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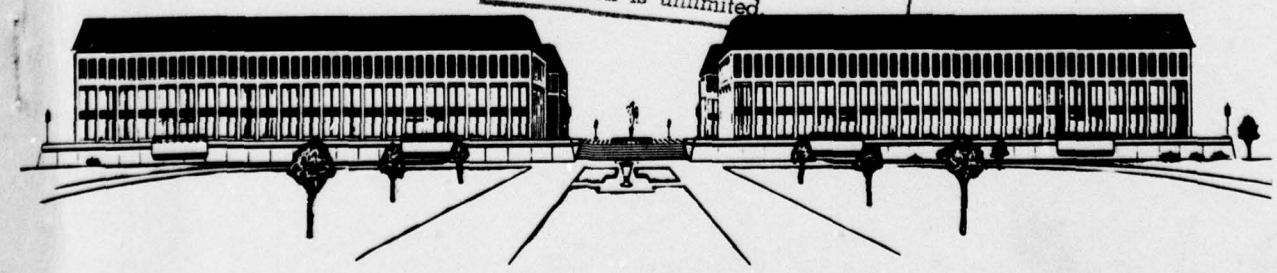

OFFICE OF NAVAL RESEARCH

Contract NR 384-923

FINAL REPORT

October 1978

Lawrence A. Crum

Department of Physics

U.S. Naval Academy

Annapolis, MD 21402

Nucleation, Stabilization, and Growth of Microbubbles in Water

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This final report of a study of nucleation, stabilization and growth of microbubbles in water reviews the advances made and external communications originating from the author's research.

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Introduction:

This final report constitutes a review of the contributions and advances made under contract NR 384-923 of the Office of Naval Research. The primary thrust of the study was toward the nucleation, stabilization, and growth of microbubbles in water. During the course of the study other topics were also examined and these areas are also reviewed in this report.

Studies in Nucleation and Stabilization:

One of the most significant aspects of the phenomenon of acoustic cavitation is that the observed dynamic tensile strength of water is very much less than the predicted strength. The observed cavitation threshold for degassed, distilled water is about 5 atmospheres; the theoretical valve is close to 1000 atmospheres. Attempts to explain this phenomenon have been concerned essentially with the concept of a nucleus or an inhomogeneity that establishes a preferential site for liquid rupture. Many theories exist for the nature of this beast: rigid skins, charged interfaces, surfactant skins and particulate matter. Although all the nuclei stabilization techniques have some merit, the overwhelming evidence supports the hypothesis that gas may be stabilized in small crevices in solid particulate matter. Using this hypothesis, we have examined the dependence of the acoustic cavitation threshold on surface tension -- because it is the shape of the air-water interface in a crevice in a mote that determines the strength of a liquid. Our contributions along this

line are presented in communications Nos. 7 and 14 (see list of external communications, Appendix I), which we feel to be major contributions to the state of the art in cavitation research.

Studies in Bubble Growth:

Once a nucleus has been created, the growth of this cavity or air bubble is also very important. Since the nucleation and stabilization aspect of this study involved the liquid surface tension, we had occasion to examine the effect of surface tension on the growth of bubbles by rectified diffusion. These studies uncovered an effect that we thought to be an original one - that surfactants on the surface of an air bubble can retard diffusion. However, we have just recently seen some communications that have independently verified and corroborated our discovery[1,2] and have led us to believe that this effect - diffusion retardation - can be of extreme importance in physiological areas. Our contribution along this line is contained in communications Nos. 6 and 8.

Studies in Bubble Pulsation:

Cavitation damage results from the asymetrical collapse of a vapor cavity near a rigid boundary. The collapsing cavity develops a liquid jet that penetrates the bubble, strikes the boundary, and damages the interface. Some work that we had started earlier was extended under the funds supplied by this contract and were reported at international meetings on cavitation and non-linear acoustics. Communications Nos. 2,4, and 9 were

issued to detail the discoveries made concerning cylic liquid jet development.

Studies in Bioacoustics:

Most of the funds expended under this contract were used to supplement the salary of the principal investigator; during the period of 9/77 to 9/78, he was priviledged to spend a sabbatical year in the laboratory of Dr. W.T. Coakley, at the University of Wales. Dr. Coakley is known internationally for his work in bioacoustics and a very fruitful year of collaboration was enjoyed by the principal investigator and Dr. Coakley. The primary area of interest was wave-like behavior in heated human erythrocytes (red blood cells), although work was also carried out in surface wave development on pulsating air bubbles. The former area resulted in communications Nos. 3,5,10,12 and 13; the latter in communication No. 11. We also extended a study of air bubble growth in insonated root tips, communication No. 7.

Financial Report:

The duration of the first contract period was 1 June 1977 to 31 December 1978; the second contract period extended from 1 January 1978 to 30 September 1978. The funds committed during the first period amounted to $12,476; those committed during the second were $13,970. Total expenditure of funds during the contract period was $26,446.

Summary:

This contract resulted in the issuance of 14 external communications

during the contract period with a total expenditure of funds of $26,446. Also during the period the principal investigator was able to travel extensively in Europe to exchange ideas with several colleagues and fellow researchers. The principal investigator gratefully acknowledges the support of the Office of Naval Research and hopes his contributions are of value to the Navy.

List of References

1. "Improved method for studying the surface chemistry of bubble formation", J.S. D'Arrigo, Aviation, Space, and Env. Med. (Feb. 1978) pp. 358-361.

2. "Physics of surfactant stabilized gas nuclei", T.D. Kunkle, Undersea Biomed. Res. 4, A33 (1977).

APPENDIX I

LIST OF EXTERNAL COMMUNICATIONS ORIGINATED UNDER CONTRACT

A. Papers presented:

1. "Acoustic levitation -- a useful experimental technique". Invited paper presented at 92nd meeting of Acoustical Society of America, J. Acoust. Soc. Amer. 60, S21 (1976).

2. "Cyclic liquid jet behavior in pulsating bubbles". Invited paper presented at Conference on Acoustic Cavitation, sponsored by British Institute of Physics, Bournemouth, England (Dec. 1977).

3. "Some effects of heat on red blood cell morphology", paper delivered at Conference on Hyperthermia, Imperial College, London (May, 1978), with W. T. Coakley, J. O. Deeley, and A. J. Bater.

4. "Liquid jet production in pulsating air bubbles", paper delivered to 8th International Symposium on Non-linear Acoustics, Paris (July, 1978).

5. "Fragmentation of heated Erythrocytes", paper delivered to 6th International Biophysics Conference, Kyoto, Japan (Sept. 1978) with W. T. Coakley, J. O. Deeley and A. J. Bater.

B. Technical reports submitted:

6. "Measurements of the growth of air bubbles by rectified diffusion", Tech. Report No. 377, Office of Naval Research Contract NR 384-923 U. S. Naval Academy (August, 1977).

7. "Acoustic cavitation inception in water and in insonated root tips", Tech. Report No. 178, Office of Naval Research Contract NR 384-923, U. S. Naval Academy (September, 1978).

C. Articles submitted for publication:

8. "The growth of air bubbles by rectified diffusion", submitted to Journal of Acost. Soc. Amer. accepted for publication with minor revision.

9. "Cyclic liquid jet production in pulsating bubbles", to be published by British Institute of Acoustics as part of Proceedings of Conference on Acoustic Cavitation.

10. "Morphological changes, haemolysis and microvesicularization of heated human erythrocytes", J. Thermal Biology (to be published) with W. T. Coakley, A. J. Bater and J. O. Deeley.

11. "Surface oscillations and jet development in pulsating bubbles", J. de Physique (to be published).

12. "Instability development in heated human erythrocytes", Biochimica et Biophysica Acta (submitted for publication) with W. T. Coakley and J. O. Deeley.

13. "The influence of temperature and incubation time on deformability of human erythrocytes", Biochimica et Biophysica Acta (submitted for publication) with W. T. Coakley and J. O. Deeley.

14. "The tensile strength of water", Nature (submitted for publication).

APPENDIX II

ABSTRACTS OF EXTERNAL COMMUNICATIONS ORIGINATED DURING CONTRACT PERIOD

Acoustic Levitation--A Useful Experimental Technique

L. A. Crum

Department of Physics

U. S. Naval Academy

Annapolis, MD 21402

The nonzero, time-averaged acoustic force exerted by a sound field on a local inhomogeneity allows liquid, solid, and gaseous particles to be suspended in a fluid medium relatively free of physical restraints. This splendid isolation allows studies to be made of radiation pressure, rectified diffusion, damping constants, acoustic streaming, surface waves, and physical parameters of metastable states, as well as many other phenomena, that would be nearly impossible without it. A review will be given of the general technique, and of various ways it can be utilized to obtain experimental data. (Work supported by ONR).

Presented at 92nd meeting of Acoustical Society of America, San Diego, California (Nov. 1976).

Cyclic Liquid Jet Behaviour in Pulsating Bubbles

Lawrence A. Crum*

Department of Physics

U. S. Naval Academy

Annapolis, MD 21402

Cyclic liquid jets have been observed in pulsating air bubbles that are trapped against a platform in a vibrating vessel. The ambient pressure above the liquid was reduced to near that of the vapour pressure and thus the bubbles driven near resonance size were a few millimeters in diameter and large enough to be readily observable visually. Motion pictures were made of the collapse of the bubble and the development of the jet under stroboscopic illumination at low frame rates and under steady illumination with a high speed camera. A movie will be shown of the cyclic jet behaviour and associated effects. (Work supported in part by Office of Naval Research).

*Currently in the Department of Microbiology, University College, Cardiff, Wales.

Presented at Conference on Acoustic Cavitation held at Bournemouth, England (Dec. 1977).

Some Effects of Heat on Red Blood Cell Morphology

W. T. Coakley, L. A. Crum*, J. O. T. Deeley, and A. J. Bater

Department of Microbiology, University College

Cardiff

Studies of the effect of elevated temperatures on the response of human erythrocytes to flow stress will be reviewed. We describe a system for the controlled heating of erythrocytes on a microscope stage. Human erythrocytes heated to temperatures at which spectrin, the form-maintaining endoskeleton of the red cell undergoes a thermal transition, fragment in a manner which depends on heating rate and the proximity of boundaries if exposed to liquid flow stresses or to mechanical shocks. The patterns of fragmentation of the erythrocytes have features which suggest that the expression of liquid-like properties of the cell and its membrane when the form maintaining structures such as spectrin are weakened. Particular attention is paid to the regular breakup of long membrane bound cylinders of haemoglobin by the growth of instabilities on the membrane surface. The periodicity of the breakup is compared with theoretical predictions for liquid cylinder breakup under capillary forces. We also show that careful handling of the cells, minimizing mechanical stress can increase the temperature for spontaneous haemolysis of the cells to 15° above previously reported values.

*On leave from Dept. of Physics, U. S. Naval Academy, Annapolis, Maryland, U.S.A.

Presented at Conference on Hypothermia held at Imperial College, London (May, 1978).

Liquid Jet Production in Pulsating Air Bubbles

Lawrence A. Crum*

Department of Physics

U. S. Naval Academy

Annapolis, MD 21402

A simple method for producing liquid jets in pulsating air bubbles will be presented. It has been found that liquid jets will develop each cycle in air bubbles that are driven near their resonance frequency in a container mounted on a low frequency vibration table, provided the ambient pressure above the liquid is reduced to near that of the vapor pressure. The liquid jets that develop each cycle can be filmed with modest photographic requirements, and if the vibration table is driven at a sufficiently low frequency, the jet can even be made to appear "motionless" with stroboscopic illumination. A film will be presented that shows low speed liquid jets that develop cyclically on air bubbles of approximately 3mm in diameter and are driven at a frequency of 60 Hz. The film shows the familiar pattern of jet production and development but also other aspects that have not been previously reported, such as inverted air jets and microbubble production. Further, small air bubbles produced by the pulsating bubble can be seen to grow rapidly and then recombine with the large bubble resulting in an intricate display of surface wave formation. (This work was supported in part by the U. S. Office of Naval Research).

*Current address - Dept. of Microbiology, University College, Cardiff, Wales, U.K.

Presented at 8th International Symposium on Non-linear Acoustics in Paris (July, 1978).

Fragmentation of Heated Erythrocytes

W. T. Coakley, L. A. Crum*, J. O. T. Deeley, and A. J. Bater

Department of Microbiology, University College

Cardiff, U.K.

Human erythrocytes have been heated in a controlled manner on a microscope stage at temperatures from 30° to 90°. The cells fragment at about 49° which is close to the first thermal transition of the structural protein, spectrin. Cinephotomicrographs and scanning electron micrographs will be presented which illustrate details of the fragmentation processes. The character of the morphological changes observed depends strongly on heating rate. The influence of mechanical shock and local liquid flow on the initiation of fragmentation suggests that the breakup occurs by the unstable growth of disturbances of the cell surface. Cells which are attached to the inner surfaces of microcapillaries and are heated at elevated temperatures for critical times can be detached by the sudden flow of phosphate buffered saline along the capillary. Some cells are restrained in the flow by a long membrane-bound tether which maintains a connection with the attachment site on the glass. The regularly spaced varicosities which develop on some of these tethers were examined by scanning electron microscopy. The development of such vesicle chains by instability growth will be discussed. Similar vesicle formations have been observed in other systems when the form maintaining

structures have been weakened. Changes in the circular dichroism of membrane-bound spectrin heated at regimes which lead to cell fragmentation will be presented.

*On leave from U.S. Naval Academy, supported in part by Office of Naval Research.

Presented at 6th Internation Biophysics Conference in Kyoto, Japan (Sept. 1978).

Measurements of the Growth of Air Bubbles by Rectified Diffusion

Lawrence A. Crum

Department of Physics

U. S. Naval Academy

Annapolis, MD 21402

Measurements are reported of the growth of air bubbles by rectified diffusion at 21.6 kHz. Values of the threshold acoustic pressure amplitude were obtained as a function of bubble radius and liquid surface tension and show good agreement with theory. Measurements of the rate of growth of bubbles by rectified diffusion as a function of acoustic pressure amplitude for varying surface tension show agreement only for high surface tension. When the surface tension is lowered by the addition of a surfactant, the observed growth rates become much larger than predicted. Surface wave activity that could increase the growth rate by acoustic streaming was not observed at low radii and was discounted as the responsible mechanism. A possible explanation for the large growth rates is given in terms of a retardation of outward gas diffusion by an organic monolayer present on the surface of the air bubble.

Technical Report No. 377 for ONR Contract NR 384-923 (August, 1977).

Acoustic Cavitation Inception in Water and in Insonated Root Tips

Lawrence A. Crum

Department of Physics

U. S. Naval Academy

Annapolis, MD 21402

An analysis of two aspects of air bubble nucleation is presented. Part I describes the role of particulate matter in acoustic cavitation inception; Part II describes the growth of air bubbles by rectified diffusion in insonated root tips. It was discovered that a modification of some earlier analyses of cavity nucleation from motes leads to a correct prediction of the variation of the acoustic cavitation inception threshold for kilohertz frequencies in water for a range of physical variables. It was also discovered that acoustic emissions from insonated root tips could be attributed to the growth of air bubbles by rectified diffusion

Technical Report No. 178 for ONR Contract NR 384-923 (Sept. 1978).

The Growth of Air Bubbles by Rectified Diffusion

Lawrence A. Crum

Department of Physics

U. S. Naval Academy

Annapolis, MD 21402

The growth of air bubbles by rectified diffusion has been studied as a function of surfactant concentration for a frequency of 21.6 kHz. It was observed that predicted growth rates are in agreement with experimental measurements only for pure water. When varying amounts of surfactant are added, the observed rate of growth is larger than the predicted rate. The disparity between experimental and predicted values becomes greater as the amount of surfactant is increased. An explanation for the increased rate of growth is given in terms of an added rectification by the surfactant molecules.

Accepted for publication with minor revision in Journal of Acoustical Society of America. Currently being revised.

Morphological Changes, Haemolysis and Microvesicularization of Heated Human Erythrocytes

W. T. Coakley, A. J. Bater, L. A. Crum and J. O. T. Deeley

Department of Microbiology, University College

Cardiff, Wales

1. A system for the controlled heating of erythrocytes on a microscope stage at temperatures from 30 to 90°C is described.
2. The influence of heating rate, proximity of boundaries, local liquid flow and mechanical shock on the type of morphological change observed at 49°C (the transition temperature of spectrin) was examined.
3. Cinephotomicrographs and scanning electron micrographs illustrate details of the fragmentation processes.
4. ATP pool size did not fall during heating. The diacylglycerol content of the membrane increased.
5. Cells protected against flow effects could be heated to 62°C before lysing.
6. Microvesicularization occurred on heating to temperature in excess of 70°C.

Accepted for publication in Journal of Thermal Biology.

Instability Development in Heated Human Erythrocytes

Lawrence Arthur Crum , William Terence Coakley , and
John Owen Thomas Deeley

Microbiology Department,
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Newport Road,
Cardiff CF2 1TA
Wales, U.K.

Heated human erythrocytes gradually lose their form-maintaining structure as the temperature is increased to 50° and can behave essentially as a viscous fluid. We have developed a technique for heating and stressing these cells that is novel, simple and quantitatively precise. We have applied this technique to heated human erythrocytes and have measured instability development in the cells. We have employed instability growth theory to calculate a value for an effective surface tension which, in contrast to other methods of membrane surface tension measurement sought to minimize the effects of membrane supporting structural elements. The value obtained for the surface tension of the heated erythrocyte membrane was 0.9×10^{-6} N/m with a range of variation from 0.4×10^{-6} N/m to 1.4×10^{-6} N/m. The methods described may be useful for determining fundamental physical parameters such as internal viscosity and interfacial tension in other systems.

Submitted to Biochimica et Biophysica Acta for publication.

The Influence of Temperature and Incubation Time on Deformability of Human Erythrocytes

John Owen Thomas Deeley,
Lawrence Arthur Crum and William Terence Coakley
Microbiology Department, University College, Newport Road,
Cardiff CF2 1TA, Wales, U.K.

Human erythrocytes have been heated and stressed in a novel and controlled manner using rectangular microcapillaries. Heated cells attached to the capillary wall were stressed by liquid flow. Under particular conditions of stress, temperature and incubation time the body of the cell could be pulled in the flow, retaining a connection with the glass by means of a narrow attachement or tether. The tethers appear as: regularly beaded, irregularly beaded or without beads depending upon the incubation conditions. We have outlined the incubation regimes necessary to achieve these different responses in the temperature range 47° - 55°C. The cells become less deformable as the incubation is continued beyond an optimum time. The behavior of the tether is compared with that of a viscoelastic liquid. Circular dichroism studies of ghost membranes show that the denaturation of membrane proteins is partially reversible when incubation times are similar to those required to bring about a loss of deformability.

Submitted to Biochimica et Biophysica Acta for publication.

The Tensile Strength of Water

Lawrence A. Crum

Department of Physics

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The measured tensile strength of water has long been known to be significantly less than theoretical predictions due to the presence of nuclei that serve as preferential sites for liquid rupture. A theoretical analysis of the nucleation problem is given that accounts for the motion of an air-water interface in a solid impurity. This analysis gives rise to an equation that correctly predicts the observed dynamic tensile strength of water as determined by the inception of acoustic cavitation.

Submitted to Nature for publication.

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