Union, we must continue to expand our research.

There is also one new problem we have to face. It is probably right to assume that the attempt to create a free trade system in Europe, in spite of the present difficulties, will ultimately lead to a positive result. Although this as a whole must be favorably received, it will nevertheless create problems for certain branches of our industry. We must expect that some of these industries, now protected by customs duties, will be unable to compete in a free market. They will thus have to be replaced by industries in fields where we have special natural conditions—such as, for instance, the electrometallurgical and electrochemical fields. Since these particular industries, however, require heavy capital investments, we must also look for other possibilities. Here I think "brain" industries would be a good answer. To make such changes in our industries successfully, and in time, will require, to my way of thinking, some early and wise decisions in the fields of education and research.

Summary

By way of a summary, may I say that I believe we now have in Norway an over-all pattern for the administration of research which fits our present situation reasonably well. There are weaknesses, as I have pointed out, but there are also signs that we have a fair chance of putting them right. The system is sufficiently flexible to allow for initiative, and we know we shall have to make changes to fit our future needs. We are in the happy situation of having our youth show an increasing interest in research work, so if we can sucessfully master our training problems we should have the good recruitment we consider essential for progress in research. The problem of "being small" can probably be solved, or at least remedied, through a combination of concentration and international collaboration.

In think it has now become clear to the greater part of our population, and specifically to a great number of influential persons, that if we in Norway are to maintain and develop our spiritual and material culture, we shall have to continue the progress in research. We thus hope that in the future, through a united effort of our Government, our trades and industries, and our research, we shall be able to contribute our share to the common fund of knowledge and shall be able also to use this knowledge to expand the social and economic life of our nation and contribute to the security of all free nations.

Spectroscopic Evidence of Metabolic Control

Rapid measurements of intracellular events afford new evidence on mechanisms for metabolic control.

Britton Chance and Benno Hess

The interaction of glucose and oxygen metabolism has been the subject of study ever since Pasteur's discovery 100 years ago of the metabolic response of glucose utilization which now bears his name (see *1, 2*). Recent interest has been stimulated by Warburg's hypothesis that irreversible damage to the respiratory mechanism and a consequent increase of glycolysis are associated with cancerous growth (*3*). With greater knowledge of the enzymatic pathways for glucose and oxygen metabolism and of the significance of intracellular levels of substrates, coenzymes, phosphate, and adenosine phosphates, general principles and specific mechanisms for the Pasteur reaction (*4–11*) have been proposed. Increasing awareness of the structural relationships of enzyme systems within the cell (*11, 12*) adds, however, to the problem of ordinary chemical analysis a dimension with which it is not currently able to cope, namely, the control of metabolism by redistribution of rate-limiting substances among the intracellular structures. Such changes in concentration might well remain undetected in chemical analyses of the average concentration of such components (*2, 11*). It is an appropriate time, therefore, to describe results of the measurements of changes of concentrations of possible control substances at the site of their action within the cell.

The development of methods for the direct spectrophotometry of intracellular respiratory pigments and their applications to the measurements of the kinetics of oxidation of cytochromes, flavoprotein, and pyridine nucleotide, together with the adaptation of rapid flow techniques to rapid reaction kinetics of intact cells, were described several years ago (*13*). The advantages of ascites tumor cell suspensions for experimental study with these new methods have been pointed out from the standpoint of physiology and biochemistry (*3, 14*) and also from the standpoint of the requirements of the spectrophotometric technique (*13*). It has further been found that ascites tumor cells are remarkable material for the study of interactions between glucose and oxygen utilization, overbalanced in favor of glycolysis. The cells furthermore show not only a Pasteur and a Crabtree effect (*15*), but also a short-lived and intense metabolic response to glucose addition which sheds considerable light on possible mechanisms of metabolic regulation (*16*).

Even though it was suggested some time ago (*17*) that it is not the enzymes but their interactions that are responsible for tumor cell metabolism, explanations for the relatively low respiratory activity of some types of tumor cells have been advanced on the basis of low cytochrome *c* and low cytochrome oxidase activities (for a summary, see *18*).

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Recently Warburg has proposed damage to the respiratory system (*3*). However, spectrophotometric studies of cytochromes of the ascites tumor cell show no deficiency of cytochrome *c* relative to cytochrome oxidase (*19*) and no damage to the respiratory carriers (*16*). This conclusion is so greatly at variance with the chemical determination of cytochrome *c* and cytochrome oxidase activities by extraction procedures in various types of tumor material that further study of the nature of the respiratory chain in the ascites tumor cell is reported here. The pathway of electron transfer, both in the intact cell and in the mitochondria isolated therefrom, has been investigated, and quantitative studies of the cytochromes of the cell and of the mitochondria have been made. These results clearly indicate the high capabilities of the respiratory chain of the ascites tumor cell for rapid electron transfer and efficient phosphorylation.

The remarkable response of the ascites tumor cell to glucose addition consists of a momentary stimulation, followed by a considerable inhibition of respiration and glucose utilization. This observation of a latent high-respiratory activity of these cells suggests that metabolic control, and not irreversible damage (*3*), is responsible for their low respiratory activity under physiological conditions. Spectroscopic studies of effects coincident with the stimulation of respiration suggest that the control of respiration is being exerted at the level of the mitochondria by the glucose phosphorylating process and, reciprocally, that some property of the mitochondria is controlling the metabolism of glucose.

The Pasteur effect has previously focused attention upon glycolytic-respiratory activity interactions, as has the converse effect found by Crabtree (*15*). Much discussion of the role of phosphate and phosphate acceptor has appeared and has been incorporated in theories of metabolic control (*4–6*), especially by Belitzer (*7*), Johnson (*10*), Lynen and Koenigsberger (*11*), and Potter (*9*). Experimental data previously available to support these theories are largely based upon chemical analysis of extracts of intact cells such as yeast and, more recently, of the ascites tumor cells by Racker (*20*) and others. In these studies, the identification of the control substance in the ascites cell requires careful consideration. Lynen (*2*) and Racker (*20; 20a*) suggest that phosphate is of importance. Our spectroscopic and chemical studies of the ascites tumor cell indicate that increases and decreases of the intracellular concentration of adenosine diphosphate (ADP) are responsible for the changes of respiratory and glucose metabolism observed therein. This leads to the general view that a low intracellular concentration of ADP is responsible for the low rate of respiratory metabolism in these cells.

We present here a critical analysis of the components of the respiratory chain of the ascites cells and a study of the phosphorylation mechanism and its efficiency in isolated mitochondria, together with measurements of the above-mentioned responses of the intact cells to glucose addition.

Methods of Preparation

The preparations of ascites tumor cells and of mitochondria will be described in detail elsewhere (*21, 22*). It should suffice here to say that a six-day growth of tumor cells was used either directly suspended in the ascitic fluid or suspended in a "saline phosphate" solution (*21*) after differential centrifugation (and sometimes differential lysis) in order to free them from erythrocytes. The mitochondria were prepared by high-speed mechanical disintegration with subsequent differential centrifugation, a process which was developed for yeast granules (*23*). The reaction medium was that customarily used for the assay of oxidative phosphorylation (*24*), except that fluoride was omitted.

The chemical methods for assay of intermediates in the cells were mainly enzymatic reactions (*25*). Measurement of the activated phase of respiration of the cells requires a rapidly responding technique, and the vibrating platinum electrode was found to be excellently suited for this purpose (*26*).

The kinetics of spectroscopic changes were followed by a double-beam (two-monochromator) spectrophotometer (*13*), and difference spectra were plotted by a split-beam recording instrument (*13*).

Pathway of Electron Transfer in the Intact Cell

A method for determining the photochemical action spectrum for relief of carbon monoxide-inhibited respiration in the ascites tumor cell based upon the platinum microelectrode has shown clearly that cytochome a_3 is the terminal oxidase of this cell (*27*). Suggestions that the antimycin-A-insensitive pathway is of importance in certain tumor cells have been afforded by the work of Reif and Potter on the Flexner-Jobling carcinoma (*28*). This result would suggest that the respiratory pathway of the tumor cell would be largely a nonphosphorylating one. The report of the anomalous response of the succinate-oxidase pathway of the tumor cell (*29*) requires further investigation. In fact, the whole concept of "respiratory enzyme balance" (*30*) may be critically reevaluated.

Our investigation of the respiratory pathway is based upon the use of specific inhibitors: carbon monoxide for the terminal oxidase (*31*), antimycin A for the contribution of the cytochrome b_5 pathway (*24, 32*), and amytal for the relative importance of diphosphopyridine nucleotide and the succinate-linked mechanisms (*32–34*). The nature of the spectroscopic responses to these inhibitors also gives evidence for the sequence of cytochromes along the respiratory pathway.

Amytal almost completely blocks respiration of the ascites tumor cell: over 97 percent of the endogenous or glucose-activated respiration is inhibited by the addition of 2 mM amytal. The spectroscopic changes that accompany this inhibition correspond to a reduction of pyridine nucleotide and an oxidation of flavoprotein and cytochrome *b*. Thus the crossover point (*35*) for inhibition by amytal (its site of action) is between reduced pyridine nucleotide and flavoprotein. This is in agreement with the crossover point for amytal inhibition of isolated liver mitochondria (*34*), and suggests that this portion of the electron-transfer pathway of the intact ascites tumor cell is the same as that of isolated, actively phosphorylating mitochondria.

The fact that amytal treatment causes oxidation of the cytochrome components of the respiratory chain of the ascites cell that are largely reduced in the steady state of metabolism enables us to obtain a spectrum representing the difference between the fully oxidized and the fully reduced cytochrome components of the respiratory chain, as illustrated in Fig. 1. A comparison of the absorption bands of cytochromes *b*, *a* ($+a_3$), and $c+c_1$ with those of highly respiring cell suspensions such as baker's yeast (*36*) gives a qualitative idea of the normal balance of the components of the respiratory chain.

In the intact cells, antimycin-A treatment causes respiratory inhibition of

more than 95 percent. The titration of the cells with antimycin A clearly indicates that the reduction of cytochrome *b* proceeds after some nonspecific binding of antimycin A. Due to this nonspecific binding, the titration has a slope of roughly eight antimycin-A equivalents to one of cytochrome *b* (*21, 37*). The crossover point for antimycin-A inhibition is between cytochromes *b* and $c + c_1$, as would have been expected from studies of isolated mitochondria. It is interesting to note that no measurable shift of the α band of cytochrome *b* is caused by addition of antimycin A to the anaerobic tumor cell suspension, and thus the "modified cytochrome *b*" found in nonphosphorylating Keilin and Hartree preparations (*38*) is not present in these cells.

Spectroscopic studies of the absorbancy changes caused by dithionite addition to the anaerobic ascites tumor cell suspension suggest that no measurable amount of cytochrome b_5 is present in these cells. This is in accordance with the failure of attempts to isolate cytochrome b_5 from these suspensions (*39*).

The nearly complete inhibition of electron transfer in the intact cell by amytal and by antimycin A suggests that the electron-transfer pathway studied in isolated liver mitochondria (*40*) operates in the intact tumor cell. There is no evidence for the cytochrome b_5 or other antimycin-A-insensitive pathways for respiration of these cells. The abrupt and complete inhibition of respiration by amytal suggests that the pool of succinate in the ascites tumor cell is very small and that the respiratory pathway utilizes reduced pyridine nucleotide to a very great extent. The lack of respiratory stimulation upon addition of succinate to the amytal-treated cells is apparently due to their impermeability to this substrate rather than to a lack of succinate oxidase activity, and thus may provide an explanation of previous work (*29*).

Cytochromes of Whole Cells and of Mitochondria

A second method of studying the cytochrome content of the electron-transfer pathway of the cell is to isolate the mitochondria. The ascites tumor cells also afford an unusually favorable opportunity to compare the cytochromes of the whole cell with those of the mitochondria, since direct spectroscopic observations of both types of material are possible at room and liquid-air temperatures. A comparison of the "apparent absolute" spectra of substrate-reduced cytochromes of the azide-inhibited cells and their mitochondria is given in Fig. 2. The sharp absorption bands due to cytochromes *c*, c_1, *b*, and $a + a_3$-azide are clearly shown. All the components of the intact cells are present in the mitochondria. On the basis that the tightly bound cytochrome *a* has been retained in the mitochondria, we find that nearly all the cytochrome *c* of these cells is likewise retained by the mitochondria. Thus, speculation on respiratory pathways which depend upon the presence of cytochrome *c* in the cytoplasm, such as the cytochrome b_5 pathway, receives no support from these experiments. Dithionite treatment of the cells and their mitochondria show no specific absorbancy increases at low temperatures that could be attributed to cytochrome b_5, in confirmation of the results mentioned above.

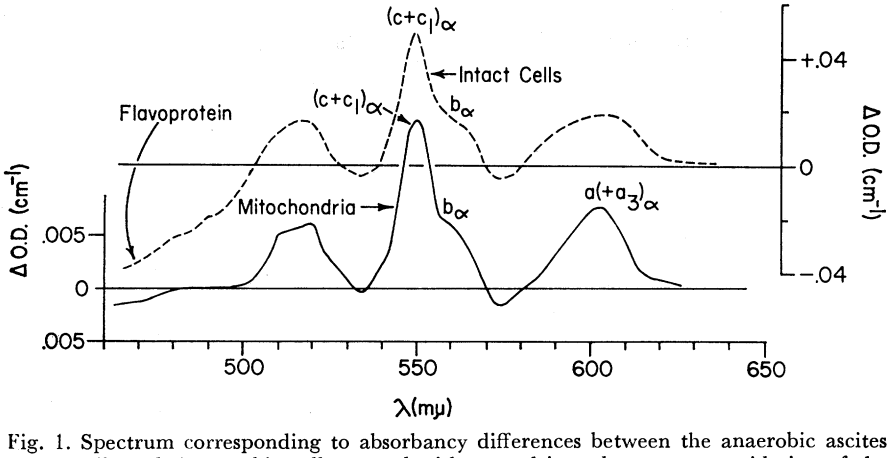

Fig. 1. Spectrum corresponding to absorbancy differences between the anaerobic ascites tumor cells and the aerobic cells treated with amytal in order to cause oxidation of the cytochrome components. In this difference spectrum, note that cytochrome *b* stands out clearly. For comparison, the difference spectrum of cytochrome components of the mitochondria isolated from the tumor cell is included. (Exp. 675e.)

There appears, however, a broad absorption band with a peak at approximately 560 mμ upon dithionite treatment of the enzymatically reduced material. This material does not appear to be a component of the respiratory chain and its possible identification with "mitochrome" (*41*) should not be overlooked.

Cytochrome Concentration: Respiratory Activity Relationships

On the basis of two types of spectrophotometric determinations of the cytochromes of the ascites tumor cells indicated by Figs. 1 and 2, we are now in a position to estimate any possible deficiency of cytochrome *c* and cytochrome oxidase in these tumor cells. Most of the previous conclusions have been based upon extraction procedures or enzymatic assays of solid tumors. It would appear that the direct determination of the respiratory components in intact cells and in isolated mitochondria of a homogenous population of freely suspended cells would have certain advantages over other procedures and other materials. In fact, a number of tumor cell suspensions derived from single clones (*42*) were placed at our disposal through the kindness of T. S. Hauschka (*43*). The results of assays of cytochromes of respiratory enzymes in ascites and other cells are given in Table 1. The relative amount of cytochromes appears to be independent of the ploidy. The amounts of the respiratory components computed relative to cytochrome *a* are typical of actively respiring cells, as indicated by comparison with yeast. Cytochrome *b* appears to be an exception, but the spectra are those corresponding to the transition from the aerobic steady state to the anaerobic state and therefore underestimate cytochrome *b* by a factor of about 3, as indicated by the studies with amytal and antimycin A. When this correction factor is applied, the content of cytochrome *b* much more nearly approaches that of other systems of highly active respiration. This type of correction is also necessary for evaluation of the total reduced pyridine nucleotide concentration.

In making up this table, the choice of materials has largely been set by their availability and suitability for the experimental method. The selection of yeast and muscle does not, therefore, represent our estimates of the "normal material" with which the ascites tumor cell should be compared, but instead represents selections of actively respir-

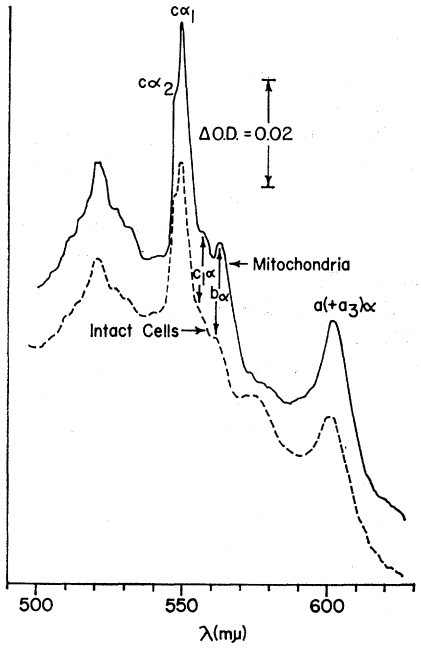

Fig. 2. Low temperature spectra of cytochrome components of intact cells and mitochondria isolated from them. These are "apparent" absolute spectra in which the reference material is the frozen solvent. The cytochrome components of the two types of material are appropriately designated. (Exp. 675e.)

ing electron-transfer systems on which data are available.

When cytochrome concentration is calculated on a weight basis instead of on a relative basis, it is seen that the ascites cells lie between baker's yeast and frog muscle. On this basis, it is apparent that the cells have not only an appropriately proportioned cytochrome system, but also a relative sufficiency of these enzymes in the cell. An examination of the mitochondria prepared from the ascites tumor cells shows that the cytochrome content per milligram protein is very nearly the same for the three types of materials assayed here. This leads to the noteworthy conclusion that mitochondria derived from these three different sources have essentially the same constitution, with the exception of the values for reduced pyridine nucleotide, which will vary somewhat because of the variable loss of reduced pyridine nucleotide in the preparation of mitochondria.

Of greatest interest are the data on the turnover number of cytochromes, especially those comparing the turnover number of cytochromes in the intact cell and in the isolated mitochondria. It is clear that the turnover number of cytochrome *a* in the intact cell is low with respect to both that of its isolated mitochondria and that of other types of cells. For example, mitochondria isolated from ascites cells have the same turnover number as mitochondria isolated from rat liver (10 sec^{-1}) (*24*), but cytochrome *a* in the intact cell turns over at only 3 sec^{-1}. This leads to the conclusion that while the concentration of cytochrome in the intact cell is adequate, the respiratory system is not used to the extent of its capabilities. This result independently suggests that, instead of an "unbalanced" enzyme system, there is a control mechanism imposed upon the respiratory activity of the intact ascites tumor cell.

Properties of Mitochondria Prepared from Ascites Tumor Cells

A key point in any consideration of metabolic control by the mitochondria is a determination of whether their electron transfer can actually be controlled by the concentration of phosphate and phosphate acceptor, as has been demonstrated for mitochondria isolated from liver by Lardy and Wellman (*44*) but not for mitochondria from ascites cells (*45*). Although the preparation procedure requires vigorous shaking with glass beads to rupture the cell, it has been possible to demonstrate respiratory control in these mitochondria. We find respiratory control ratios to average threefold and to exceed sixfold with succinate as substrate. The average P/O value with succinate as substrate is 1.8, as compared with previous values of 1.4 (*43*). Thus these mitochondria show good respiratory control and phosphorylation efficiency. The high phosphorylation efficiency of ascites mitochondria is suggested by indirect data of Quastel and Bickris (*46*).

A further finding of studies of the effect of ADP upon the isolated mitochondria is their spectroscopic response, illustrated by Fig. 3. Here, addition of ADP to the substrate-treated mitochondria (state 4) causes a disappearance of the absorption bands of reduced pyridine nucleotide, flavoprotein, cytochrome *b*, and cytochrome *c* (see *35*). (The decreases of absorbancy are plotted as an upward deflection in order to aid in the recognition of the absorption bands.) For comparison, the difference spectra corresponding to those absorption bands which appear in anaerobiosis are included.

In summary, experimental results show that respiration of mitochondria isolated from ascites tumor cells can be readily activated by adding ADP. Furthermore, characteristic spectroscopic changes are observed which could be used to identify changes of ADP concentration within the intact cells.

Respiratory Response to Glucose

A response similar to that of the isolated mitochondria to the addition of ADP is found under certain conditions with the intact cells. As indicated above, it is clear that the respiratory activity proceeds at a low rate under endogenous

Table 1. Respiratory components of cells and mitochondria.

Material	Designation	Chromosome No.	Cytochrome *a*		Relative amounts of respiratory enzymes						
			Amount	Turnover No. (sec^{-1})	a	b	c + c_1	fp	a_3	RPN	Expts.
Ascites cells	E L stock	46	7*	3	1	0.3†	2.2	4.4	0.9	6†	225
Ascites cells	E-1	84	9*	3	1	0.4†	2.6	3.5		6†	232
Yeast cells	"Bakers"		20*	52	1	1.4	2.5	1.5	1.6	6	911
Excised muscle	Frog sartorius		2*		1	0.6†	1.8	3	0.8	20†	W-1
Mitochondria	Ascites cells (E L stock)		2‡	10§	1	1	3.1			10	662
Mitochondria	Rat liver		2‡	9§	1	0.9	1.7	3.6		19	

* Units: moles/g cells × 10^9. † Steady-state oxidized only; considerably more of the component is present. ‡ Units: moles/mg pr × 10^{10}. § Glutamate as substrate.

metabolism and that the mitochondria have a capability of at least a threefold acceleration over this rate. Addition of glucose to a suspension of the intact cells freshly drawn from the mouse and suspended in the ascitic fluid or in the saline phosphate medium shows a rapid acceleration of respiration (Fig. 4), varying from two- to sixfold and depending largely upon the nature of the endogenous metabolism.

A second respiratory response is indicated by Fig. 4. It shows that after approximately a minute of accelerated respiration, a severe inhibition sets in which reduces the respiratory rate below that previously obtained with endogenous substrate. The record further shows that this inhibited respiration is largely relieved by the addition of an uncoupling agent such as dicoumerol (Fig. 4*A*) or dinitrophenol (Fig. 4*B*). The amount of oxygen taken up during the accelerated phase of respiratory metabolism in the presence of excess glucose is linearly related to the number of cells used (*47*).

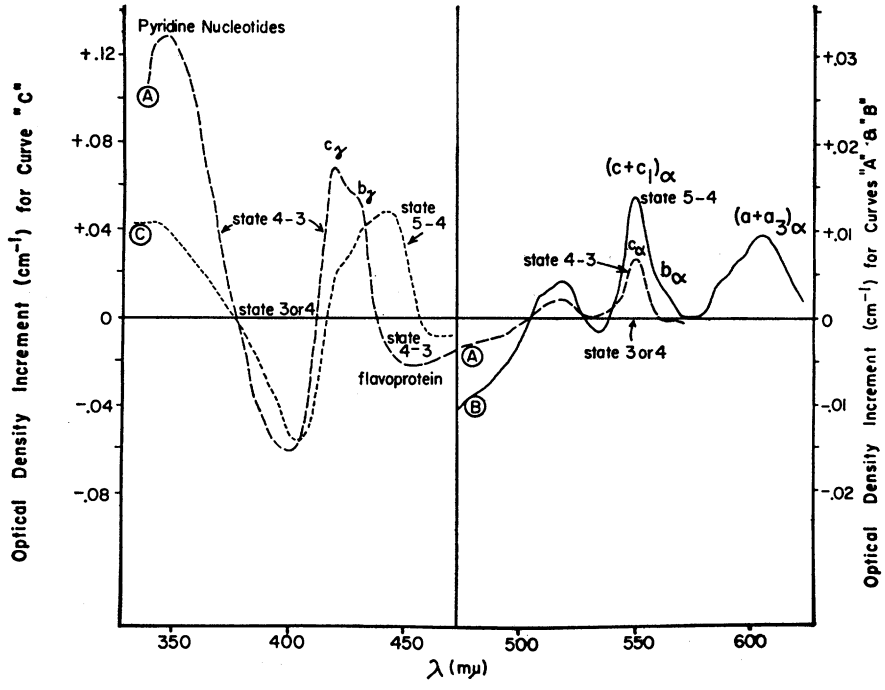

Fig. 3. The spectroscopic response of a suspension of mitochondria isolated from ascites tumor cells to an exhaustion of added ADP (trace A state 4–3 transition). Upon exhaustion of added ADP, absorption bands appear corresponding to the reduction of the pyridine nucleotides, flavoprotein, and cytochromes *b* and *c*. For comparison, the absorption changes that occur when the aerobic mitochondria become anaerobic are included (traces B and C, state 5–4). (Exp. 662d-3.)

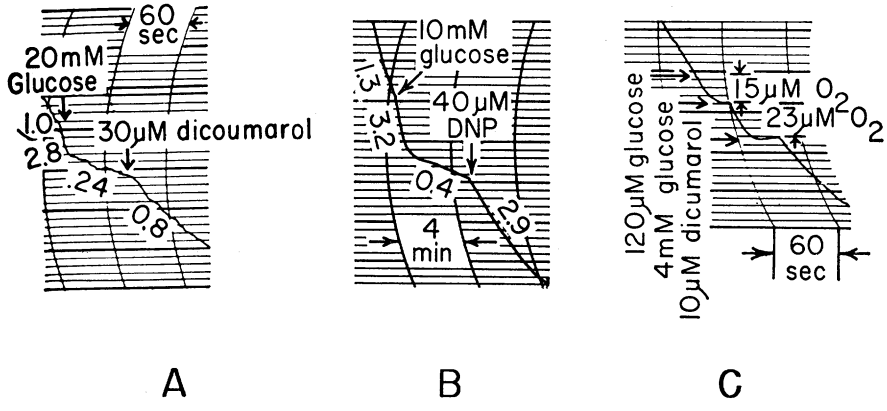

Fig. 4. Typical examples of the respiratory response of ascites tumor cell suspensions to glucose addition as measured by the vibrating platinum microelectrode. *A* and *B* represent the acceleration and inhibition of endogenous respiration of ascites tumor cell suspensions caused by the addition of glucose together with the restarting of this inhibited respiration by the addition of uncoupling agents (dicoumarol, *A*; dinitrophenol, *B*). In *C*, a rather low concentration of glucose (120 μ*M*) is added, which soon leads to an inhibition of respiration. The addition of an excess of glucose restarts the respiration, which again is inhibited after a short period. Respiration may be further restarted by addition of dicoumarol. (Exps. 647e, 677a.)

Some pertinent data on the nature of this effect follow. The acceleration of respiration does not depend upon the activity of the Embden-Meyerhof sequence of enzymes; it can be obtained in the presence of sufficient iodoacetate to inhibit the glycolysis. Thus the acceleration does not depend upon additional substrate for the respiratory chain, but instead upon an intermediate produced from the glucose-phosphorylating enzymes—presumably ADP. This observation further suggests that the inhibition of the glucose-activated respiration is due to a depletion of the intracellular store of adenosine triphosphate (ATP) available to the glucose-phosphorylating enzymes. Furthermore, the inhibition of respiration does not depend upon the expenditure of ADP by phosphoglycerate kinase, not only because of the relatively higher affinity of the mitochondria for ADP but also because the inhibition is observed in the presence of iodoacetate.

Further evidence for the cause of the inhibition is provided by the fact that the addition of an amount of glucose smaller than the intracellular store results, nevertheless, in respiratory inhibition after a brief interval of oxygen uptake (Fig. 4*C*). Respiration may be started again by a second addition of glucose. In this case, the same intracellular store is depleted, for the total oxygen uptake due to the two additions of glucose is equal to that caused by the addition of one large excess of glucose. This reactivation of respiration by a further addition of glucose is of considerable significance in interpreting the control mechanism. Since ADP from the glucose-phosphorylating enzymes is the logical cause of the reactivation of respiration, it may be concluded that lack of ADP is the cause of the inhibition. It is further reasonable to conclude that when respiration is blocked about a minute after addition of an excess of glucose, lack of ADP is also the cause. Thus, increases and decreases of the intracellular concentration of ADP appear to be responsible for the increases and decreases of the respiratory rate caused by glucose addition.

Spectroscopic Response to Glucose

The preceding data suggest an ADP control of metabolism in the intact ascites tumor cell, and an incisive confirmation of this can be obtained from studies of the spectroscopic response of the mitochondria of the intact cell to the addition of glucose. One of the clearer records of this phenomenon is indicated by Fig. 5, in which both respiratory and spectrophotometric methods are shown in the top two traces. The former record shows the usual phenomenon of acceleration and inhibition of respiration about a minute after adding glucose. The spectroscopic trace shows an abrupt upward deflection corresponding to an oxidation of cytochrome *b* which persists during the activated phase of respiration and subsides as the respiratory activity subsides. This would appear to be the characteristic response of the respiratory chain to an increase of ADP concentration produced during the phosphorylation of glucose (see Fig. 3 at 430 mμ).

Two of the cytochrome components affected by glucose addition are suggested by studies of the difference spectrum obtained with a rapidly recording split-beam spectrophotometer. It is found that glucose addition causes the fused Soret bands of both cytochromes *b* and *c* to diminish in intensity. Studies similar to those of Fig. 5 verify that these absorbancy changes also occur in the visible region of the spectrum (562 and 550 mμ). In summary, reduced pyridine nucleotide, flavoprotein, cytochromes *b* and *c*, and in some cases cytochrome *a* are more oxidized during the glucose-activated phase of respiration.

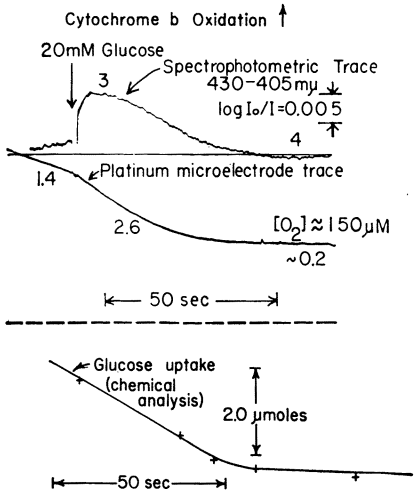

Fig. 5. Spectroscopic response accompanying acceleration and inhibition of respiration caused by glucose addition to a suspension of ascites tumor cells. The spectrophotometric trace is recorded with the double-beam spectrophotometer at 430 mμ (405 mμ as a reference). (The changes of respiratory rate are measured by the platinum microelectrode.) In a separate experiment, with a different suspension of ascites tumor cells, chemical analysis shows glucose uptake to proceed rapidly following the addition of glucose and to be inhibited about a minute after its addition. (Exp. 498b, 0-92.)

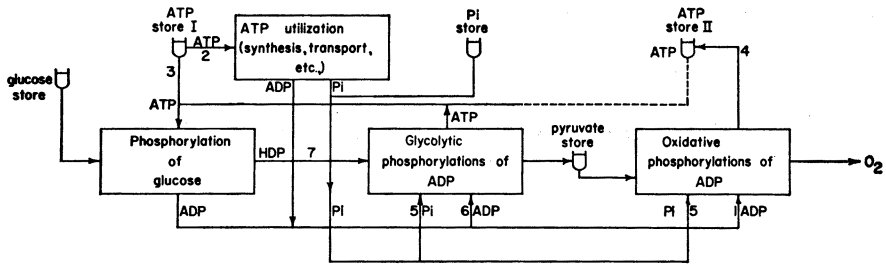

Fig. 6. Possibilities for metabolic control in ascites tumor cells: a schematic representation of interaction of glucose phosphorylating enzymes, glycolytic phosphorylations of ADP, and oxidative phosphorylations of ADP. In the interests of simplicity, many other interactions are arbitrarily omitted. The numbers refer to points at which control of metabolism may be exerted as follows: (1) Control of respiration by ADP concentration. (2) Control of ATP utilization by endogenous processes by the ATP concentration. (3) Control of the rate of glucose phosphorylation (either hexokinase or phosphohexokinase) by the ATP level. (4) A reservoir in which ATP formed in mitochondrial oxidative phosphorylation may be retained in such a way that it is not readily available for glucose phosphorylation. (5) Control of glycolytic or oxidative phosphorylation by the inorganic phosphate level. (6) Control of glycolytic phosphorylation by the ADP level. (7) Control of glycolytic phosphorylation by the substrate level (hexosediphosphate). (MD-65.)

These data identify a crossover point (*35*) above cytochrome *c* for the activation of the respiratory chain of the ascites tumor cell caused by glucose addition. This is in agreement with the experiments on isolated mitochondria where the same components were observed to be oxidized on ADP addition. In combination with the data of Fig. 4, we put forward strong evidence for the supposition that ADP produced in phosphorylation of glucose momentarily activates the mitochondria of the cell and allows these slowly respiring cells to show their latent respiratory activity.

Regulation of Metabolism

Some responses of the cell to a change of its environment involve metabolic regulations. A well-known example of such a response is the Pasteur effect, in which the change in environment may be from oxygen to nitrogen, and vice versa. The metabolic responses to these changes are manifestations of the underlying mechanisms. Some responses may give incisive information about the mechanism, others may not. The Pasteur response has been studied in detail for many years, but the lack of definitive results suggests that the consequences of initiation or cessation of oxygen metabolism are manifold and not easy to interpret. Initiation of glucose metabolism involving the Crabtree response appears to be more easily understood, and two new responses, described below, are found most useful in the study of mechanisms of metabolic control. Ascites tumor cell suspensions are found to give four types of responses, two of which clearly differ from the Pasteur and Crabtree effects; the third has a feature in addition to the Crabtree effect; and the fourth is a type of Pasteur reaction. These responses have been studied in detail in order to reveal the steps in the reaction mechanism that can control metabolism.

The sequences of chemical reactions involved in glucose and oxygen metabolism of the ascites tumor cell are schematically represented from the standpoint of possible pathways of metabolic control in Fig. 6. The Embden-Meyerhof sequence of glycolytic reactions is divided into two parts, "phosphorylations of glucose" and "glycolytic phosphorylations of ADP," to emphasize ATP utilization and synthesis. The Krebs cycle and the respiratory chain are lumped together as "oxidative phosphorylation of ADP" to emphasize ATP synthesis by these enzymes. The hexosemonophosphate shunt is not included because it does not exert a direct effect upon the ATP level, and TPNH formed in this pathway is not an essential intermediate in these regulations. The utilization of ATP by the cell for synthesis, transport, and other functions is indicated as a single function. Reserves of ATP, pyruvate, and glucose are relevant to the discussion that follows; other

substances are omitted for the sake of simplicity.

In cells freshly withdrawn from the mouse at about the sixth day of tumor growth, we find an endogenous respiration that is not greatly increased by pyruvate addition. Figure 6 indicates a "pyruvate store" in which are lumped the Krebs cycle substrates which are apparently in excess in the endogenous condition. Glucose is present in low concentrations. ATP is accumulated in a store available to hexokinase.

The first response to glucose addition is an activation of glucose phosphorylation, and the ADP formed thereby activates respiration (line 1, Fig. 6). In terms of the responses of isolated mitochondria to ADP addition, the state 4–3 transition occurs (*35*).

The second response occurs where the added glucose concentration is less than the store of ATP and consists of an inhibition of endogenous respiration after the added glucose has been expended in phosphorylation reactions. The excess ADP formed during glucose phosphorylation is also expended by the mitochondria, and respiration slackens because of lack of ADP—a typical state 3–4 transition in terms of the response of isolated mitochondria to a decrease of ADP concentration. The most striking feature of this second response is the decrease of respiratory rate to a level considerably below that characteristic of endogenous metabolism (see Fig. 4*C*).

A possible explanation for this inhibition is that the ATP utilization associated with endogenous metabolism has a low ATP affinity and a small decrease of the ATP store I (Fig. 6) is inhibitory. For example, the utilization of acetate has clearly been shown to be sensitive to dinitrophenol (*48*), and although it is not possible to identify the ATP-utilizing reactions involved here, a sensitivity to the ATP concentration is not unexpected.

The third response occurs in the presence of an excess of glucose and consists of an inhibition of both glucose utilization and respiration. In this case, the first response lasts longer, and accelerated respiration and glucose utilization continue for about a minute, depending upon the condition of the cells, that is, upon the amount of their ATP in store I (Fig. 6). When this ATP store has been expended, respiration and glucose utilization decrease very markedly (see Fig. 5). A slackening of respiration after glucose addition has been observed in long-term experiments by Crabtree (*15*). The very intense, short-term effects recorded here are clearly due to a lowering of the intracellular ADP level that leads to a state 3–4 transition and to a system in which ADP is rate-limiting for respiration. However, the long-term Crabtree effect need not be a consequence of the same metabolic control; it is not necessary that all metabolic controls be exercised by the same chemical.

The slackening of glucose metabolism in the presence of excess glucose (bottom trace, Fig. 5) is a novel part of the third response and requires special consideration. The above-mentioned reduction of the ATP store is unexpected because the ADP formed in glucose phosphorylation is largely rephosphorylated at ATP in the mitochondria (top trace, Fig. 5, and line 4, Fig. 6). The simplest hypothesis is that the ATP formed in the mitochondria is not directly available for glucose phosphorylation. It would appear that a compartment (see Lynen and Koenigsberger, *11*; see also *16, 21, 49*) or an equivalent system for the retention of this newly formed ATP (line 4, Fig. 6) may interrupt the flow of ATP back to the glucose-phosphorylating enzymes and thereby control glucose utilization. The unavailability of the ATP to glucose phosphorylation is apparently subject to change with time; we find that the inhibition diminishes (see also *20a, 50*) and that uncoupling agents abolish the inhibition as discussed below.

A fourth response is the release of inhibition of respiration and of glucose utilization by the addition of an uncoupling agent. Figures 4*A* and 4*B* clearly illustrate the reversal of respiratory inhibition, and separate chemical studies, similar to those of Fig. 5 (see *25*; see also *20, 20a*), show the activation of glucose utilization. The explanation of the respiratory effect is straightforward: the mitochondria no longer require ADP (or phosphate) for respiration in the presence of an uncoupling agent (lines 1 and 5 of Fig. 6 are not needed). The effect upon glucose utilization is a typical Pasteur response (rapid aerobic glycolysis in the presence of various uncoupling agents). A hypothesis that is consistent with an explanation of the three responses described above is that a redistribution of ATP occurs upon addition of the uncoupling agent. In this redistribution, more ATP becomes available for the phosphorylation of glucose and more ADP (and phosphate) becomes available to the glycolytic phosphorylations (see *11*).

While these four responses of the schematic representation of Fig. 6 are given here in qualitative terms, a complete representation of the kinetics of 26 chemical components in 17 equations has been accomplished by means of a special chemical kinetics program for Univac I (*51, 52*). These solutions, which will be discussed in detail elsewhere (*53*), show a quantitative correspondence to the four responses observed experimentally. Thus, the three features of this hypothesis—(i) an overriding ADP affinity at the mitochondria (*8–10, 26, 54*) which are under control of the ADP level (*44*), (ii) a compartmentalization (*11*) of newly phosphorylated ATP (*16*) which can be released by uncoupling agents or by anaerobiosis (*11, 20*), and (iii) a sensitivity of ATP utilization to the intracellular ATP level—appear to be adequate for an over-all description of our currently available experimental data. The agreement of the computer solutions and the experimental data does not of itself eliminate other hypotheses for metabolic control, and they are discussed below.

The possibility of a control of the activity of phosphohexokinase has been suggested from time to time. Recently Aisenberg and Potter have suggested that mitochondria exert this control (*55*), and their conclusion would appear to be applicable to the studies reported by Lynen (*2*). While it is apparently true that liver mitochondria, when mixed with the supernatant fluid from a brain homogenate, exert an inhibitory influence on glycolysis (*55*), the direct experiment on the ascites tumor cell indicates that the relationship of this enzyme to the mitochondria under physiological conditions is such that an inhibition is not noticeable. Prior to the addition of glucose under the experimental conditions in Fig. 5, the cells are in an excellent physiological state for the postulated inhibition to apply; they are respiring fairly actively, thus the concentration of any inhibitor (such as Y-phosphate) would be expected to be high (*55*). Nevertheless, added glucose is rapidly utilized in direct contradiction to the proposed inhibition mechanism. This experimental observation also makes it unlikely that the accumulation of glucose-6-phosphate is a cause of the inhibition of respiration, since, in this case, the second addition of glucose (Fig. 4*C*) would not accelerate respiration to the same extent as the first addition. Thus the explanation of ATP retention after its phosphorylation by the mitochondria, together with the decrease

of ATP available to hexokinase, is a hypothesis that is presently in agreement with the experimental data on the whole cell.

Ibsen, Coe, and McKee (*20a*) propose that Seikevitz and Potter's (*56*) hypothesis for ATP-AMP control of respiration applies here. However, the isolated mitochondria from the ascites tumor cell respond rapidly and directly to ADP alone, and liver mitochondria respond five times more rapidly to ADP than to AMP (*57*). In addition, the chemical data (*20a*) show responses of ADP that are much more appropriate to metabolic control by ADP than by AMP (see Fig. 8 in *20a*).

Chemical analysis of the changes of ATP and ADP during the first and third responses are in good qualitative agreement with the mechanism described (*20a, 25*). In the phase of activated metabolism the ATP level falls and the ADP level rises, and vice versa in the inhibited phase of metabolism. Phosphate falls somewhat in the activated phase due to the phosphorylative activity, but rises somewhat in the inhibited phase, in agreement with our conclusion that lack of phosphate does not control the respiratory rate at this time (*47*).

In the inhibited state of respiration after the third response, the levels of ATP, ADP, and inorganic phosphate as analyzed after disruption of the cell show average concentrations which are all in excess of the Michaelis constants for glucose phosphorylation or for respiration. Lynen (*2*) finds a related paradox in his chemical studies of the Pasteur effect in yeast cells. The response of the intracellular hexose phosphates to increased oxygen clearly indicates metabolic control in the glucose-phosphorylating enzymes, probably due to a decrease of ATP concentration, yet the chemical data show no measurable change of the average ATP concentration. Furthermore, chemical analyses of muscle in the resting state give results that are obviously inconsistent; the average ADP and phosphate levels are sufficient to give maximal respiratory activity (*58*), but the mitochondria are shown by spectroscopic observations of the respiratory carriers to be substantially free of phosphate or phosphate acceptor (*59*). It would appear, therefore, that chemical analysis without intracellular localization can at best be only suggestive of the concentration of a rate-limiting component and cannot be used to appraise metabolic responses involving a redistribution of nucleotides.

There have been two recent attempts to reconstruct the interactions indicated by Fig. 6 by mixtures of enzymes and mitochondria (*55, 60*). Such systems can be arranged to be dependent upon any one of the lines that interconnect the functions of Fig. 6 and are most useful for a demonstration of these properties of the system. However, two or more properties of the intact cell may be lacking. First, the retention of newly phosphorylated ATP may not occur; and second, the control of respiration in the mitochondria by the phosphate and phosphate acceptor level is a very labile effect which may be impaired by the conditions of the experiment. The remaining metabolic controls would be due to phosphate or phosphate acceptor at the level of substrate phosphorylations or to the substrate concentration itself at any point in the system. The fact that Wu and Racker (*60*) find inorganic phosphate (line 5, Fig. 6) and Aisenberg and Potter (*55*) find hexose diphosphate (line 7, Fig. 6) to be controlling under certain conditions in the reconstituted system is not unexpected.

In different types of intact cells, the integrated action of the components of the system of Fig. 6 may not be expected to involve identical controls. Yeast and ascites tumor cells which have been studied by these methods demonstrate quantitatively different responses. In yeast, respiratory changes attributable to the retention of ATP can readily be demonstrated, but are less marked, probably because the energy utilization by endogenous processes is less sensitive to changes in the ATP concentration. It is also possible that the control which ADP can exert over electron transfer in the mitochondria of the intact yeast cell is less marked than in mammalian cells, since isolation of yeast mitochondria in a state that demonstrates respiratory control has not yet been accomplished. In microorganisms, evidence for respiratory control in isolated mitochondria is also lacking. However, the Pasteur effect, for example, does not require a universal mechanism; cells without mitochondrial respiratory control would show aerobic inhibition of glucose uptake simply by retention of ATP (see Fig. 6). Such cells would not be expected to show a Crabtree effect, and so far this effect is found only in tumor cells. On the other hand such cells might employ the alternative pathways for metabolic control indicated by Fig. 6.

Since the mitochondria cannot be used as indicators of the ADP and phosphate levels in the anaerobic cell, this experimental approach affords no new information under these conditions, and anaerobic glycolysis may be controlled by the alternate pathways of Fig. 6.

Summary

The Pasteur and Crabtree effects demonstrate that changes at the beginning of the metabolic sequence for glucose metabolism give rise to effects at the end, and vice versa. We have presented here three additional responses of the ascites tumor cell suspensions, and presumably more will be uncovered. Each one of these responses is a manifestation of factors in the underlying mechanism that are in the nature of chemical feedback of a linear or nonlinear nature. The metabolic reactions are sufficiently complex that it is unlikely that any single component or step need control metabolism in different types of cells or under all conditions for a particular cell. However, it is due to a favorable circumstance that, in an appropriate type of cell and with the use of a direct intracellular indicator for changes in ADP concentration, we can state that the respiratory metabolism of the ascites tumor cell suspension, as freshly withdrawn from the mouse abdomen, is limited by the intracellular ADP concentration, and that this is why these cells show a predominance of glycolytic over respiratory activity. The response of the metabolism to small and large additions of glucose illustrates aspects of the metabolic mechanism which involve control of endogenous metabolism and compartmentalization of ATP formed in oxidative phosphorylation, the net result being a depression of the respiratory activity. The results of this approach emphasize the importance of chemical assays of localized portions of the living cell in its physiological state (*61*).

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News of Science

Science Advisory Committee's Recommendation for Science Council Being Implemented by Executive Order

Rapid progress is being made on the implementation of the proposal of the President's Science Advisory Committee for the establishment of a Federal Council for Science and Technology. Informed observers in Washington indicate that an executive order establishing the new council and giving its membership can be expected very soon. The interagency council will have responsibility for promoting coordinated science policy planning and more effective management of federal programs in science and technology. The recommendation for the council was made last December in the report "Strengthening American Science" issued by the President's Science Advisory Committee.

Major Problems Solved

At this writing, the executive order that will bring the council into existence is being reviewed by the Justice Department for any legal or jurisdictional problems that might have been overlooked by its framers in the executive department. Customarily, this review is the last step before an executive order is signed. Both the quality of the Advisory Committee's original report and the early solution of the thorny problem of council membership have contributed to the rapid progress of the work, according to various governmental sources. The membership problem offered one of the greatest difficulties. How many of the governmental agencies doing scientific work should be represented on the council? The committee report called for a membership of nine persons —the chairman and eight representatives from the various major governmental agencies doing scientific and technological work.

The agencies, which were selected primarily on the basis of their expenditures for scientific activity, were the National Science Foundation, the Atomic Energy Commission, the National Aeronautics and Space Administration, and the departments of Defense, Interior, Commerce, Agriculture, and Health, Education, and Welfare. The committee's recommendation was accepted, and representatives of these agencies will constitute the council. In the case of three agencies, the National Science Foundation, the Department of Defense, and NASA, the representatives are known. In order, they are Alan Waterman, Herbert York, and T. Keith Glennan. All but two of the remaining representatives are said to have been decided upon. It is expected that these persons will not be given new positions in their departments, but rather, they will be the existing secretary, one of the assistant secretaries, or a special assistant. In all cases the object is to have one man with general policy responsibility to represent effectively all the technical activities of his department. The position of Director of Defense Research and Engineering in the Defense Department exemplifies the type of representation the council needs. This position, now held by Herbert York, was defined recently by Secretary McElroy as the top research and development position in the Department of Defense.

A second problem that has been treated successfully by the Bureau of the Budget personnel working on the executive order is that of reconciliation of previous executive acts with the new one. Orders which gave the National Science Foundation authority to coordinate governmental scientific activity and which established the Interdepartmental