Spectrophotometry of Intracellular Respiratory Pigments

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PHYSICAL methods that are chemically specific, that are fast enough to measure biochemical processes, and that permit observations to be made on living cells are rare, and spectroscopy has only recently been developed to the point where extensive studies of a wide range of material are possible. Such observations of the integrated action of intracellular enzyme systems are essential complements to the intensive biochemical researches that have succeeded in breaking down the cellular enzyme systems into their soluble components and in reconstructing many important biochemical processes in solution. But the success of these reconstructions and the theories that evolve from them can be evaluated only by comparison with the direct measurements of the *in vivo* system. The physical method is limited in this case to biochemical substances that change their absorption spectra a sufficient amount in response to biological function to permit a satisfactory measurement. In the sequence of respiratory enzymes of mammalian cells we can study in detail the reactions of six components. Table 1 gives the wavelengths at which the six components are most satisfactorily measured. Other cell pigments that do not change their absorption in response to metabolic activity are compensated by suitable methods so that they do not interfere with the study of the active ones.

Methods. Visual spectroscopy of cell pigments in the visible spectrum has been done in detail by MacMunn (*1*), Keilin (*2*), Warburg (*3*), and Japanese workers (*4*). This method is still extremely useful for the rapid identification of the types of cytochrome in various microorganisms. However, this method is best suited for the study of the α and β bands of cytochromes (650 to 500 mμ); the 5- to 10-fold stronger γ bands that lie in the region 440 to 400 mμ cannot be studied effectively by visual spectroscopy (*5*), nor can any measurements be made of reduced pyridine nucleotide (340 mμ).

Spectrographic studies of cytochromes of microorganisms revealed their γ bands (*6*), but the larger light-scattering effects at these shorter wavelengths were superimposed upon the light absorption to give a considerably distorted record.

Millikan's work (*7*) in 1937 pointed the way to our current instruments for the sensitive, selective, and rapid recording of small optical density changes in living cell suspensions and tissues. He used in very simple form two essential features: (i) a differential colorimeter (following Tyndall's principle, *8*) that was responsive only to a change in absorption at two closely spaced wavelength bands, one at the peak of the absorption band of the pigment to be studied; (ii) a biological system that could exist in two clearly defined states, and in which the transition from one to the other could be made rapidly and at will. Thus the relative intensities of the two light beams are adjusted initially to give zero response in one state, the biological system is shifted to the other state, and the magnitude of the absorption of the cell pigment is recorded directly.

Millikan's apparatus was suitable only for measurements of hemoglobin and myoglobin, and the two states studied were aerobic and anaerobic muscle. By using two monochromators and a vibrating mirror for flickering light of two selected wavelengths through the sample and then upon a photocell (*9*), we have made this method suitable for measurements of both the sharp bands of the cytochromes in the visible region and the reduced pyridine nucleotide in the ultraviolet region (*10*) (Fig. 1).

The choice of wavelengths and the method of eliminating interference from other pigments is illustrated by Fig. 2. The absolute absorption spectra for the oxidized and reduced states are shown on the left, but since we record only the changes of absorption that occur upon a change between the two states, the curve on the right is also relevant. Thus wavelengths set at

Table 1. Appropriate wavelengths for measurement of respiratory enzymes. The figures in parentheses refer to neutral reference wavelengths used in double-beam spectrophotometry.

O_2 ← cytochrome a_3	← cytochrome a	← cytochrome c	← cytochrome b ←
445 mμ (460)	605 mμ (630)	550 mμ (541)	564 mμ (575)

flavoprotein ←	reduced pyridine nucleotide	← substrate
465 mμ (510)	340 mμ (374)	

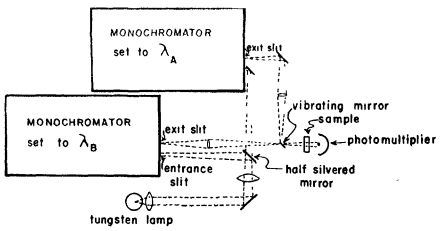

Fig. 1. Double-beam spectrophotometer using quartz or grating monochromators. The sample volume is 0.8 to 10 ml. The vibrating mirror is mounted in a Brown Instrument Co. "Converter" and oscillates at 60 cy/sec.

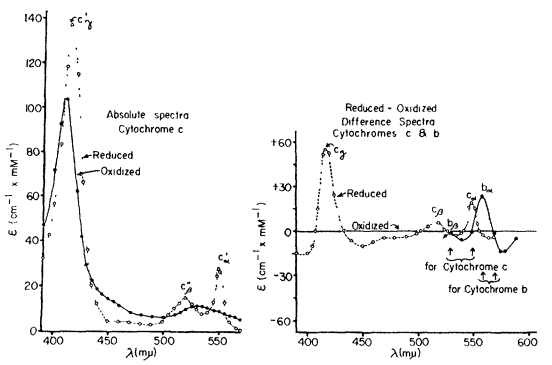

Fig. 2. The spectra of oxidized and reduced cytochrome *c* on the left are subtracted to give the reduced-oxidized difference spectrum on the right. The appropriate wavelengths for measuring cytochrome *c* in the presence of cytochrome *b*, and vice versa, are indicated.

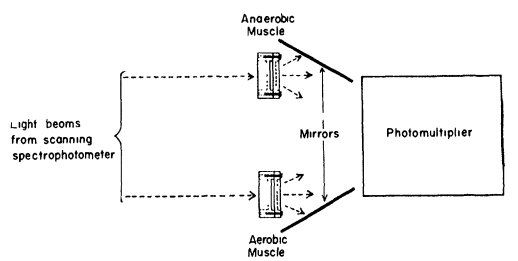

Fig. 3. Optical arrangement suitable for measuring the reduced-oxidized spectrum for the respiratory pigments of a pair of frog sartorius muscles.

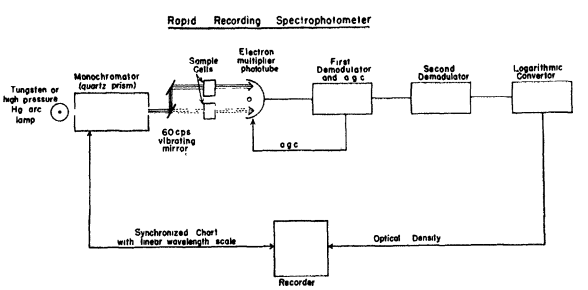

Fig. 4. Split-beam recording spectrophotometer constructed to measure difference spectra. The vibrating mirror is mounted on a Brown "Converter" and its switch contacts select wave forms of the photomultiplier output corresponding to the two-beam positions.

550 and 541 mμ are suitable for cytochrome *c* and will be insensitive to cytochrome *b*, which shows no significant change in absorption for these two wavelengths. If, on the other hand, cytochrome *b* is to be measured, 564 and 575 mμ are a suitable pair of wavelengths. The pairs of wavelengths in Table 1 are chosen to give optimal selectivity in the measurement of these six respiratory enzymes.

With a large number of bacterial suspensions, it has been possible to use a single-beam technique in which the photocurrent caused by the transmitted light is "bucked out" by an adjustable voltage (*9*). The optical density change is recorded when the aerobic suspension becomes anaerobic (*11*). This method is satisfactory for material that does not settle rapidly and does not change its light scattering on the transition from aerobiosis to anaerobiosis. These conditions are apparently fulfilled with suspensions of bacteria, yeast, and muscle homogenates; many measurements of cytochromes have been made in this way (*12*). In fact, intracellular reduced pyridine nucleotide was first recorded by this method (*11*).

A method (*13*) that is particularly suited to recording the spectrum representing the differences of light absorption between two metabolic states of a living system is illustrated by Fig. 3. In this case the paired sartorius muscles differ in oxygen tension—one is aerobic, the other anaerobic—and they are illuminated with the light of the same wavelength. Variation of the wavelength of light gives the difference spectrum, provided that electronic or other means are supplied for maintaining constant photocurrent when the light falls upon only one sample. A method for accomplishing this by control of the dynode voltage of the photomultiplier is illustrated by Fig. 4 and has been described by Yang and Legallais (*14*).

Instead of recording the difference between two biochemical states of the same material, some investigators record the difference of absorption between the biological material and a substance that approximates its light-scattering properties such as filter paper (*15*) or oiled filter paper (*16*) (Duysens has apparently not yet taken advantage of this principle in his apparatus, *17*). In this case the absorption of metabolically inactive pigments is also recorded. The difference spectrum resulting from two metabolic states must be obtained by subtraction of two separate recordings from such a device, and the selectivity that can be obtained is diminished.

Limitations. The performance of current methods (Fig. 1) in which a reasonably large sample is available (~10 ml) is set largely by the brightness of the light source (steradiance), the accuracy with which the light intensity can be stabilized (there is intrinsic "noise" in the light from tungsten lamps, *9*), the aperture and dispersion of the monochromator, and the sensitivity of the photocathode (*18*). "Head-on" types of photomultipliers have been found to be very suitable for gathering the light from the sample. Good results are obtained for 10-ml samples with a cuvette area approximately equal to the area of the

photosurface (10 cm²). On the other hand, samples of 0.8 ml have been used in a number of studies where the material available is limited. In fact, the thorax of a housefly, crushed and suspended in 1 ml, gives adequate deflections for spectroscopic studies of rapid enzyme reactions.

Controls. The light absorption of respiratory enzymes in cells is considerably lower than that of hemoglobin in the erythrocyte (*19*) or chlorophyll in the chloroplast (*17*), and the complex optical situation due to light losses in the pigment granules has not been observed. Probably the most direct control on this point is provided by the following experiment. A difference spectrum of a turbid homogenate containing cytochrome *c* attached to cell fragments is recorded by the method of Fig. 4. Sodium cholate is then added to both samples. The turbidity nearly completely disappears, and cytochrome *c* is now in true solution, as is shown by chemical tests. The difference spectrum is again recorded and is essentially unchanged. A similar control has been carried out by comparing the difference spectra of bacteria before and after treatment with lysozyme to dissolve the cell wall—again no significant change is observed (*20*). Even in the case of measurements at 340 mμ, where light-scattering effects would be expected to be severe, the assay of the reduced pyridine nucleotide content of baker's yeast agrees with chemical assays (*11*).

Accessory techniques. Since the most incisive method involves the difference between two biochemical states of the living cells, accessory methods to determine whether those states have been established are highly desirable for, and in some cases essential to, a successful result. In the case where aerobic and anaerobic states are compared, a platinum microelectrode is inserted directly into the cuvette, and oxygen concentration is measured polarographically (*21*). A vibrating electrode (*22*) has a more rapid recovery time following stirring of the solution and is illustrated in Fig. 5 (*23*).

In cases where the two biochemical states differ with respect to rates of fermentation, the glass electrode may be used to measure the rate of acid production in lightly buffered suspension mediums of appropriate *p*H, as is illustrated by Fig. 5.

An illustration of the use of these two accessory techniques in the study of the reactions of reduced pyridine nucleotide enzyme systems in a suspension of yeast cells is afforded by Fig. 6 (*10*). Here there are three metabolic transitions: (i) Addition of glucose to the starved, quiescent yeast initiates rapid respiration, as indicated by the abrupt slope of the platinum microelectrode trace. This respiration is caused by a reduction of pyridine nucleotide. (ii) The initiation of aerobic fermentation is indicated by the delayed rise of the *p*H recording. (iii) The onset of anaerobiosis is marked by the inflection of the platinum microelectrode trace. The spectrophotometer then records a large increase in the reduction of pyridine nucleotide.

Thus this combination of accessory methods records the metabolic state of the cell suspension, and the spectrophotometric method records fundamental changes in the pattern of enzymatic activities that may control the metabolism. In short, this method provides the features of Warburg's techniques for measuring the over-all processes of respiration and fermentation and, at the same time, gives a direct assay of the steady-state levels of various respiratory catalysts involved in these processes.

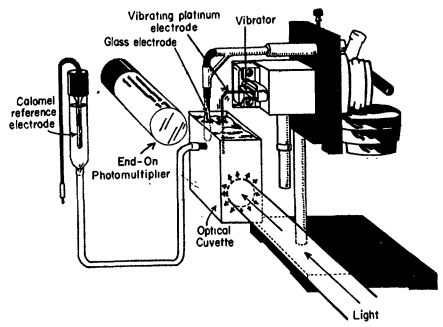

Fig. 5. Arrangement permitting simultaneous measurement of respiration with the platinum electrode, fermentation by means of the change of *p*H in a lightly buffered medium as recorded by the glass electrode, and changes in the steady-state levels of the respiratory enzymes by the double-beam spectrophotometer.

Identification of intracellular respiratory enzymes. It has been possible to survey the types of respiratory enzymes in a wide variety of materials by the methods of Figs. 1 and 4. As an example of our results on mammalian cells, Fig. 7 (left) shows the difference spectrum of aerobic and anaerobic ascites tumor cells. These cells are one of the most convenient sources of a homogeneous suspension of the mammalian type. The peritoneal cavity of one cancer-bearing mouse contains adequate material for a number of measurements. The difference spectrum shows clear absorption bands due to cytochromes a_3, a, and c, flavoprotein,

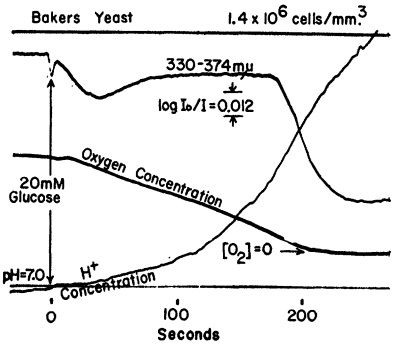

Fig. 6. Recording obtained with the combined methods of Fig. 5. Respiration and subsequently fermentation are initiated in an aerobic starved yeast cell suspension by adding glucose. The downward deflection of the spectrophotometric trace (optical density change at 330 mμ minus that at 340 mμ) shows the reduction of intracellular pyridine nucleotide.

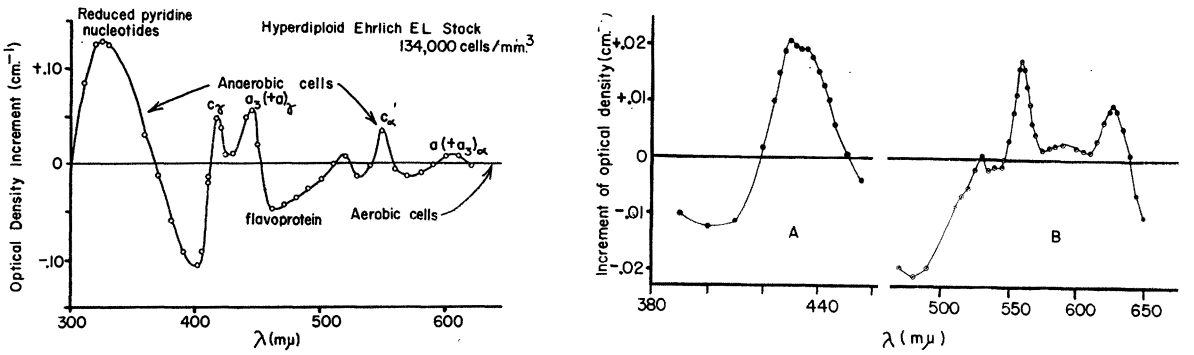

Fig. 7. (Left) Reduced minus oxidized difference spectrum for ascites tumor cells obtained by the method of Fig. 4 (Ehrlich hyperdiploid cells obtained through the kindness of T. S. Haushka). The absorption bands of the respiratory enzymes are identified on the figure. (Right) Reduced-oxidized spectrum for a suspension of *Aerobacter aerogenes*. The cell concentration in *B* is 4 times that in *A*.

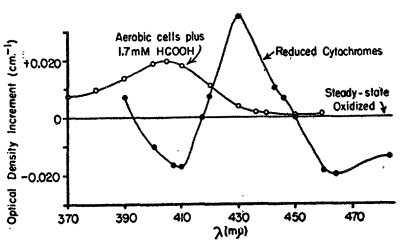

Fig. 8. Evidence for the existence of the primary catalase hydrogen peroxide complex in cells of *Micrococcus lysodeikticus*. Addition of 1.7 mM formate cause the decomposition of the complex and the characteristic rise of absorption at 405 mμ. The reduced-oxidized difference spectrum is included for comparison.

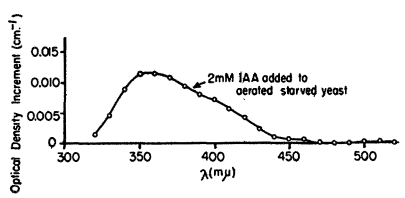

Fig. 9. Evidence for the compound of DPN and glyceraldehyde-3-phosphate dehydrogenase in aerobic starved yeast cells. Addition of IAA causes this compound to decompose with the disappearance of the characteristic absorption band shown here. The optical density decreases upon IAA addition.

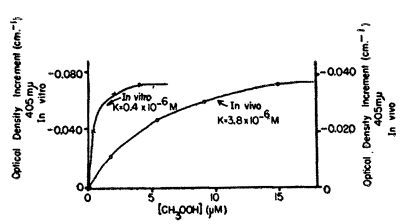

Fig. 10. Comparison of the methyl hydrogen peroxide titration of intracellular catalase of *M. lysodeikticus* with the titration of pure crystalline enzyme from these bacteria. See text for explanations for the diminished affinity of the enzyme *in vivo*.

and reduced pyridine nucleotide at the appropriate wavelengths (Table 1). Cytochrome *b* is present at a low concentration in these tumor cells and is difficult to detect without poisoning the cells with antimycin-A (*24, 25*).

A rather different example is represented by the difference spectrum of aerobic and anaerobic *Aerobacter aerogenes* (Fig. 7, right). In the visible region, peaks are due to cytochrome a_2, a_1, and *b*, at 630, 590, and 560 mμ, respectively. The γ bands of these pigments fuse to make a broad peak at 430 mμ (*12*).

Identification of intracellular enzyme-substrate compounds. Based upon *in vitro* studies of enzyme-substrate compounds (*26*), it seemed likely that such compounds would exist as reactive intermediates in metabolizing cells. This expectation is realized in the experiment recorded in Fig. 8. The catalase hydrogen peroxide complex has been found to react rapidly with formate *in vitro*. The complex is decomposed in this reaction, and the free enzyme is released. If the complex exists in the living cell, a similar reaction is to be expected and is verified by the increase of optical density caused by adding formate to aerobic bacterial cells. The spectroscopic shift is that expected on the basis of *in vitro* data and other chemical tests (reactions with methyl hydrogen peroxide and with azide identify the catalase complex positively, *27*). Such a demonstration is very important in our understanding of the level and the metabolism of hydrogen peroxide in the living cell.

In vitro it has been found that glyceraldehyde-3 phosphate dehydrogenase combines with diphosphopyridine nucleotide (DPN), and the enzyme-DPN compound has a characteristic absorption peak at 365 mμ (*28*). A similar *in vitro* reaction has been observed for the yeast enzyme (*29*). The presence of this enzyme-DPN compound may be readily detected *in vitro* by its rapid decomposition by iodoacetic acid (IAA) which causes the 365-mμ absorption band to disappear. Figure 9 shows the application of this test to a suspension of aerobic starved yeast cells. The spectroscopic change caused by IAA *in vivo* is very similar to the *in vitro* effect and leads us to the conclusion that about 20 percent of the intracellular DPN of the yeast

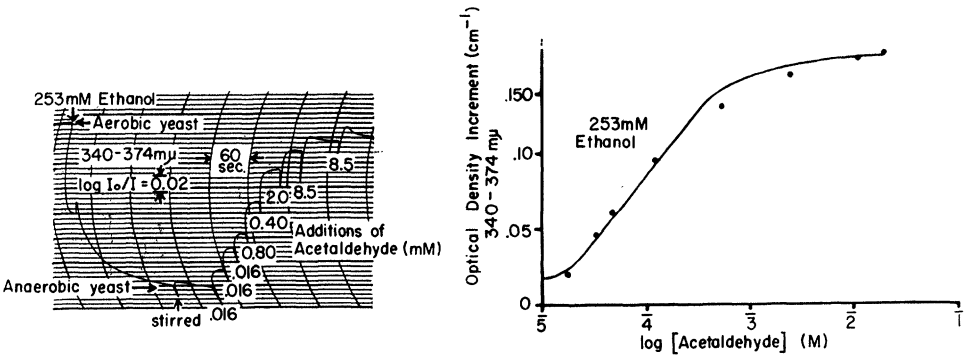

Fig. 11. (Left) Reduction of pyridine nucleotide of baker's yeast caused by ethanol addition followed by the oxidation of the intracellular pyridine nucleotide caused by a series of additions of acetaldehyde. (Right) Evidence that the oxidation of intracellular reduced pyridine nucleotide by acetaldehyde (points) follows a simple mass law equation (solid line).

cell may be bound to this enzyme (*27*). This result gives some insight concerning the intracellular localization of DPN on a molecular basis.

Quantitative studies of intracellular enzyme systems. The methods we now use are of sufficient stability and sensitivity that it is possible to carry out detailed studies of intracellular enzyme systems. For example, intracellular catalase may be titrated with peroxide to form the catalase complex I (*26*) as is illustrated by Fig. 10. In this case we may compare the result with the *in vitro* titration. More peroxide is required to form the intracellular compound, probably because the cell contains hydrogen donors and, in addition, the cell wall may provide a diffusion barrier to the peroxide.

The DPN system of anaerobic cells becomes completely reduced when an excess of ethanol is added to the starved cells, as is indicated by the downward deflection of the trace of Fig. 11 (left). A titration with aldehyde causes oxidation of the DPN system that is influenced by the alcohol dehydrogenase equilibrium (*10*). These titration data (points of Fig. 11, right) closely follow the solid curve drawn from simple equilibrium considerations. Similar titrations may be carried out with acids and bases that also influence the DPN-DPNH equilibrium, and the possibility that such shifts in the steady-state levels of the pyridine nucleotide system may govern pathways of metabolism can now be directly investigated (*10*).

Metabolic correlations. Since these methods permit a simultaneous recording of respiration (as oxygen disappearance measured by the platinum microelectrode) and fermentation (as acid production measured by the glass electrode), it is possible to correlate changes in the enzymatic systems with these metabolic activities. An interesting correlation in a suspension of ascites tumor cells is illustrated by the three traces of Fig. 12. The ascites cells as harvested from the mouse have a slow respiration and very little fermentation, as is indicated by the platinum and the glass electrode traces. Upon addition of glucose, the reduction of pyridine nucleotide occurs (as is indicated by the downward deflection of the spectrophotometric trace) and within a minute rapid fermentation is initiated (as is indicated by the rise of the glass electrode trace). The increased reduction of pyridine nucleotide does not, however, increase the respiration; instead, a decrease of respiration is observed. The amount of reduced pyridine nucleotide clearly does not set the slow pace of respiration in these tumor cells.

Photochemical reaction kinetics. The kinetics of photochemical reactions can be followed in cell suspensions with the modification of the apparatus of Fig. 1 illustrated in Fig. 13. The cuvette containing the cell suspension is illuminated with photodissociating light, and the resulting spectroscopic changes are measured by the double-beam spectrophotometer that is rendered unresponsive to the constant photodissociating light because of the capacitance coupling in the amplifier and because of the color filters in front of the photocell (*13*). A typical record of the kinetics of photodissociation of the carbon monoxide compound of cytochrome a_3 in yeast cells and its recombination in the dark is shown in Fig. 14 (*30*). By a comparison of the kinetics of these reactions with those of myoglobin-CO for which the molecular ex-

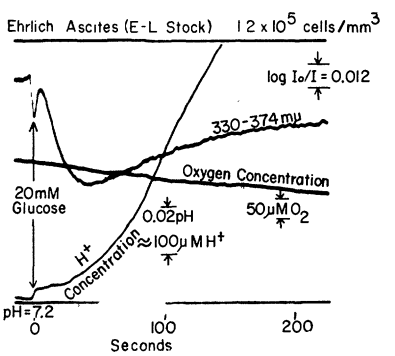

Fig. 12. Interrelationships of pyridine nucleotide reduction, and changes in respiration and fermentation caused by adding glucose to a suspension of aerobic ascites tumor cells. Data measured as indicated by Fig. 5.

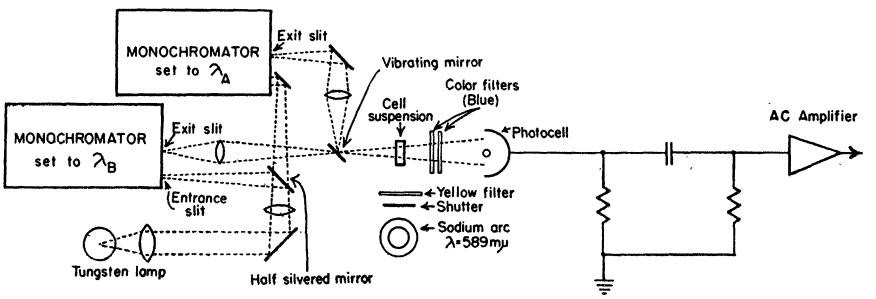

Fig. 13. A modification of the apparatus of Fig. 1 for spectroscopic measurements of the kinetics of photochemical reactions. The capacitance coupling in the amplifier, together with the blue filters, renders the measurement circuit insensitive to the illumination of the cell suspension with yellow light.

tinction coefficient (*31*) and quantum yield (*32*) are known, the molecular extinction coefficient for the carbon monoxide compounds of cytochromes a_3 and a_1 have been computed (*30*).

Photosynthetic reactions in purple bacteria, for example, *Rhodospirillum rubrum,* are excited by infrared radiation beyond the response of the Cs-Sb photosurface, and in this case no protecting filter in front of the photocell is required. Figure 15 shows the measurement of the kinetics of oxidation of respiratory pigments of an anaerobic suspension of *Rhodospirillum rubrum* upon illumination with infrared light, followed by a reduction in the dark. The kinetics of this reaction are diphasic and, in agreement with our action spectrum for this effect, lead us to the conclusion that the whole chain of respiratory enzymes is involved in this photochemical effect (*33*)—a result contrary to that of Duysens who first demonstrated this reaction (*34*). Lundegårdh has also reported an action spectrum involving cytochromes upon illumination of *Chlorella,* but his technique does not permit the recording of rapid reactions (*35*).

Studies of very rapid intracellular reactions. It has recently been possible to apply the accelerated- and stopped-flow methods (*36, 37*) to the measurement of the kinetics of rapid intracellular enzyme reactions in the time range 5 to 30 msec. An intracellular reaction of particular interest is the oxidation of reduced cytochrome oxidase by molecular oxygen. These measurements are most efficiently carried out in the regenerative flow apparatus shown in Fig. 16. In a typical experiment of this type the anaerobic suspension of yeast cells is driven downward from the large upper chamber into the mixing chamber and observation tube at flow velocities of 1 to 5 m/sec. The small syringe, driven simultaneously downward, contains an oxygenated solution that is mixed with the yeast just before it reaches the observation chamber, which has a 1-cm² cross section and is 1 cm long.

The material passing out of the observation tube is collected in a large syringe that provides quick stopping for the flow as used by Gibson (*37*). Since the yeast cells use up the added oxygen in a short time, they may be transferred into the upper syringe and used in subsequent experiments—a very important feature when the volume of reactants is limited.

Figure 16 includes a typical experimental record; the lower trace represents the spectrophotometric data, the upper one the flow velocity. The anaerobic yeast in the observation tube is replaced by the mixture of oxygen and anaerobic yeast when the flow is started (as indicated by the downward deflection of the upper trace). The oxidation of intracellular cytochrome *c* by oxygen and cytochrome oxidase has proceeded to about half-maximal completion at the value of flow velocity used—corresponding to a first-order velocity constant of 70 sec^{-1}. When the flow stops the reaction reaches the maximal steady-state oxidation of cytochrome *c,* which is maintained for 10 sec, when the added oxygen is used up.

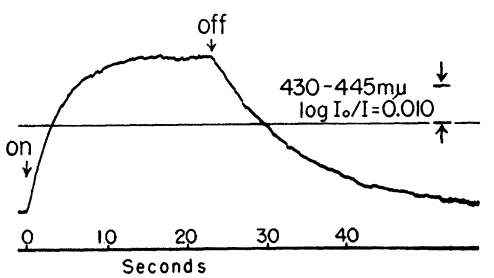

Fig. 14. The kinetics of photodissociation and dark recombination for the CO compound of cytochrome a_3. The yeast cell suspension was illuminated at 589 mμ as is shown in Fig. 13. An upward deflection of the trace corresponds to a decrease in optical density at 430 mμ. The calculated value of the molecular extinction coefficient for the a_3-CO compound is 12 $cm^{-1} \times mM$ at 589 mμ.

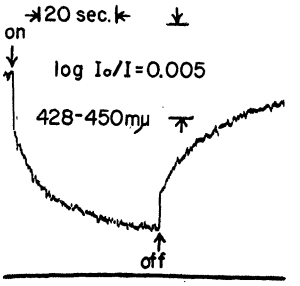

Fig. 15. The kinetics of the oxidation of cytochromes in an anaerobic suspension of *R. rubrum* caused by infrared illumination. A downward deflection of the trace corresponds to a decrease in optical density at 428 mμ. Upon the cessation of illumination the dark reduction was initiated and proceeded to completion after the recording shown here was stopped.

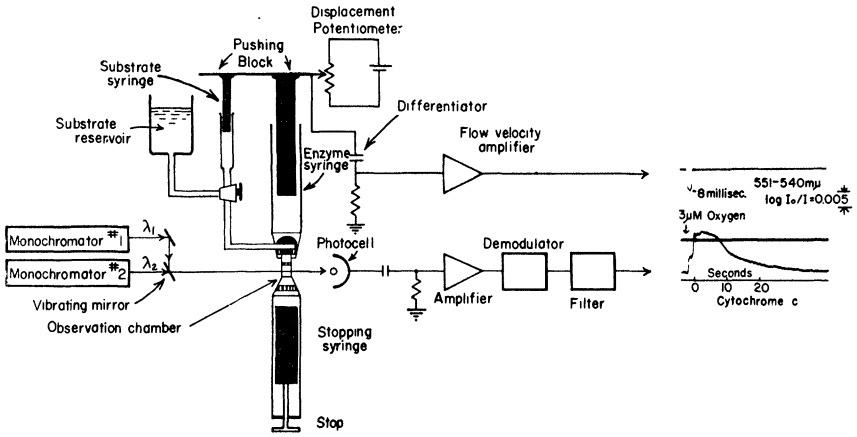

Fig. 16. The regenerative flow method for the measurement of very rapid chemical reactions in suspensions of intact cells in the particular experimental result shown at the right, anaerobic yeast cells are mixed with 3 μM oxygen, and the oxidation of the intracellular ferrocytochrome *c* is recorded by the double-beam spectrophotometer.

This flow apparatus has adequate speed to permit detailed studies of the relative speeds of oxidation of the members of the respiratory sequence of various cells (Table 1). It has been found that the oxidation reaction proceeds extremely rapidly through cytochrome *c* and somewhat more slowly through cytochrome *b*, flavoprotein, and reduced pyridine nucleotide, the oxidation of cytochrome *b* being a rate-limiting step, for example, in baker's yeast.

It is often desired to record the difference spectrum between anaerobic cytochromes and those oxidized a short time after adding oxygen in order to determine whether any labile intermediate compounds involved in the oxidation reactions of the respiratory chain can be observed. An apparatus similar in principle to that of Fig. 16, but with larger volumes of reactants, permits a flow of about 30-sec duration—long enough to permit the recorder of Fig. 4 to plot the difference spectrum corresponding to the extent of oxidation of the respiratory pigments 30 msec after adding 23 μM of oxygen (Fig. 17). This record clearly shows that the cytochromes oxidized in this short time are *a*, *c*, and a_3; the bands of flavoprotein at 465 mμ and reduced pyridine nucleotide at 340 mμ are conspicuously absent at the shorter time.

Spectroscopic studies of intact tissues. Lundegårdh has made a number of interesting observations of the cytochromes of cut bundles of wheat roots (*15*). The roots are held in a circular tube about 1 cm in diameter that is illuminated with monochromatic light, and the light scattering is compensated by a piece of filter paper. The roots are bathed in various solutions to cause metabolic changes. The results are, at the present time, restricted to the cytochromes; no data on pyridine nucleotides have been published.

It has recently been possible to make detailed kinetic studies of the effect of contraction upon the steady state of pyridine nucleotide enzyme systems of frog sartorius muscle (*38*). The flow chamber for the muscle is illustrated by Fig. 18. The perfused muscle is held between two perforated Lucite sheets that allow the muscle to be bathed continuously in Ringer's solution

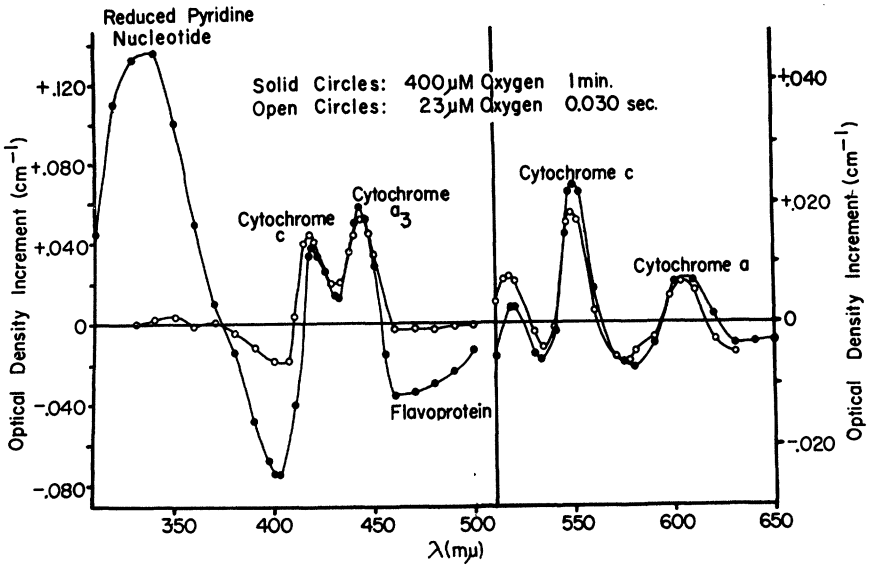

Fig. 17. Recording of the respiratory pigments that are oxidized 30 msec after adding 23 μM of oxygen to anaerobic yeast cells (open circles). This spectrum may be compared with the result obtained 1 min after adding 400 μM of oxygen to anaerobic cells (solid circles). To assist in the recognition of the absorption bands of the reduced pigments that disappear upon addition of oxygen, the data are plotted as a usual reduced minus oxidized difference spectrum and may therefore be directly compared with other data. These results were obtained with a combination of the apparatus of Fig. 4 and a modification of that of Fig. 16.

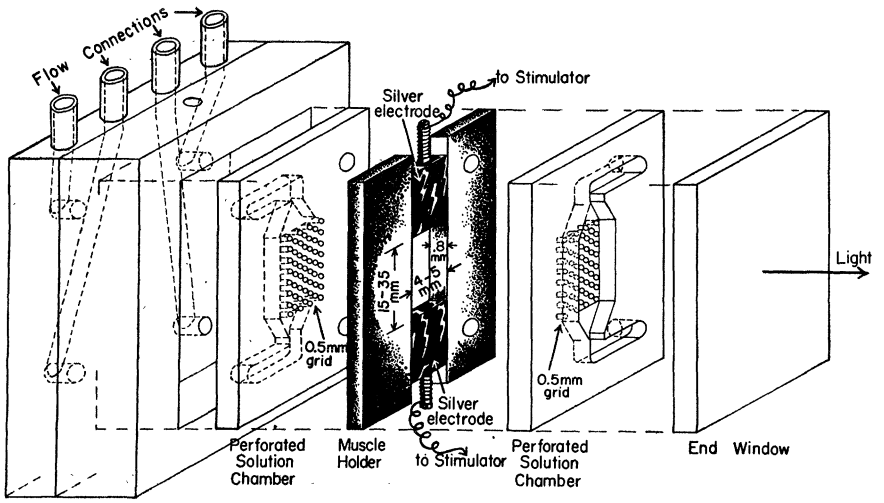

Fig. 18. Flow chamber for spectroscopic observations of changes in the steady-state level of reduced pyridine nucleotide enzyme systems in aerobic frog sartorius muscle. The flow chamber is made of Lucite (free of ultraviolet-absorbing dyes).

containing various other reagents as desired. The muscle ends are in contact with two silver electrodes for electric stimulation. The muscle is illuminated by the double-beam spectrophotometer of Fig. 1, which is not appreciably affected by slight shifts in the position of the muscle caused by contraction, or by light-scattering changes in the muscle when the two wavelengths used are closely spaced.

When aerobic muscle contracts, current theories state that adenosine diphosphate (ADP) produced in the contraction is converted back to adenosine triphosphate by the oxidative phosphorylation system of muscle mitochondria or sarcosomes. In liver mitochondria, it has been found that oxidative phosphorylation of ADP causes an oxidation of the steady state of reduced pyridine nucleotide (*39*). The effect of muscular contraction upon the steady state of pyridine nucleotide of aerobic frog sartorius muscle during a series of twitches at low temperature is recorded by the double-beam technique, as is shown in Fig. 19. The abrupt upward deflection of the trace upon initiation of the contractions corresponds to an oxidation of reduced pyridine nucleotide; this persists as long as the contractions are maintained. Thereafter a slow recovery to the previous steady state occurs. Although this technique is only in its preliminary development, the particular reaction of Fig. 19 may be of some use in evaluating how rapidly ADP or inorganic phosphate released in muscular contraction reaches the oxidative phosphorylation system. The study of the steady state of intracellular respiratory pigments of muscle and other tissue in which hemoglobin and myoglobin do not interfere with the spectroscopic observation presents some fascinating possibilities for the future.

Future developments. There are a number of important problems that cannot be adequately solved with present spectroscopic methods because the optical density change is either too small to measure or occurs too rapidly. When the amount of material available is limited, such problems can be taken up only when brighter and more stable light sources, better monochromators, and more sensitive photosurfaces become available. Thus future developments in this field are closely tied to physical aspects of the techniques.

The importance of proper use and selection of biological material can scarcely be overemphasized. Since the philosophy of the method is centered about the measurement of spectrophotometric changes that accompany changes in the biological state, it is important to know how to cause such changes and which organisms respond maximally. In this respect it should be pointed out that a suitable change in the biological state might well be caused by adaptations in microorganisms.

There are at the present time a number of interesting possibilities for extending the scope of the present studies with currently available equipment. The ability to make rapid and accurate assays of the steady state and the total content of the respiratory enzyme sequence may be of considerable practical importance in evaluating growth and metabolic patterns of cells

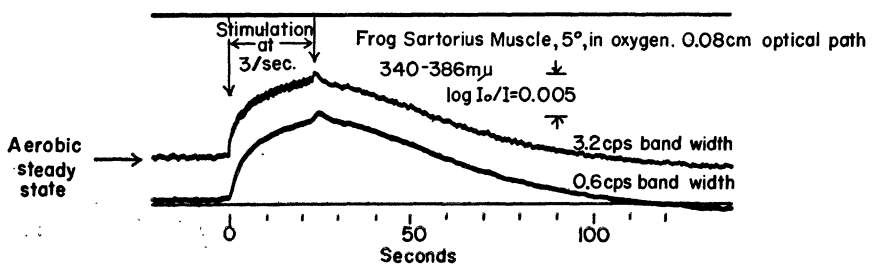

Fig. 19. A spectroscopic change caused by the contraction of frog muscle. The upward deflection of the trace corresponds to a decrease of optical density at 340 mμ and is found to have an action spectrum corresponding to the oxidation of reduced pyridine nucleotide.

and tissues, and possibly in monitoring the steady state appropriate to a particular metabolic function.

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Abstracts of Papers Presented at the Autumn Meeting

8–10 November 1954, Columbia University, New York

Semiempirical Study of the H_2Cl Transition Complex through the Use of Hydrogen Isotope Effects

Jacob Bigeleisen and Max Wolfsberg,
Brookhaven National Laboratory

Linear and triangular structures for the H_2Cl transition complex have been analyzed in terms of normal vibration theory and by an evaluation of the experimental data on the effect of hydrogen isotope substitution on the rate of reaction of hydrogen molecules and chlorine atoms. The frequencies of the bending vibrations are probably small enough that their contribution to the relative rates can be expressed in terms of a small quantum correction of the order of $(hc/kT)^2\,(\omega_H{}^2 - \omega_{D^2,T}/24)$. The near classical behavior of the bending frequencies for both linear and triangular structures of the H_2Cl complex serves to reduce the ratio of the frequency factors, $A_H/A_{D,T}$, in the Arrhenius equation from that expected from structural considerations alone. Quantitative agreement is found between the caluculated and experimentally determined frequency factor ratios.

The "symmetrical" stretching frequencies, for both linear and triangular complexes, respectively, have been evaluated from the relative rates of reaction of H_2 and HT with chlorine atoms at 0°C and the known properties of the isotopic hydrogen molecules. This empirically evaluated parameter together with thermodynamic data and an estimate of the tunnel correction suffice to calculate the relative rates of reaction of the isotopic hydrogen molecules with chlorine atoms as a function of temperature. Good agreement is found between the calculated rates for both linear and triangular structures and the available experimental data on HD, D_2, and HT.

Work was performed under the auspices of the U.S. Atomic Energy Commission.

Diffusion and Sedimentation of a Weak Polyelectrolyte, Carboxymethyl Amylose (CMA)

Charles O. Beckmann and Anthony G. Scott,
Columbia University

The diffusion curve (dc/dx vs. x) of CMA solution against water is not of the ideal Gaussian form but possesses a shoulder on the solvent side of the boundary. This anomaly has also been observed for the nucleic acids. Analysis shows the diffusion coefficient (D) to be a complicated function of the concentration, rising sharply as the concentration increases from zero, going through a maximum, decreasing to a minimum and then rising very slowly.

These variations stem from the superposition of two effects that arise from the increase in ionization with decreasing concentration. On the one hand, the increase in negative charge on the polymer molecule causes its