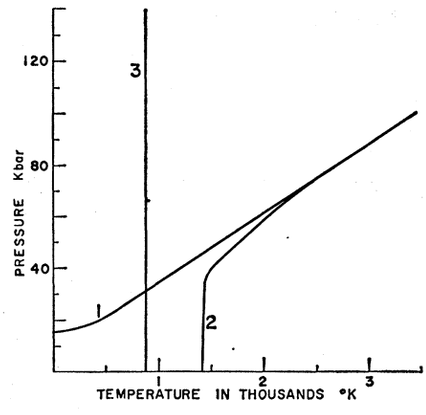
Fig. 2. Diamond sintering regions. Line 1 is the thermodynamic equilibrium boundary for diamond and graphite. Line 3 is a somewhat arbitrary minimum practical sintering temperature for diamond particles. Line 2 is the metastability line for conversion of diamond powder to nondiamond carbon. The location of line 2 depends on time, but the form of the curve for other time periods is similar to the 30-minute curve which is shown.



line 2 can yield a product ranging in color from white through gray to black, and synthetic carbonado of good quality can be produced throughout this color range. Debye-Scherrer x-ray diffraction patterns of the black product show lines characteristic of nondiamond carbons in addition to the diamond lines. The black product is a fair electrical conductor, whereas the white product is insulating. There has been some surface transformation of the diamond particles into nondiamond carbon in the black product. But when the amount of transformation is carefully controlled, a well-bonded black product with excellent physical properties can be produced. The nondiamond carbon is quite effective in bonding the mass of particles together. It always requires a longer sintering time and lower temperature to make an acceptable white product. Increasing the amount of diamond decomposition can produce a soft product that will wear away more rapidly in abrasive use. The softness and accompanying rate of wear can be controlled by manipulation of the pressure and temperature time variables.

The sintered products made from diamond powder thus far discussed are self-bonded. That is to say, the agencies responsible for bonding the mass of particles together come from the diamond itself. It is also possible to produce a synthetic carbonado from diamond powder, wherein a bonding agent or binder is added to the diamond powder. I have found that powders of hard refractory substances, including borides, carbides, nitrides, and oxides, will serve as suitable binders.

The time considerations for pressure and temperature discussed for self-bonded diamond also apply when binders are used.

Two examples for the preparation of synthetic carbonado are given below.

1) A cubic (hexahedral) press was used to generate pressure. The square faces of its tungsten carbide anvils were 0.953 cm on edge. The cubic pyrophyllite sample cell was 1.19 cm on edge and contained a graphite sample tube with sample space 0.254 cm in diameter by 0.475 cm long. This space was filled with natural diamond powder (1 to 5 μm particle size) and was heated by an electric current passed through the graphite tube. Pressure was increased to 85 kb (room temperature calibration; no correction for elevated temperature) then temperature was increased to about 2440°K and held for 3 minutes. Heating current was then switched off, whereupon the sample cooled to near room temperature in about 10 seconds. Pressure was released, and a near-white synthetic carbonado was removed. It was cylindrical in form, being 0.218 cm in diameter by 0.376 cm long; it weighed 0.25 carat (5 carats = 1 g). Its pycnometric density was 3.48 g/cm^3.

2) A cubic press with anvils 1.27 cm on edge was used. The heater and sample container loaded with 1- to 5-μm diamond particles was a molybdenum tube with interior sample space 0.775 cm in diameter by 0.525 cm long. This was centered within a pyrophyllite cube 1.58 cm on edge. Pressure was 65 kb (room temperature calibration) and temperature was held at about 2500°K for 21 seconds. The product was a dark steel-gray cylinder 0.498 cm long by 0.643 cm in diameter, weighing 2.5 carats and having a pycnometric density of 3.09 g/cm^3.

If, in a run like example 2 above, some of the 1- to 5-μm diamond powder is replaced with 5- to 40-μm particles, the product density can be increased significantly. Synthetic diamond powders produced by explosive means as well as by static pressure methods can also serve as starting material for the production of synthetic carbonado.

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15 July 1970

Hemolysis Near an Ultrasonically Pulsating Gas Bubble

Abstract. *A small volume of an erythrocyte suspension was subjected to the action of a manipulated gas bubble set into stable oscillation at 20 kilohertz. Release of hemoglobin occurred when the oscillation amplitude exceeded a critical threshold. Hydrodynamic stresses resulting from acoustically induced small-scale eddying motion near the bubble may be the mechanism of hemolysis.*

The sonic interactions with biological suspensions characteristically occur by means of cavitation, a complicated phenomenon involving sonically activated bubbles. To understand the mechanism, the situation may be simplified by maintaining a single stable oscillating gas bubble in a suspension of cells within a Plexiglas vessel attached to a vibrating bar. Observations in this system led to two primary conclusions: (i) stresses associated with acoustic streaming constitute an important mode of action for sonic effects on cell suspensions, and (ii) measurement of the critical threshold for release of cell contents provides a method for determining the mechanical strength of cell membranes.

The gas bubble is formed in a stainless steel tube (260 μm inside diameter by 2.0 cm) which is connected to a gas reservoir through a 30-cm length of stainless steel tubing (50 μm inside diameter). The latter small tubing prevents large volume changes of the bubble by providing resistance to rapid changes in pressure. By observing with a microscope the operator adjusts the pressure in the gas reservoir so that the gas-liquid interface is hemispherical. Upon application of the sound field the bubble starts to oscillate and also flattens as the enclosed air tends to retreat up the tubing. The reservoir pressure is then increased until the bubble is again hemispherical. In this system the increase in reservoir pressure can be

used to determine the radial oscillation amplitude ξ_0 of the hemispherical air-liquid interface (*1*).

Upon application of the sound field one observes the onset of small-scale vortex motion near the bubble, called acoustic microstreaming (*2*), as shown in Fig. 1. A thin boundary layer is present near the bubble, and, although acoustic streaming velocities are not large, a high velocity gradient exists in this boundary layer (*2*).

In earlier experiments, with the use of bubbles trapped in holes drilled in the face of an oscillating metal bar, difficulties were encountered because of instabilities. These instabilities seemed to be related to the onset of surface waves on the bubbles and the ejection of microbubbles (*3*). In order to avoid these complications a 13 percent solution of dextran 500 in physiological saline, which stabilizes the bubble by damping surface waves, was used; possibly also the dextran acts by forming a "skin" on the bubble, in line with suggestions by Elder (*2*) and Fox and Herzfeld (*4*). This solution has a viscosity of 0.31 poise at 25°C as measured with a capillary-type viscometer. To 10 ml of the dextran-saline solution 0.05 ml of whole blood was added.

In order to study the effects of a single bubble it was necessary to remove gas from the sample, which might exist in the form of unwanted bubbles or bubble nuclei in the sound field. The degassing was accomplished by placing the irradiation vessel under reduced pressure (a few centimeters of mercury) for 20 minutes. Any fluid that was lost was replaced, and the sample was degassed for an additional 10 minutes. The 0.2-ml Plexiglas vessel (internal dimensions, 1.0 cm by 0.2 cm horizontally and 1.0 cm in height) was set into vertical oscillation for 5 minutes at 20 khz by attachment to a Branson Sonifier transducer excited at low amplitudes. After sonation, the irradiation vessel was centrifuged for 20 minutes at 1150*g*. The supernatant was then drawn off and placed in a microcell of a spectrophotometer, and the absorbance was measured at 552 nm. This absorbance was proportional to the concentration of released hemoglobin.

Absorbance of the supernatant of the treated cells was studied as a function of the amplitude ξ_0 of bubble oscillation, with results as shown in Fig. 2. There was no significant release of hemoglobin at low amplitudes; above a fairly well-defined threshold the absorbance rises steeply. If the experimental procedure was repeated when the bubble was absent, there was a maximum absorbance of 0.07 for ξ_0 values up to 29 μm. With no sound, the background absorbance was 0.03. Thus the absorbance was much greater when the bubble was present, for the amplitudes used in these experiments. An extrapolation of the rising part of the curve yields a threshold of 18.2 μm for the amplitude ξ_0.

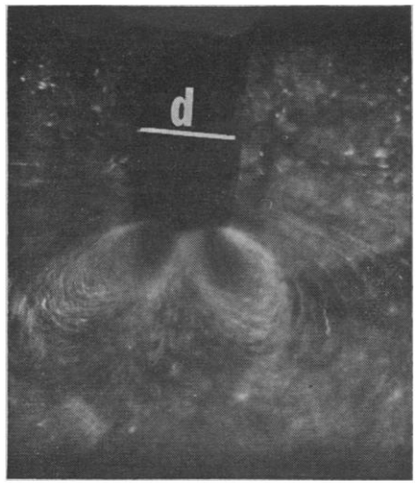

Fig. 1. Bubble-associated streaming pattern obtained by timed-exposure of side-lighted polystyrene spheres (7 to 14 μm in diameter). Amplitude ξ_0 is 26 μm. Dimension *d* shown on photograph gives the tube diameter (520 μm). See Fig. 2 insert for line drawing of arrangement.

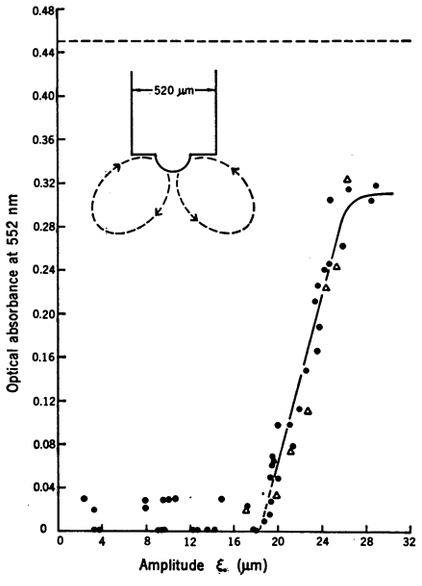

Fig. 2. Hemolysis resulting from a single stable bubble. Dashed line on top of figure indicates complete osmotic hemolysis. In the insert the direction of streaming is shown by the arrows. The circles and triangles are for canine and human erythrocytes, respectively.

A plateau in the absorbance which corresponds to less than complete hemolysis is reached at higher amplitudes. In explanation of this, it is observed that there is relatively little transfer of material from the top and bottom volumes of the sample into the region of the bubble. Thus, the plateau seems to result from depletion of intact cells in the active region of the bubble. For the situation described by Williams, Hughes, and Nyborg (*5*), a similar plateau was not observed. There a slow streaming motion was observed along the length of the vibrating wire which aids in the transfer of material within the sample.

An experiment was done to test whether the metal tubing itself (without a bubble) could affect the cells. For this purpose a stainless steel rod 400 μm in diameter was placed in the cell suspension. A slow acoustic streaming motion occurred near the tip of the rod but only the background absorbance (about 0.03) was observed.

Tests for the presence of sonically produced free iodide radicals were made with 4*N* methyl iodide in dextran-saline solution containing 1 percent soluble starch. When cavitation was present the starch was colored by the sonically produced iodine and the effect was readily observed (*6*). This means that the free radical concentration produced sonically was much greater than the minimum concentration detectable (10^{17} radical/ml) by this method (*7*). Under the acoustic conditions of the present experiment no free radicals were detected.

Because the bubble remained stable, no shock waves or pronounced local maxima in temperature ("hot spots") occurred in the sample. At these amplitudes of bubble oscillation, gross heating should not be important. Experiments were done at 25°C, and sample temperatures never rose more than 2°C during sonic irradiation.

It is believed that stresses resulting from acoustic streaming are the important mechanism in this case. Theory does not exist for the specific kind of acoustic streaming described here. The acoustic streaming velocity gradients and stresses may be estimated from the theory derived by Nyborg, who gives an expression (Eq. 147, in *8*) for the acoustic streaming velocity $u(z)$ tangential to the end surface of the metal tube holding the bubble as a function of z, the distance above the surface. Differentiating u with respect to z and letting $z = 0$ the velocity gradi-

ent G at points near the surface of the bubble is

$$G = 2\pi f \xi_0^2 / a\delta \quad (1)$$

Here δ is the boundary layer thickness defined below, a is the bubble radius (130 μm), and f is the frequency.

The boundary layer thickness can be calculated from the expression

$$\delta = (\eta/\pi\rho f)^{\frac{1}{2}} \quad (2)$$

where η and ρ are, respectively, the shear viscosity and the fluid density. If $\eta = 0.31$ poise, $\rho = 1.0$ g/cm^3, and $f = 2 \times 10^4$ hz, then $\delta = 22.0$ μm. The viscous stress is given by ηG. For the critical threshold of hemolysis, G is 1.4×10^4 sec^{-1} and the viscous stress S_c is 4500 dyne/cm^2. Taking into account experimental errors, one would expect that the standard deviation for S_c would be about 5 percent. However the velocity gradient is nonuniform for the acoustic streaming situation. As a result the maximum uncertainty in S_c is greater, very likely of the order of 1500 dyne/cm^2.

A comparison of values of critical stress for hemolysis obtained by the ultrasonic technique can be made with those obtained with hydrodynamic methods. Samples of blood treated with heparin have been sheared in a closed concentric cylinder viscometer with the bottom of the bob machined to a conical shape. Using such a device, Nevaril *et al.* (*9*) found a threshold stress for hemolysis of 3000 dyne/cm^2. In other experiments Blackshear *et al.* (*10*) have injected jets of saline into suspensions of red cells. The critical velocity gradients observed are of the order of 10^6 sec^{-1}, from which a lethal stress for normal erythrocytes of 40,000 dyne/cm^2 was calculated. Thus, my results fall within the range of values obtained by others.

Williams *et al.* (*5*) describe results for hemolysis caused by acoustic streaming near a vibrating wire. Similarity of results by both ultrasonic techniques demonstrated that details of the ultrasonic interaction with biological materials occurring in a stable bubble field can be elucidated using the vibrating wire apparatus. The comparable results also support the hypothesis that viscous stresses associated with acoustic microstreaming are the important mechanisms involved, since no bubble activity is present near the vibrating wire.

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11. Supported in part by a NASA traineeship [NSG(T)28-S3] and by NIH research grant GM 08209.

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Hemolysis Near a Transversely Oscillating Wire

Abstract. *Erythrocyte suspensions were subjected to hydrodynamic forces generated by a partially submerged tungsten wire set into transverse oscillation at 20 kilohertz. Free hemoglobin appears in solution when the oscillation amplitude exceeds a critical threshold value. The hemolysis probably results from stresses exerted on cell by a microstreaming field established near the wire.*

Rooney has shown that hemoglobin is released from both human and canine erythrocytes when they encounter small scale acoustic streaming in which velocity gradients are sufficiently high (*1*). In his experiments these gradients were produced near an oscillating gas bubble of about 250 μm diameter under conditions where undesirable concomitants of cavitation (such as the production of shock waves and high temperature "pulses") were avoided. Specifically, it was found that erythrocytes in physiological saline containing 13 percent dextran 500 require a minimum velocity gradient (G) of 14,300 sec^{-1} for hemolysis. For this dextran-saline solution, the shear viscosity coefficient (measured by capillary viscometry) is about 0.31 poise, so that the critical shear stress (ηG) becomes about 4500 dyne/cm^2.

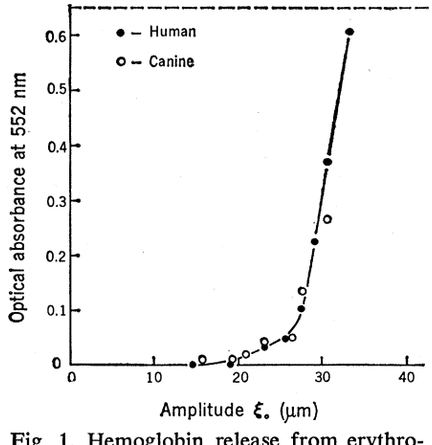

Fig. 1. Hemoglobin release from erythrocytes in suspension, brought about by a partially immersed tungsten wire set into transverse vibration for 5 minutes at a frequency of 20 khz. Osmotic hemolysis in distilled water yields an absorbance value of 0.65, as shown by dashed line.

In view of these results, we were led to consider other arrangements with which one might obtain similar results. An acoustic streaming situation which has probably received more theoretical attention than any other is that which occurs near a transversely vibrating cylinder [see, for example, Schlichting (*2*); Holtzmark, Johnson, Sikkeland, and Skavlem (*3*); Raney, Corelli, and Westervelt (*4*); and a review by Nyborg (*5*)]. Near a transversely oscillating cylinder eddying motions are established (in planes perpendicular to the axis, for an infinite rigid cylinder), with relatively high velocity gradients in a boundary layer very near the cylindrical surface, in the absence of any form of bubble activity. If only the simple approximate expression given by Schlichting is considered, the magnitude G of the maximum velocity gradient at the boundary is

$$G = 2\pi f \xi_0^2 / a\delta \quad (1)$$

Here f is the frequency in hertz, a is the radius of the cylinder, and ξ_0 is the displacement amplitude of the cylindrical surface; the parameter δ is equal to $(\eta/\pi f\rho)^{\frac{1}{2}}$, where ρ and η are, respectively, the density and the shear viscosity coefficient for the liquid. This velocity gradient applies to fluid motion along the boundary, the gradient being directed perpendicular to it. Thus if the velocity parallel to the surface is $u(z)$ at any distance z from the boundary, then G in Eq. 1 refers to the derivative $\partial u/\partial z$ at $z = 0$. The expression for G in Eq. 1 has precisely the same form as that used by Rooney (*1*) for microstreaming near a vibrating bubble. This