(0.8*M*), flash evaporated into the cavities (*7*).

The experimental setup is described in Fig. 1. The observation region has been studied with the use of three nozzles, two nozzles pound a water jet on the water surface, and the third is immersed about 20 mm below surface. The flow rates were 240 ml sec^{-1} for the wide nozzle (pressure difference, 8 mm-Hg) and 150 ml sec^{-1} for the narrow nozzle (pressure difference, 300 mm-Hg). The water impinged at a rate of 370 ml sec^{-1} cm^{-2} and 1500 ml sec^{-1} cm^{-2} for the wide and narrow nozzles, respectively (corresponding to a linear velocity of 3.7 and 15 m sec^{-1}, respectively). The sonoluminescence was measured with the multichannel pulse height analyzer, and the background count was subtracted for each channel (background was measured under identical conditions immediately before and after each measurement with the water flow stopped).

The results summarized in Table 1 are presented in arbitrary units of channel number and pulse count. A rough estimate would set the actual number of cavities in the impact area, in which excited sodium atoms were formed, about 100 times the recorded number. (This includes geometry and photomultiplier efficiency.) The scintillation pulse height spectrum, taken several times over periods of 100 or 200 seconds, was bell-shaped and ranged from channel 3 to about channel 50 (over a range of 200 channels). Each pulse in the pulse height spectrum represents a number of scintillations which occur within the preset resolution time of the analyzer and which presumably originate from the collapse of one and the same cavity. The results show that the overall sonoluminescence (i) increases with the linear velocity of the water, (ii) decreases in the presence of oxygen (compared with argon), (iii) is totally quenched in presence of 60 m*M* methanol, and (iv) is absent altogether when the water flows at the same linear velocity but passes the observation region without hitting a new water surface. From this behavior, which is characteristic of sonoluminescence induced by high frequency transducers (*6*, *7*), it may be inferred that cavitation takes place when water impinges on water at relatively low linear velocities.

The linear velocity of water in collapsing waves in the ocean is roughly estimated to be about 5 m sec^{-1} and above, which is the range of velocities investigated in this study. In view of the established parallelism between sonoluminescence and sonolysis in water (*8*) these results provide strong supporting evidence for the occurrence of sonochemical processes in the ocean. The latter conclusion, if verified under natural conditions, implies an additional route for nitrogen fixation as well as a probable pathway to the formation of simple and complex organic compounds in the primordial ocean.

MICHAEL ANBAR

Stanford Research Institute,
Menlo Park, California 94025

References and Notes

1. M. Anbar, *New Sci.* **30**, 365 (1966).
2. H. Beathe, *Z. Physik. Chem.* **163A**, 161 (1963); A. I. Virtanen and N. Ellfolk, *Acta Chem. Scand.* **4**, 93 (1950); *ibid.* **6**, 660 (1952); *ibid.* **11**, 230 (1957); H. Gabrecht, *Z. Physik* **135**, 85 (1953).
3. H. C. Urey, *The Planets* (Yale Univ. Press, New Haven, Conn., 1952).
4. M. Anbar, paper presented at ACS meeting, Atlantic City, Sept. 1968.
5. ——— and I. Pecht, *J. Phys. Chem.* **68**, 352, 1460, 1462 (1964); *J. Chem. Phys.* **40**, 608 (1964); *J. Phys. Chem.* **71**, 1246 (1967).
6. I. E. Elpiner, *Ultrasound: Physical, Chemical and Biological Effects*, F. L. Sinclair, Trans. (Consultants Bureau, New York, 1964).
7. P. Gunther *et al.*, *Z. Naturforsch.* **11a**, 882 (1956), *ibid.* **12a**, 521 (1957); *Z. Elecktrochem.* **61**, 188 (1957); *Ber. Bunsenges. Phys. Chem.* **63**, 43 (1959).
8. R. O. Prudhomme, *Bull. Soc. Chem. Biol.* **39**, 425 (1957).
9. I thank W. Gunter, Instrumentation Division, Ames Research Center, for assistance in constructing the scintillation measurement device; and J. Carter for technical assistance.
10. This work was carried out at the NASA Ames Research Center, Moffett Field, California, where M. A. was a senior research associate of the National Research Council, the National Academy of Sciences, Washington, D.C.

30 July 1968

Inhibitor of Bacterial Growth Released by Human Cells in Culture

Abstract. *Used medium from cultured human cells contains a factor that inhibits growth of the "less virulent" strains of pathogenic bacteria, but only retards growth of the "more virulent" strains. The factor is heat-stable, dialyzable, and unaffected by change in pH; it chromatographs as material of molecular weight between 700 and 1500. There is evidence that this factor is an α-ketoaldehyde attached to a carrier.*

During studies of the host-parasite relations of tissue-culture cells and bacteria, we noticed that certain strains of some bacterial species did not grow when inoculated into HeLa-cell monolayer cultures in antibiotic-free Medium 199 containing 10 percent calf serum, and that the tissue-cell sheet remained intact and healthy; there was no evidence of phagocytosis. All bacterial species tested could, however, grow in Medium 199 with or without 10 percent calf serum, the indication being that a serum constituent was not responsible for the inhibition of growth. Some strains of these bacteria could not survive in the used tissue-cell medium after it had been separated from the tissue cells that it had nourished; this finding was taken to indicate that the inhibition did not depend on the physical presence of the cells. The tissue cell–conditioned medium (CCM) therefore either lacked some nutrient essential for growth of the bacteria or contained an inhibitor of bacterial growth (BGI). Preliminary experiments proved the latter alternative to be true.

For observation of inhibition of growth, suspensions of susceptible strains of *Staphylococcus aureus* were prepared by washing of 18-hour growths from Columbia blood agar base medium (*1*) with Medium 199. These suspensions, in appropriate dilutions, provided the inocula for the test. A series of five tubes, each containing 1.0 ml of CCM and 1.0 ml of Medium 199 with serum, and a control series containing 2.0 ml of Medium 199 with serum, were prepared. In volumes of 0.1 ml, 10^7 bacteria (*2*) were inoculated into the first tube of each series, and 10^6 bacteria were inoculated into the second, with one-tenth as many successively into each remaining tube of each series—down to 10^3 bacterial cells. The tubes were incubated at 37°C and observed at specified times for growth. In this fashion a rough "titration" of the growth-inhibitive activity of the CCM was shown by comparison with growth in the controls.

Inhibition of growth depends on the concentration of the inhibitor and on the number of bacteria present. The BGI was present in CCM that had supported tissue-cell monolayer growth for periods ranging from 1 to 4 days. Filtrates from older cultures were not tested.

A survey was performed to determine whether BGI was active for different bacterial genera. Two series of tubes were inoculated as before. Table 1 lists some bacterial strains whose growth was or was not inhibited by BGI obtained from CCM that had supported the growth of HeLa cells for 66 hours. One should note that, apart

from *Neisseria gonorrhoeae*, growth was detected at 24 hours and always occurred in the control Medium 199 with calf serum added.

Clinical staphylococcal infections which are more resistant to chemotherapy are caused by organisms belonging primarily to *Staphylococcus aureus* phage groups I and III (*3*). Strains from these groups were not inhibited by BGI; nor was *Escherichia coli* serotype O26, which has been classed as pathogenic for infants by ecological implication (*4*), or a strain of *Salmonella typhi*. All the *Neisseria* strains tested to date, except for some virulent strains of gonococci (*5*), were inhibited.

Staphylococcus aureus strains 2919 (from phage group I) and 2695 (from phage group II) were used for assay of BGI in experiments designed to determine its distribution and some of its physicochemical characteristics.

To see whether Medium 199, containing 10 percent calf serum, was essential for the production of the factor by HeLa cells, a monolayer was grown for 48 hours in nutrient broth with yeast RNA (*6*). After supporting cell growth for 48 hours this medium could inhibit the growth of *S. aureus* strain 2695 but not that of strain 2919; both grew in fresh nutrient broth plus yeast RNA.

Other tissue cell lines were cultured in Medium 199 plus 10 percent calf serum, and the conditioned media were assayed for the presence of BGI. Strain 2695 was inhibited by a factor from human amnion FL cells, primary human amnion cells, human embryonic intestine cells, human embryonic kidney cells, human kidney cells, or (to a very small extent) rhesus-monkey kidney cells. A BGI was not detected in African-green-monkey kidney cells. Growth of strain 2919 was not inhibited by supernatants from these described media. Separate lines of HeLa and FL cells that were contaminated with pleuropneumonia-like organisms produced no detectable BGI.

From HeLa CCM the BGI activity against strain 2695 was stable when individual samples were held for 24 hours within a *p*H range of 3.0 to 11.0. The activity did not decrease in individual samples maintained at temperatures ranging from −20° to 100°C for 24 hours before assay at 37°C. The factor also proved stable to autoclaving at 121°C for 15 minutes. Such stability suggested that the factor may have a relatively low molecular weight; indeed it was found to be dialyzable.

Table 1. Growth of bacteria in medium previously used by HeLa cells. Growth after 66-hour incubation in 1 ml of CCM plus 1 ml of Medium 199 is shown by +; − indicates no growth.

Item	Strains tested (No.)	Growth
Staphylococcus aureus		
Phage group I	3	+
Phage group II	8	−
Phage group III	3	+
Phage group IV	1	−
Miscellaneous	3	−
Neisseria meningitidis		
Serotype A	2	−
Serotype B	2	−
Serotype C	2	−
Neisseria gonorrhoeae		
Colony type I	2	+
Colony type III	2	−
Escherichia coli		
Serotype O14	1	−
Serotype O26	1	+
Salmonella typhi	1	+

A primary purification procedure was carried out. HeLa CCM was freeze-dried, reconstituted to 10 percent of its original volume with water, and separated into fractions of 4.0-ml volume by gel filtration on a column (2.8 by 40 cm) of Sephadex G-50, with water as the eluent. Three distinct peaks were obtained by determination of the optical densities of the fractions at 280 mμ. The tubes of eluate comprising each of the peaks were pooled by fractions, concentrated by freeze-drying, and reconstituted to 4.0 ml with either Medium 199 and calf serum for assay, or water for further purification. The material from the third peak contained BGI activity. The controls for this procedure were the three corresponding peaks obtained when a Sephadex G-50 fractionation, of fresh Medium 199 and calf serum, was treated the same way. No BGI activity was observed in this material.

The third G-50 peak of the conditioned medium that contained BGI was passed through a column of Sephadex G-25. Two principal peaks were eluted with water. The activity, residing mainly in the first peak, appeared after elution of the void volume. After passage of the first G-25 peak through a column of Sephadex G-10, the void-volume peak displayed the activity indicating that the molecular weight exceeded about 700. When this material was passed through Sephadex G-15, the BGI was eluted immediately after the void volume, the indication being a molecular weight below 1500.

Because at this point it seemed that some of the properties of our factor resembled those of "retine" (*7*), we further tested for the presence of a ketoaldehyde. The activity is reversed by cysteine, which is known to react with α-ketoaldehydes. Arsenious oxide releases the activity into methanol, the suggestion being that the ketoaldehyde is bound to polypeptide material through sulfhydryl groups. The reactions of the methanol-arsenate extract are characteristic of α-ketoaldehydes. A 2,4-dinitrophenylhydrazine derivative forms quickly in the cold; the crystals are dark red and become blue-violet in alcoholic-KOH—an indication of a bis-2,4-dinitrophenylhydrazone. The infrared spectrum confirms this structure.

Among the genera tested, the more virulent strains are inhibited less by BGI than are strains of lesser virulence (*8*), so that BGI may provide a basis for the grading of bacterial virulence (*9*). The presence of a ketoaldehyde in man (*10*) suggests that he has yet another line of defense against bacterial infection.

C. Paul Kenny
Brian G. Sparkes*
Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada

References and Notes

1. P. D. Ellner, C. J. Stoessel, E. Drakesford, F. Vasi, *Amer. J. Clin. Pathol.* **45**, 502 (1966).
2. All bacterial counts were determined with a Coulter model-B counter fitted with a 30-μ aperture tube at the settings described by C. P. Kenny, F. E. Ashton, B. B. Diena, L. Greenberg, *Bull. World Health Organ.* **37**, 569 (1967).
3. I. B. R. Duncan and R. Comtois, *Can. Med. Ass. J.* **94**, 879 (1966); W. R. McCabe, *Yale J. Biol. Med.* **38**, 323 (1966).
4. W. H. Ewing, B. R. Davis, T. S. Montague, in *Studies on the Occurrence of* Escherichia coli *Serotypes Associated with Diarrheal Disease* (National Communicable Diseases Center, Atlanta, Ga., 1963).
5. D. S. Kellogg, Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, C. I. Pirkle, *J. Bacteriol.* **85**, 1274 (1963).
6. C. P. Kenny and H. Lees, *Can. J. Biochem.* **43**, 933 (1965).
7. A. Szent-Györgyi, L. G. Egyud, J. A. McLaughlin, *Science* **155**, 539 (1967).
8. Since *Neisseria meningitidis* seems to be an exception, we should point out that only laboratory strains whose virulence was questionable were available.
9. The ratio of BGI concentration to number of bacteria is important for the grading of virulence. We observed in preliminary experiments that "virulent" pathogens in very small numbers grew more slowly in the presence of BGI than in control medium, and that very heavy inocula of "less virulent" pathogens could grow in the presence of the inhibitor, although more slowly than in control medium. The chemical nature of BGI will be detailed elsewhere.
10. L. G. Egyud, *Biochem. J.* **96**, 19c (1965).
11. We thank Mrs. B. Aris for technical assistance.
* Present address: McGill University Cancer Research Unit, McIntyre Medical Sciences Building, 3655 Dummond Street, Montreal, P.Q.

24 July 1968