(30 nsec) they performed a similar experiment with very much more intense light, with a focused power density on the order of a million megawatts per square centimeter. In this case, the frequency-shifted light is so intense as to materially enhance the probability that other frequency-shifting collisions will occur, a phenomenon recognized in Einstein's theory of stimulated emission. Once a certain threshold intensity is exceeded, as it is in this experiment, there is, in effect, positive feedback that results in an avalanche of phonons. In the case of compressional waves, the process may be viewed as the generation of sound waves by electrostriction of the medium (in Townes and Chiao's experiment, quartz or sapphire) under the action of the intense electric field of the incident light wave. The sonic pulse lasts about 30 nsec and has a calculated peak power of about a kilowatt; the frequency is determined jointly by the wavelength of the incident light and the line along which the observation is made; in the experiments reported, the sonic frequency was in the neighborhood of 60,000 Mc/sec.

Quate and his colleagues reported a complementary experiment, in which they observed the effect on light of a high-frequency elastic wave generated by conventional means. Measuring the fraction of a laser beam diffracted by the sonic wave, they determined the coupling constants for the photon-phonon interaction. The stage is now set for the generation of intense beams of microwave phonons through the interaction of two beams of light.

The generation of sonic waves with frequencies higher than 10^9 per second has already been accomplished for several years by subjecting a piezoelectric material to the rapidly alternating electric field in an electrically resonating cavity driven by a microwave oscillator. J. deKlerk and E. F. Kelly (Westinghouse Research Laboratories) and N. F. Foster (Bell Telephone Laboratories) gave details of a technique for depositing piezoelectric layers of cadmium sulfide onto other solids, which can then be excited at high frequency even though they are not piezoelectric. The interest in sound waves of such high frequency comes in large part from their utility as probes of the solid state. However, because they correspond to the microwave region of the electromagnetic spectrum, they will probably find use in communication and radar technology. R. Pohl (Cornell University) spoke on what may be called the spectroscopy of phonons. The scheme uses the thermal vibrations of the lattice as a source and the thermal conductivity as a measure of transparency. The dominant lattice frequency is proportional to the temperature; at 0.3°K, it is 5000 Mc/sec. For alkali halide crystals doped with CN^- ions, thermal conductivity plotted as a function of temperature has dips at certain temperatures. One of these can be interpreted as the temperature at which the rotary motion of the CN^- ion changes from libration to rotation; a dip at low frequency is ascribed to a resonance absorption associated with tunneling between two equilibrium orientations of the ion.

On the communications side, J. E. May (Bell Telephone Laboratories) discussed design considerations for the amplifying of electronic signals by means of sound waves. This amplification, startling when first conceived, is possible in semiconductors that are piezoelectric. If an electric field established in such a material is large enough to make the electrons drift faster than sound travels, then the electrons can transfer their energy to a sound wave; the effect can be more than large enough to offset the ordinary acoustic attenuation, and the result is a sonic amplifier. Though conversion of an electronic signal into a sound wave and back again introduces some loss, overall gain can be achieved. F. S. Hickernell (Motorola) described a gallium arsenide amplifier that has net gain at frequencies in the neighborhood of 100 Mc/sec. The outlook for development of this type of amplifier is good.

The symposium was enlivened by a session devoted to medical problems. It dealt chiefly with the improvement of techniques for mapping internal organs by means of ultrasonic echograms. Typically, such work employs frequencies of about 1 Mc/sec. Advances in design were put forward by G. Kossoff (Commonwealth Acoustical Laboratory, Sydney, Australia) with applications to the pregnant uterus, and by R. A. Brinker (Presbyterian Hospital, New York) with applications to the detection of brain tumors by noting displacements of the ventricles from their normal positions. An ingenious instrument for both scanning and surgery was described by D. Gordon (West End Hospital for Neurology and Neurosurgery, London), who has the distinction of being the first ultrasonic radiologist appointed as such to a hospital staff. His system is built around a focusing transducer well controlled in its position and orientation with respect to reference planes in the patient. The focus of the transducer, located by maximizing the echo from a 1.5-millimeter metal sphere, is known in position to a few hundredths of a millimeter. The device is first used as a sonar set, emitting pulses at low power, to map the subject. The result is a sequence of silhouette-like outlines of coronal or sagittal sections of the brain. A blood vessel 1 mm in diameter can be located to within about 30 μ. When the desired locus has been established, it can be irradiated with high continuous-wave ultrasonic power, which destroys localized groups of cells at the focus. The technique permits the destruction of tumors that cannot be reached without going through some organ that must not be harmed.

The meeting showed that the older phases of ultrasonics are moving into broader use in technology, and that there is an impressively expanding interplay between ultrasonics and solid-state physics. Because the methods of generating ultrasounds are different in the regions above and below about 500 Mc/sec, some feel that the field of ultrasonics will undergo fission. I believe, however, that unity of concept will win out over diversity of technique, and that ultrasonics will grow as a single increasingly well-knit body.

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Biological Nitrogen Fixation

The fundamental role of nitrogen fixation among the earth's life-dependent processes has stimulated the interest of scientists for many years. Recent successes in obtaining N_2 reduction with cell-free preparations have led to examination of this process at the enzyme level, studies which, it is hoped, will lead to an understanding of the mechanism. A group of scientists active in this field attended the 1964 Colloquium on Biological Nitrogen Fixation held at Butternut Lake, Wisconsin,

1–4 October, under the sponsorship of the Charles F. Kettering Research Laboratory. The meeting provided an opportunity for free exchange of information, critical discussion of experimental observations, and correlation of information obtained with different nitrogen-fixing systems.

Presentation and discussion of experimental results were preceded by a historical review of the field by P. W. Wilson (University of Wisconsin) who placed special emphasis on the relation between hydrogenase and nitrogen fixation and on the frustrating efforts over many years to obtain cell-free preparations that would fix nitrogen. The remainder of the program was devoted to discussion of the N_2-fixation systems of anaerobic, aerobic, and symbiotic organisms and to related studies of nitrification and denitrification.

Cell-free extracts of the anaerobe *Clostridium pasteurianum* can reduce N_2 to ammonia when supplied with a suitable reductant and a system for generating adenosine triphosphate (ATP). With crude extracts, pyruvate can supply both requirements. L. E. Mortenson (Purdue University) described studies on pyruvic dehydrogenase in which thiamin pyrophosphate (TPP) was removed from the enzyme by prolonged alkaline dialysis and its reinsertion required the presence of pyruvate. The synthesis of acetolactate required both TPP and coenzyme A (CoA), which suggests that high-energy acetolactyl CoA is an intermediate in acetoin synthesis.

Reductants other than pyruvate can function in the *C. pasteurianum* system. R. W. F. Hardy (E. I. duPont de Nemours and Co.) reviewed data obtained in collaboration with A. J. D'Eustachio and E. Knight, Jr., which show that KBH_4, H_2, or reduced diphosphopyridine nucleotide will serve as reductants with either acetyl phosphate or creatine phosphate plus creatine phosphokinase as ATP generators.

Cofactors required for fixation by extracts of *C. pasteurianum* include Mg^{++}, CoA, ADP or ATP, and ferredoxin. R. H. Burris (University of Wisconsin) reported that his colleague Munson had reduced the activity of these extracts virtually to zero by removal of cofactors on an anaerobic column of Sephadex and had obtained almost complete reactivation of pyruvate-supported fixation by restoration of ATP, CoA, and Mg^{++}. Ferredoxin is also essential. Hardy showed that dialysis or treatment with diethylaminoethyl-cellulose, charcoal, Dowex-1, or Dowex-50 inactivates the system when KBH_4 or H_2 is used as a reductant and one or more of Mg^{++}, adenosine diphosphate (ADP), or ferredoxin restores activity.

The possible function of ATP received considerable attention. M. Dilworth (University of Wisconsin and University of Western Australia) reported that removal and replacement studies with the *C. pasteurianum* system suggest the existence of two sites of action of ATP between H_2 and N_2. Results with the N_2-fixation inhibitors CO and N_2O support this scheme. Mortenson presented the hypothesis that ATP is required to activate "reduced nitrogenase," to which N_2 is then adsorbed and reduced *in situ*. This hypothesis is based on the findings that: (i) utilization of ATP depends upon the presence of reduced ferredoxin; (ii) with preparations from cells grown on medium containing NH_3, little ATP is utilized even in the presence of reduced ferredoxin; and (iii) the removal of a protein component that will not hydrolyze ATP itself from extracts of cells grown in N_2 renders them incapable of N_2 fixation or utilization of ATP unless the component is restored. The addition of this component to an extract of cells grown in the presence of NH_3 resulted in highly active N_2 fixation and ATP utilization. Hardy reported results on the exchange of phosphate and pyrophosphate with ATP and suggested that the utilization of ATP might result in the release of pyrophosphate. The DuPont group has found a clostridial pyrophosphatase that is stimulated about 40-fold by a reductant.

There is still no evidence for free intermediates in the N_2 fixation reaction. Burris reported that his colleagues Garcia and Winter employed $^{15}N_2$ to examine the possibility that hydroxylamine, hydrazine, diimide, and carbamyl phosphate might be intermediates in N_2 fixation by *C. pasteurianum* extracts, but their results were negative. Klucas was unable to demonstrate any N_2 exchange reaction with this system. Hardy described a dialysis-fixation system permitting absolute N_2 fixations up to ten times those obtained with the conventional closed system. It appears, however, from the fact that both systems had similar initial rates that dialyzable intermediates are not involved.

In discussing inhibitors, Burris reported Lockshin's finding that H_2, CO, and N_2O are clearly competitive inhibitors of N_2 fixation in *C. pasteurianum* extracts, as in other N_2 fixing agents. Results were less clear with NO, but it appeared to be competitive also.

Dua (University of Wisconsin) observed that the N_2-activating enzyme from *C. pasteurianum* is cold labile. He achieved a 121-fold purification of the enzyme.

Nitrogen fixation with cell-free preparations from the aerobe *Azotobacter vinelandii* was discussed. W. A. Bulen (Kettering Laboratory) reported that this system required both an electron donor and a source of ATP. The occurrence of the reaction under strongly reducing conditions rules out the formation of an oxidized intermediate by reaction with O_2. The product of the reaction was shown to be ammonia. Both H_2 and $Na_2S_2O_4$ served as electron donors; the former required a preparation of crude hydrogenase plus ferredoxin (from *C. pasteurianum*). The $Na_2S_2O_4$ system gave the best rates, and optimal conditions for this assay were presented. The N_2-reducing activity was found to be concentrated in a fraction obtained by prolonged centrifugation at 144,000*g*, a fraction previously shown to contain a large portion of ^{99}Mo taken up by the cells. The N_2 reducing activity of these preparations was stable in air at 0°C. Evolution of H_2 accompanied the formation of ammonia and increased about fourfold if argon replaced N_2 in the atmosphere, demonstrating the presence of an ATP-dependent hydrogenase in the preparations.

The properties of the ATP-dependent hydrogenase activity were presented in detail by R. C. Burns (Kettering Laboratory). The similarity between the requirements and optimal conditions for this activity and those for N_2 reduction suggests that both activities may be catalyzed by the same enzyme complex. This possibility led to the suggestion that electrons activated by ATP either are transferred to N_2 or evolve as H_2.

Several interesting aspects of fixation by symbiotic systems were reported. W. S. Silver (University of Florida) reported that the leaf-nodule symbiosis occurring in *Psychotria bacteriophila* is somewhat unique in that N_2 is fixed by the isolated endophyte as well as by the nodulated plant. The endophyte, a species of *Klebsiella*, fixes nitrogen anaerobically. Fixation by cell-free extracts resembled the clostridial system,

since pyruvate was required. Although the nature of the reductant is not known, ferredoxin is ruled out, for it is not present in the organism. The nature of the reductant and the requirement for ATP are being investigated.

H. J. Evans (Oregon State University) reviewed investigations, conducted in collaboration with S. Ahmed, R. Lowe, M. Kliewer, G. Johnson, A. DeHertogh, and P. Mayeux, of the requirement and role of cobalt in nitrogen-fixing organisms. It was concluded that soybean plants forced to fix N_2 require cobalt, but no conclusive evidence was obtained that leguminous or nonleguminous plants supplied with adequate fixed nitrogen require cobalt. *Azolla folliculoides* grown in symbiotic association with *Anabaena azollae* exhibits a requirement for cobalt when cultured in nitrogen-free medium. The cobalt requirement for symbiotically grown *Azolla* can probably be accounted for by the requirement of the blue-green alga living in symbiotic association with the fern. The cobalt requirement exhibited by *Rhizobium* species grown in pure culture indicates that the requirement of symbiotically grown legumes reflects that of the nodule bacteria. Leguminous nodules and pure cultures of *Rhizobium* spp. contain 5,6-dimethylbenzimidazolylcobamide coenzyme, and the quantity synthesized is proportional to the cobalt supply. The only enzyme so far identified in the rhizobia that requires the B_{12} coenzyme is the methylmalonyl mutase. Both bacteroids from leguminous nodules and cells from pure culture contain enzymes that activate propionate, carboxylate propionyl CoA, and convert methylmalonyl CoA to succinyl CoA. Cobalt-deficient rhizobia fail to oxidize propionate and exhibit little or no methylmalonyl CoA mutase in cell-free extracts. The addition of 5,6-dimethylbenzimidazolylcobamide coenzyme to extracts of deficient rhizobia restores methylmalonyl mutase activity.

In discussing the localization of fixation in soybean nodules, Burris reported Klucas's finding that the soluble portion of soybean nodules consistently contains the highest concentration of ^{15}N after short exposures to $^{15}N_2$, contrary to Bergerson's hypothesis that fixation occurs on the membrane. A round-table discussion resulted in little or no agreement on the role of nodule leghemoglobin.

C. C. Delwiche (University of California, Davis) made brief reference to work being done at the University of California on the relation of cobalt to nitrogen fixation. He went on to describe related studies of nitrification and denitrification which may have some bearing on problems of nitrogen fixation.

Recently it has been clearly established that *Nitrobacter* sp. requires molybdenum. Concentrations of 10^{-8} molar molybdenum as molybdate are adequate for normal cell growth and nitrification. No direct dependence of the nitrification reaction on molybdenum was demonstrable with cell-free preparations or with growing cells, nor could it be shown that molybdenum is required for nitrate or nitrite reduction.

The ammonium ion supported a much greater incorporation of nitrogen into cell material than did any other ^{15}N-labeled nitrogen source employed.

Studies of the denitrification reaction were described in which the conversion of aberrantly labeled nitrous oxide to N_2 was observed by means of mass spectrometry. An isotopic equilibration was observed which indicated that in its conversion to N_2 nitrous oxide is equilibrated with a one-nitrogen compound.

A uniform method for reporting quantities of nitrogen fixed was the topic of a general discussion session. Participants agreed that the unit millimicromoles of N_2 fixed per minute per milligram of protein conforms to the system adapted by the International Commission on Enzyme Nomenclature and will be used in future publications.

A summary and suggestions of profitable areas of future effort were provided by J. R. Postgate (University of Sussex).

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Calorimetry

The exchange of views on mutual problems and techniques, the development of cooperative schemes for the acquisition and dissemination of thermodynamic data, and visits to calorimetric laboratories were all accomplished at the 19th Calorimetry Conference, Washington, D.C., 13–16 October 1964. Joint sponsors for this conference were the National Bureau of Standards and the National Naval Medical Center of Bethesda, Maryland. This was the first time that a conference on calorimetry has been cosponsored by an institution primarily interested in the life sciences. Appropriately, thermodynamics provided a link between the physical and biological sciences.

The first day of the conference was devoted to tours of the many laboratories in the Washington area of interest to calorimetrists. Participants were able to see calorimeters of almost every known kind, from calorimeters for studies at very low temperature to those for studies at very high temperatures; from calorimeters for studies of inorganic reactions to those for studies of living processes.

The opening paper of the conference was the Huffman Memorial Lecture entitled, "Heats of Biochemical Reactions," by J. M. Sturtevant (Yale University). The subject chosen by Sturtevant was particularly appropriate because Huffman himself was an outstanding pioneer in the measurement of heats of biochemical reactions. Sturtevant illustrated his talk with examples of heats of biochemical reactions from his own work on the heats of hydrolysis of peptide and amide bonds in several compounds of known structure. The effect of structure in reaction heat was also illustrated in the enthalpy changes for three transmethylation reactions leading to the formation of methionine. Sturtevant also pointed out that results from enthalpy studies in vitro often do not yield the real enthalpies in vivo because of the variation of enthalpies with the *p*H of the media in which they are studied. He illustrated this with studies of some enthalpies of ionization measured with various buffers in the solution. Following the lecture, T. H. Benzinger (National Naval Medical Center) discussed key biothermodynamic data and described a calorimeter capable of measuring energies as small as 4 millicalories.

During a special session on the driving forces behind the process of life, R. E. Davis (University of Pennsylvania) discussed the metabolism and use of proteins, fats, and carbohydrates in furnishing energy to life processes and the very important role played in metabolism in all forms of life by the compound adenosine triphosphate (ATP). The interactions of ATP liberate energy that is used for many chemical syntheses, for secretion, for osmotic work, for the production of light, for muscle contraction, and for many other energy functions in living cells. The synthesis and utilization of ATP are crucially involved in making energy