326
3-14-65

BROOKHAVEN LECTURE SERIES

Chemical-Biochemical Signal and Noise - Resolution at Low Temperatures

SIMON FREED

MASTER

Number 39

September 23, 1964

PATENT CLEARANCE OBTAINED. RELEASE TO
THE PUBLIC IS APPROVED. PROCEDURES
ARE ON FILE IN THE RECEIVING SECTION.

BROOKHAVEN NATIONAL LABORATORY

Associated Universities, Inc.

under contract with the

United States Atomic Energy Commission

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

FOREWORD

The Brookhaven Lectures, held by and for the Brookhaven staff, are meant to provide an intellectual meeting ground for all scientists of the Laboratory. In this role they serve a double purpose: they are to acquaint the listeners with new developments and ideas not only in their own field, but also in other important fields of science, and to give them a heightened awareness of the aims and potentialities of Brookhaven National Laboratory.

Before describing some recent research or the novel design and possible uses of a machine or apparatus, the lecturers attempt to familiarize the audience with the background of the topic to be treated and to define unfamiliar terms as far as possible.

Of course we are fully conscious of the numerous hurdles and pitfalls which necessarily beset such a venture. In particular, the difference in outlook and method between physical and biological sciences presents formidable difficulties. However, if we wish to be aware of progress in other fields of science, we have to consider each obstacle as a challenge which can be met.

The lectures are found to yield some incidental rewards which heighten their spell: In order to organize his talk the lecturer has to look at his work with a new, wider perspective, which provides a satisfying contrast to the often very specialized point of view from which he usually approaches his theoretical or experimental research. Conversely, during the discussion period after his talk, he may derive valuable stimulation from searching questions or technical advice received from listeners with different scientific backgrounds. The audience, on the other hand, has an opportunity to see a colleague who may have long been a friend or acquaintance in a new and interesting light.

The lectures are being organized by a committee which consists of representatives of all departments of the Laboratory. A list of the lectures that have been given and of those which are now scheduled appears on the back of this report.

Gertrude Scharff-Goldhaber

The drawing on the cover is taken from a 5th Century B.C. relief on the Acropolis in Athens, the "Dreaming Athena," by an unknown sculptor.

BNL 893.(T-363)

(Chemistry; Biology and Medicine - TID-4500, 36th Ed.)

Chemical-Biochemical Signal and Noise - Resolution at Low Temperatures

SIMON FREED

BROOKHAVEN LECTURE SERIES

Number 39, September 23, 1964

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report; or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

PRINTED IN USA
PRICE \$1.00

Available from the
Clearinghouse for Federal Scientific and Technical Information
National Bureau of Standards
U.S. Department of Commerce
Springfield, Virginia

February 1965

1900 copies

INTRODUCTION

Our speaker is Dr. Simon Freed. When Simon asked me to introduce him tonight I was quite pleased. I accepted with considerable trepidation because I doubted that I could do justice to him. He is a very unusual and interesting person. He does very unusual and interesting things, and I suspect that a just introduction would not be compatible with due brevity. But I'll tell you a little about him.

Commencing with college days, or perhaps just before, Simon had a venture into semi-pro baseball. I think he played catcher. I don't know how successful this was, but I believe he dropped it after he went to MIT. Dropped the venture, that is, not the ball. He also studied at the Sorbonne and at the University of California at Berkeley, where he took his doctorate with G.N. Lewis. His career has taken him to a number of other places: first, after Berkeley, to Leiden to the Kammerlingh-Onnes Cryogenic Laboratory; then to the University of Chicago, where he spent many years in teaching and research; then to the SAM Labs at Columbia; then to Oak Ridge. In 1949 he came to Brookhaven. I'd like to interpolate the personal remark that I have found these past fifteen years of association most stimulating, pleasant, and rewarding.

Both before coming to Brookhaven and after he came here, Simon has been interested in quite a diverse variety of scientific subjects. To indicate the range let us say that it goes from the splitting of spectral lines by crystal fields to reactions of enzymes. Currently he is also very much interested in the chemistry of learning.

Rather than recite his accomplishments in these works I would like to remark instead on impressions which I have about Simon's style as a scientist – the nature of his approach, his attitude toward scientific research. Let me just say two things. First, while all research men are in some sense explorers, I think Simon is really an explorer entitled to that name. I mean that he is not content to till the nearby field; he likes to look at the horizon. His imagination is fired by the distant range, what's behind the distant range waiting to be found. The second point is about his approach to experiment. He has been consistently much concerned with the question of resolution, with the resolving power of observation. Simon wants to see the discrete states of systems. If the system isn't behaving discretely he will take steps to make it do so. What I mean is that, not so much following the route of improving instruments, Simon takes the course of putting the system which he is studying into such a situation that its signals are raised relative to its noise. If possible, noise is converted into signal, into meaningful information. This is the line of thought which, of course, accounts for his continued interest in chemistry at low temperatures; and it furnishes the theme of his lecture tonight, which is entitled "Chemical-Biochemical Signal and Noise – Resolution at Low Temperatures."

RICHARD W. DODSON

Chemical-Biochemical Signal and Noise – Resolution at Low Temperatures

Suppose you have acquired a radio of very high sensitivity and selectivity, and you find that a station is coming in indistinctly or that two stations are interfering with each other. You may surmise that your inability to receive the messages clearly is due not to your radio but rather to the sending stations. A somewhat similar situation holds in chemical research. An apparatus that is extremely sensitive and discriminating, of high signal-to-noise ratio, is being used to examine some property of a mixture of substances. Suppose the accuracy with which one of these substances responds to the apparatus is very poor, and that the qualities of two other substances are so close together that the contribution of each separately can be measured only with inadequate precision. Consider the possibility that the limitation to better precision is not the apparatus but rather the behavior of the chemical substances. What is there in the nature of these substances that can be used to produce sharper signals, so to speak? In the radio reception sharper tuning on the part of the sending stations could improve the signal-to-noise ratio of the feeble band sufficiently to communicate a message, a series of numbers, for example, with high precision; likewise with sharper tuning silence could be achieved between the two interfering stations.

Even a single chemically pure substance is a mixture of different states, each of which has pre-

cise properties. Figure 1 shows a spectrum of an imaginary atomic gas. The spectral lines either in absorption or emission originate in the various levels or energy states. From the differences in vertical length or in frequency of the spectral lines are derived the spacing of the corresponding levels in energy. At room temperature and even more so at increasingly higher temperatures, atoms occupy levels of higher energy. The property of the gas as measured is an average over all the properties of the atoms in various levels weighted according to the fraction in each particular level. The same kind of distribution holds for more complex systems, polyatomic molecules, crystals, etc. Imagine the situation reversed: cooling brings a redistribution of the atoms among the energy levels from higher to lower states, and in the extreme limit all the atoms are in the lowest or ground level. The average of the properties is now extremely sharp since it is linked with only one state.

Magnetic properties are indicated at the right by decomposition of most of the levels by the magnetic field into sublevels observed as multiplets of spectral lines. These atoms or molecules oriented by the field are known as paramagnetic. The second level is virtually inert toward the field, and the atoms of this level are diamagnetic. An electric field evokes somewhat similar responses. The precise pattern into which a given level and a given line are decomposed reflects the symmetry of the electric field. Figure 2 is a photograph of the decomposition of an emission line of helium in electric fields generally of increasing intensity downward. The original line in zero field is single at the top. (The σ - and π -components are irrelevant to this discussion.)

Imagine a crystal of a salt consisting of positive and negative ions and of water molecules with positive and negative parts, an example of so-called electric dipoles. The colored positive ions are exposed to the electric fields from the negative charges, from the electric dipoles, and more remotely and so perhaps negligibly from other positive ions. The charges and dipoles are in motions

Figure 1. Spectrum of an imaginary atomic gas.

Figure 2. Decomposition of an emission line of helium by electric fields.

of high degree of randomness and are thus at a given instant at various distances from the central positive ions and are exerting upon them fields of various intensities and directions which differ from one central ion to another.

The light beam as it traverses the crystal will find a configuration of charges about the first positive ion randomly different from that about the second, and so on. The superposition of the effects on the positive ions has some resemblance to the projection of all the decompositions of Figure 2 onto a horizontal line, say the bottom one, which will sum up to diffuse bands such as occur in the spectra of a crystal, as shown in Figure 3. These spectra may be regarded as due to two color centers, positive ions, in the crystal. The uppermost strip was taken with the crystal at room temperature, the second at -196°C , the temperature of liquid nitrogen, and the third at -253°C , that of liquid hydrogen. As the temperature is reduced the diffuse band or bands acquire structure and end by becoming discrete. Absorption concentrates into narrow regions or lines at the expense of background, which becomes so depleted at the lowest temperature as to be transparent. One might say that the signal is strengthened at the expense of the noise. Further lowering of the temperature sharpens and in some instances further resolves the diffuse structure into lines. Not only are the formerly uncertain two bands of the color centers

clearly separate, but each band has been resolved into characteristic line structure. This behavior may be linked with the fewer types of motion of the negative ions and dipoles which perturb the positive ions. Because of the discrete character of the information, a kind of characteristic fingerprinting holds at the lower temperature and makes possible easier recognition and sharper identification of the composition of the centers in their environment. Improved sensitivity has been reached in qualitative chemical analysis of the substances.

Since in most cases the total intensity of absorption is not appreciably changed by variation in temperature, the concentration of the absorption into sharp discrete regions implies that, within the narrower frequency range of absorption, the density of absorption is greater at the low temperature. The amount of material in the light path at low temperature can be reduced to produce the same density of absorption as at the higher temperature. Since it is the density of absorption which is utilized in quantitative determination of substances, detection of smaller quantities is possible at low temperatures and, as a consequence, a more sensitive quantitative analysis is available. A direct mechanism contributing to this increase in sensitivity is evident in the presence of some lines at the temperature of liquid nitrogen and their absence at the temperature of liquid hydrogen. These lines originate in the thermally excited states populated at the higher temperature; at the lower temperature this population joins others already present at the lower level and increases the density of absorption arising from the lower level.

The numbers of lines within the separate groups characterize the microscopic symmetry of the electric fields about the positive ions in the crystal.

Figure 3. Spectra of a crystal at various temperatures. Top, room temperature; second, at -196°C ; third, at -253°C .

Figure 4 shows that the microscopic symmetry can similarly be determined about ions in solutions. The fluorescence spectra of Figure 4 arise from solutions of a rare earth chosen only because of the supposed possibility of counting numbers of lines in the groups. It is understandable that the spectra are in general more diffuse from a solution than from a crystal with its higher order in placement of ions and more ordered motions. The upper spectrum arises in the solution at room temperature, the second at -80°C , the third at -196°C , and the lowest spectrum arises in a solution at -253°C which had congealed into a glass. (The lowest photograph happens to have been underexposed.) If the lines had been counted at the higher temperature despite their broadness, the fewer numbers would have led to assignments of several possible indistinguishable symmetries all consistent with the observed number of lines in the various sets. The implied assumption would have been made that such a line would merely sharpen and remain single at lower temperature. The actual resolution into additional lines at low temperatures reduced the number of possible symmetries consistent with the sets of lines in all the groups. The number of microsymmetries about the positive ions was reduced to virtually one in

Figure 4. Spectra of ions in solutions at various temperatures. Top, room temperature; second, at -80°C ; third, at -196°C ; fourth, in congealed solvent at -253°C .

one solvent and to a different symmetry in another solvent.¹ This brings out the point that less information, in this instance, number of possible symmetries, is required to specify the state of the system at the lower temperature. Moreover the reduced amount of information is more discrete in character, a valuable coincidence for analyzing properties of fewer states.

In preparing solvents fluid at the temperature of liquid nitrogen, the chemist has considerable latitude to match the character of the solute. For a hydrocarbon solute such as carotene, the components of the solvent were hydrocarbons, propane and propene, gases at ordinary temperatures and pressures but liquids at lower temperature which remained fluid to below -200°C . Salts² were first dissolved in propyl or ethyl alcohol, the temperature was reduced to -80°C , and equal portions of propane and propene were condensed into the alcohol. A very mobile homogeneous fluid was created by these diluents. Such a solution containing about 20% isopropyl alcohol (or 20% isopropyl ether) was sufficiently fluid, although extremely viscous, to permit the bubbling of hydrogen gas through it at 50°K (-223°C), about 13° below the freezing point of nitrogen.

A piece of apparatus frequently used at the start of these experiments is a transfer tube for mixing and sometimes filtering solutions at low temperatures (Figure 5). It is a straightforward siphon tube surrounded by a vacuum-jacketed space for a coolant such as liquid nitrogen. The latter surrounds the central siphon tube almost to the bottoms of the two arms, one of which is provided with a sintered glass filter. The tubes containing the liquids to be mixed are filled with helium gas while still attached to a vacuum line where the solutions are prepared. The tubes, always surrounded by liquid nitrogen, are quickly fitted onto the ground joints on the two arms of the transfer tube. If nitrogen gas or argon were used instead of helium, it would condense to become part of the solvent.

Figure 6 shows what a solution looks like at -196°C . Only the lower portion of a fused quartz Dewar flask is shown. In its center is a sample tube also of fused quartz containing a salt, uranium tetrachloride, dissolved in the usual mixture of propane, propene, and propyl alcohol. The curved meniscus of the liquid is visible as well as bubbles from the boiling nitrogen surrounding the sample tube. While the absorption spectrum of

Figure 5. Transfer tube for solutions at low temperatures.

this green solution was being taken it gradually acquired a red color. At the temperature of liquid nitrogen the new spectrum was so sharp that it could be recognized as that of uranium trichloride, a product of the photochemical reaction. At room temperature the diffuseness of the spectrum would have made the identification of the salt difficult if not impossible.

Increasingly complex systems will be discussed to show that at low temperature sharpening of response or signal and increase in resolution are generally to be expected, particularly of the more stable forms. Should one be interested in thermally excited states, it is advisable first to reduce the temperature as far as the system permits in order to ascertain the properties of the more stable forms, since they are always present at some con-

Figure 6. A solution of salt fluid at temperature of boiling nitrogen, -196°C . Two views of the same tube.

centration and their contributions to the measurement may be subtracted from the total response. Raising the temperature brings about the emergence of the excited state, but at the same time even higher energy states may make their appearance. Since the molecules in the latter states act as sources of error in the desired measurement, the problem is to optimize the signal-to-noise ratio. In a good number of instances further refinement of the responses of molecules in the excited state may be obtained by utilizing non-equilibrium conditions, which will be illustrated in the discussion of chlorophyll.

In Figure 7 are shown the spectra of carotene dissolved in the hydrocarbon heptane at room temperature and in an equi-volume mixture of propane and propene at that of liquid nitrogen. At temperatures in between, where both solvents can be employed, the spectra are probably indistinguishable. Observe the greater degree of contrast between absorption and transparency at the lower temperature. For quantitative spectroscopic analyses, a beam of light of the frequency of one of the maxima at the lower temperature will permit the determination of smaller quantities than a beam at the corresponding frequency at room temperature.

Figure 7. Absorption spectra of carotene (90% α + 10% β). A. In heptane at room temperature. B. In equi-volume mixture of propane and propene at -196°C .

The fluorescence spectrum of tryptophan in nine parts methyl alcohol-one part ethyl alcohol exhibits a single broad band at room temperature (Figure 8, curve I). At -196°C the fluorescence is resolved into two peaks (curve II) actually of greater total intensity. (The two solutions, I and II, of the figure are not of the same concentrations.) The lower temperature has thus made available more information in discrete form about tryptophan. At -196°C , the solvent is congealed into a glass whose rigidity makes possible the appearance of a phosphorescence band (left portion of II, amplified in III) resolved into discrete structure.

Chemists have long recognized that a chemical reaction usually consists of a succession of partial reactions, and that little understanding in terms of chemical structure is possible merely by knowing what ingredients they put in at the beginning and what products they analyze at the end. The problem is to resolve over-all reactions into intermediate stages. Since each state of a molecule may be expected to have its own reactivity, progress has been made when a substance is resolved according to differences in energy of its states. Those in a

Figure 8. Fluorescence and phosphorescence spectra of tryptophan in 9 parts methyl alcohol-1 part ethyl ether. I, Fluorescence spectrum at room temperature; II, phosphorescence and fluorescence spectra at -196°C ; III, amplification of the phosphorescence portion of curve II.

paramagnetic state would react differently from those in a diamagnetic state. Further resolution follows from differences in the threshold energies required before a reaction occurs. These are the so-called activation energies, which must be supplied by the thermal energy of the system characterized by its temperature. The magnitudes of the activation energies differ from one partial reaction to another, and resolution among them becomes possible by progressively increasing the temperature from the lowest possible value.

An illustration of such resolution is given in Figure 9, where is represented the reaction between propene and iodine. It is known that when benzene and iodine react they do not immediately form the final colorless product, iodobenzene, but initially undergo a milder molecular addition, one molecule of benzene to one of iodine. The brown iodine color still persists, but a new absorption band characteristic of the addition compound appears in the ultraviolet region. Would propene also form such a compound? Actually, as propene gas condenses on iodine powder at about -50°C ,

Figure 9. Resolution among activation energies for the reaction between propene and iodine. Formation of molecular addition compound.

Figure 10. Partial reactions of iodine with propene and with isoprene. Formation of molecular addition compound. iodine-isoprene and its irreversible decomposition.

Figure 11. Spectra of chlorophyll *b* in ether diluted with hydrocarbons. Equilibria.

Figure 12. Spectra of chlorophyll *b* in 15% isopropylamine + 1:1 propane-propene. Equilibria and subsequent irreversible reaction at higher temperatures.

it forms at once a colorless compound, presumably diiodopropane.

Below -50°C , iodine dissolves in liquid propene producing a brown solution which is accompanied by a new absorption band in the ultraviolet region of the spectrum close in frequency to that of the 1:1 benzene-iodine addition compound. The intensity of this band continues to increase as the temperature is lowered; that is, the concentration of the molecular addition compound appears to grow reversibly to about -196°C . If the solvent is pumped off at -80°C , iodine remains behind as a powder. The original reactants are then reversibly restored. But if the temperature is raised to about -50°C , the solution decolorizes irreversibly: reduction in temperature will not restore the brown color, nor will iodine be regenerated by pumping off the solvent. This is an example of an over-all irreversible reaction resolved into two partial reactions, the first of which is reversible.

Both these partial reactions are represented as curves in Figure 10. Prior to this experiment it had been noted that when isoprene, a hydrocarbon gas, was condensed on iodine powder at -80°C , a deep brown solution formed at the surface of the iodine, but by the time it diffused a few millimeters the solution had become decolorized. It was natural to suppose that an attempt to observe the formation of an isoprene-iodine addition compound was likely to be more fruitful in a solution dilute in isoprene and iodine and at temperatures below -80°C . The main solvent was propane, an inert substance; some propene was added to increase the solubility of isoprene at -196°C , which was to be the temperature of mixing. Propane and propene in the same proportion composed the solvent for the iodine. It should be remembered that this solution already contained the propene-iodine addition compound of Figure 9 with its absorption band in the ultraviolet region, and this had to be differentiated from any new absorption which might arise should isoprene and iodine form an addition compound. The separate solutions of isoprene and of iodine were mixed and held at -196°C . Only the band of propene-iodine was in evidence for a day. The temperature was then raised rather quickly. At 146°K (-127°C) the 1:1 propene-iodine band was still the only origin of absorption; at 4°C higher (a matter of minutes in warming) a band with two maxima appeared, and at a further 4° higher decoloration

occurred irreversibly. The indication is clear that the irreversible iodination of isoprene requires prior formation of the 1:1 molecular addition compound at these temperatures. The decoloration was not complete in the experiment, since the propene-iodine band still persisted and associated with this a brown solution in the visible, but the intensity of the brown had been substantially reduced. Subtraction of the 1:1 propene-iodine band from the middle curve leaves the net absorption due to the intermediate isoprene-iodine addition compound.³

Reversible reactions of another kind are illustrated by chlorophyll *b* in ether, diluted with hydrocarbons to give fluidity at about -200°C . At room temperature the diluent was heptane, unreactive in the present context, while at temperatures below -45°C it was an equi-volume mixture of propane and propene. The absorption band studied in Figure 11 is the well-known Soret band in the blue region of the spectrum. At the left is the band from the solution at room temperature and on the extreme right, at -196°C . But for the shift in wavelength, the shapes are little changed except for detail, a shoulder at long wavelength at -196°C . At intervening temperatures there are equilibria. At the highest temperature one chlorophyll molecule is combined with presumably one molecule of the active component of the solvent, ether, and at the lowest temperature with two molecules. At each temperature readjustment occurs in the relative concentrations. One form increases at the expense of the other. They co-exist in about equal concentrations at -93°C as shown by the roughly equal intensities of the two spectra resolved at this temperature.

In an experiment to accentuate the assumed effect on chlorophyll by the negative polarity of the ether molecule, a more basic substance, isopropylamine, was tried as solvent. When chlorophyll as powder dissolved in this colorless solvent at dry ice temperature, it formed a red solution. It was disconcerting to see chlorophyll, the embodiment of greenness, become red, especially under what were accepted to be the mild conditions of low temperature. However, when the temperature of the solution was raised slowly, a green color grew in, and at about -50°C yielded the characteristic spectrum of chlorophyll, as may be seen in Figure 12. The new absorption at dry ice temperature between 4500 and 5550 Å accounts for the red color. These transformations proved to be re-

versible with temperature provided it was not raised appreciably above -40°C , when the green solution acquired a yellow cast that on cooling never reverted back to the red color or the color of chlorophyll. If a tube containing the green solution in reversible equilibrium with the red is suddenly quenched into liquid nitrogen, the green color is maintained at the low temperature. Now raising the temperature, rather than lowering it, transforms the green solution into the red one and upon further warming into the original green color again. Thus, a thermally excited state can be quenched-in if its equilibration is inhibited for lack of proper activation energy at the low temperature. It can now be examined in greater detail than at the higher temperature. Also it may be compared with the form normally stable at the lower temperature in precisely the same solvent, concentration, and temperature, should it be desired to obtain subtle distinctions by means of diagnostic chemical reagents.

Some quantitative aspects of these phenomena are considered in the table. The table is abstractly the same for equilibria and for chemical rates. First, consider the effect of temperature on the ratio of concentrations in two forms in equilibrium. Assume the two forms (or reactants and products) to be present in equal concentrations at room temperature. If the heat per mole evolved or required to transform one form into the other is 4.6 kcal, the form more stable at the lower temperature would at -73°C (200°K) be 46 times, and at -123°C (150°K) 2000 times, as plentiful as the form less stable at low temperatures. If the energy per mole between the two forms was about 9.0 kcal, the more stable form would at -73°C be 2000-fold, and at -123°C 4,000,000-fold, as concentrated as the less stable form. If the difference in energy proved to be 18.4 kcal per mole, similar reductions in temperature would produce differences greater by orders of magnitude in the ratios of the concentrations of the two forms. Thus, reduction in temperature is a way of concentrating forms stable at lower temperature when they are present in unfavorably low concentrations at higher temperatures.

The same ratios hold for relative reaction rates at the corresponding temperatures. In the table, the rate of the chemical reaction is arbitrarily normalized to 1 at room temperature, and the table expresses how much more slowly it would go at the lower temperature. If the threshold or activation

Effect of Temperature on Ratios of Concentrations at Equilibrium and on Ratios of Reaction Rate Constants (Ratio of concentrations or of rates = 1 at 300°K)			
$T, ^{\circ}\text{K}$	ΔH or ΔE , cal/mole		
	4600	9200	18400
200	46	2.1×10^3	$(2.1 \times 10^3)^2$
150	2.1×10^3	$(2.1 \times 10^3)^2$	$(2.1 \times 10^3)^4$
100	$(2.1 \times 10^3)^2$	$(2.1 \times 10^3)^4$	$(2.1 \times 10^3)^8$

energy per mole of reactants is 9.2 kcal, the rate would be 2000-fold slower at -73°C (200°K) than at room temperature and 4,000,000-fold slower at -123°C . If the activation energy is 18.4 kcal per mole, at -123°C (150°K) the rate would be reduced by a factor of 10^{-13} compared with the rate at room temperature. Reactions too rapid at room temperature may be within range for measurement at the lower temperature.

Examples of such phenomena appeared to be manifold in biochemistry. One of the less involved technically seemed to be in the field of enzyme chemistry, since catalytic activity before and after reactions could serve as a criterion as to what degree enzymic character has been irreversibly lost. The enzyme α -chymotrypsin has a molecular weight close to 23,000 and consists of more than 1000 atoms. It catalyzes the fragmentation of proteins in specific regions of molecules by hydrolysis, that is, by reaction with water. First the enzyme combines with the protein substrate to form the enzyme-substrate complex, and then the chain of reactions proceeds until the protein is hydrolyzed. Figure 13 relates⁴ the heats evolved when one mole of α -chymotrypsin combines with one mole of hydrocynammic acid as the degree of acidity changes. This acid is not a substrate but an inhibitor against the attachment of the substrate. The heat evolved in formation of the enzyme-inhibitor complex is usually of the same general magnitude as in that of the enzyme-substrate complex. At a degree of acidity $\text{pH } 6.5$, 9 kcal/mole was evolved and at $\text{pH } 7.5$, 18 kcal/mole. These are approximately the values used in the table, which lists how the relative concentrations of products and reactants vary with temperature and shows in what measure the concentration of the enzyme-substrate compound increases relative to that of the separate reactants at low temperature.

The general trends in properties with change in temperature already sketched for relatively simple chemical systems hold also for systems that include enzymes or other macromolecules. The high degrees of specificity for which enzymes are renowned may well be exceeded at low temperatures, observable not only in the over-all enzymic reaction but also in the intermediate stages into which it may be resolved. Substrates which are catalytically transformed at about equal rates at room temperature would be expected to be transformed at unequal rates at decidedly lower temperatures. The structure of the enzyme molecule would then be more compact; its vibrations, spreading of bonds, and configurational changes would be less active at the low temperatures. As an extreme example, the active site of the enzyme that may afford little constraint against attachment by the substrates at room temperature may prove to be practically inaccessible to one but not to the other substrate at very low temperature.

In the early experiments, α -chymotrypsin was merely another molecule. It was dissolved in solvents which biochemists would look upon with amusement or horror. Would they denature the enzyme? Could the irreversibility be avoided at the low temperature? To what degree could catalytic activity be maintained at low temperatures? Various derivatives of ammonia were tried.⁵ Somewhat similar substances have been thought to compose the atmosphere when life began on this planet. For the most part, the solvents denatured the enzyme rapidly at room temperature

but not at some lower temperature. The enzyme never made contact with the solvent except at low temperatures. Some of the rather bizarre solvents first used, such as amines, produced significant changes in the spectra of the enzymes which indicated disorganization between the constituent amino acid residues composing the enzyme. Yet when the amine was pumped off at -80°C (dry ice temperature) substantial enzymic activity, if not all, was found intact at room temperature. If denaturation had occurred at the low temperature it was reversible in character.

Finally some contact was made with the big body of biochemistry by including water in the solvent. To have such a solvent fluid below 0°C , mixtures of alcohols and water were chosen rather arbitrarily. Methyl alcohol freezes at -98°C , ethyl alcohol at -117°C . For example, a mixture of 20% water, 55% methyl alcohol, and 25% ethyl alcohol is still fluid although very viscous at -150°C .

The usual method for preparing solutions at low temperatures was simple. A solution of the enzyme in water cooled to 0°C was injected with a micropipette as a fine spray into the alcohol-water mixture previously cooled, for example to -80°C . The spray freezes at once as floating particles of ice in which the enzyme is dissolved. The solution becomes clear within fractions of a second

Figure 13. Heat evolved when 1 mole of α -chymotrypsin combines with 1 mole of hydrocyanamic acid as a function of acidity.

Figure 14. Enzymic catalysis by α -chymotrypsin in 80% methanol at -33°C .

to a matter of hours depending on the temperature and the concentration of alcohol. A homogeneous solution of α -chymotrypsin in 80% methyl alcohol–20% water was kept at -40°C for 40 days and also at -80°C for this duration without any sign of precipitation and without deterioration of enzyme activity. Rates of producing nuclei of crystals of so complex a substance as an enzyme are extremely slow under these conditions compared with the rates of many chemical reactions it may experience.

A 42% methanol–water mixture denatures about half the α -chymotrypsin irreversibly in about 7 minutes at room temperature. Depicted in Figure 14 is the course of the enzymically catalyzed reaction by α -chymotrypsin in 80% methyl alcohol–water, which would have destroyed the enzyme at great speed at room temperature. At -33°C , the enzymic catalysis⁶ proceeds smoothly and linearly with time for the duration of the run, 1000 min. A next stage in the development of the subject is to resolve or at least detect other transformations concurrent with the over-all enzymic reaction. It is axiomatic that the rate of the over-all reaction is determined by the slowest intermediate step. The more rapid steps may be sufficiently slow at low temperature to be followed. Under favorable conditions quenching at appropriate stages may permit the characterization of intermediates more or less at leisure. Our present methods have not been adequate as yet in detecting intermediates of catalyzed hydrolyses of the specific substrates of α -chymotrypsin. The enzyme lactate dehydrogenase

formed an intermediate product with great speed at -80°C . The partial reaction was already completed in a few seconds.

The high dielectric constant of water is regarded as critical for biochemical processes. The dielectric constant determines the degree of interaction of the charged portions of a molecule and their separation, and consequently affects the size and shape of the molecule. Dielectric constants⁷ of alcohol and water mixtures are given in Figure 15 with their dependence on temperature. Quite generally, the lower the temperature the higher the dielectric constant. Consider curve 3, for 70% methyl alcohol and 30% water. At 190°K (-83°C) its dielectric constant equals that of water at room temperature. The dielectric constants of the solvents used in the enzymic reactions of α -chymotrypsin approximate that of water.

To proceed a step farther in the complexity of systems, consider a recently discovered phenomenon *in vivo* whereby a change in temperature brings about a resolution in the rates of living processes. If a planarian, a small fresh water flatworm, is transected across its length, regeneration sets in to create two complete planarians. At the cut of each portion cells gather and grow into a cluster which will later differentiate in the tail portion to be the cells of the future eyes, nerve ganglia, and head of the new planarian, and in the head portion to be the future tail. But if the transection is made at 5°C or lower, only the first stage of the regenerating process occurs.⁸ Cells are observed to gather at the cut in each section, but the process of differentiation to acquire new characteristics becomes so slow as hardly to occur. The biochemical processes including enzymic reactions required for differentiation are greatly retarded at the reduced temperature, and yet planarians are lively below 5°C . In fact at 2°C they grow eggs which hatch into new generations.

I would like to venture anticipations of some future applications of biochemistry at low temperatures in the medical sciences. The development of appropriate biochemical analyses at low temperatures will be assumed. In view of the previous discussion these may be expected to be less disruptive of structure and more discriminating and sensitive than the analyses of conventional biochemistry.

Considerable progress has been made after much empirical experimentation in the preservation at low temperatures of various kinds of cells,

Figure 15. Dielectric constants of alcohol-water mixtures as a function of temperature.

red blood cells, tumor cells, and some organs such as rat kidneys. Red blood cells may be kept at -80°C for a year but with some irreversible deterioration. At -196°C (liquid nitrogen temperature) the cells remain intact for this time and are as good for transfusion as fresh red blood cells. These achievements have come after many failures, presumably because of a lack of knowledge of biochemistry at low temperatures.

Pharmacology and toxicology would appear to be natural fields for application of biochemistry at low temperatures with a view to discovering intermediate reactions between drugs and tissue and observing reversibilities before the toxic or lethal quantities of the chemicals overwhelm the recuperative chemistry of the body. An approach which recommends itself would embrace a comprehensive study of the biochemical transforma-

tions induced in cells of tissue cultures, of invertebrates, and of cold-blooded and hibernating animals at as low a temperature as possible, then at progressively higher temperatures, to clarify the sequence of intermediate reactions which lead to toxicity. Knowledge of the chemistry of intermediate reactions permits possibilities of chemical control at each stage that are entirely hidden in the rapid irreversible reactions at room temperature.

The sharper discriminations in reactivities when the temperature is markedly reduced may be utilized to achieve greater contrasts between the reactions of different cells to chemical reagents. Tumor cells and mammalian cells in tissue cultures immediately suggest themselves for comparative biochemical studies at low temperatures where carcinogenic drugs and other pharmacological reagents may be effective without unfavorable effects which would be toxic or even lethal under ordinary conditions of temperature and concentration. Precautions should be taken, of course, to remove the reagent at the lower temperature; in this way a cryopharmacology may be developed. This approach appears to be timely now with the emergence of cryosurgery and the related need for information. More time for carrying out surgical operations is gained by retarding physiological and biochemical processes by reducing the temperature. Only recently a group at Western Reserve Medical School⁹ in order to remove a tumor lowered the temperature 28°C below normal in the brain of a man and stopped the blood supply for several hours. Not only was the operation successful, but no unfavorable consequences resulted from the reduction in temperature.

Aside from the successful resuscitation of organs from low temperatures, the only experimental encouragement I can call attention to at present, and that by analogy, is the behavior of the enzyme α -chymotrypsin. An unexpectedly mild reduction in temperature was found sufficient to retard irreversible denaturation for a considerable time. In Figure 16 are shown incubation experiments at various temperatures with α -chymotrypsin in 42% methanol-water mixtures. At 25°C half the activity of the enzyme was lost irreversibly in about 7 min. At 23.8°C half the activity was lost in 50 min. At 23°C after an initial drop in activity no further loss occurred throughout the 90 min of the experiment. At a still lower temperature (15°C) no deterioration was detected. Such marked

Figure 16. Effect of temperature on loss of activity of α -chymotrypsin in 42% methanol. Incubation times versus activity at various temperatures.

change in rate over a small temperature interval is characteristic of transformations of highly ordered systems. In cells and organs the degree of order is of course much higher than in solutions.

The effects of radiation on living matter may also be considered from the point of view of biochemistry at low temperatures as an aid in resolving some of the complex chemistry. The initial products of irradiation immediately propagate further reactions in straight and branched chains with such speed at room temperature that most of them are over before analysis has started. It has proved possible to retard the initiation of the chains at the temperature of liquid nitrogen and to build up sufficient concentration of the initial products, fragments known as free radicals, to measure their number and sometimes to characterize them. At this temperature or as close to it as possible, where appreciable concentration of free radicals still exists, biochemical analysis of solutions of components of the tissue should be started and continued at gradually higher temperatures. Knowledge of intermediate products and reactions offers prospects of control and modification of subsequent effects.

The last aspect of medical research to be touched upon is the endogenous chemistry of learning, the chemical transformations that take place within the animal while it is learning – indeed during all the recognized conditioning processes. Positive results of this emerging science were obtained by subjecting rats to Pavlovian conditioning, to pairing of the stimuli of electric shock and of noise from a buzzer. The animal was placed in a cage with a metallic grid as floor which could be released magnetically to drop into liquid air. After a given number of electric shocks by way of the metallic grid, the floor was released and the animal fell into the liquid air (at about -190°C). Presumably the chemical reactions were completely arrested, and the brain retained the chemical situation induced by the shocks. These difficult, pioneering experiments¹⁰ with their controls were carried out with painstaking care. Analysis showed that the ammonia content of the brain had been increased above that in unshocked animals and above that in the brains of animals stimulated only by buzzing. After the Pavlovian association had been established between shock and buzzing, buzzing alone elicited approximately the same increase in the ammonia content as had been induced by shock. Here was chemical correspondence to the physiology of conditioning.

The correspondence must be regarded as partial. More biochemical information will doubtless become available when analyses are devised to be carried out at temperatures so low that all spontaneous chemical reactions within the tissue are virtually arrested. Because of high specificity and hence selectivity of reactions at such low temperatures, appropriate reagents can be envisioned to exist which cleave molecular bonds of definite character and yet leave intact detailed structure of the information-containing macromolecules. Clearly, analyses at these temperatures would more closely approximate the initial biochemical representation of the conditioned, physiological experience of the animal. Efforts to devise the analytical procedures seem eminently worth while.

SUMMARY

A reduction in temperature of a chemical system effects a redistribution of the molecules from higher to lower energy states so that practically all the molecules occupy fewer states. At the same time they are exposed to fewer kinds of random, fluctuating perturbations from the environment such as a solvent. Since molecules in each state have precise properties except for the diffuseness induced by the perturbations, a sharpening in the magnitudes of properties relative to background has been acquired at low temperatures.

As examples, behaviors of solutions are described which range in complexity of solute from inorganic salt to enzyme, of solvent from nonpolar to strongly polar, and in scale of temperature from about $+25^{\circ}\text{C}$ to -200°C , in some instances. The discussion includes the following features: discreteness of information, specificity, resolution of properties according to energy states of a pure substance, resolution of properties of different substances, resolution of reactions into intermediate reactions, equilibria among intermediates, avoidance of irreversibilities, properties of substances in excited states with minimal perturbations, and sensitivities of qualitative and quantitative analyses at low temperatures. Some anticipated applications to medical sciences are briefly indicated.

Acknowledgments: It is a pleasure to state that many of the results I have discussed here are due to the participation of Kenneth Sancier, Edward Sayre, and Benon Bielsky.

REFERENCES

1. E. SAYRE, D.G. MILLER, AND S. FREED, *J. Chem. Phys.* **26**, 109 (1957).
2. S. FREED AND C. J. HOCHANADEL, *J. Chem. Phys.* **18**, 780 (1950).
3. S. FREED AND K.M. SANCER, *J. Am. Chem. Soc.* **74**, 1273 (1952).
4. W. J. CANADY AND K. J. LAIDLER, *Can. J. Chem.* **36**, 1289 (1958).
5. S. FREED, J.H. TURNBULL, AND W. SALMRE, *Nature* **181**, 1731 (1958).
6. B.H. J. BIELSKI AND S. FREED, *Biochim. Biophys. Acta* **89**, 314 (1964).
7. G. ÅKERLÖG, *J. Am. Chem. Soc.* **54**, 4125 (1932).
8. A. BRÖNDSTED AND H.V. BRÖNDSTED, *J. Embryol. Exptl. Morphol.* **9**, 159 (1961).
9. R. J. WHITE, M.S. ALBIN, AND J. VERDURA, *Science* **141**, 1060 (1963); *The New York Times*, June 8, 1964, p. 1.
10. E.A. VLADIMIROVA, *Physiol. J. SSSR* **25**, 930 (1938); *Dokl. Akad. Nauk SSSR* **106**, 937 (1956); *Proc. 5th Intern. Congr. Biochem., Moscow, 1961*, Vol. 9, p. 269, Pergamon, Oxford, 1963.

LIST OF BROOKHAVEN LECTURES (Continued)
(Those with BNL numbers given have been published or are in press)

23. Neutrino Physics, BNL 787
Leon M. Lederman, Physics Department	January 9, 1963
24. The Use and Misuse of the Atmosphere, BNL 784
Maynard E. Smith, Instrumentation and Health Physics Department	February 13, 1963
25. The Nucleus Today
Denys Wilkinson, Physics Department	March 6, 1963
26. Trace Metals: Essential or Detrimental to Life, BNL 828
George C. Cotzias, Medical Department	April 10, 1963
27. The Early Days of the Quantized Atom
Samuel A. Goudsmit, Physics Department	May 15, 1963
28. Catalysis in Life and in the Test Tube
Daniel E. Koshland, Jr., Biology Department	June 19, 1963
29. Collisions of "Elementary" Particles With Protons at High Energies
Samuel J. Lindenbaum, Physics Department	September 25, 1963
30. Chemistry of Isotopes, BNL 870
Jacob Bigeleisen, Chemistry Department	October 16, 1963
31. The Nuclear Reactor Comes of Age, BNL 838
Jack Chernick, Nuclear Engineering Department	November 13, 1963
32. Radio Galaxies
David Heeschen, National Radio Astronomy Observatory	January 15, 1964
33. The Impact of Isotopic Tracers on Physiological Concepts, BNL 857
James S. Robertson, Medical Department	February 12, 1964
34. The Biology of Aging, BNL 854
Howard J. Curtis, Biology Department	March 18, 1964
35. The Problem of Development
Ernst W. Caspari, University of Rochester	April 15, 1964
36. Bubble Chambers: Instruments for High Energy Physics Experiments
William B. Fowler, Physics Department	May 6, 1964
37. Bubble Chamber Experiments – Alpha to Omega Minus
Nicholas P. Samios, Physics Department	May 20, 1964
38. "Hot Atoms" and Their Reactions With Organic Compounds
Alfred P. Wolf, Chemistry Department	June 17, 1964
39. Chemical-Biochemical Signal and Noise – Resolution at Low Temperature, BNL 893
Simon Freed, Chemistry Department	September 23, 1964
40. Oriented Nuclei, BNL 903
Vance L. Sailor, Physics Department	October 14, 1964
41. The Gases of the Blood, BNL 898
Donald D. Van Slyke, Medical Department	November 18, 1964
42. Antibodies: The Key to Immunity
Marian E. Koshland, Biology Department	December 9, 1964
43. The Ordered-Bed Breeder Reactor
Warren E. Winsche, Nuclear Engineering Department	January 20, 1965
44. Talking to Computers
John E. Denes, Applied Mathematics Department	February 24, 1965

LIST OF BROOKHAVEN LECTURES

(Those with BNL numbers given have been published or are in press)

1. Radioastronomy and Communication Through Space, BNL 658
Edward M. Purcell, Physics Department	November 16, 1960
2. Current Ideas on the Endocrine Regulation of Cellular Processes, BNL 685
Irving Schwartz, Medical Department	December 14, 1960
3. Inside the Protein Molecule, BNL 649
Werner Hirs, Biology Department	January 11, 1961
4. Nuclear Chemistry Research With the Cosmotron
Gerhart Friedlander, Chemistry Department	February 15, 1961
5. Neutron Physics Of and With the High Flux Beam Research Reactor, BNL 664
Herbert Kouts, Nuclear Engineering Department	March 15, 1961
6. High Energy Accelerators, BNL 747
Ernest Courant, Physics Department	April 12, 1961
7. Dislocations in Crystal Lattices
George H. Vineyard, Physics Department	May 17, 1961
8. The History of Cosmic Rays and Meteorites, BNL 779
Oliver A. Schaeffer, Chemistry Department	June 14, 1961
9. The Physics of Semiconductor Radiation Detectors, BNL 699
G.L. Miller, Instrumentation and Health Physics Department	September 27, 1961
10. Theory of the Gene, BNL 739
Milislav Demerec, Biology Department	October 18, 1961
11. Fundamental Particles of Physics
Maurice Goldhaber, Director, Brookhaven National Laboratory	November 15, 1961
12. Excessive Salt Intake and Hypertension: A Dietary and Genetic Interplay, BNL 733
Lewis K. Dahl, Medical Department	December 13, 1961
13. Galaxies, BNL 710
Otto Struve, National Radio Astronomy Observatory	January 17, 1962
14. A Computer Learns To See, BNL 725
Paul Hough, Physics Department	February 14, 1962
15. Wet Electrons – The Radiation Chemistry of Water
A.O. Allen, Chemistry Department	March 14, 1962
16. Fundamental Studies of Radiation Damage in Graphite, BNL 745
Donald G. Schweitzer, Nuclear Engineering Department	April 17, 1962
17. The Role of the Cell Nucleus in Determining Radiosensitivity, BNL 766
Arnold H. Sparrow, Biology Department	May 16, 1962
18. Accelerators of the Future, BNL 741
John P. Blewett, Accelerator Department	June 13, 1962
19. The Renewal of Cells and Molecules – The Fountain of Youth
Walter L. Hughes, Medical Department	September 19, 1962
20. A Neutron's Eye View of Magnetic Materials
Julius M. Hastings, Chemistry Department	October 17, 1962
21. Landscaping the Groves of Academe
R.C. Anderson, Director's Office	November 14, 1962
22. Chemical Communication Systems in the Cell
Henry Quastler, Biology Department	December 12, 1962

(Continued inside back cover)