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Pathways of Intracellular Hydrogen Transport

Absence of enzymatic hydrogen-carrying systems is a factor in aerobic glycolysis of malignant tissue.

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The formation of lactic acid from carbohydrate sources in the presence of an ample supply of oxygen—that is, aerobic glycolysis—is one of the most general and distinctive metabolic features of malignant tissues (*1*). The various hypotheses proposed to explain the imbalances between glycolysis and respiration in malignant tissues have been critically reviewed by Kit and Griffin (*2*) and concern four aspects of carbohydrate metabolism; (i) the concentration and activity of the glycolytic enzymes; (ii) deficiencies in the mitochondrial electron transport chain; (iii) relative inability of the mitochondria to oxidize pyruvate by way of the citric acid cycle; and (iv) failure of the normal regulatory mechanisms that control glycolysis and respiration. There are, undoubtedly, differences between many normal and malignant tissues in these areas, but the following summarizing statement of Kit and Griffin is still correct: "Although the detailed mechanism of the cancer [metabolic] imbalance remains unknown, its reality is not questioned. In particular, the imbalance between respiration and glycolysis first emphasized by Warburg [*1*] remains the foundation stone of biochemical investigation in cancer."

The basic question is still unanswered: Why do most malignant tissues aerobically form appreciable quantities of lactate while most normal tissues do not? Both types of tissues qualitatively have the enzymatic equipment (lactic dehydrogenase) for the reduction of pyruvate to lactate, as well as the capability to metabolize pyruvate by way of the citric acid cycle.

This article (*3*) summarizes experimental data that support the suggestion of Weinhouse (*4*): "The extent to which pyruvic acid, a common intermediary in both respiration and glycolysis, competes for electrons held by the pyridine nucleotides with those factors that transport electrons to oxygen—namely, the flavoproteins and cytochromes—should be a crucial factor in determining the degree of aerobic glycolysis."

Diphosphopyridine Nucleotide in Glycolysis

A condensed version of the glycolytic pathway from glucose to pyruvate is presented in Fig. 1. Two aspects of glycolysis need to be emphasized.

1) Evidence both from cell-fractionation studies (*5*, *6*) and from studies of intact cells (*7*, *8*) support the generally accepted concept that the enzymes and cofactors involved in glycolysis are primarily located in the cell sap—that is, the soluble extramitochondrial portion of the cytoplasm—of mammalian cells. The implications of this separation of glycolysis from the terminal oxidative reactions of carbohydrate metabolism which occur in the mitochondria have been discussed by Lynen (*7*) and by Chance and Hess (*8*), as well as by others, and these authors have stressed the permeability barrier of the mitochondria as a major regulatory factor in cell metabolism.

Several investigators have reported that isolated mitochondria, primarily from brain (*9*, *10*) and from mouse melanoma (*11*, *12*), catalyze glycolysis. In most cases, however, only a small fraction of the total glycolytic activity remains with the mitochondria, even after a single washing (*9*, *11*). These results indicate that the enzymes and cofactors are either loosely bound or are contaminants of the mitochondria (see *13*). Irrespective of whether the enzymes (or perhaps only a few of the enzymes) are associated with the mitochondria or are strictly soluble in the cytoplasm of the intact cell, the evidence demonstrates (*8*) that the enzymes of glycolysis function with soluble extramitochondrial cofactors, particularly the coenzymes and adenine nucleotides.

2) In the glycolytic chain, a single oxidative reaction occurs—the oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglyceric acid. Diphosphopyridine nucleotide (DPN) is the obligatory acceptor of electrons in this reaction. Since DPN is present in only catalytic amounts in both normal and malignant mammalian cells (*14*), the presence of efficient mechanisms for the continuous oxidation of the reduced DPN (DPNH) generated is essential for the continued flow of carbon through this step and thus for the maintenance of glycolysis. The reduction of pyruvate to lactic acid by DPNH is one such mechanism, but the normal cell apparently must have other systems available which effectively compete with the lactic dehydrogenase reaction. Such systems in normal tissues, capable of competing with lactic dehydrogenase for the oxidation of extramitochondrial DPNH, are described later

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in this article, and the lack of these systems in malignant tissues is shown to favor the accumulation of lactic acid.

It should be emphasized, however, that the route of reoxidation of DPNH does not necessarily regulate glucose utilization in either normal or malignant tissues, since the activity of specific enzymes (for example, hexokinase) or the availability of phosphate (*6*) or adenosine di- and triphosphate (*8*) may control the rate of glycolysis.

Oxidation of DPNH by Mitochondria

Molecular oxygen is the ultimate acceptor of reducing equivalents from the oxidation of metabolic intermediates. The terminal transfer of reducing equivalents from substrates to oxygen is carried out within the mitochondria by various dehydrogenases (some of which are DPN-linked) in conjunction with the electron transport system. Mitochondrial oxidation of DPNH formed in the cell sap appears to be the most direct route for the transfer of reducing equivalents from the cell sap to oxygen. Lehninger (*15*), however, demonstrated that carefully isolated "intact" rat liver mitochondria do not oxidize added DPNH, and thus, extramitochondrial DPNH is presumably not a substrate for oxidation by way of the electron transport system. The inability to oxidize externally added DPNH is attributable to the impermeability of the mitochondrial membrane to this substrate, and is, in fact, a most sensitive criterion for the "intactness" of isolated liver mitochondria. Alteration of the mitochondrial membrane due to the isolation procedure, osmotic imbalances (*15*, *16*), uncoupling agents, ageing, and chemical (*17*, *18*) or mechanical (*19*) damage permits oxidation of external DPNH by mitochondria.

The observations of Lehninger with mitochondria from rat liver have been confirmed by other investigators (*16*) but have not been extended to mitochondrial preparations from many other tissues (*18*, *20*). The results of a survey of the ability of isolated mitochondria from various tissues to oxidize externally added DPNH are presented in Table 1 (*21*). The values presented for oxygen uptake were determined polarographically with a microplatinum electrode, and with mitochondrial concentrations that manifested a high rate of oxygen uptake with succinate as substrate. Similar results were observed spectrophotometrically by measuring the oxidation of DPNH at 340 millimicrons (*21*). With untreated mitochondria from normal and malignant tissues the rate of oxygen uptake was small, in many instances zero. When the mitochondrial structure was altered by pretreatment in a hypotonic medium, the rate of oxidation of DPNH was greatly increased, demonstrating that the respiratory mechanisms of these mitochondrial preparations were unimpaired.

Since all preparations, whether from normal or from malignant tissues, manifested a greater rate of DPNH oxidation after treatment in a hypotonic medium, it may be assumed that mitochondria from most tissues are impermeable to exogenous DPNH. The low rate of DPNH oxidation observed with most of the mitochondrial preparations before treatment indicates that only minimal damage to the mitochondria occurred during isolation. The relatively high rate of DPNH oxidation by "intact" heart mitochondria is probably a reflection of the lability of the mitochondrial membrane (*22*). Nevertheless, increased DPNH oxidation was observed after hypotonic treatment. Direct mitochondrial oxidation of external DPNH is, therefore, not available in most normal and malignant tissues, and the continual oxidation of DPNH necessary for maintenance of carbon flow through the glycolytic pathway must be catalyzed by other enzymatic systems.

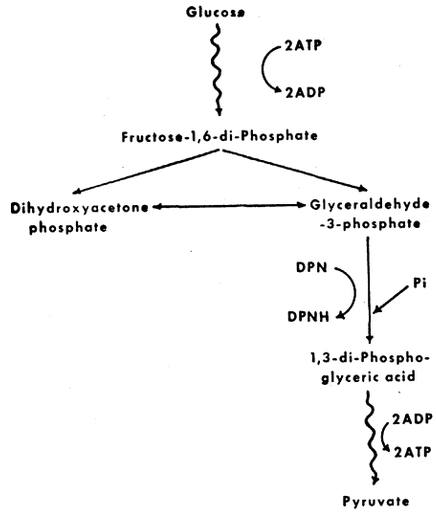

Fig. 1. A condensed version of the glycolytic pathway. *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *Pi*, inorganic phosphate; *DPN*, oxidized diphosphopyridine nucleotide; *DPNH*, reduced diphosphopyridine nucleotide.

Glycerophosphate "Shuttle"

Electrons can be carried from extramitochondrial DPNH to the intramitochondrial electron transport chain by indirect routes. In principle, any metabolite that can be reduced in the cell sap by DPNH to a product that is a substrate for mitochondrial oxidation can serve as a link in the transport of reducing equivalents across the mitochondrial membrane. To be effective, (i) such an acceptor must be formed continuously before or simultaneously with DPNH in carbohydrate metabolism, or (ii) the oxidized and reduced substrates must not be appreciably metabolized by the tissue, the compounds being thus permitted to function catalytically.

A system of the first type is the so-called "glycerophosphate cycle" or, preferably, "shuttle," which has recently been studied in detail in both normal and malignant tissues (*23–26*). Dihydroxyacetonephosphate and glyceraldehyde-3-phosphate are formed in equimolar amounts as the products of the action of aldolase on fructose-1,6-diphosphate. The very active triosephosphate isomerase establishes an equilibrium between these products which, by a ratio of 20 to 1, favors dihydroxyacetonephosphate, which can, in the presence of the soluble α-glycerophosphate dehydrogenase of the cell sap, accept electrons from DPNH to form L-α-glycerophosphate. Since DPNH is formed during the oxidation of glyceraldehydephosphate, the two enzymes, glyceraldehyde-3-phosphate and glycerophosphate dehydrogenase, form an effective dismutation system that leads to the continuous regeneration of DPN (*27*). Glycerophosphate also fulfills the second requirement for a system for intracellular hydrogen transport, since it is the substrate for an intramitochondrial enzyme that oxidizes glycerophosphate by way of the phosphorylating electron transport chain (*28*). Whereas the equilibrium of the soluble, DPN-linked glycerophosphate dehydrogenase greatly favors (by a ratio of 10^4 to 1) reduction of dihydroxyacetonephosphate, the equilibrium of the insoluble, intramitochondrial, flavin-linked glycerophosphate oxidase greatly favors oxidation of glycerophosphate. The alternate reduction of dihydroxyacetonephosphate and oxidation of glycerophosphate thus form an effective system for shuttling reducing equivalents from extramitochondrial DPNH to the intramitochondrial electron transport system.

In the metabolically very active musculature of a number of insects, the glycerophosphate "shuttle" provides a major route of hydrogen transport, particularly since these tissues have very low levels of lactic dehydrogenase, that can function as an extramitochondrial hydrogen-accepting system (*23*). The enzymatic equipment for this shuttle in normal mammalian tissues has been demonstrated for brain by Sacktor *et al.* (*29*), and for other tissues by Klingenberg and Slenczka (*30*). Ciaccio and Keller (*26*) demonstrated the dynamic functioning of this cycle with mitochondria isolated from the liver of hyperthyroid rats, which have exceptionally high glycerophosphate oxidase activity.

A corollary of the function of glycerophosphate dehydrogenase in intracellular hydrogen transport is that glycerophosphate should accumulate, together with lactic acid, as an end product of anaerobic carbohydrate metabolism. This has been demonstrated by Ciaccio *et al.* (*31*) for homogenates of normal tissues and for the anaerobic glycolysis of normal tissues *in situ*. Even under aerobic conditions, glycerophosphate is found in considerable concentration in most normal tissues (*32*), since the mitochondrial oxidase has a relatively low substrate affinity ($K_m \sim 1.0 \times 10^{-3} M$) and effective oxidation of glycerophosphate does not occur below this concentration.

Lack of Glycerophosphate "Shuttle" in Malignant Tissues

In most malignant tissues, glycerophosphate dehydrogenase activity, the component of this "shuttle" found in the cell sap, is either lacking or very low. Holzer *et al.* (*33*) first observed this lack in Ehrlich ascites and Yoshida hepatoma cells and stressed the significance of a change in the ratio of the two cytoplasmic hydrogen accepting systems, glycerophosphate dehydrogenase and lactic dehydrogenase. The ratios of activities of these enzymes (lactic to glycerophosphate dehydrogenase) in a variety of normal tissues of rodents (mice, rats, and hamsters), including regenerating liver and embryonic tissue, was found by Boxer and Shonk (*25*) to vary between 0.5 and 7.0 to 1, while this ratio ranged from 10 : 1 to several hundreds to 1 in a large series of tumors of rodents and human beings. The malignancies studied included some 30 transplanted tumors, four human tumors growing in cortisonized hamsters, virus-induced malignancies of mice, and carcinogen-induced tumors of rats.

Similar changes were observed by Delbrück *et al.* (*34*) in other animal tumors, and by Sacktor and Dick (*35*) in a comparison of bone marrow and spleen with cells from myelogenic and lymphatic leukemias. In general, ascites forms of rodent tumors do not contain any measurable glycerophosphate dehydrogenase activity, and this suggests the possibility that the residual activity of solid tumors rests in the supporting stroma rather than in the malignant cell itself. Only histochemical localization of the enzyme can clarify this point.

The change in the ratio of lactic to glycerophosphate dehydrogenase was due, in each instance, to a drastically lowered activity in glycerophosphate dehydrogenase, although the imbalance was in some instances accentuated by a moderate increase in lactic dehydrogenase. The low glycerophosphate dehydrogenase activity, or complete lack of such activity, in malignant tissues is not due to the presence of any free inhibitor, since the addition of tumor extracts did not inhibit glycerophosphate dehydrogenase activity in normal tissues (*25*). The mitochondrial counterpart to the soluble dehydrogenase of the cell sap, the flavin-linked glycerophosphate oxidase, was found to be present in mitochondria from all malignant tissues tested. As compared to the activity of succinic oxidase, the activity of this enzyme was either normal or somewhat higher than in normal tissues (*36*).

Changes in enzymatic activities, even drastic ones, do not necessarily lead to changes in metabolic pattern, particularly if the individual enzyme activities are greatly in excess of the activity required to maintain the observed rate of metabolite flow, as is the case in glycolysis (*6*). Ciaccio *et al.* (*31*), however, demonstrated that there is also a drastic change in the end products of anaerobic glycolysis of tumors. Not only is glycerophosphate lacking in these tissues, but glycerophosphate is not produced by homogenates or by glycolysis *in situ* of malignant tissues. Furthermore, with homogenates of tumors, glycerophosphate formation can be restored by the addition of crystalline dehydrogenase to the level found in normal tissue.

Two exceptions to the pattern described should be noted: Essentially normal ratios of lactic to glycerophosphate dehydrogenase were observed in the ascitic Ehrlich-Lettré tumor of the mouse and the Morris hepatoma 5123 of the rat (*37*). While the Ehrlich carcinoma in the ascites form is free of any measurable glycerophosphate dehydrogenase activity, the Ehrlich-Lettré mutant of this ascites tumor has essentially "normal" activity of the enzyme. For reasons which are not as yet understood, the Ehrlich-Lettré tumor does not form glycerophosphate on glycolysis in vitro or *in situ*. The Morris hepatoma 5123 has low activity for both enzymes but in an essentially normal ratio, and on anaerobic glycolysis in vitro, this tissue does form glycerophosphate. This hepatoma is apparently an exceptional tumor, since Woods (*38*) has observed that the rate of anaerobic lactic acid formation, measured manometrically, is in the same range as in liver and not as high as in most tumors. But both the Ehrlich-Lettré and the Morris hepatoma 5123 tumors show a striking abnormality in another system for intracellular transfer of reducing equivalents.

Acetoacetate-β-Hydroxybutyrate "Shuttle"

Another pathway for the transfer of reducing equivalents from extramitochondrial DPNH to molecular oxygen, involving acetoacetate and β-hydroxybutyrate, has been described by Devlin and Bedell (*39*). In manometric experiments, the presence of catalytic quantities of acetoacetate increased, four- to sixfold, the oxidation of DPNH by freshly isolated "intact" mitochondria from rat liver. As shown in Fig. 2*A*, a stimulatory effect of acetoacetate has also been observed when oxygen uptake is measured polarographically with a platinum electrode. The presence of DPNH did not increase the respiration above that recorded for the endogenous rate. When acetoacetate was added, the low rate of oxygen uptake remained unchanged for several minutes, at which time the rate was increased four- to fivefold. The dashed curve of Fig. 2*A* is the respiration in the presence of DPNH if acetoacetate is not added. With a duplicate sample measured spectrophotometrically at 340 millimicrons, the lack of oxidation of DPNH was confirmed. The addition of acetoacetate, however, initiated a rapid oxidation of DPNH, with no time

delay, such as that observed in Fig. 2*A* for oxygen uptake. The lag observed in measurements of respiration is apparently due to the fact that only when enough β-hydroxybutyrate has accumulated to be oxidized by the mitochondria is there an increase in respiration.

Catalytic quantities of D(-)-β-hydroxybutyrate were as effective as acetoacetate in stimulating DPNH oxidation, whereas the L(+) isomer was inactive, demonstrating that the reaction was catalyzed by the D(-)-β-hydroxybutyrate dehydrogenase (*40*). There was a concomitant increase in phosphate uptake with the increase in respiration, yielding ratios of phosphate uptake to oxygen consumption in the range of 1.5:1 to 2.4:1.

A similar stimulatory effect by acetoacetate was observed in a system where DPNH was continuously generated by an alcohol dehydrogenase system. Other mitochondrial substrates (malate, α-ketoglutarate, citrate, and so on) were without effect on DPNH oxidation (*39*), indicating that the increase in respiration observed when acetoacetate was added was not a nonspecific effect due to the presence of a substrate.

The interpretation that emerges is quite analogous to that given previously for the glycerophosphate "shuttle." Extramitochondrial DPNH reduces acetoacetate to D(-)-β-hydroxybutyrate, which is then oxidized intramitochondrially to acetoacetate by an intramitochondrial dehydrogenase coupled to the phosphorylating electron transport chain. There is a difficulty with this interpretation in that β-hydroxybutyrate dehydrogenase is a tightly bound mitochondrial enzyme and there is no known soluble counterpart to the mitochondrial enzyme in the cell sap (*41*). The results become understandable, however, if the presence of β-hydroxybutyrate dehydrogenase activity in two locations in the mitochondrion is assumed: (i) at a locus that permits reaction with substrate and DPN external to the mitochondrion, and (ii) at a locus permitting interaction with the internal electron transport chain. The latter activity is the well-established intramitochondrial DPN-linked D(-)-β-hydroxybutyrate dehydrogenase (*41*). The extramitochondrial activity of the dehydrogenase has also been demonstrated spectrophotometrically, as described above, where external DPNH did not react with the intramitochondrial electron transport system but was oxidized by acetoacetate. Studies of the stimulatory effect of added DPN and cytochrome *c* on β-hydroxybutyrate oxidation in the presence of the respiratory inhibitors Amytal and antimycin A also suggest the presence of two β-hydroxybutyrate dehydrogenase activities (*40*). Thus, the mitochondria apparently contain two different enzymes, spatially separated, reacting with the same substrate.

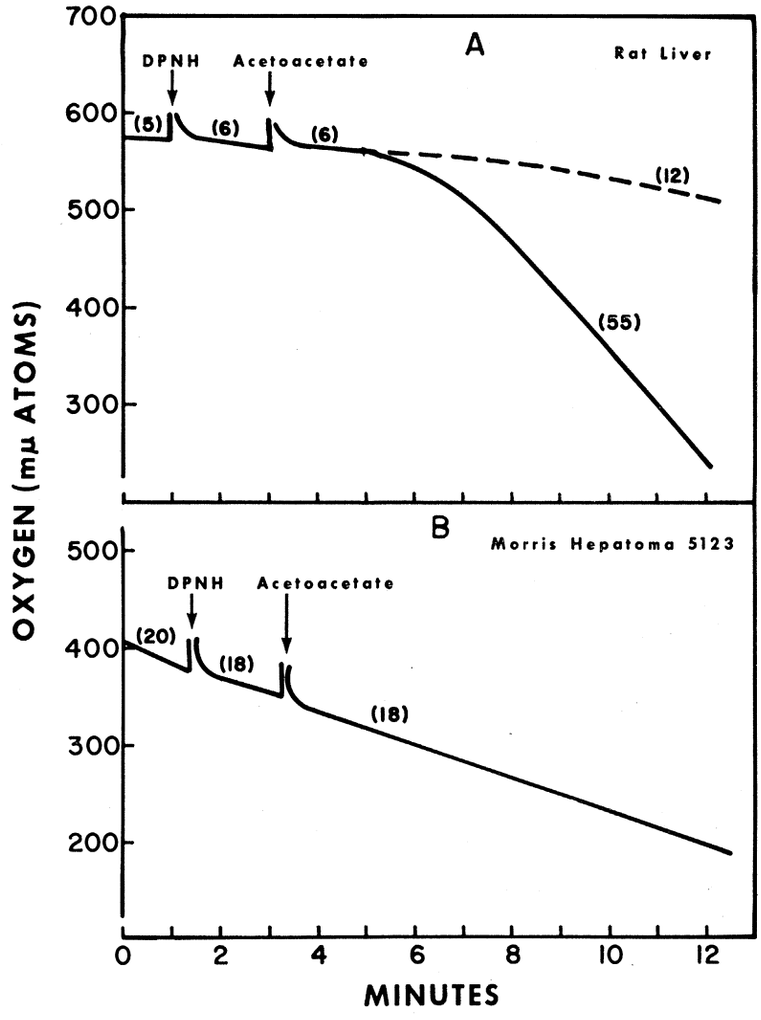

Fig. 2. Effect of acetoacetate on the oxidation of DPNH by mitochondria from rat liver and Morris hepatoma 5123. The incubation medium (1.5 ml), including KCl (0.10*M*), as in Table 1. Oxygen uptake was measured polarographically. At the times indicated, DPNH (0.5 μmole) and acetoacetate (1.5 μmole) were added. The figures in parentheses represent oxygen uptake in millimicroatoms per minute.

In a recent report, Krebs *et al.* (*42*) have suggested that in liver the activity of the acetoacetate "shuttle" would be adequate for the oxidation of DPNH generated in the cell sap by the glyceraldehyde-3-phosphate dehydrogenase.

Oxidation of DPNH by mitochondria from ten transplanted and two induced rodent tumors was not stimulated by the addition of catalytic quantities of either acetoacetate or β-hydroxybutyrate (*43*). This is illustrated in Fig. 2*B* for mitochondria from the Morris hepatoma. As noted above, the Morris hepatoma 5123 does have the enzymatic capability to catalyze the α-glycerophosphate "shuttle" but does not catalyze the acetoacetate pathway.

Whereas in the case of the glycerophosphate "shuttle" it was in all instances the cytoplasmic enzyme that was deleted in the neoplasm, the situation is more involved in the case of the acetoacetate "shuttle." Results indicate that either the "external" or the "internal" enzyme activity may be deleted, and that in some cases both activities are missing. The necessity for isolating fresh, well-preserved mitochondria, in sufficient quantity to permit detailed analysis of the enzyme deleted, makes the investigation of a large number of tumors difficult.

Other Pathways

The existence of other pathways to molecular oxygen for the oxidation of DPNH formed in the cell sap cannot be ruled out. As mentioned previously, any substance that can be reduced extramitochondrially by a pyridine-nucleotide-linked reaction and yields a product that is a substrate for mitochondrial oxidation can serve to transfer reducing equivalents. The potential importance of enzyme systems acting on the same substrates in the cell sap and the mitochondria has been stressed by Delbrück *et al.* (*44*), who proposed the term *enzymes of type III* for these systems. Among these, the malic and glutamic dehydrogenase systems are of particular interest as possible pathways. Both of these systems have been tested in normal tissues with the methodology described, but neither one functions as an intracellular hydrogen transport system.

Conover and Ernster (*45*) have proposed that the cytoplasmic nonspecific pyridine nucleotide diaphorase may serve in this capacity, the electron transfer being mediated by a quinone (vitamin K_3 or coenzyme *Q*). In a preliminary communication they reported that the oxidation by mitochondria of exogenous DPNH and reduced triphosphopyridine nucleotide is stimulated by the addition of purified diaphorase and vitamin K_3. The relationship of this system to the well-established stimulation of DPNH oxidation by cytochrome *c* and the extramitochondrial cytochrome *c* reductase has yet to be established. Kaplan *et al.* (*46*), have suggested that a specific DPN–DPN transhydrogenase may serve to link extramitochondrial DPNH with the intramitochondrial electron transport system. Stein *et al.* (*47*), however, have reported that the activity of the DPN–DPN transhydrogenase is increased by digitonin treatment of the mitochondria, a finding which indicates that the enzyme may be located within the mitochondrion and have a specific function intramitochondrially, as previ-

Table 1. Oxidation of DPNH by isolated mitochondria from various tissues. The incubation medium (1.5 ml) contained phosphate buffer (*p*H, 7.4; 10 m*M*); Tris buffer (*p*H, 7.4; 10 m*M*); $MgCl_2$ (6.9 m*M*); adenosine diphosphate (0.3 m*M*); and DPNH (1 μmole). With untreated mitochondria, KCl (0.10 *M*) was also added. Oxygen uptake was measured polarographically with a platinum electrode (temperature, 24°C). Untreated mitochondria were isolated and suspended in 0.25 *M* sucrose or 2.5-percent polyvinylpyrrolidone-0.25 *M* sucrose and used immediately. In experiments with hypotonically treated mitochondria, the preparation was suspended in water and allowed to stand for 20 to 30 minutes at 24°C.

Tissue	Oxygen uptake (μatom/hr per milligram of N)	
	Untreated mitochondria	Hypotonic treatment of mitochondria
Liver, rat	0	7.4
Kidney, rat	1.2	14.4
Heart, rat	11.6	39.0
Novikoff hepatoma, rat	0.6	8.0
Dunning hepatoma, rat	0	7.9
Morris hepatoma 5123, rat	0	5.6
Crabb sarcoma, hamster	0	4.0
Walker carcinosarcoma 256, rat	0.8	2.5
Mammary tumor, rat (3-methylcholanthrene-induced)	0.1	5.6
Ehrlich-Lettré ascites, mouse	0.4	9.2
Novikoff ascites, rat	0.8	10.1

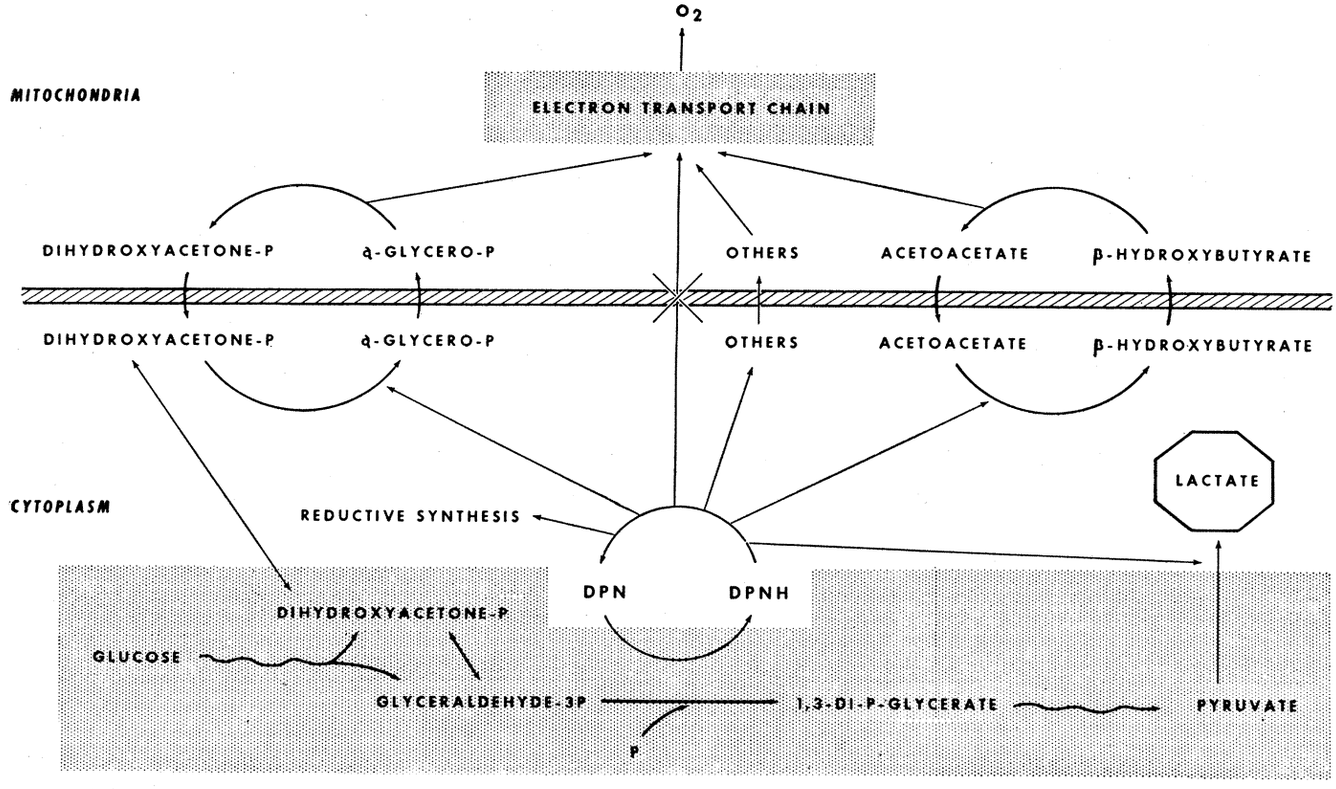

Fig. 3. Pathways of intracellular hydrogen transport.

ously proposed (*46*, *48*). In addition, the results in Table 1 also exclude the proposed extramitochondrial link.

Other intracellular hydrogen transport systems can be theoretically constructed from known enzymatic oxidation-reduction reactions and are under active investigation.

Oxidation of Cytoplasmic DPNH

In Fig. 3 are summarized the various pathways which are potentially available for the oxidation in normal tissues of DPNH in the cell sap. Figure 3 emphasizes the compartmentalization of various phases of carbohydrate metabolism within the cell, and the importance of intracellular hydrogen transport systems in regulation of aerobic lactic acid formation. The central role of DPN in the maintenance of the flow of carbon through the glycolytic path and the known transmission belts of electrons from the oxidation of glyceraldehyde-3-phosphate to the phosphorylating electron transport chain inside the mitochondria are indicated. The lack of direct reaction of DPNH with the mitochondrial oxidation system in either normal or malignant tissues is denoted by the intercepted direct (vertical) arrow.

It should be emphasized that the relative importance of any one of the pathways presented in the electron flow has yet to be determined for each specific normal tissue. It is quite conceivable that a given tissue may have only one pathway of importance, the other being of low activity or nonexistent. An absolute determination of the contribution of each of these systems will require techniques not available at this time.

Irrespective of the existence of "other" pathways, at least three routes by which extramitochondrial DPNH can be oxidized are available to cells from normal tissues: (i) the glycerophosphate system, (ii) the acetoacetate system, and (iii) the reduction of pyruvate to lactate. Since at least two of the three pathways are not available to malignant cells, the reduction of pyruvate to lactate would become a metabolic necessity for the malignant cell in resupplying DPN for steady-state glycolysis. Under anaerobiosis the first two pathways are cut off in the normal cell, and it too forms lactic acid, but on recovery, the reoxidation of lactic acid is possible, since mitochondrial systems are open for regeneration of DPN.

The reduction of pyruvate to lactate is not the only cytoplasmic anaerobic system that can utilize DPNH. A number of reactions in fat, amino acid, and nucleotide synthesis require reducing equivalents. It is of considerable interest that the malignant cell is poised in a metabolic equilibrium that is favorable to reductive synthesis and that could, therefore, provide the metabolic climate for one of the most striking pathological features of the malignant cell—the potential for growth. Many of the synthetic reductive reactions are, however, TPN-linked rather than DPN-linked. An effective DPN–TPN transhydrogenase enzyme has been described by Kaplan *et al.* (*46*), but it is an intramitochondrial enzyme and, furthermore, has been reported to be deleted in at least one tumor (*49*). The effectiveness of the steroid-coupled transhydrogenase systems that have been described by Talalay and Williams-Ashman (*50*), as well as by others, remain to be investigated in malignant tissues.

Consequence of Altered Pathway

The failure of malignant tissues to oxidize DPNH by way of one of the described "shuttles" to the phosphorylating electron transport chain entails the loss of at least two high-energy phosphate bonds available from the glycolytic system. This loss is, however, of minor importance as compared to the energy potential lost when pyruvate becomes an obligatory electron acceptor, since each mole of lactic acid formed withdraws one mole of a three-carbon unit from end oxidation, and thus 15 mole equivalents of high-energy phosphate are lost.

Lactic acid is one of the rare dead ends in a mammalian metabolic path, since it is not directly oxidized by the mitochondria, is not aminated or decarboxylated, and the only way for the three carbons to return to the main stream of metabolic events is by reoxidation to pyruvic acid by way of the DPN-dependent lactic dehydrogenase in the cell sap. This route, of course, requires systems for the effective reoxidation of DPNH which are limiting in the malignant cell.

As noted earlier, there is no indication that the enzymes of the citric acid cycle, or the condensing enzyme in particular, are lacking in tumors (*51*), although the number of mitochondria per cell is usually smaller than in normal tissues (*2*). In spite of the presence of the enzymes required for oxidation of pyruvate, Busch (*52*) observed that injected labeled pyruvate (2-C^{14}) was primarily converted to lactic acid by tumor tissue, in contrast to findings in a number of normal tissues. In experiments with tissue slices, the percentage of added pyruvate that was converted to lactate increased in the presence of added glucose in tumors but not in normal tissues (*53*). This again would be the expected result if increased flow through the glycolytic path requires increased oxidation of DPNH, for such oxidation can be achieved in normal tissues by way of aerobic pathways, but in the malignant tissue it has to be accomplished primarily by reduction of pyruvate to lactate.

Inhibition of lactic dehydrogenase formation has been proposed in cancer chemotherapy, primarily on the basis that lactic acid formation is a characteristic feature of malignant cells (*54*). The reduction of pyruvate to lactate is, however, a metabolic *necessity* for the malignant cell, as indicated by the data here summarized, whereas in the normal cell it is only one of at least three pathways available under aerobic conditions (Fig. 3). It would seem to be a reasonable working hypothesis that effective inhibition of lactic dehydrogenase would embarrass the normal cell only to a limited degree but would have major metabolic consequences in malignant cells.

Finally, the data presented have some bearing on the question of whether aerobic lactic acid formation is the cause or the effect of the malignant transformation. It is the deletion of enzymes, in the sense proposed by Potter (*55*), that leads to the failure of hydrogen transport within the internal structure of the malignant cell. If, therefore, it is the genetic information for the formation or control of synthesis of some of the enzymes involved in intracellular hydrogen transport that is deleted or altered, it would follow that aerobic lactic acid formation is the effect of a change—for whatever reason—in the chromosomal structure of the malignant cell.

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Cause and Effect in Biology

Kinds of causes, predictability, and teleology are viewed by a practicing biologist.

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Being a practicing biologist I feel that I cannot attempt the kind of analysis of cause and effect in biological phenomena that a logician would undertake. I would instead like to concentrate on the special difficulties presented by the classical concept of causality in biology. From the first attempts to achieve a unitary concept of cause, the student of causality has been bedeviled by these difficulties. Descartes's grossly mechanistic interpretation of life, and the logical extreme to which his ideas were carried by Holbach and de la Mettrie, inevitably provoked a reaction leading to vitalistic theories which have been in vogue, off and on, to the present day. I have only to mention names like Driesch (entelechy), Bergson (élan vital), and Lecomte du Noüy, among the more prominent authors of the recent past. Though these authors may differ in particulars, they all agree in claiming that living beings and life processes cannot be causally explained in terms of physical and chemical phenomena. It is our task to ask whether this assertion is justified, and if we answer this question with "no," to determine the source of the misunderstanding.

Causality, no matter how it is defined in terms of logic, is believed to contain three elements: (i) an explanation of past events ("a posteriori causality"); (ii) prediction of future events; and (iii) interpretation of teleological—that is, "goal-directed"—phenomena.

The three aspects of causality (explanation, prediction, and teleology) must be the cardinal points in any discussion of causality and were quite rightly singled out as such by Nagel (*1*). Biology can make a significant contribution to all three of them. But before I can discuss this contribution in detail, I must say a few words about biology as a science.

Biology

The word *biology* suggests a uniform and unified science. Yet recent developments have made it increasingly clear that biology is a most complex area—indeed, that the word *biology* is a label for two largely separate fields which differ greatly in method, *Fragestellung*, and basic concepts. As soon as one goes beyond the level of purely descriptive structural biology, one finds two very different areas, which may be designated functional biology and evolutionary biology. To be sure, the two fields have many points of contact and overlap. Any biologist working in one of these fields must have a knowledge and appreciation of the other field if he wants

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