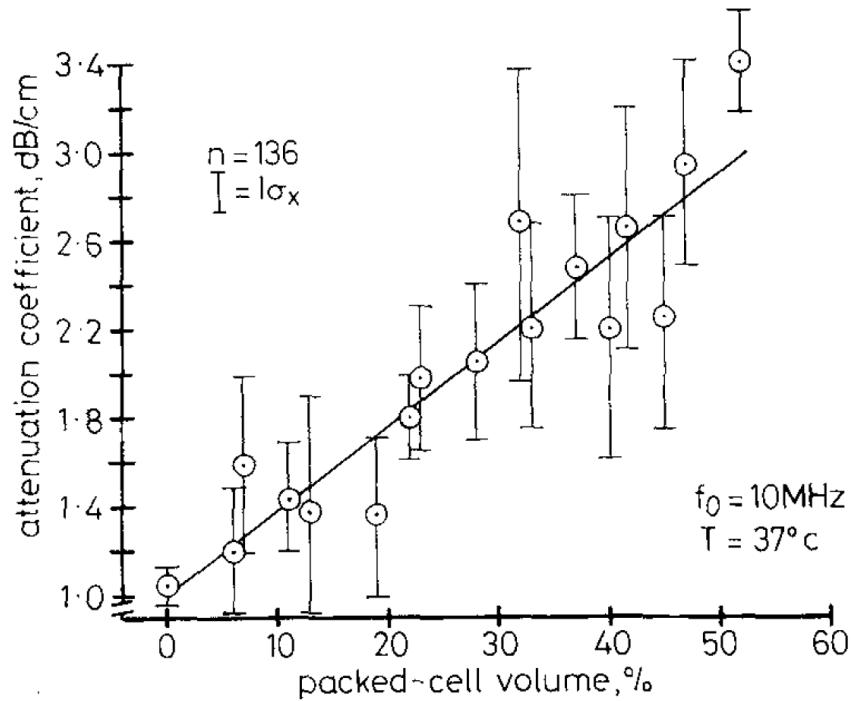


Fig. 4 Dependence of attenuation coefficient in blood on packed cell volume

Fig. 5 presents the speed of 10 MHz ultrasound in canine blood for various packed cell volumes. Note the suppressed zero and error bars indicating ± 1 standard deviation. The speed of ultrasound in blood is slightly dependent on packed-cell volume and may vary up to 7% over the range of PCVs from 0 to 53%. (Note the suppressed zero on the vertical axis.) The curved line represents a regression function obtained from the wave equation model, as will now be described.

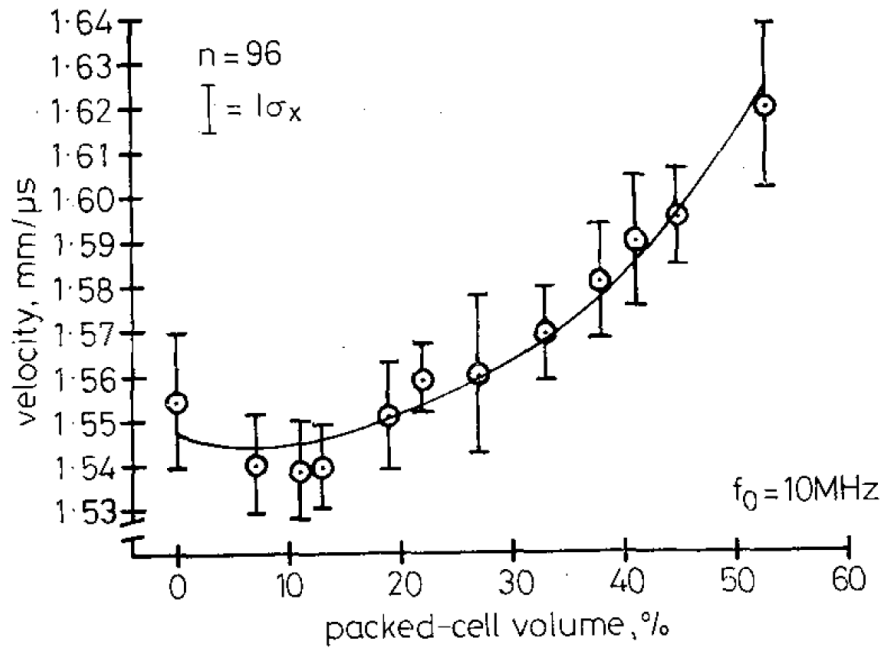


Fig. 5 *Dependence of speed on 10 MHz ultrasound in blood on packed cell volume*

4 Discussion

The speed of 10 MHz ultrasound in blood is slightly dependent on packed cell volume, as shown in Fig. 5. The shape of the dependence appears to be parabolic with a minimum speed at PCVs in the neighbourhood of 10%. These results agree with those of ANGELSEN (1975), who, using a different measurement technique, found a minimum in speed for blood at PCV = 12%.

The nature of the dependence of sound speed in a fluid suspension of particles has been investigated previously by URICK (1947). He reasoned that the nature of the dependence was determined by the differences in density and in adiabatic or isothermal compressibility between the fluid and the suspended particles. Consider the equation for speed of sound, c , in a medium with mean density, ρ_0 , and mean adiabatic compressibility, κ_0 ,

$$c^2 = \frac{1}{\rho_0 \kappa_0}$$

Urick proposed that ρ_0 and κ_0 can be described for a fluid suspension by linear combinations of the corresponding fluid and particle properties according to their volume fractions. If ρ_1 and κ_1

denote the compressibility and mass density of the plasma, and if ρ_2 and κ_2 denote the corresponding values for individual erythrocytes and PCV denotes packed cell volume, then

$$\kappa_0 = \kappa_2 PCV + \kappa_1 (1 - PCV)$$

and

$$\rho_0 = \rho_2 PCV + \rho_1 (1 - PCV)$$

Substitution of these expressions for the density and compressibility of whole blood gives an expression relating speed of sound c to PCV as follows:

$$\begin{aligned} 1/c^2 &= \{\rho_2 PCV + \rho_1 (1 - PCV)\} \\ &\quad \times \{(\kappa_2 PCV + \kappa_1 (1 - PCV))\} \\ &= \rho_1 \kappa_1 + PCV (\rho_2 \kappa_1 + \rho_1 \kappa_2 - 2\rho_2 \kappa_1) \\ &\quad + PCV^2 (\rho_1 - \rho_2)(\kappa_1 - \kappa_2) \end{aligned}$$

Thus, according to Urick's model, the squared inverse of sound speed ($1/c^2$) is a parabolic function of PCV with coefficients depending on the relative densities and compressibilities of the suspended cells and the suspending medium.

Equations of best fit for this wave equation model were computed for the measured $1/c^2$ against PCV data just described using the least square parabolic curve fit of MOOD and GRAYBILL (1963). The calculated regression function was

$$\begin{aligned} 1/c^2 &= 0.418 + 2.09 \times 10^{-4}(PCV) \\ &\quad - 1.75 \times 10^{-5}(PCV^2) \end{aligned}$$

or

$$\begin{aligned} 1/c^2 &= 0.418(1 + 5.0 \times 10^{-4} PCV \\ &\quad - 4.19 \times 10^{-5} PCV^2) \end{aligned}$$

The constant $0.418 = 1/c^2$ for $PCV = 0$ corresponds to a calculated plasma speed of 1.546 mm/ μ s, which agrees well with the measured values reported by AUBERT et al. (1978), who found, respectively, plasma speeds of 1.53 to 1.55 for plasma protein concentrations of 1.29 to 5.163 g/100 ml at 37 °C. Solving the simultaneous equations for the regression coefficients of PCV and PCV^2 , namely

$$5.00 \times 10^{-4} = \rho^2/\rho^1 + \kappa^2/\kappa^1 - 1$$

$$4.19 \times 10^{-5} = (1 - \rho^2/\rho^1)(1 - \kappa^2/\kappa^1)$$

gives ratios of best fit for

$$\rho^2/\rho^1 = 1.007 \text{ and } \kappa^2/\kappa^1 = 0.993$$

These values suggest that the cells in dog blood are slightly more dense but slightly less compressible than the plasma. It is of interest that only small differences in density and compressibility of cells against plasma need be assumed to explain the parabolic dependence of ultrasound velocity PCV which we have observed.

The data of the present study also indicate that the attenuation coefficient of blood is strongly dependent on the PCV. Signal losses in blood at 10 MHz are chiefly due to absorption of energy by macromolecular proteins (CARSTENSEN, 1950); moreover, the energy of the return echo is also decreased by scattering of acoustic waves from the surfaces of suspended cell membranes. Since the most abundant protein constituents of whole blood are contained within cells, both the number of suspended particles and the protein concentration of whole blood are directly related to the PCV. It is reasonable, therefore, to expect the attenuation coefficient in turn to be directly related overall to cellular protein concentration and/or the concentration of formed elements, as measured by the PCV. In fact, an apparently linear relation between the attenuation coefficient of 10 MHz ultrasound and PCV was demonstrated in the present study (Fig. 2). Attenuation increases over a threefold range as PCV is increased from 0 to 53 %.

5 Conclusions

Both the attenuation coefficient and the speed of 10 MHz ultrasound in blood are dependent on the packed cell volume (PCV). Attenuation is strongly dependent on the PCV; this fact can be explained by absorption of ultrasonic energy by intracellular macromolecules and by scattering of ultrasonic energy by blood cell membranes. The speed of ultrasound in blood is also dependent on PCV to a lesser degree; this fact can be explained by slight differences in the densities and the compressibilities of cells as compared to plasma, as predicted by the wave

equation model of Urick. Accurate measurements of blood-filled chambers, such as the heart and great vessels, using ultrasonic techniques may require knowledge of, and correction for, the packed cell volume of blood.

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