charts (though it would not be difficult to extend the system so that these materials could be presented on lantern slides or generated by the computer on its oscilloscope screen). The affective quality of the computer's comments is also a matter for his decision.

About 30 hours were required for the doctor and the computer programmer to prepare the medical case shown. Much of this time was spent in choosing a problem with characteristics that would provide a full but economical demonstration of the system. An additional 30 hours of computer programming and clerical transcription were needed, as well as several hours spent in composing and editing the English prose. With this experience, and with the aid of compiler programs developed since, comparable cases can be designed and programmed for the computer in much less time. Present computer-programming efforts should further reduce the time of problem preparation: we are developing a teacher-oriented compiler to permit a subject-matter specialist, who is unacquainted with computers, to prepare a case program without assistance from a computer programmer.

Three other computer-based systems that use the case method of instruction have come to our attention; all three were developed to aid in teaching medical diagnosis. None of these systems places a comparable emphasis on conversational interaction. Two of them are similar in structure to the alphabet guessing game illustrated above; they incorporate a statistical approach to problem generation which permits generation of large numbers of cases by the computer (*2*). In the third system the student presses one of nine buttons to choose among, and carry out, a variety of activities: he can see a film, answer questions, experiment with the patient, check the patient's condition, check laboratory norms, check a dictionary, or receive help in answering the questions (*3*).

Three other existing systems resemble the Socratic System in providing an automated context for student-controlled exploration. One of them teaches relations between symbolic and graphical representation of mathematical functions. The student can vary the coefficients of an equation and observe corresponding changes in the graph displayed on the oscilloscope screen, or he can sketch a graph on the screen and view the best-fitting function, together with its equation (*4*). In another system, for language teaching, the student speaks phrases in imitation of standard phrases stored in the computer. The computer displays the magnitude and direction in which the imitation must be modified—in intensity, intonation, and rhythm—to decrease its deviation from the standard (*5*). The third system was devised to conduct experiments on perceptual learning. It allows the subject to choose among several modes of practice, and to regulate the introduction of new material (*6*).

Conclusion

The Socratic System was programmed for the Digital Equipment Corporation's PDP-1 computer, a moderately fast, medium-sized machine. The program operates in the time-sharing mode, which permits several students to be served simultaneously and independently. Facilities for computer time-sharing are proliferating rapidly. This development will make possible the economic use of computer-aided instructional systems on a large scale in the near future.

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Chemical-Biochemical Signal and Noise

Resolution of properties at low temperatures may be utilized in the medical sciences.

Simon Freed

As in the reception of signals, the relative prominence of background in a measurement affects the differentiation between contributions from various substances of a chemical system. The relative magnitude of the background affects also the precision with which the property may be measured.

Suppose that the apparatus has high signal-to-noise ratio and introduces negligible error or background in the measurement. Reduction in the background must then be sought in the nature of the chemical system itself. Greater contrast between the contributions stems from the recognition that even a pure substance is, in a sense, an equilibrium mixture of components residing in energy states in each of which the substance has characteristic and precise properties. The magnitude of the measurement constitutes, then, a superposition from the components originating in appreciably occupied states weighted according to the fraction of the substance in each state.

The fractions vary with the temperature, of course—that is, with the thermal energy that maintains the populations of molecules in the activated states. Cooling brings about a redistribution of the populations from higher to lower states, and, at the extreme limit, all the molecules are in the lowest or ground state. The averages are now extremely sharp, since all the molecules are precisely alike.

Imagine a crystal of a salt consisting of positive and negative ions and of

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water molecules with their positive and negative parts, examples of so-called electric dipoles. The positive ions are exposed to the crystalline electric fields from the negative ions, from the electric dipoles, and, more remotely and so perhaps negligibly, from other positive ions. The ions and dipoles have motions of a high degree of randomness and are thus, at a given instant, at various distances from the central positive ions and exerting upon them fields of various intensities and directions which differ from one central ion to another.

A light beam as it traverses the crystal will encounter a configuration of charges about the first positive ion which is randomly different from that about the second, and so on. The spectra of Fig. 1 may be regarded as due to two color centers (positive ions) in the crystal. The uppermost spectrum was observed with the crystal at room temperature; the second, at −196°C, the temperature of liquid nitrogen; the third, at −253°C, the temperature 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. 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, which makes it easier to recognize the composition of the centers in their environment. Greater sensitivity has been achieved 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 path of the light beam 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 in 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 other populations already present at the lower level and increases the density of absorption arising from the lower level.

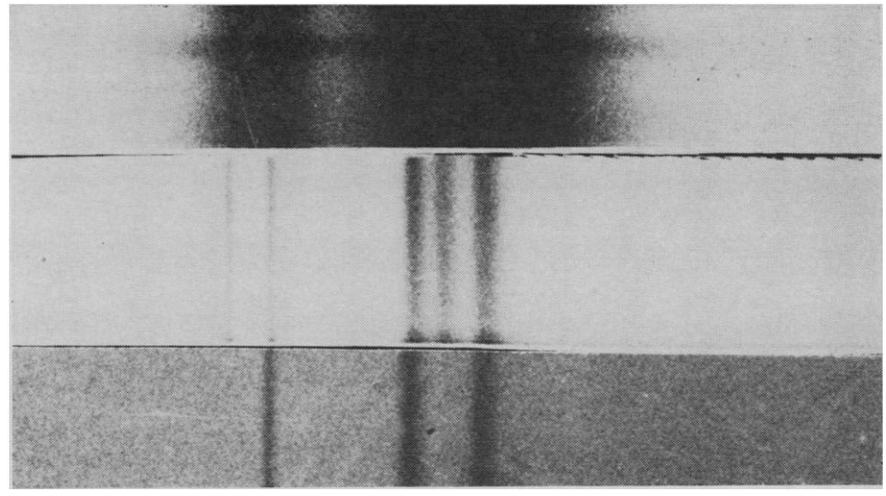


Fig. 1. Spectra of a crystal at various temperatures: (top) at room temperature; (middle) at −196°C; (bottom) at −253°C.

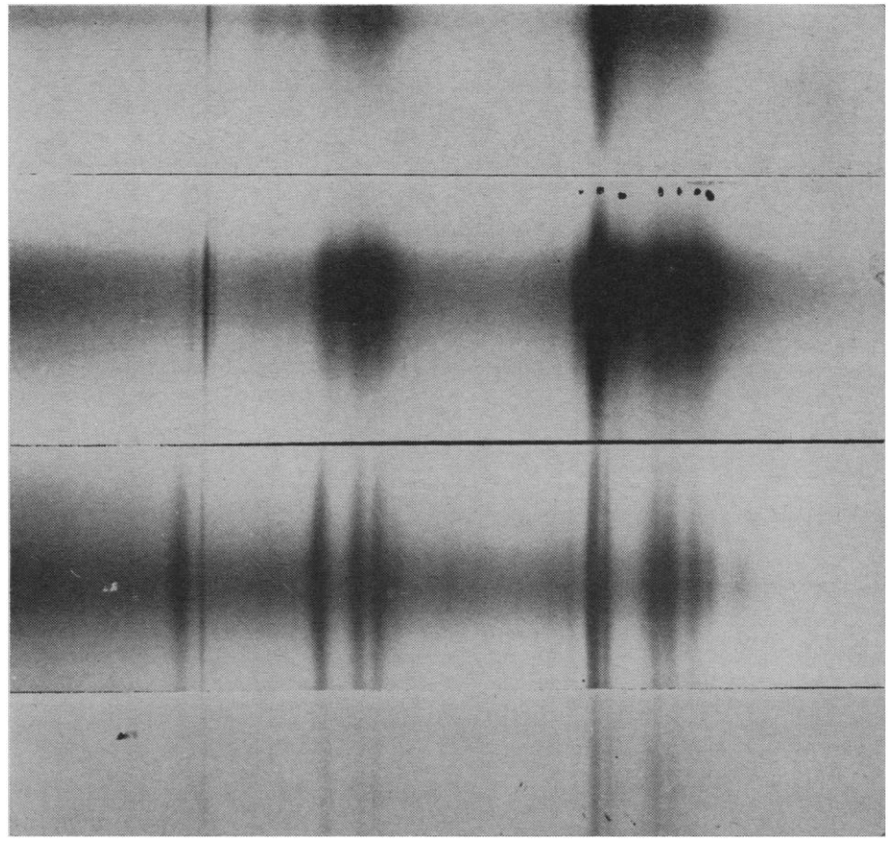


Fig. 2. Spectra of ions in solutions at various temperatures: (top) at room temperature; (second from top) at −80°C; (second from bottom) at −196°C; (bottom) in congealed solvent at −253°C.

The numbers of lines within the separate groups characterize the microscopic symmetry of the electric fields about the positive ions in the crystal. Figure 2 shows that the microscopic symmetry of the fields about ions in a solution can be similarly determined. The fluorescence spectra of Fig. 2 arose 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 from a solution are in general more diffuse than those from a crystal, since the crystal is characterized by greater order in placement of ions and by more ordered motions. The top spectrum in Fig. 2 arose in a solution at room temperature; the second, in a solution at −80°C; the third, in a solution at −196°C; and the bottom spectrum, in a solution at −253°C which had congealed into a glass (the bottom photograph was 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. It would have been assumed that the lines 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 was reduced to virtually one particular symmetry in one solvent, and to a different symmetry in another solvent (*1*). This brings out the point that less information (in this instance a smaller number of definite symmetries) is required to specify the state of the system at the lower temperature. Moreover, the information is more discrete in character, a valuable coincidence in analyzing properties of fewer states.

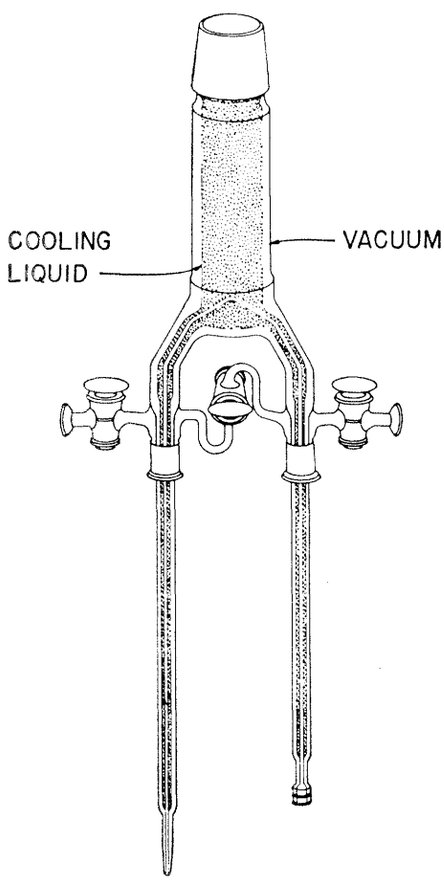


Fig. 3. Transfer tube for solutions at low temperatures.

Procedure

In preparing solvents that are fluid at the temperature of liquid nitrogen, the chemist has considerable latitude in matching the character of the solute. For a hydrocarbon solute such as carotene, the selected components of the solvent were hydrocarbons propane and propene. These compounds are gases at ordinary temperatures and pressures but liquids at lower temperature; the mixed solvent remained fluid to below −200°C. Salts (*2*) 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. A solution containing about 20 percent isopropyl ether in equal proportions of propane and propene, although extremely viscous, was sufficiently fluid to permit the bubbling of hydrogen gas through it at −223°C, about 13° below the freezing point of nitrogen.

A piece of apparatus frequently used at the start of these experiments was a transfer tube for mixing and sometimes filtering solutions at low temperatures (Fig. 3). The tube was surrounded by a coolant, such as liquid nitrogen, within a vacuum-jacketed space which extended almost to the bottoms of the two arms, one of which was provided with a sintered glass filter. The tubes containing the liquids to be mixed were filled with helium gas while still attached to a vacuum line where the solutions were prepared. The tubes, always surrounded by liquid nitrogen, were quickly fitted onto the ground joints on the two arms of the transfer tube. If nitrogen gas or argon had been used instead of helium, it would have condensed, becoming part of the solvent.

Figure 4 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 are bubbles from the boiling nitrogen surrounding the sample tube. While the absorption spectrum of this green solution was being observed, the solution 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. The diffuseness of the corresponding spectrum at room temperature would have made identification of the salt difficult if not impossible.

Resolution of Properties

I shall now discuss increasingly complex systems to show that, at low temperature, sharpening of response or signal and increase in resolution are generally to be expected. Should one wish to investigate 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 these forms are always present at some concentration, 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 molecules in these higher energy states are sources of error in the desired measurement, the problem is to optimize the signal-to-noise ratio. In a number of instances further refinement of the responses of molecules in the excited state may be obtained by utilizing nonequilibrium conditions, as discussed later.

In Fig. 5 are shown the spectra of carotene dissolved in the hydrocarbon heptane at room temperature (Fig. 5*A*) and dissolved in an equi-

volume mixture of propane and propene at the temperature of liquid nitrogen (Fig. 5*B*). At temperatures in between, where both solvents can be used, 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 permits determination of smaller quantities than a beam at the corresponding maximum at room temperature.

The fluorescence spectrum of tryptophan in methyl alcohol (nine parts) and ethyl alcohol (one part) exhibits a single broad band at room temperature (Fig. 6, 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 Fig. 6 are not of the same concentration.) The lower temperature has thus made available more information about tryptophan in discrete form. At −196°C the solvent is congealed into a glass whose rigidity makes possible the appearance of a phosphorescence band (left portion of curve *II*, amplified in *III*) resolved into discrete structure.

Resolution of Reactions

Chemists have long recognized that a chemical reaction usually consists of a succession of intermediate reactions. The problem is to resolve overall 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. Molecules in a paramagnetic state would react differently from those in a diamagnetic state. Further resolution may be achieved by utilizing 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 characterized by the temperature of the system. The magnitudes of the activation energies differ from one intermediate reaction to another, and resolution among them becomes possible through progressive increase of the temperature from the lowest possible value.

An illustration of such resolution is available in 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 combining with one of iodine. The brown iodine color still persists, but a new aborption band characteristic of the addition compound appears in the ultraviolet region. Actually, as propene gas condenses on iodine powder at about −45°C, it forms at once a colorless solution, presumably that of 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 the frequency of the band associated with the 1 : 1 benzene-iodine addition compound. The intensity of the band continues to increase as the temperature is lowered; that is, the concentration of the molecular addition compound appears to increase reversibly as the temperature is lowered 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 −45°C, the solution decolorizes irreversibly: reduction in temperature will not restore the brown color, nor will iodine

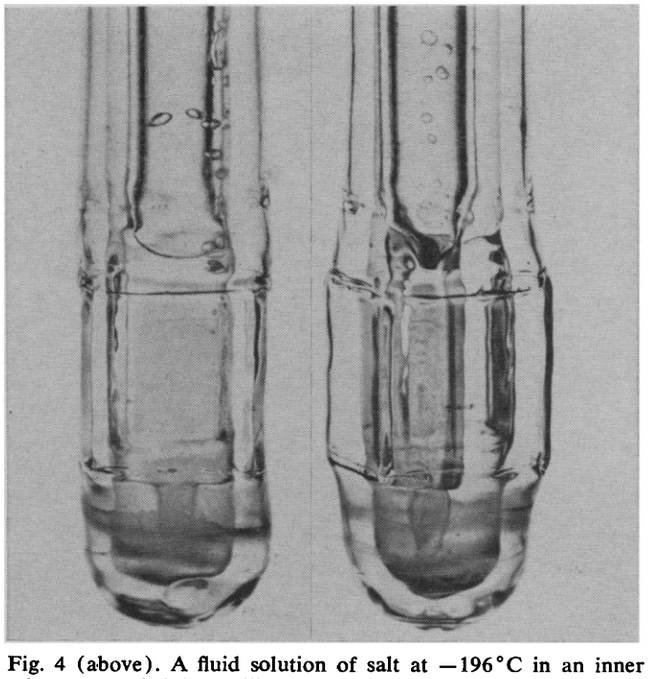


Fig. 4 (above). A fluid solution of salt at −196°C in an inner tube surrounded by boiling nitrogen (two views of the same tube).

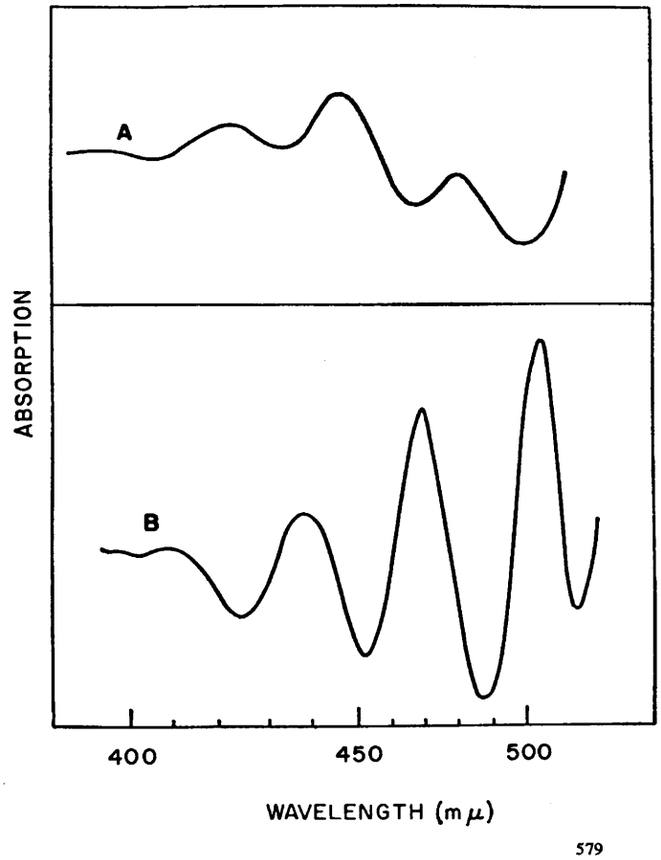


Fig. 5 (right). Absorption spectra of carotene (90 percent α-carotene + 10 percent β-carotene), (*A*) in heptane at room temperature; (*B*) in an equivolume mixture of propane and propene at −196°C.

be regenerated by evaporation of the solvent. This is an example of an overall irreversible reaction resolved into two intermediate reactions, the first of which is reversible at the lower temperature.

Similar partial reactions are represented as curves in Fig. 7. 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 the solution had diffused away a few millimeters, it had become decolorized. It was natural to suppose that the reversible formation of an isoprene-iodine addition compound would be more readily observable at temperatures below −80°C and in dilute solutions. The main solvent used 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, with its absorption band in the ultraviolet region, and that this band 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. For a day, only the propene-iodine band was in evidence. The temperature was then raised rather quickly. At −127°C (146°K) the 1 : 1 propene-iodine band was still the only absorption band observed; at a temperature 4°C higher (warming the mixture was a matter of minutes), a band with two maxima appeared, and, at a temperature 4° still higher, decoloration occurred irreversibly. The indication is clear that the irreversible iodination of isoprene requires prior formation of the molecular addition compound at these temperatures. The decoloration was not complete in the experiment, since the propene-iodine band persisted at −119°C (154°K) and associated with it was a brown solution, but the intensity of the brown color had been substantially reduced. Subtraction of the 1 : 1 propene-iodine band from the middle curve of Fig. 7 leaves the net absorption due to the intermediate isoprene-iodine addition compound (*4*).

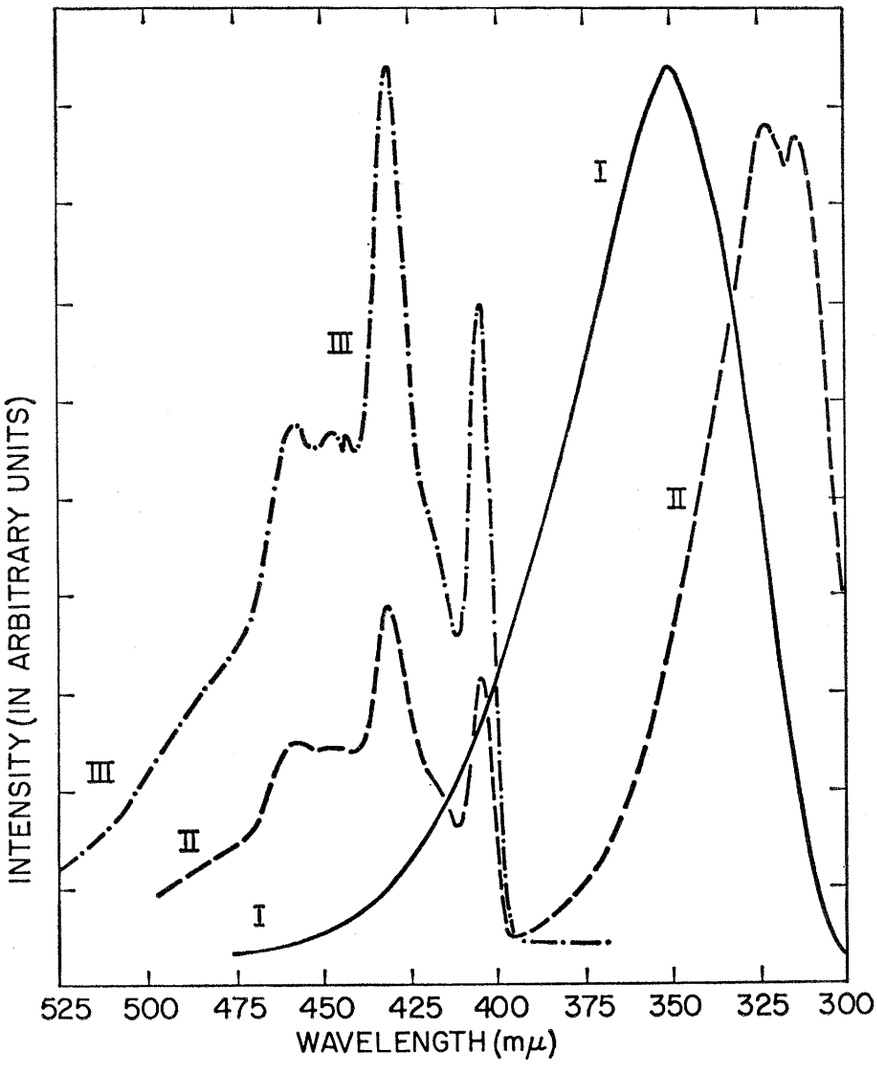


Fig. 6. Fluorescence and phosphorescence spectra of tryptophan in a mixture of methyl alcohol (nine parts) and ethyl alcohol (one part). (*I*) Fluorescence spectrum at room temperature; (*II*) phosphorescence and fluorescence spectra at −196°C; (*III*) amplification of the phosphorescence portion of curve *II*.

Equilibria among Intermediates

A reduction in temperature of a solution of chlorophyll *b* in ether made evident two spectra whose relative intensities varied reciprocally with change in temperature. The spectra were interpreted as due to two compounds of chlorophyll and ether in equilibrium. In an experiment made to intensify the assumed effect of the negative polarity of the ether molecule on chlorophyll, a more basic substance was tried as solvent, isopropylamine. At dry-ice temperature this colorless liquid dissolved chlorophyll *b* powder and produced a red solution. To see chlorophyll, the embodiment of greenness, become red was disconcerting, especially under what were accepted as being the mild conditions of low temperature. However, when the temperature of the solution was raised slowly, a green color reappeared, and at about −40°C (230°K) the solution yielded the characteristic spectrum of chlorophyll, with peak absorption at 470 mμ, as may be seen in Fig. 8. The new absorption between 4500 and 5550 angstroms, at dry-ice temperature, accounted for the red color. These transformations proved to be reversible with temperature, provided the temperature was not raised appreciably above −40°C; at that point the green solution acquired a yellow cast and, on cooling, never reverted back to the red color or to the color of chlorophyll. If the green solution in reversible equilibrium with the red is suddenly "quenched-in" by plunging the tube into liquid nitrogen, the green color is maintained at the low temperature. In this case rais-

ing the temperature, rather than lowering it, transforms the green solution into the red one and, upon further warming, into the original green. Thus, a thermally excited state can be "quenched-in" if its equilibration is inhibited through lack of adequate activation energy at the low temperature. The thermally excited state can now be examined in greater detail than it could be at the higher temperature. Also, it may be compared with the form normally stable at the lower temperature in precisely the same solvent and at the same concentration and temperature, should it be desired to obtain subtle distinctions by means of diagnostic chemical reagents.

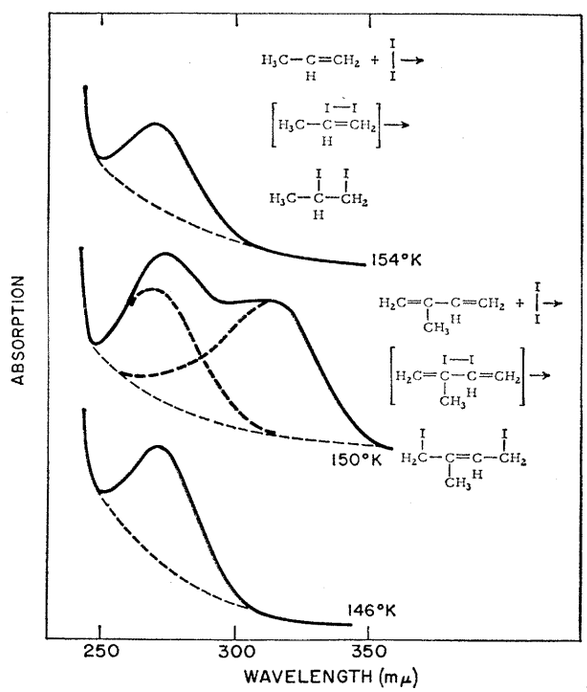
Quantitative Considerations

Some quantitative aspects of these phenomena are considered in Table 1. The ratios in the table are the same for equilibrium conditions and for chemical reactions. First, let us consider the effect of temperature on the ratio of concentrations in two forms in equilibrium when the two forms (or reactants and products) are 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 kilocalories, the form more stable at the lower temperature would, at −73°C (200°K), be 46 times as plentiful as the form less stable at low temperatures; at −123°C (150°K) it would be 2000 times as plentiful. If the energy required per mole to transform one form into the other was about 9.0 kcal, the more stable form would, at −73°C, be 2000 times as plentiful as the less stable form; at −123°C it would be 4 million times as plentiful. If the evolved energy proved to be 18.4 kcal per mole, similar reductions in temperature would produce differences greater by a number of 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 low concentrations at higher temperatures.

The same ratios hold for relative reaction rates at the corresponding temperatures. In Table 1 the rate of the chemical reaction is arbitrarily normalized to 1 at room temperature, and the table indicates how much more slowly the reaction would go at the lower temperature. If the threshold or activation energy per mole of reactants were 9.2 kcal, the rate would be 2000 times slower at −73°C (200°K) than at room temperature and 4 million times slower at −123°C (150°K); at −173°C (100°K) the rate would be reduced by a factor of 10^{-13} compared with the rate at room temperature. Reactions too rapid to be measured at room temperature may be within range for measurement at the lower temperature.

Applications to Biochemistry

Examples of such phenomena appeared to be manifold in biochemistry. Investigations of enzyme chemistry seemed to be less involved technically than studies in other biochemical fields since catalytic activity before and after reactions could serve as a criterion for judging the degree to which enzymic character had been irreversibly lost. The enzyme α-chymotrypsin, with a molecular weight close to 23,000, catalyzes the fragmentation of proteins in specific regions of molecules by hydrolysis. 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 9 relates (*4*) the heats evolved when 1 mole of α-chymotrypsin combines with 1 mole of hydrocinnamic acid as the degree of acidity changes. This acid is not a substrate but is an inhibitor against



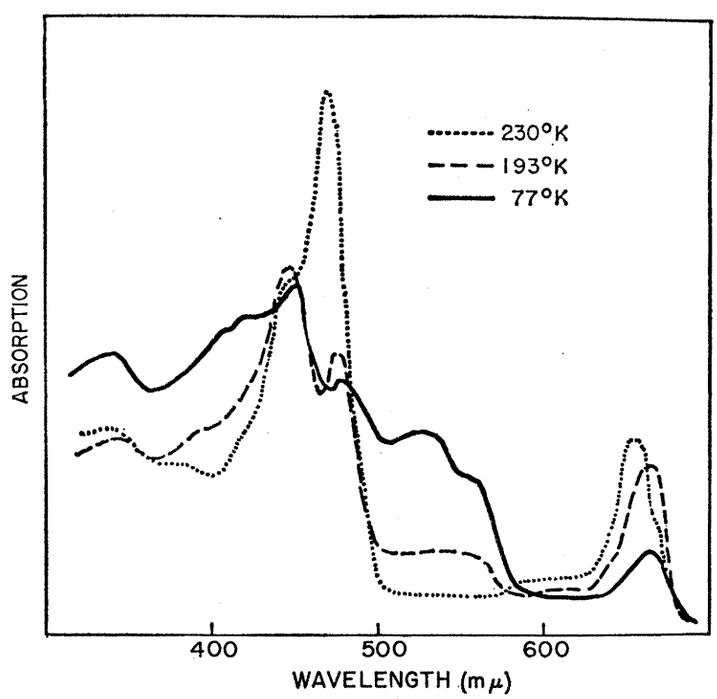


Fig. 7 (left). Intermediate reactions of iodine with propene and with isoprene: formation of the molecular addition compound iodine-isoprene at 150°K and its irreversible decomposition at 154°K. Fig. 8 (right). Spectra of chlorophyll *b* in isopropylamine (15 percent) dissolved in a 1:1 mixture of propane and propene. Equilibria, prior to subsequent irreversible reaction at higher temperatures, which is not depicted.

attachment of the substrate. The heat evolved in the formation of the enzyme-inhibitor complex is usually of the same general magnitude as that evolved in the formation of the enzyme-substrate complex. At *p*H 6.5, 9 kcal/mole was evolved, and at *p*H 7.5, 18 kcal/mole. These are approximately the values used in Table 1, 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 the concentrations 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; this fact is evident not only in the overall enzymic reaction but also in the intermediate stages into which it may be resolved. At low temperatures the structure of the enzyme molecule would then be more compact and its vibrations, spreading of bonds, and configurational changes less active. To give an extreme example, the active site of the enzyme, which may afford little constraint against attachment by two 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 dissolved in fluids having little connection with present biochemistry. Various derivatives of ammonia were tried (*5*). It has been thought that somewhat similar substances may have composed 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), a substantial amount of enzymic activity, if not all, was found intact at room temperature. If denaturation had occurred at the low temperature, it was reversible.

Table 1. Effects of temperature T and of heat of reaction (ΔH) on the ratio of concentration of product to concentration of reactant at equilibrium, relative to a ratio of 1 at 300°K. Also, effects of temperature T and of heat activation (ΔE) on the ratio of reaction rate constants relative to a reaction rate constant of 1 at 300°K (see text).

T (deg K)	ΔH or ΔE (kcal/mole)		
	4.6	9.2	18.4
300	1	1	1
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	$(2.1 \times 10^3)^2$	$(2.1 \times 10^3)^4$	$(2.1 \times 10^3)^8$

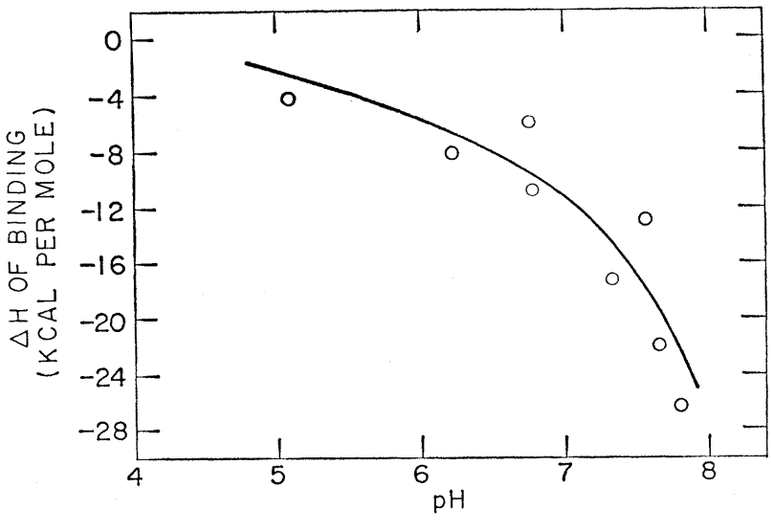
Finally, some contact was made with the big body of known biochemistry by including water in the solvent. To have such a solvent fluid below 0°C, mixtures of alcohols and water were chosen rather arbitrarily. Methyl alcohol freezes at −98°C; ethyl alcohol, at −117°C. For example, a mixture of water (20 percent), methyl alcohol (55 percent), and ethyl alcohol (25 percent) 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, by means of a micropipette, as a fine spray into the alcohol-water mixture previously cooled—for example, to −80°C. The spray froze at once as floating particles of ice in which the enzyme was dissolved. The solution became clear within times which varied from fractions of a second to hours, depending on the temperature and the concentration of alcohol. Much of the original hydration of the enzyme appears to persist at low temperatures even in predominantly alcoholic solvents. A homogeneous solution of α-chymotrypsin in a mixture of methyl alcohol (80 percent) and water (20 percent) was kept at −40°C for 40 days and also at −80°C for 40 days without any sign of precipitation and without deterioration of enzyme activity. The rates at which nuclei of crystals of so complex a substance as an enzyme are produced are extremely slow under these conditions as compared with the rates of many chemical reactions the enzyme may experience.

A mixture of methanol (42 percent) and water denatures about half the α-chymotrypsin irreversibly in about 7 minutes at room temperature. Depicted in Fig. 10 is the course of the reaction enzymically catalyzed by α-chymotrypsin in a mixture of methyl alcohol (80 percent) and water at −33°C, which would have destroyed the enzyme at great speed at room temperature. The enzymic catalysis (*6*) proceeds smoothly and linearly with time for the duration of the experiment, 1000 minutes. A next stage in the development of the subject is to resolve other transformations which are concurrent with the overall enzymic reaction. It is axiomatic that the rate of the overall reaction is determined by the slowest intermediate step. The more rapid steps may be sufficiently slow at low temperature to be followed by the investigator. Under favorable conditions quenching at appropriate stages may permit him to characterize the intermediate steps more or less at leisure. Our methods of detecting the intermediates of catalyzed hydrolyses of the specific substrates of α-chymotrypsin are not adequate as yet. However, the enzyme lactate dehydrogenase formed an easily detected intermediate product with great speed at −80°C. The intermediate reaction was completed within 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 constants (*7*) of alcohol and water mixtures are given in Fig. 11, with their dependence on temperature. Quite generally we find a relationship as follows: the lower the temperature the higher the dielectric constant. Consider curve 3, for methyl alcohol (70 percent) and water (30 percent). At 190°K (−83°C) the dielectric constant of the mixture equals that of water at room temperature.

To proceed a step farther in the complexity of systems, let us 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 freshwater flatworm, is transected across its length, regeneration sets in, creating two complete planarians. At the cut of each portion, cells gather and grow into a cluster; these later differentiate in the tail portion to become the cells of the future eyes, nerve ganglia, and head of the new planarian and in the head portion to become the future tail. But



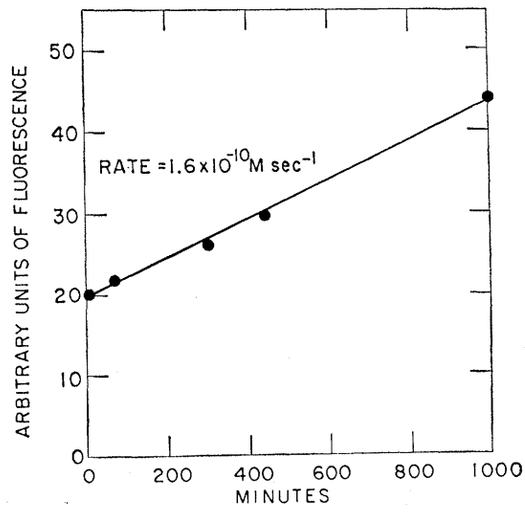


Fig. 9 (left). Heat evolved when 1 mole of α-chymotrypsin combines with 1 mole of hydrocinnamic acid, as a function of acidity. Fig. 10 (right). Enzymic catalysis by α-chymotrypsin of the hydrolysis of *N*-acetyl-L-tryptophan ethyl ester (in 80-percent methanol at −33°C).

if the transection is made at temperature of 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 becomes too slow to be observable. The biochemical processes, including enzymic reactions required for differentiation, are greatly retarded at the reduced temperature; yet planarians are lively below 5°C. In fact at 2°C they grow eggs which hatch into new generations.

Possible Future Applications

I would like to venture a prediction of some future applications of biochemistry at low temperatures in the medical sciences, assuming that appropriate biochemical analyses at low temperatures will be developed. In view of the preceding discussion these may be expected to be less disruptive of structure and more discriminating and sensitive than methods of conventional biochemical analyses.

Considerable progress has been made, after much empirical experimentation, in the preservation at low temperatures of various kinds of cells, such as red blood cells and tumor cells, and of some organs, such as rat kidneys. Red blood cells may be kept at −80°C for a year, but there is some irreversible deterioriation. At −196°C (liquid-nitrogen temperature) the cells remain intact for this length of time and are as good for transfusion as fresh red blood cells. These achievements have come after many failures, presumably the result of a lack of knowledge of biochemistry at low temperatures.

Pharmacology and toxicology would appear to be natural fields for the application of biochemistry at low temperatures with a view to discovering intermediate reactions between drugs and tissue and to observing reversibilities before toxic or lethal quantities of the chemicals overwhelm the recuperative chemistry of the body. One approach would be a comprehensive study of the biochemical transformations 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 opens up 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 as subjects for com-

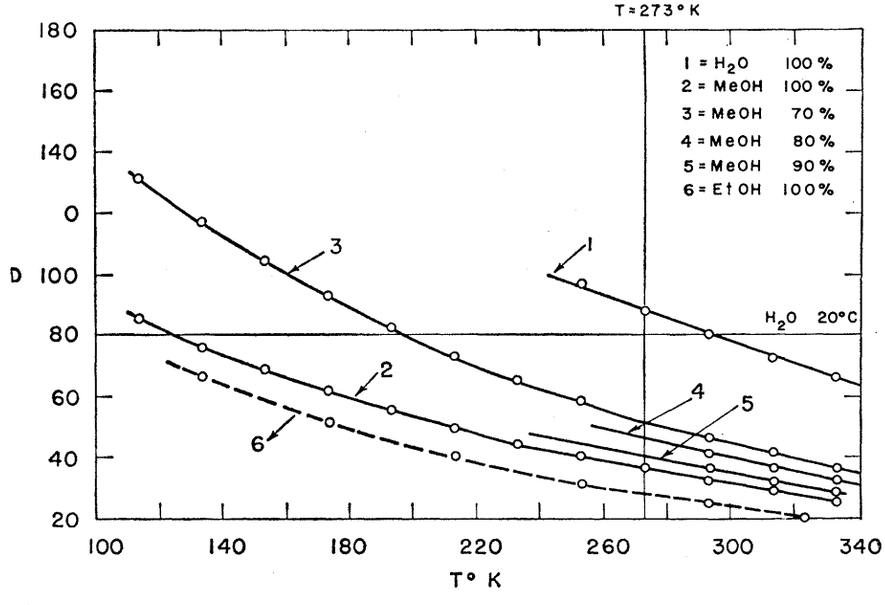


Fig. 11. Dielectric constants (*D*) of alcohol-water mixtures as a function of absolute temperature.

parative biochemical studies at low temperatures where carcinogenic drugs and pharmacological reagents, which would be toxic or even lethal under ordinary conditions of temperature and concentration, may act selectively without unfavorable effects. 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 through retarding physiological and biochemical processes by reducing the temperature. Recently a group at Western Reserve Medical School (*9*), 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.

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 the reactions 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 that I shall touch upon here 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 in this emerging science were obtained by subjecting rats to classical 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 the rat 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 liquid air (at about −190°C). Presumably the chemical reactions were completely arrested, and the brain retained the chemical situation induced by the shocks. Analysis (*10*) 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 between shock and buzzing had been established, 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.

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

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