Stimulated Brillouin scattering in tropocollagen solutions

S. N. Bagrov, Yu. V. Dolgopolov, G. G. Kochemasov, S. M. Kulikov, A. V. Osipov, S. P. Smyshlyaev, S. A. Sukharev, and S. N. Fedorov

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We have performed experiments on stimulated Brillouin scattering (SBS) in a collagen solution. We demonstrate, partly based radiation scattering spectra, that hypersound (with a frequency of 3.8 GHz) is generated within a small volume located at a distance from the surface many times greater than the decay length of hypersound. We investigated the energy characteristics of SBS in solutions of different concentration. The experimental results are satisfactorily explained within a simple model that takes into account the extinction of light.

INTRODUCTION

Ultrasound is widely employed in medicine for diagnostics and therapeutic treatment as well as in surgery.[1] In therapeutic treatment ultrasound is used for performing micromassage and local heating of tissue as well as for activation of exchange processes. In surgery ultrasound is used for cutting tissue by means of intense acoustic vibrations. Applications of high-frequency ultrasound are limited by its high absorption (proportional to the squared frequency) in biological tissues. Thus the absorption coefficient in water—the main component of biological tissue—is 1 cm^{-1} at 40 MHz (wavelength $\lambda_s = 3.7\cdot 10^{-3}$ cm). The upper limit on the frequency limits the possibilities of this method of action on tissue. In particular, the characteristic spatial scale of local dynamical action (equal to approximately the wavelength of the sound) cannot be significantly decreased.

Research at the MNTK "Eye Microsurgery" has shown that the effect of refractive operations on the cornea is determined by structural changes occurring in the collagen fibers, which form the main framework of the cornea. In particular, in frontal radial keratotomy old collagen fibers are destroyed and new fibers, which, according to electron microscopy, have a smaller diameter and are more closely packed, are formed at the location of the cut. The newly formed collagen is highly elastic. This elasticity is associated with the high degree of regularity of the spatial structure of the microfibrils and the absence of rigid transverse intermolecular bonds.[2,7] Postoperative processes are, in a certain sense, the counterpart of aging processes in an organism, when tissue elasticity decreases and the collagen fibers shrink.

For treatment of near- and far-sightedness it would be helpful to have nonsurgical methods for treating the cornea, which, by influencing the microstructure of the collagen and by changing the corneal elasticity, would produce the necessary refraction effect. The method of ultrasonic treatment mentioned above possesses the required properties. As shown in Ref. 4, irradiation of fibroplasts with ultrasound of a certain intensity and frequency increases collagen synthesis, and the arrangement of the collagen fibers in the scar tissue becomes more ordered.

From the physical standpoint it is of interest to use for collagen treatment sound with wavelength comparable to molecular dimensions, i.e., $\sim$300 nm. This corresponds to hypersound with frequency of 5 GHz. In this case, a nearly resonant, i.e., more effective, effect on collagen microstructure is possible. Hypersound is not employed in medical practice because of the extremely strong damping ($\sim$10 000 cm^{-1}) noted above, which limits the treatment depth to surface layers ($\sim 1\,\mu$m). In addition, hypersound generation by standard transducers (based on the piezoelectric or magnetostriction effect) is a difficult problem.[5]

For phototransparent media, which eye tissues are, hypersound can be efficiently generated with the help of stimulated Brillouin scattering (SBS) of laser beams.[6,7] With the help of SBS it is possible to achieve, by focusing light inside the medium, hypersonic action on collagen fibers at any depth and not only in a layer close to the surface of the medium. It is obvious that this method of local action is also possible for other laser-transparent media.

In order to assess the opthalmological applications of SBS we have initiated an investigation of SBS in collagen solutions. In this work hypersound has been generated for the first time within the volume of a collagen solution. We present below the first results of these investigations.

In Sec. 1 we briefly review information about collagen molecules and the properties of the tropocollagen solution which we employed. In Sec. 2 the acoustic properties of collagen, namely, sound speed, dispersion, and absorption in the range 25–200 MHz, are investigated. The results of Sec. 2 give helpful additional information for analyzing the data presented in Sec. 3 on SBS in a tropocollagen solution. Finally, in Sec. 4 we give a preliminary discussion of the results obtained.

1. PROPERTIES OF COLLAGEN AND TROPOCOLLAGEN SOLUTION[3]

Most multicellular organisms contain collagen. Collagen is a fibrous protein. Mammals have especially large amounts of collagen: It comprises one-fourth of all proteins and it is the main fibrillar component of skin, bones, tendons, cartilage, blood vessels, and teeth. Collagen is distinguished by the fact that it is capable of forming highly elastic, insoluble fibrils.

Collagen can be extracted in solution form from tissues of young animals, since in young animals there are relatively few cross-links. The absence of covalent cross-links in immature collagen makes it possible to separate the main structural unit—tropocollagen.

The molecular mass of tropocollagen is about $3\cdot 10^5$

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times the mass of a hydrogen atom. It consists of three polypeptide chains of the same size (~ 1000 amino acid residues), linked with one another by hydrogen bonds. Intertwining in a three-stranded helix, these chains form a chain-like elastic macromolecule ~ 300 nm long and ~ 1.5 nm in diameter. In live organisms collagen fiber is formed by longitudinal and transverse aggregation of tropocollagen molecules and it is very strong—a force of 10 kg is required in order to break a fiber 1 mm in diameter.

When a collagen solution is heated, the physical properties of the collagen change significantly at a characteristic temperature (otherwise called the melting temperature). The viscosity drops sharply, indicating loss of fibrillar structure. Judging from the change in the optical rotation, separate molecules lose their helical structure. Hence it follows that thermal motion overcomes the forces which stabilize the three-stranded helix, consequently forming a torn structure—gelatin, whose molecules have a tangled, clump-like configuration.

In our experiments we employed a slightly acidic solution of tropocollagen, obtain at the "Eye Microsurgery" MNTK. It consists of a gel containing 1.8% collagen, 1.5% acetic acid $C_2H_4O_2$, and 96.7% water. It is believed that a collagen molecule in such solutions has the shape of a long, slightly bent, rigid stick [the persistent length is 170 nm (Refs. 8 and 9)]. It is also believed that the gel nature of the solution is due to the cross-linkage of the molecules, which creates a three-dimensional network-like structure in the solution. A single tropocollagen molecule has a mass of $4.56\cdot 10^{-19}$ g, so that 1 cm^3 of solution contains $4\cdot 10^{16}$ collagen molecules, and the specific volume per molecule is $2.5\cdot 10^{-17}$ cm^3. Approximately 1000 randomly oriented molecules, linked with one another by hydrogen bonds, fit into a cube with 300 nm long edge, equal to the linear size of the molecule. This example gives an idea of the complexity of the structure of the medium under study.

Different effects occur when laser radiation propagates in a solution of tropocollagen. These include absorption (predominantly in the water), scattering, and rotation of the polarization plane. As the radiation power increases, different nonlinear phenomena can occur: self-focusing, optical breakdown, and different types of stimulated scattering.

All these effects can influence, to one degree or another, the stimulated Brillouin scattering process. Prerequisites for realizing SBS in a collagen solution are that the SBS threshold is lowest in most transparent condensed media and that the main component of the solution is water, for which the SBS process has been studied quite well (see, for example, Ref. 7).

2. ACOUSTIC PROPERTIES OF TROPOCOLLAGEN SOLUTION AT FREQUENCIES 25–200 MHz

The character of the propagation of sound waves in biological tissue is determined by the inertial and elastic properties of the medium as well as by the loss mechanisms operating in it. In order to make the most effective use of ultra- and hypersound and to determine the SBS threshold it is necessary to know the acoustical parameters of collagen (sound speed, dispersion, and absorption) and their dependence on the frequency, amplitude, temperature, tissue structure, etc.

We measured the speed and absorption coefficient of ultrasound (25–200 MHz) in the collagen solution described in Sec. 1. The acoustical parameters were measured by an opticoacoustic method, i.e., by observing light diffraction by ultrasonic waves excited in the medium.[5] A light beam directed into the ultrasound propagation region will be diffracted by the traveling acoustic grating, the first Bragg diffraction order containing a significant fraction of the deflected light. The deflection angle is determined by the wave speed and the intensity of the diffracted light is proportional to the intensity of the sound wave.

Four different media were investigated:

1. Distilled water, as the main component of biological tissue and as a standard liquid.

2. Acetic acid (mass concentration $M = 1.5\%$), as a collagen solvent.

3. Tropocollagen (collagen solution with mass concentration $N = 1.8\%$ in acetic acid).

4. Tropocollagen subjected to thermal denaturation at $T = 50$ °C for 25 min.

Plots of the sound speed versus the sound frequency for the media investigated are displayed in Fig. 1 (the measurements were performed at $T = 18$ °C). For water and acetic acid the sound speed is constant (within the limits of measurement error) in the frequency range 25–200 MHz: $V = 1472 \pm 3$ m/sec in water and 1483 ± 3 m/sec in acetic acid.

For tropocollagen the sound speed exhibits weak dispersion, probably due to the heating of the medium when the

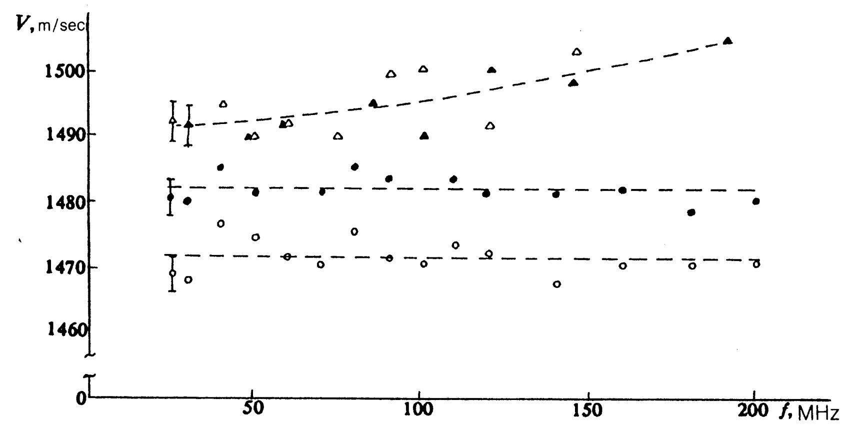

FIG. 1. Sound speed in the medium versus the sound frequency: ○—distilled water; ●—acetic acid ($N = 1.5\%$); ▲—tropocollagen ($N = 1.8\%$); △—denatured tropocollagen ($N = 1.8\%$). The dashed line is the approximation of the experimental points.

ultrasound is absorbed: $V = 1490 \pm 3$ m/sec at $f = 25$ MHz and 1505 ± 3 m/sec at $f = 200$ MHz.

The frequency dependence of the ultrasonic absorption coefficient, normalized to the squared frequency (α/f^2), is shown in Fig. 2. Similar frequency dependences, obtained from published data, for water and 10% hemoglobin solution are also displayed in Fig. 2 for comparison.[1]

For water and acetic acid the normalized absorption coefficient is frequency-independent, and the measured values are close to existing data for water. For tropocollagen the absorption coefficient is approximately three times higher than the value for water at the lower limit of the experimental frequency range. As the frequency increases, the normalized absorption coefficient of tropocollagen solution decreases, in agreement with similar data for protein solutions. Tropocollagen subjected to thermal denaturation is distinguished by appreciably weaker absorption.

Extrapolating the frequency dependence of the ultrasonic absorption coefficient of the tropocollagen solution into the hypersonic range ($f > 1$ GHz) shows that hypersound absorption will be mainly determined by absorption in water. This increases the changes for obtaining SBS in the collagen solution. Moreover, the most important characteristics of SBS in collagen solution can be estimated on the basis of the well-known data for water.[7]

3. EXPERIMENTS ON SBS IN COLLAGEN SOLUTION

One of the most reliable methods for investigating the hypersonic characteristics of a nonlinear medium is to study the characteristics of the scattered light and its Stokes component. These investigations are usually conducted with scattered light energies constituting an appreciable fraction ($\gtrsim 0.01$) of the energy of the excitation radiation. Although for SBS applications in medicine significantly lower hypersound amplitudes may be more useful, we performed the first experiments using the standard method of nonlinear optics.

The experiments were performed with collagen at the nominal concentration [1.8% solution of collagen in acetic acid (see Sec. 1)] as well as solutions with lower concentration ($N = 0.7\%$ and $N = 0.35\%$).

The excitation radiation source was a neodymium glass laser whose parameters were adequate for investigating SBS in water: wavelength $\lambda = 1.06\ \mu$m, energy $E = 1.5$ J, pulse width $\tau_p = 30$ nsec, and beam divergence $\theta = 3 \cdot 10^{-4}$ rad. The experimental arrangement is shown in Fig. 3. The recording methods enabled us to measure the energy and the power of the radiation directed into the cell holding the collagen as well as the energy, power, and spectral composition of the reflected radiation. In addition, during the laser flash the cell was photographed with two cameras (*6*) in visible light and at the laser wavelength.

The Stokes radiation reflected by the cell containing the collagen could be recorded either directly at the exit from the cell (calorimeter, *8*) or after amplification in the amplifier (*3*) in the case when the energy was too low to be recorded by the calorimeter (*8*). These measurements were performed under conditions which were identical for all solutions: the focal length of the lens 14 was $f = 10$ cm and the distance from the lens to the entrance window of the cell was $l = 5$ cm.

The main results of the measurements can be formulated as follows.

1. Stimulated Brillouin scattering is excited comparatively easily in dilute solutions ($N = 0.35\%$ and $N = 0.7\%$). Spectral measurements performed using a Fabry–Perot etalon with a dispersion range of $0.5\ \text{cm}^{-1}$ showed (Fig. 4) that the reflected radiation is frequency-shifted into the Stokes (long-wavelength) region with respect to the laser line by an amount $\Delta\nu = 0.125\ \text{cm}^{-1}$. According to the classical theory of SBS, this shift corresponds to sound speed $V = c\Delta\nu/$

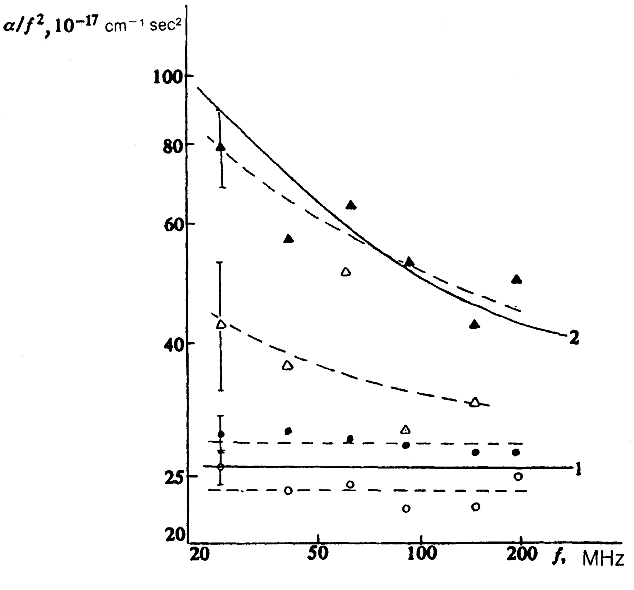

FIG. 2. Frequency dependence of the normalized hypersound absorption coefficient: ○—distilled water; ●—acetic acid ($N = 1.5\%$); ▲—tropocollagen ($N = 1.8\%$); △—denatured tropocollagen ($N = 1.8\%$); *1*—water (data of Ref. 1); *2*—hemoglobin solution ($N = 10\%$) (Ref. 1). The dashed line is an approximation of the experimental points.

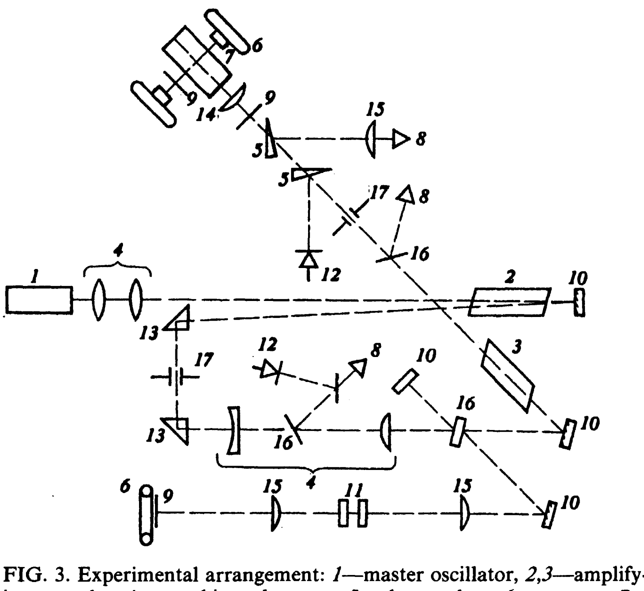

FIG. 3. Experimental arrangement: *1*—master oscillator, *2,3*—amplifying cascades, *4*—matching telescopes, *5*—glass wedges, *6*—cameras, *7*—cell with containing the collagen solution, *8*—calorimeters, *9*—IR filters, *10*—dielectric mirrors, *11*—Fabry–Perot etalon, *12*—coaxial photocells, *13*—rotating prisms, *14, 15*—focusing lenses, *16*—glass plates, *17*—diaphragms.

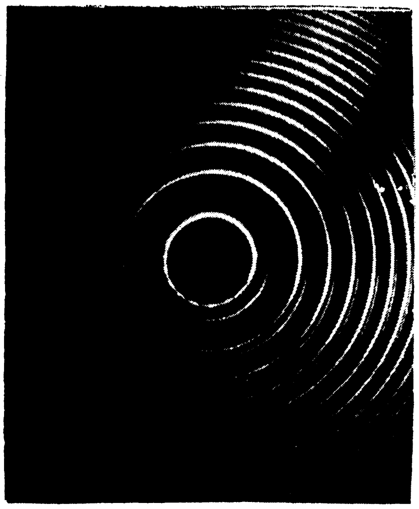

FIG. 4. Spectrograms of the excitation and Stokes radiation in tropocollagen.

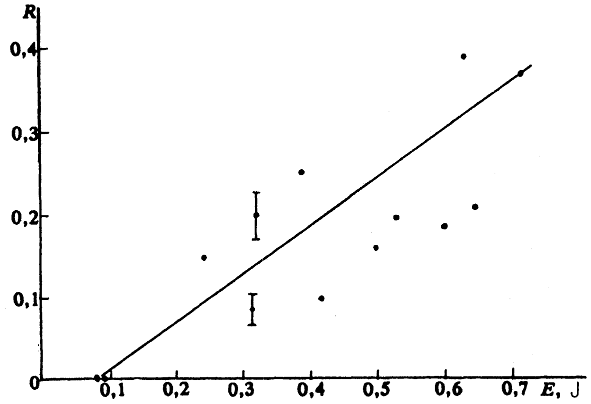

FIG. 6. SBS reflection coefficient versus the excitation radiation energy (tropocollagen concentration $N = 0.35\%$).

$2nv = 1490 \pm 50$ m/sec and frequency $f = 2Vn/\lambda = 3.8$ GHz.

2. Stimulated Brillouin scattering has a threshold. This is illustrated by the oscillograms of the excitation and Stokes radiations (Fig. 5).

3. The reflection coefficient for SBS under identical pumping conditions fluctuates appreciably in different realizations. Figure 6 displays the reflection coefficient versus the laser radiation energy for a solution with concentration $N = 0.35\%$. The fluctuations are probably associated with initial and laser-induced nonuniformities of the medium in the region of the focal constriction.

4. The threshold energy of SBS, determined by extrapolating the energy dependences $R(E)$ and with the help of oscillographic methods, was 0.02–0.04 J in the solvent (acetic acid) and 0.05–0.10 J in all collagen solutions, irrespective of the concentration.

5. The maximum reflection coefficients (the energy of the radiation admitted into the cell $E = 0.6$–0.7 J) reached the following values: $R = 0.2$–0.4 (with concentration $N = 0.35\%$), 0.15–0.2 ($N = 0.7\%$), and 0.05 ($N = 1.8\%$).

6. The strength of the reflected signal depends on the distance between the entrance window of the cell and the focal plane of the focusing lens. As this distance decreases from 10 cm to 6.5 cm, the reflection coefficient increases linearly by approximately a factor of 10. This behavior is related, in all probability, to the strong extinction of radiation in the collagen solution. In this sense the energy characteristics presented above are contingent. In contrast to optically transparent media, they are valid for a fixed distance $l = 6.5$ cm from the cell entrance to the focal plane.

7. As the laser radiation propagates in the cell, gas bubbles initially present in the solution are affected and new bubbles are formed. Both the light and the hypersound can participate in the formation of new bubbles. Indeed, the continuity of the medium can be disrupted at locations of rarefaction, i.e., a cavitation bubble can appear.[10] Photographs

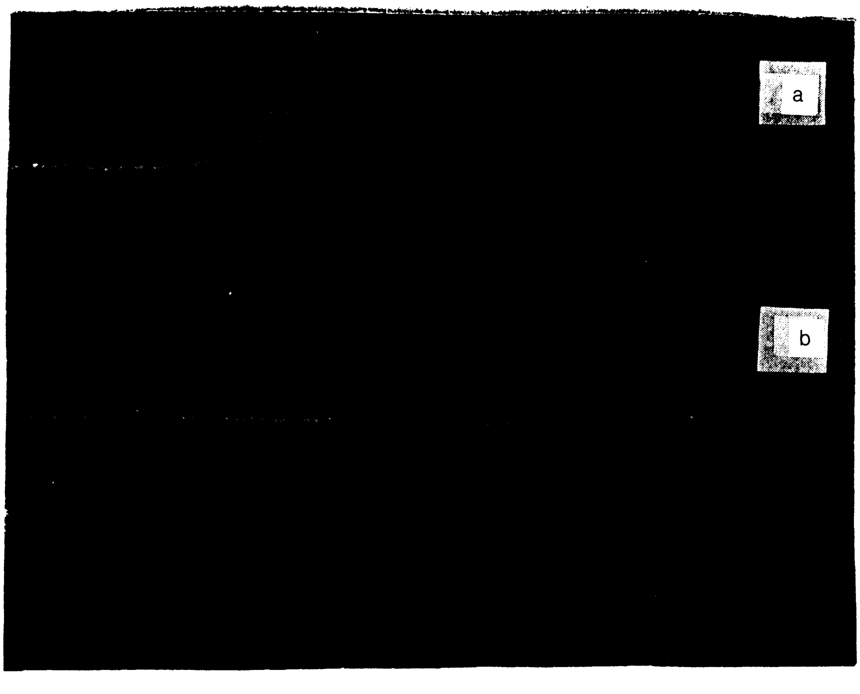

FIG. 5. Oscillograms of the excitation (a) and Stokes (b) radiation in tropocollagen.

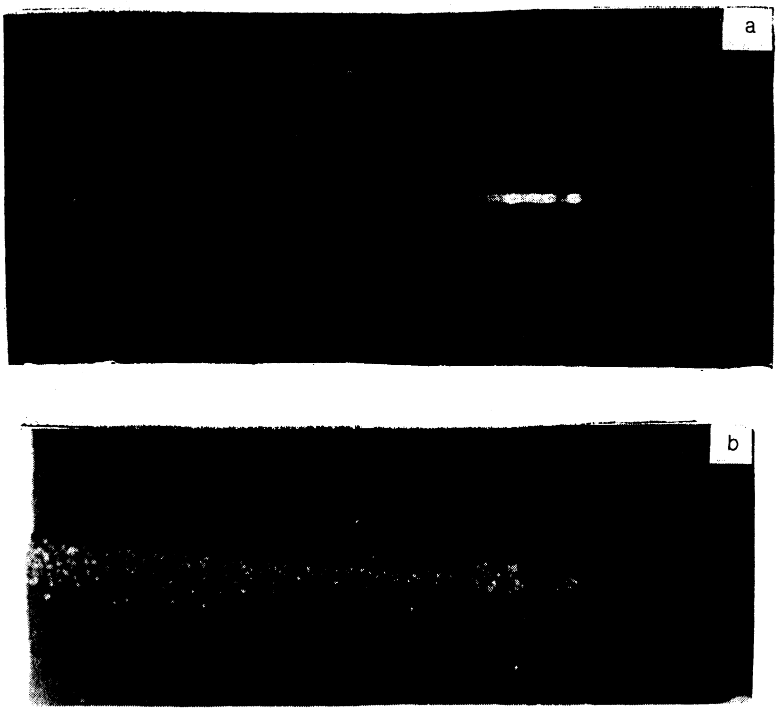

FIG. 7. Photographs of the cell containing tropocollagen. The photographs were made in a) the laser radiation ($\lambda = 1.06\ \mu$m) and b) visible light during the action of the laser radiation.

of the cell containing the collagen solution are presented in Fig. 7. These photographs were taken at a wavelength of 1.06 μm and in visible light during the action of the laser radiation. Strong scattering of the laser pulse is present at all energy levels and collagen concentrations. In visible light a bright flash is observed in the focal region, and the volume of the bubbles formed is greatest here. Scattering of the laser beam is not observed in the acetic acid solution.

4. DISCUSSION

Stimulated Brillouin scattering is a process in which laser photons "decay" into a paired phonon and a lower-frequency Stokes photon. The SBS threshold corresponds to amplification of the noise Stokes waves by a factor of 10^{10}–10^{11}. For this reason, the threshold power is[6]

$$P_{\mathrm{thr}} \approx 30\theta D/4g, \tag{1}$$

where θ is the divergence of the laser beam, D is the diameter of the beam at the focusing lens, and g is the gain with SBS.

In our case we have $\theta = 3\cdot 10^{-4}$ rad and $D = 1$ cm. The value of g for water is well known:[7] $g = 4.3\cdot 10^{-3}$ cm/MW for $\lambda = 1.06\ \mu$m. Substituting these values of the parameters gives $P_{\mathrm{thr}}(H_2O) = 0.5$ MW. Therefore, the computed threshold energy is $E_{\mathrm{thr}}^{\mathrm{theor}} = P\tau_p = 0.015$ J, which is somewhat less than the experimental threshold for water and acetic acid $E_{\mathrm{thr}}^{\mathrm{exp}} = 0.02$–$0.04$ J.

The relation (1) is valid for isotropic nonabsorbing and nonscattering media. Actually, water, acetic acid, and the collagen solution absorb radiation. Measurements showed that the extinction coefficient (which in the case of water and acetic acid is also the absorption coefficient) is $k = 0.13$ cm^{-1} at $\lambda = 1.06\ \mu$m. As a result of radiation absorption, the excitation laser radiation in the focal plane is attenuated by a factor of $\exp(kl)$, where l is the distance from the entrance window of the cell to the focal plane. Taking all this into account, we find that the threshold energy is approximately

$$E_{\mathrm{thr}} \simeq \tau_p \frac{30\theta D}{4g} \exp(kl). \tag{2}$$

Since $l = 6.5$ cm, we obtain $E_{\mathrm{thr}}^{\mathrm{theor}} = 0.035$ J for water and acetic acid, which agrees, to within the limits of the measurement error, with the experimental value.

In order to estimate E_{thr} from the relation (2) for a collagen solution, it is necessary to know k and g. Measurements showed that the extinction coefficient for a collagen solution with the nominal concentration is $k = 0.27$ cm^{-1}. Assuming now that the parameter g for water is close to the value for the collagen solution, we find $E_{\mathrm{thr}}^{\mathrm{theor}} = 0.087$ J for the nominal collagen solution, which likewise agrees, to within the limits of the measurement error, with the experimentally measured value.

How justified is the assumption that the difference in the values of g for water and the collagen solution is small? According to Ref. 7

$$g[\mathrm{cm/MW}] = \frac{5{,}3\cdot 10^4 n(n-1)^2}{\rho V^2 \lambda^2 \alpha}. \tag{3}$$

Here n is the index of refraction, ρ is the density, V is the sound speed, λ is the laser wavelength, and α is the hypersonic absorption coefficient.

Since the index of refraction and the density of the vitreous body of the eye are close to those of water,[11] we can neglect the difference in n and ρ for water and the collagen solution. The sound speeds are also close. The observed weak dispersion of the sound speed $V = 1490$ m/sec at $f = 25$ MHz and $V = 1505$ m/sec at $f = 200$ MHz; see Sec. 2) suggests that the speed is not likely to change by more than several percent.

The difference in the hypersonic absorption coefficients is the most difficult question. It is well known that at comparatively low frequencies absorption is determined by the expression[12]

$$\alpha = \frac{2\pi^2 f^2}{\rho V^3}\left[\left(\frac{4}{3}\eta + \zeta\right) + \varkappa\left(\frac{1}{C_v} - \frac{1}{C_p}\right)\right] \equiv \alpha_0 f^2. \quad (4)$$

Here the coefficients η and ζ characterize the dynamical shear and volume viscosity and $\varkappa$ is the thermal conductivity. For water the term containing the thermal conductivity is a few percent of the term containing η and it can be neglected. It is well known that in the frequency range 7–250 MHz the value of α for water, calculated from Eq. (4) with $\zeta = 0$, is approximately three times smaller than the experimental value. This is customarily attributed to the structural relaxation of the water (transition of a water molecule from a tetrahedrally coordinated structure into a close-packed structure[13]). The restructuring process is apparently characterized by a time constant of approximately 10^{-12} sec, and for this reason α_0 can be expected to remain constant right up to gigahertz frequencies.

In a 1.5% solution of acetic acid there exist additional mechanisms of structural relaxation, which increase the bulk viscosity ζ. But, since the difference of the absorption coefficient of water and acetic acid solution is small (Fig. 2) and it should decrease with increasing frequency, we can neglect the difference in α for hypersound.

Proceeding now to the collagen solution, we first consider low frequencies. We assume that the solution consists of randomly oriented rods with length-to-diameter ratio $L/d = 200$. It is well known that the macroscopic viscosity of a suspension can be expressed by the following equation:[8]

$$\eta' = \eta(1 + \varepsilon\varphi), \quad (5)$$

where η is the viscosity of the solvent, φ is the ratio of the volume of all particles of the dispersed phase to the total volume of the system, and the coefficient ε takes into account the shape of the particles. Long rod-shaped collagen molecules can be approximated by elongated ellipsoids with semiaxis ratio $a/b = \sqrt{2/3}(L/d) = 160$ (L and d are the length and diameter of the rod). For an elongated ellipsoid $\varepsilon = (a/b)^2[15\ln[2(a/b)] - 3/2]$. In the case of interest to us $\varepsilon = 400$. For volume concentration $\varphi = 0.02$ at low frequencies the viscosity of the suspension should be nine times greater than the viscosity of the solvent. At the same time, ultrasonic damping in the collagen solution at 25 MHz is only three times greater than in water. This is probably due to the fact that in the collagen solution processes which decrease the effective viscosity come into play as the frequency increases. First, it is hypothesized that structural relation occurs due to disturbances of the hydrate shells of the macromolecules by the ultrasound, the characteristic relaxation times being quite long: $\tau \sim 10^{-7}$ sec.[1] Second, at high frequencies the collagen molecules do not follow exactly the deformation of the solvent.[13] It can be conjectured that at frequencies above 100 MHz, the deformation along degrees of freedom specific to collagen molecules is elastic and quasirigid, and for this reason the main damping mechanism is viscous dissipation in the water.

The attenuation of both sound and light waves can also be affected by cavitation bubbles which are formed. We intend to study this effect in future work.

We now discuss the characteristics of the scattered light above threshold. In nonabsorbing media the SBS reflection coefficient usually increases monotonically as a universal function of the excess above threshold. In our experiments the function $R(E/E_{\rm thr})$ is different for solutions with different concentration. This difference can be associated to the phase modulation of the medium due to absorption of the laser radiation and subsequent heating of the medium.[14]

CONCLUSIONS

The present work represents the first experimental realization of stimulated Brillouin scattering in a tropocollagen solution. This method can be used to produce hypersound with frequency 3.8 GHz deep in transparent biological tissues (and not at the surface of the tissue). The evidence for this is the experimentally mesured Stokes shift, corresponding to hypersound speed 1490 ± 50 m/sec.

A preliminary analysis showed that the observed experimental results are satisfactorily explained within a simple model in which light extinction is taken into account and hypersound absorption is due mainly to viscous dissipation in the solvent (water).

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