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SOURCES OF REDUCING EQUIVALENTS FOR CYTOCHROME P-450 MITOCHONDRIAL STEROID HYDROXYLATIONS IN RAT ADRENAL CORTEX CELLS

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SUMMARY

Pyruvate-supported 11β-hydroxylation of 11-deoxycorticosterone (DOC) *via* cytochrome P-450 reductase chain in incubated rat adrenal mitochondria was maximal when either traces of oxaloacetate (OAA) or 2 mM ATP were added to the incubation medium. This showed that reducing equivalents formed at the pyruvate dehydrogenase level as well as those derived from Krebs-cycle activity were needed for maximal corticosterone (B) formation from DOC. These findings were confirmed in experiments with whole cells isolated from rat adrenals designed to show the possible pathways of intramitochondrial NADPH generation for steroid hydroxylations. Whereas citrate, isocitrate, succinate and malate were not metabolized because of their impermeability to the plasma membranes of the cells, both [1-^{14}C] and [2-^{14}C]-pyruvate were efficiently utilized by the mitochondria of the cells for B formation from DOC. Whereas arsenite completely inhibited the pyruvate-^{14}C supported B formation, comparison of the data obtained with two inhibitors, 2,4-dinitrophenol (2,4-DNP) and fluorocitrate, showed that for the same per cent inhibition of $^{14}CO_2$ production the inhibition of 11β-hydroxylation of DOC was greater with 2,4-DNP than with fluorocitrate. It is concluded that operation of the Krebs-cycle is essential for optimizing the production of reducing equivalents needed for 11β-hydroxylation to occur. It is also concluded that the rate of oxidation of pyruvate in the mitochondria of the cells and the activity of the Krebs-cycle is dependent on availability of OAA. When ATP levels were reduced by the uncoupling effect of 2,4-DNP resulting in a decreased production of OAA *via* ATP-requiring pyruvate carboxylase, a concomitant inhibition in production of reducing equivalent occurred which led to a lack of B production from DOC.

Mitochondria isolated from the steroid-producing Snell adrenocortical carcinoma 494 cells (P_2T) were unable to oxidize pyruvate and several Krebs-cycle substrates. Succinate and α-glycerol phosphate which both were oxidized *via* their respective P_2T flavoprotein linked enzymes, supported high rates of O_2 uptake but had little effect on DOC conversion into B. Electron micrographs of P_2T when compared to P_2C from normal rat adrenals showed that their ultrastructure was markedly altered. The number of mitochondria per cell in the tumor tissue was also considerably lower than that found in normal adrenal cells. Many of the P_2T were elongated, had lamillar-shaped cristae and showed a much less uniformed shape than P_2C which are usually oval and contain packed vesicular cristae. Adrenodoxin reductase activity, cytochrome P-450 and NADP nucleotide levels in P_2T were also lower than those in P_2C which partly accounted for the low conversion of DOC into corticosterone.

Because (1) mitochondrial flavoprotein-linked α-glycerol phosphate dehydrogenase activity in P_2T was 10 times higher than that found in P_2C, (2) an active NAD-linked α-glycerol phosphate dehydrogenase in the cytosol of the carcinoma cell was found and (3) pyruvate is not utilized by P_2T or whole cancer cells, this suggested the possibility of an α-glycerol phosphate shuttle operating in the tumor cells. The purpose of the shuttle might be to provide some of the cellular ATP required in this rapidly growing cancer tissue.

The function of cytochrome P-450 is well established in a number of steroid hydroxylations. Most, if not all, of the steroid hydroxylations involved in the biosynthesis of steroid hormones are known to require NADPH. Some of these reactions, namely the steroid hydroxylations in rat adrenal mitochondria, have been the subject of investigation in our laboratory for a number of years. Since these mitochondria are not permeable to reduce pyridine nucleotides, it is unlikely that NADPH generated in the cytosol could be used directly to support mitochondrial hydroxylations. Therefore, we have sought mechanisms by which NADPH can be produced in the mitochondria.

Figure 1 shows most of the possible pathways of NADPH generation in the mitochondria. Oxidation of isocitrate *via* NADP-linked isocitrate dehydrogenase would yield NADPH by a one step mechanism. On the other hand oxidation of pyruvate, α-ketoglutarate and malate *via* the respective NAD-linked dehydrogenases would yield NADH which could subsequently yield NADPH *via* the energy-linked transhydrogenase [1]. Oxidation of palmitoyl CoA and succinate takes place *via* flavin-linked enzymes and, therefore, NADPH would be produced only after two energy requiring steps; one, reversal of electron transport from CoQ to NAD and, second, the transhydrogenase reaction mentioned above.

In the *in vitro* studies, large amplitude swelling of the mitochondria by high levels of calcium allows permeation of exogenously added NADPH which can support steroid hydroxylations by the cytochrome P-450 reductase chain in the mitochondria.

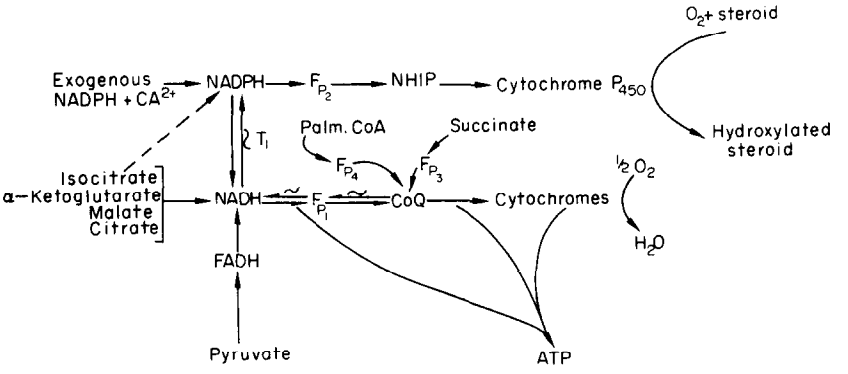

Fig. 1. Multicomponent mitochondrial system involving respiratory enzymes and an energy-linked pyridine nucleotide transhydrogenase (T_1) for producing reducing equivalents (NADPH) required for steroid hydroxylations in the rat adrenal. Fp_2 and NHIP are the flavoprotein adrenodoxin reductase, and non-heme iron protein (adrenodoxin) respectively.

Another mechanism for intramitochondrial NADPH production is the malate shuttle proposed by Simpson and Estabrook[2–4, 7]. Briefly, pyruvate in the cytosol is converted to malate by reductive carboxylation *via* $NADP^+$-linked malic enzyme. Malate then permeates into the mitochondria and is converted back to pyruvate, *via* the mitochondrial malic enzyme, thereby, generating NADPH in the mitochondria. Pyruvate, formed in the mitochondria, is released into the cytosol and the pyruvate-malate cycle can continue as long as there is sufficient NADPH in the cytosol.

Experiments with isolated rat adrenal mitochondria

A few years ago we showed that pyruvate was oxidized by the mitochondria and also supported 11β-hydroxylation of DOC [5]. Pyruvate supported respiration as well as 11β-hydroxylation was found to be stimulated by additions of small amounts of several Krebs cycle intermediates. Thus, it appeared that the small amounts of Krebs cycle intermediates which by themselves were unable to support respiration or 11β-hydroxylation, were necessary to supply a "priming" event leading to efficient utilization of pyruvate in the mitochondria. This priming event was visualized as the formation of OAA and/or ATP (see Table 1). More recently, Simpson and Boyd have confirmed that the rate of oxidation of pyruvate in the mitochondria is indeed dependent on the availability of oxaloacetate [6].

Table 1. Effect of varying concentrations of oxaloacetate and ATP on pyruvate-supported 11β-hydroxylation of DOC into corticosterone in rat adrenal mitochondria

Additions	None	OAA 50 μM*	ATP_2 mM
Pyruvate 100 μM	19.6	19.3	27.9
" 500 μM	20.9	40.5	40.5
" 1 mM	19.4	36.5	43.9
" 2 mM	17.4	31.5	40.5

Incubation conditions were those published previously (5,8). OAA = oxaloacetate. *Increasing OAA to 400 μM concentrations had no additional effect than at 50 μM. 800 μM OAA was inhibitory at all pyruvate concentrations. Values are in μg corticosterone produced from DOC in 10 minutes per 1.00 mg mitochondrial protein.

In the bovine adrenal mitochondria, arsenite was found to have little effect on the oxidation of malate as well as 11β-hydroxylation [3]. However, in rat adrenal mitochondria this inhibitor almost completely abolished malate or pyruvate supported 11β-hydroxylation of DOC [6]. The pyridine nucleotide steady states in mitochondria incubated with malate and measured with the Eppendorf fluorometer also showed a return to the oxidized state after arsenite additions. This happened regardless of whether DOC was present in the system and could not have occurred had malate been oxidized by the mitochondrial $NADP^+$-linked malic enzyme. In the latter case all $NADP^+$ and more than likely most of the intramitochondrial NAD^+ would have been kept in the reduced states. The inhibition of malate-supported 11β-hydroxylation by arsenite was not due to an inhibition of cytochrome P-450 reductase chain because it had no effect on the NADPH-supported 11β-hydroxylations [5].

If the malate shuttle was functioning in the rat adrenal, incubations of mitochondria with malate and arsenite should have led to pyruvate accumulation in the medium in accordance with the observations on beef adrenal mitochondria [3]. However, little pyruvate was found to accumulate in the incubation medium [5]. Other strong lines of evidence indicating lack of a malate shuttle in the rat adrenal were derived from studies carried out with respiratory chain inhibitors like KCN, antimycin A and Amytal. In all cases these substances as well as high levels of oxaloacetate strongly inhibited 11β-hydroxylation of DOC and O_2 uptake supported by malate. These results also indicated that NADPH required for steroid hydroxylations in the malate and other incubations carried out with Krebs-cycle intermediates was made available by the energy-linked transhydrogenase reaction as well as by isocitrate oxidation which is known to give rise to NADPH production in rat adrenal mitochondria [8]. Finally it is also clear from Figs. 2 and 3 derived from data of analyses of

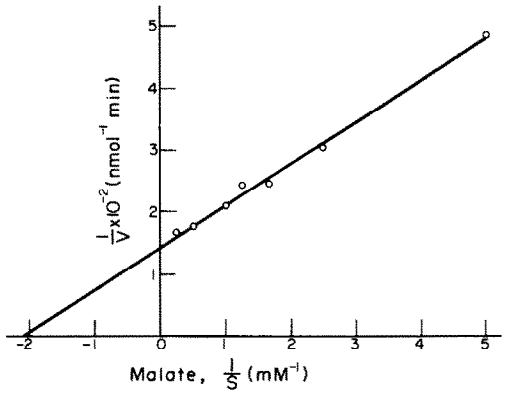

Fig. 2. Lineweaver–Burke plot of values obtained in the enzymatic assay of cytosol $NADP^+$-linked malic enzyme using malate as the variable substrate. Apparent K_m for malate was 0·50 mM. Assays carried out at room temperature in 50 mM-Tris–HCl pH 7·4, 5 mM Mn^{2+}, 0·2 mM $NADP^+$ and 0·05 ml 103,000 *g* rat adrenal homogenate supernatant (0·33 mg protein) in a final vol. of 3 ml.

the activity of cytosol $NADP^+$-linked "malic" enzyme (L-malate:NADP oxidoreductase (decarboxylating) E.C. 1.1.1.40) that malate would be more likely to be converted to pyruvate than the latter into malate. Additional analyses of mitochondrial NAD^+-linked malate dehydrogenase (L-malate:NAD oxidoreductase E.C. 1.1.1.37) showed a far greater activity of this enzyme than that found for mitochondrial $NADP^+$-linked malic enzyme (E.C. 1.1.1.40) (V_{max} for E.C. 1.1.37 with malate as substrate was 3,420 nmol/min/mg mitochondrial protein, while it was only 1·32 for the malic enzyme). These results, combined with those discussed above strongly suggest that the malate shuttle is probably non-functional in the rat adrenal cortex cell.

Experiments with isolated rat adrenal cells

Experiments carried out with isolated rat adrenal cells, prepared by collagenase-trypsin treatment of adrenal sections [9, 10] confirmed our earlier observations, on the effects of arsenite, with mitochondria and adrenal sections [11]. Thus, arsenite not only prevented the utilization of pyruvate but also abolished both the 11β-hydroxylation of DOC [5] as well as ACTH-stimulated steroidogenesis from endogenous precursors [11].

Using pyruvate labeled in position 1 or 2 and DOC as the steroid substrate we were able to obtain estimates of Krebs cycle activity as well as pyruvate dehydrogenase activity in relation to 11β-hydroxylation of DOC in the intact cells. The data showed that pyruvate dehydrogenase was quite active in these cells and did provide carbon for the operation of the Krebs cycle. For every mole of corticosterone formed from DOC, the $^{14}CO_2$ evolved from [1-^{14}C]-pyruvate and [2-^{14}C]-pyruvate was 1·9 (1·6–2·5) and 0·8 (0·7–1·0) mol respectively [12].

The close relationship of the Krebs cycle activity to 11β-hydroxylation of DOC suggested that NADPH production in the mitochondria may be dependent on the former. Indeed, fluorocitrate which effectively blocked the production of $^{14}CO_2$ from [2-^{14}C]-pyruvate (Krebs cycle activity), also produced inhibition of 11β-hydroxylation of DOC. The decrease in the Krebs cycle activity was not due to inhibition of pyruvate dehydrogenase because the inhibition of $^{14}CO_2$ production from [1-^{14}C]-pyruvate was much less compared to that from [2-^{14}C]-pyruvate.

The Krebs cycle in rat adrenal mitochondria produces both NADPH and NADH; the former by the oxidation of isocitrate *via* $NADP^+$-linked isocitrate dehydrogenase [8] and the latter by the oxidation of malate and α-ketoglutarate *via* NAD^+-linked dehydrogenases. NADH can further give rise to NADPH *via* the energy-linked transhydrogenase. The experiments with fluorocitrate cited above did not reveal the relative importance of (i) $NADP^+$-linked isocitrate dehydrogenase and (ii) the NAD^+-linked dehydrogenases in the production of NADPH. If (ii) was the major pathway for the formation of NADPH, then a reduction in ATP production would be expected to result in a decrease in the 11β-hydroxylation of DOC. Indeed, when phosphorylation was uncoupled from respiration by 2,4-dinitrophenol (2,4-DNP), there was also inhibition of corticosterone production from DOC. However, the uncoupler also inhibited the production of $^{14}CO_2$ from [2-^{14}C]-pyruvate. The inhibition of Krebs cycle activity by 2,4-DNP was an unexpected finding because this compound is known to stimulate respiration and generally the rate of substrate oxidation which is controlled by the rate of electron transport. The most plausible explanation for the inhibition of Krebs cycle activity by 2,4-DNP we can offer at this time is based on the evidence suggesting that the rate of oxidation of pyruvate in the mitochondria, and perhaps in the activity of the Krebs cycle, is dependent on the availability of oxaloacetate [5,6 and Table 1]. Thus, as a consequence of reduction in the ATP levels in the presence of 2,4-DNP there would be a decrease in

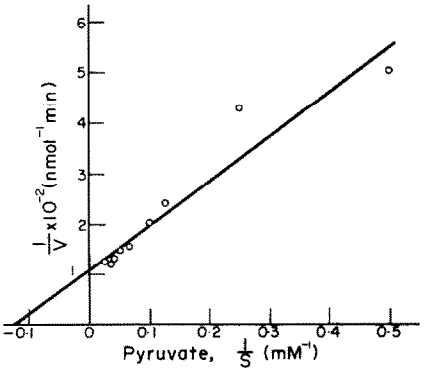

Fig. 3. Similar plot values as in Fig. 2 except that pyruvate was the variable substrate. In addition, the assays were carried out with NADPH (0·27 mM) instead of $NADP^+$. $KHCO_3$ saturated with CO_2 was also added to the assay buffer solution (final concentration 40 mM). Apparent K_m for pyruvate was 8 mM.

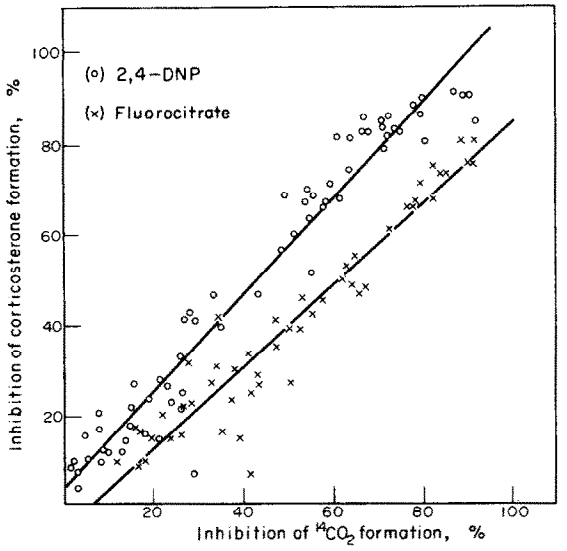

Fig. 4. Correlation between Krebs cycle activity and the 11β-hydroxylation of DOC. The points were obtained in several experiments with different concentrations of 2,4-dinitrophenol [O] or fluorocitrate [×]. In all experiments, concentration of pyruvate-2-^{14}C was 5 mM and that of DOC was 180 μM (from Lin *et al.*[12]).

the production of OAA via the enzyme pyruvate carboxylase and decreased production of oxaloacetate could result in an inhibition of the Krebs cycle activity.

A close examination of the data obtained with fluorocitrate and 2,4-DNP brings out an important fact. Results from several experiments with the two inhibitors are summarized in Fig. 4, where percentage inhibition of Krebs cycle activity is plotted against the percentage inhibition of 11β-hydroxylation of DOC. Comparison of the slopes and intercepts of the two lines showed that they were significantly different from each other ($P < 0{\cdot}01$). It is also clear that for the same percentage inhibition of the Krebs cycle activity, the percentage inhibition of 11β-hydroxylation with 2,4-DNP is greater than that with fluorocitrate. The data indicates the relative importance of the transhydrogenase pathway for the production of intramitochondrial NADPH for 11β-hydroxylation of DOC. It also shows that operation of the Krebs cycle is important for 11β-hydroxylation primarily because the NADPH produced in the cycle provides both the substrate and ATP (*via* the respiratory chain) for the energy-linked transhydrogenase.

Experiments with Snell adrenocortical carcinoma 494

Several laboratories have studied the biosynthetic potential of the adronocortical carcinoma 494 first described by Snell and Stewart in 1959 [13]. Ney and coworkers [14] showed that the tumor was able to utilize exogenous steroid substrates for production of corticosteroids although at a much lower rate compared to tissue from normal rat adrenals. In addition, the steroidogenic action of ACTH was not manifested in the tumor tissue [14] although Schorr and Ney [15] demonstrated that this trophic hormone was capable of initiating the production of fairly large amounts of cyclic AMP. Because cyclic AMP failed to stimulate corticosteroidogenesis, *in vitro*, it was concluded that reduced steroidogenesis in the tumor was primarily due to a defect beyond the formation of cyclic AMP [14].

In the initial studies carried out in our laboratory [16] it became evident as Sharma *et al.* had shown [17] that the ultrastructure of the carcinoma tissue differed from the normal adrenal cortex mainly in the mitochondria. Instead of being circular or oval in shape and containing packed vesicular cristae as mitochondria in normal rat adrenal cortex cells, these were elongated and much less uniform in shape. In addition, the mitochondria contained sparse lamellar cristae reminiscent of liver mitochondria or those visualized in adrenal cells obtained from hypophysectomized rats [18].

The observations on the ultrastructure and the fact that the carcinoma cells had a very low potential to convert DOC or other steroid substrates into corticosterone, indicated that major defect(s) existed in mitochondrial activity. Consequently, comparative studies were undertaken with mitochondria isolated from normal rat adrenals (P_2C) and carcinoma tissue (P_2T).

Succinate was found to sustain a good rate of respiration in both mitochondrial preparations. Succinate-supported 11β-hydroxylation of DOC in P_2T was, however, negligible compared to that found in P_2C (Fig. 5). Experiments carried out with other Krebs-cycle intermediates besides succinate, pyruvate or palmitoyl CoA^+ carnitine, showed that these substances were unable to sustain respiration in P_2T although they had their usual effects in supporting respiration and 11β-hydroxylation of DOC in P_2C. Exogenous NADPH, with swelling amounts of calcium, also did not support corticosterone production from DOC or respiration. On the other hand, α-glycerol-3-phosphate stimulated respiration in P_2T to a much greater extent than in P_2C (Fig. 6). As shown in Table 2 this substrate was oxidized *via* the mitochondrial flavoprotein linked α-glycerol phosphate dehydrogenase (α-DPD_m) and coenzyme Q. As in succinate experiments (not shown), Antimycin A almost completely inhibitied respiration supported by α-glycerol phosphate in P_2T.

A comparison of several mitochondrial components and enzymes in the normal adrenal and the carcinoma tissue is shown in Table 2. The concentration of cytochrome P-450 was very low in P_2T as compared to P_2C. On the other hand, the concentration of NAD^+ + NADH in P_2T was about half and that of NADP + NADPH about one-fourth of that in P_2C. Although we have no data on non-heme iron levels (adrenodoxin, NHIP) these results, combined with the finding that adrenodoxin reductase had about the same activity in P_2T as in P_2C indicated that poor steroid 11β-hydroxylation in P_2T may have been due to the low levels of cytochrome P-450. However, the fact that there are substantial amounts of

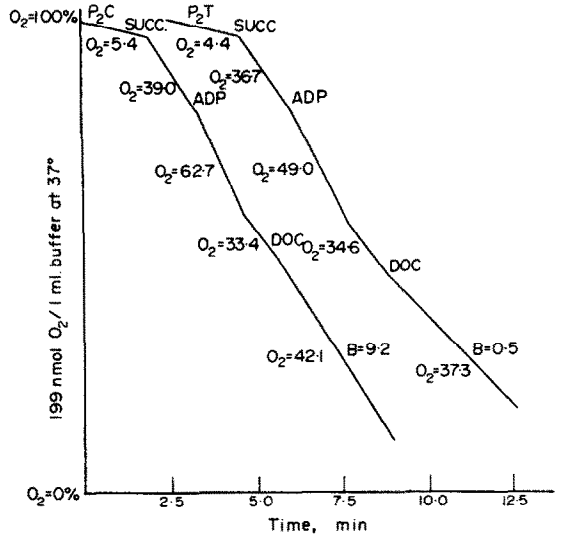

Fig. 5. Traces of O_2 uptake by mitochondria of normal adrenal tissue (P_2C) and by mitochondria obtained from the Snell adrenocortical carcinoma 494 (P_2T). Incubations of the mitochondria at 37°C were carried out as before [8] in 1·00 ml of a buffer medium, pH 7·4, having a final concentration of 14 mM Tris–HCl, 250 mM sucrose, 55 mM nicotinamide, 16 mM KCl, 16 mM NaCl, 1·5 mM P_i and 0·15% bovine serum albumin. Incubations were initiated at arrows by addition of 0·10 ml control (P_2C) and tumor mitochondria (P_2T). Other arrows indicate the addition of 5 μmol succinate (0·05 ml), 200 nmol ADP (0·02 ml) and 70 nmol DOC (0·01 ml). Values below the traces are rates of O_2 utilized and above the traces rates of corticosterone production designated as B in nmol/mg mitochondrial protein/min of incubation (from Péron *et al.*[19]).

pyridine nucleotides and adequate activity of Krebs-cycle enzymes respiratory chain enzymes, (as exemplified by NAD^+- and $NADP^+$-linked malate and isocitrate dehydrogenases as well as normal concentrations of respiratory chain NADH-dehydrogenase) does not answer the question why respiration is not supported by any of the Krebs-cycle intermediates (except succinate), pyruvate, pyruvate + ATP or pyruvate + OAA. This question becomes more complex in light of recent findings that damaged mitochondria, which presumably possess "leaky" membranes, were able to utilize exogenously added NADH in support of respiration. The latter findings indicate that P_2T either possess the usual components of the respiratory chain and the necessary coupling factors to make electron flow possible *via* NADH-dehydrogenase, CoQ and the cytochromes, or else NADH may be oxidized directly for example via cytochrome b_5.

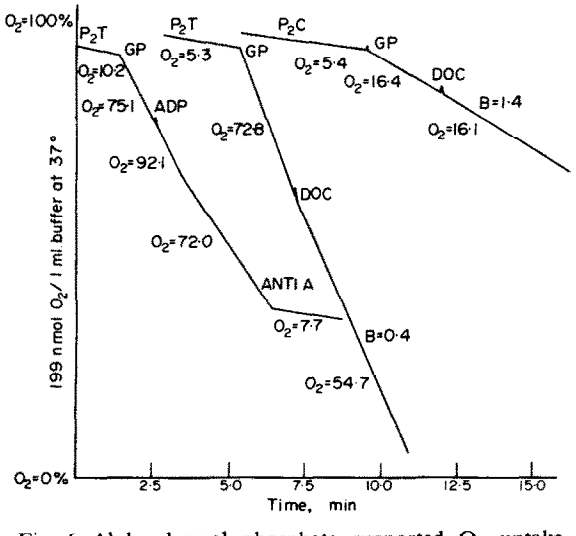

Fig. 6. Alpha-glycerol phosphate supported O_2 uptake by incubated mitochondria of normal adrenal tissue (P_2C) and those derived from Snell adrenocortical carcinoma 494 tissue (P_2T). GP = α-glycerol phosphate, 5 μmol (0·05 ml). Anti A = Antimycin A (2 μg) (from Péron *et al.*[19]).

Table 2. A comparison of some enzyme activities and pyridine nucleotide levels in normal adrenal and carcinoma tissues

Enzyme or PN* Assayed	Normal Adrenal	Cancer
1. Mitochondrial $NADP^+$-linked isocitrate D'hase	0.5	0.8
2. Mitochondrial NAD^+-linked malate D'hase	6.8	2.8
3. Mitochondrial Flavin-linked α-Glyc. PO_4 D'hase	.13	1.32
4. Mitochondrial NAD^+-linked α-Glyc. PO_4 D'hase	N.D.	N.D.
5. Cytosol NAD^+-linked α-Glyc. PO_4 D'hase	.45	.40
6. Mitochondrial Adrenodoxin Reductase	.39	.20
7. Mitochondrial Respiratory Chain NADH D'hase	.63	.66
Mitochondrial Cytochrome P-450	.34	.06
Mitochondrial NAD^+ + NADH	3.4	1.9
Mitochondrial $NADP^+$ + NADPH	2.67	.71

Enzyme activities in the different fractions were determined as published (5,19). *PN = pyridine nucleotides determined by the specific enzymic fluorometric method described by Purvis (28). Activities for 1,2,4,5 and 7 expressed as Δ absorbance at 340 nm/min/mg protein; for 3 as Δ absorbance at 500 nm/mg protein; for 6 as Δ absorbance at 600 nm/100 μg protein/min. Cytochrome P-450 and pyridine nucleotides = nmoles/mg mitochondrial protein. D'hase = dehydrogenase; α-Glyc. PO_4 = α-glycerol phosphate. N.D. = not detectable.

The finding of a cytosol NAD^+-linked glycerol phosphate dehydrogenase in the carcinoma cells (Table 2) indicated that perhaps immediate precursors of α-glycerol phosphate like dihydroxyacetone phosphate (di-OH Acetone P) or those produced via glycolysis like fructose-1,6-disphosphate (F-1,6-diP) could also bring about respiration in mitochondria when incubated with the cytosol. These two substances indeed supported a rapid rate of O_2 uptake in P_2T (Fig. 7). Thus F-1,6-diP was first converted *via* aldolase in the cytosol to di-OH Acetone P and the latter *via* cytosolic α-glycerophosphate dehydrogenase (GPDc) and NADH into α-glycerol phosphate.

The above findings clearly indicate that there are abnormal pathways of glucose and pyruvate metabolism in the carcinoma cells. However, the low glycolytic activity in these cells as compared to normal adrenal cortex cells [10, 16] may be more apparent than real. Perhaps glucose is channeled through other pathways rather than to pyruvate which does not appear to be utilized by the cells. Certainly, in view of low levels of lactate formation found in the cancer cells [16], NADH would accumulate in the cytosol as a result of pyruvate formation. This reduced nucleotide could then be used for the conversion of di-OH Acetone P in the cytosol by α-GPD_c. Indeed the cytosol has been shown to contain a considerable

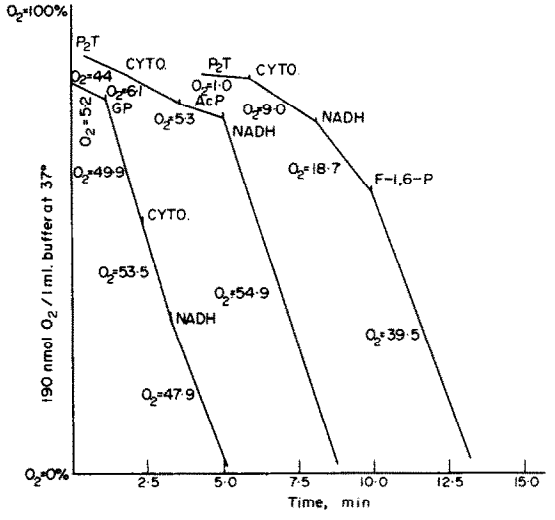

Fig. 7. Substrate supported O_2 uptake in a cell free system made up of 0·20 ml cytosol (CYTO) and 0·10 ml mitochondria (P_2T) derived from Snell adrenocortical carcinoma 494. GP = α-glycerol phosphate (3 mM), F-1,6-P = fructose-1,6-diphosphate (5 mM). Ac.P = dihydroxyacetone-3-phosphate (7·3 mM) (from Péron *et al.*[19]).

amount of α-glycerol phosphate [19]. These findings coupled to the fact that α-GPD_m in the tumor was found to be 10 times as active as that found in P_2C suggests that some of the cellular ATP requirements which must be large in this fastly growing tissue may be provided by a glycerol phosphate shuttle. Accordingly, the GPD_m of the carcinoma cell when coupled to GPD_c could as shown in Fig. 8 provide the cell with a high capacity for oxidation of extramitochondrial NADH generated during glycolysis. Provided NADH is not oxidized by the conversion of pyruvate into lactate *via* lactate dehydrogenase, the α-glycerol phosphate shuttle might be self-sustaining and lead to the production of large amounts of ATP.

Whether these proposals are indeed what occur in the living Snell adrenal carcinoma 494 cell is a matter of conjecture. Nevertheless, our present data "fit" most of the suggested reactions seen in Fig. 8 although they do not agree with those of other laboratories reporting that an α-glycerol phosphate shuttle does not exist in cancer tissue [20, 21]. On the other hand, it has been found to be operative in normal mammalian tissue [22], rat hepatomas [23] and to be necessary in the normal metabolism of insect flight muscle for ATP production [24–26].

Finally, our present findings bring out the intriguing possibility of designing experiments to interfere with the energy supply of the tumor cell which might be a way of "hitting tumor growth" as mentioned by Colowick and Nagarajan[27]. This could possibly be done by interfering with the ATP production of the cancer cell and consequently cell division and tumor growth by blocking the GPD_c with specific antibody formed in the rabbit to isolated rat GPD_c.

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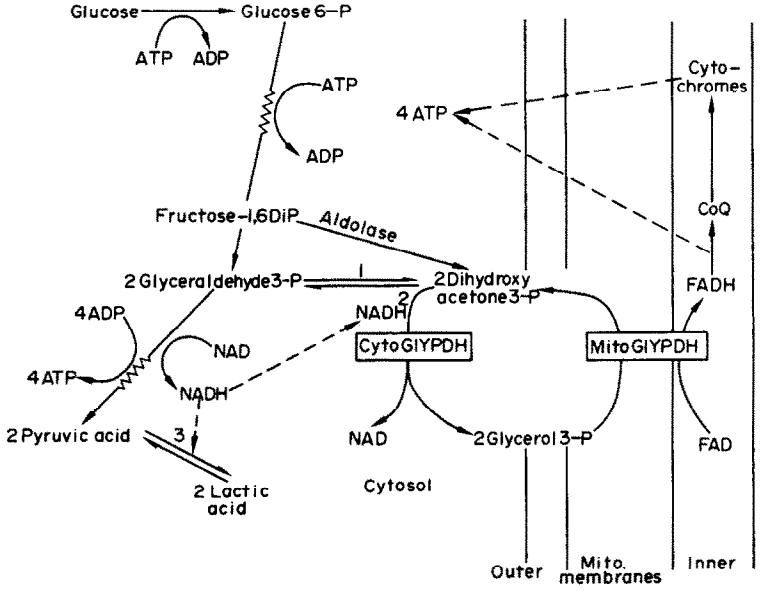

Fig. 8. Diagrammatical representation of possible metabolic pathway of glucose in Snell adrenocortical carcinoma 494. Cytosolic reduced NADH would be "shared" by both lactate and cytosol α-glycerol phosphate dehydrogenase (CYTOGLYPHD). Formation of either lactate or α-glycerol phosphate would prevail depending on activity and amount of enzymes, K_m's for NADH and availability of NADH to the enzymes. Net ATP production from glucose to pyruvate in the cytosol = 2 ATP and if the shuttle is operating = 6 ATP (from Péron *et al.*[19]).

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