Biochimica et Biophysica Acta, 1015 (1990) 258–263
Elsevier

BBABIO 43128

Investigation by amperometric methods of intracellular reduction of 2,6-dichlorophenolindophenol in normal and transformed hepatocytes in the presence of different inhibitors of cellular metabolism

Doris Mayer [1], Renate Naumann [1], Lutz Edler [2] and Peter Bannasch [1]

[1] *Institut für Experimentelle Pathologie and* [2] *Institut für Epidemiologie und Biometrie, Deutsches Krebsforschungszentrum, Heidelberg (F.R.G.)*

(Received 1 September 1989)

Key words: Oxidoreductase; Cytoplasm; Mitochondrion; Dichlorophenolindophenol; Artificial coupling site; (Hepatocyte); (Hepatoma)

The reduction of 2,6-dichlorophenolindophenol (DCIP) was measured by amperometric methods in Morris hepatoma 3924A cells, normal isolated rat hepatocytes and in mitochondria isolated from normal rat liver. The influence of aerobic and anaerobic atmospheres and of various inhibitors of cellular metabolism, especially of the respiratory chain (KCN, NaN_3, oligomycin), on DCIP-reduction were studied using glucose, succinate, β-hydroxybutyrate, α-ketoglutarate and oxalacetate as substrates. Under the influence of KCN and oligomycin the velocity of DCIP-reduction was increased in both cell types. Azide showed a similar effect on tumour cells and to a lower extent on hepatocytes. Using isolated mitochondria total $DCIP_{red}$ was increased by KCN and azide using various mitochondrial metabolites as substrates and with ADP/P_i present. The effects of KCN, azide and oligomycin could be explained by taking DCIP as an artificial coupling site in mitochondria which is only used when oxygen is absent or when the respiratory chain is blocked by inhibitors of cytochrome oxidase. Evaluation of the reaction kinetics revealed differences between normal and transformed cells in terms of the pseudo-first-order rate constants and the activity of overall oxidoreductases. The results apparently reflect quantitative differences in enzymatic equipment and the metabolic pathways predominating in normal and neoplastic cells.

Introduction

The enzymatic reduction of 2,6-dichlorophenolindophenol (DCIP) in intact cells incubated in the presence of different substrates has revealed quantitative differences between normal and transformed hepatocytes [1–3]. These differences concerned the reaction rate and the influence of the environmental atmosphere and were explained in terms of the aberrant carbohydrate metabolism in tumour cells. As shown in the accompanying paper [3], cytosolic as well as mitochondrial enzymes can be involved in intracellular DCIP reduction.

In order to gain an insight into the reactions taking place in mitochondria, DCIP reduction was investigated in the present paper in normal rat liver and MH 3924A cells under the influence of various inhibitors of cellular metabolism, particularly those affecting mitochondrial enzymes. Similar experiments were carried out with mitochondria isolated from normal liver. The reactions were followed quantitatively first with respect to their maximum amount of DCIP reduced ($DCIP_{red}$) and second with respect to their kinetic constants. The first quantity was shown earlier [1] to be related to the reducing capacity of the cells. Pseudo-first-order rate constants, on the other hand, were shown in the preceding paper [3] to reflect the overall cellular oxidoreductase activity. The higher reducing capacity of substrate-saturated tumour cells compared to normal cells – particularly under aerobic conditions – will be discussed in terms of competitive enzymatic pathways. Differences in pseudo-first-order rate constants between normal and transformed cells found under the influence of various inhibitors will be explained taking DCIP as an artificial coupling site.

Correspondence: D. Mayer, Institut für Experimentelle Pathologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, F.R.G.

Materials and Methods

Experimental set up for measurements with cell suspensions

The experimental arrangement for the measurements was the same as described previously [1–3]. When inhibitors were involved in the experiments, the reagents were added in the following order: phosphate-buffered saline (final volume 6.5 ml), glucose or succinate as substrates from 1 molar stock solutions to obtain final concentrations of 0.02 mol/l. This solution was purged with nitrogen or oxygen for 3 min in order to obtain anaerobic or aerobic conditions, respectively. Thereafter, the gas was allowed to flow over the solution in a gentle stream. Then the cells were added from a stock suspension (typically 10^7 cells/ml) to obtain $2.5 \cdot 10^6$ cell per total volume. When anaerobic conditions were used, the small amount of oxygen added together with the cells was rapidly consumed by respiration. This consumption could be monitored in the limiting current range of the cathodic reduction of oxygen at about -0.6 V. With aerobic conditions the oxygen reduction current did not disturb the measurement of the anodic current at $+0.2$ V, which indicated the formation of $DCIP_{red}$. After having prepared the cell suspension in the appropriate anaerobic or aerobic environment, the respective inhibitor was added. The final inhibitor concentrations are given in Table I. Finally, the reaction was started by adding deaerated or aerated DCIP stock solutions ($5 \cdot 10^{-3}$ mol/l) in order to obtain final concentrations ranging from 2 to $5 \cdot 10^{-4}$ mol/l (see Table I).

Experimental arrangement for measurements with mitochondria

The polarographic cell was filled with a buffer containing 250 mM sucrose, 20 mM triethanolamine (pH 7.4), 3 mM $MgCl_2$, 1 mM EDTA, 5 mM phosphate and 1 mM ADP. As substrates, 2 mM α-ketoglutarate, 20 mM hydroxybutyrate, 20 mM succinate and 4 mM oxalacetate (final concentrations) were added. These solutions were purged either with nitrogen or oxygen to obtain anaerobic or aerobic conditions. Thereafter, the solutions were kept under a nitrogen or oxygen layer and 1 ml of a mitochondria suspension typically containing between 2 and 2.5 mg protein per ml was added. In the case of anaerobic conditions the small amount of oxygen added together with the mitochondria was rapidly consumed by respiration as with the cells (see above). Inhibitors were added at concentrations given in Table III. Then the reaction was started by adding the DCIP solution, which was deaerated if necessary (final concentration $2 \cdot 10^{-4}$ mol/l).

Preparation of the cells

Normal hepatocytes were isolated from well-fed male Sprague-Dawley rats (250–350 g body weight, Zentralinstitut für Versuchstierzucht, Hannover, F.R.G) according to the method of Seglen [4] with modifications as described earlier [5]. The cells were washed and suspended in phosphate-buffered saline and kept in ice until used. Morris hepatoma 3924A cells were cultured as described in the accompanying paper [3]. Cell counts were made in a Neubauer hemocytometer.

Preparation of mitochondria

For isolation of mitochondria, the livers of male Sprague-Dawley rats were homogenized in a buffer containing 20 mM triethanolamine buffer (pH 7.4), 250 mM sucrose, 3 mM $MgCl_2$ and 1 mM EDTA. Nuclei and cell debris were spun down at $800 \times g$ in a Christ Minifuge. Mitochondria were sedimented at $8000 \times g$, resuspended in homogenization buffer and spun down again. They were taken up in the same buffer as described above and kept in ice until used. Protein was determined by the method of Heinzel [6].

Evaluation of the measurements

The formation of $DCIP_{red}$ was measured as anodic current as a function of time. The curves obtained were biphasic, modelled by pseudo-first-order kinetics in the first and Michaelis-Menten kinetics in the second phase. The rate constant, k, of the initial part was calculated according to the statistical model described in the accompanying paper [3]. Since differences between normal and transformed cells were reflected mainly in the k values, only these values are given in the present paper.

Results

The maximum amount of $DCIP_{red}$ produced in MH 3924A cells and normal isolated hepatocytes with increasing $DCIP_{ox}$ concentrations is shown in Fig. 1. All experiments were performed under aerobic and anaerobic conditions. In the experiments shown in Fig. 1A–E, glucose was used as substrate, either with no inhibitors (Fig. 1A, see also Ref. 3) or with various inhibitors present (Fig. 1B–E). Fig. 1F represents the experiments using succinate as substrate. Data points were collected from at least five cell preparations.

It is obvious from Fig. 1 that with glucose as substrate and under anaerobic conditions MH 3924A cells have a much higher reducing capacity for DCIP than normal isolated hepatocytes. Addition of KCN (Fig. 1C) increased the saturation concentration for DCIP slightly in both cell types. This means that more DCIP could be reduced by KCN-treated cells than by untreated cells. This effect was more pronounced under aerobic conditions in both transformed and normal cells. A similar effect, although less pronounced, could be observed under the influence of azide (Fig. 1B), another inhibitor of mitochondrial cytochrome oxidase.

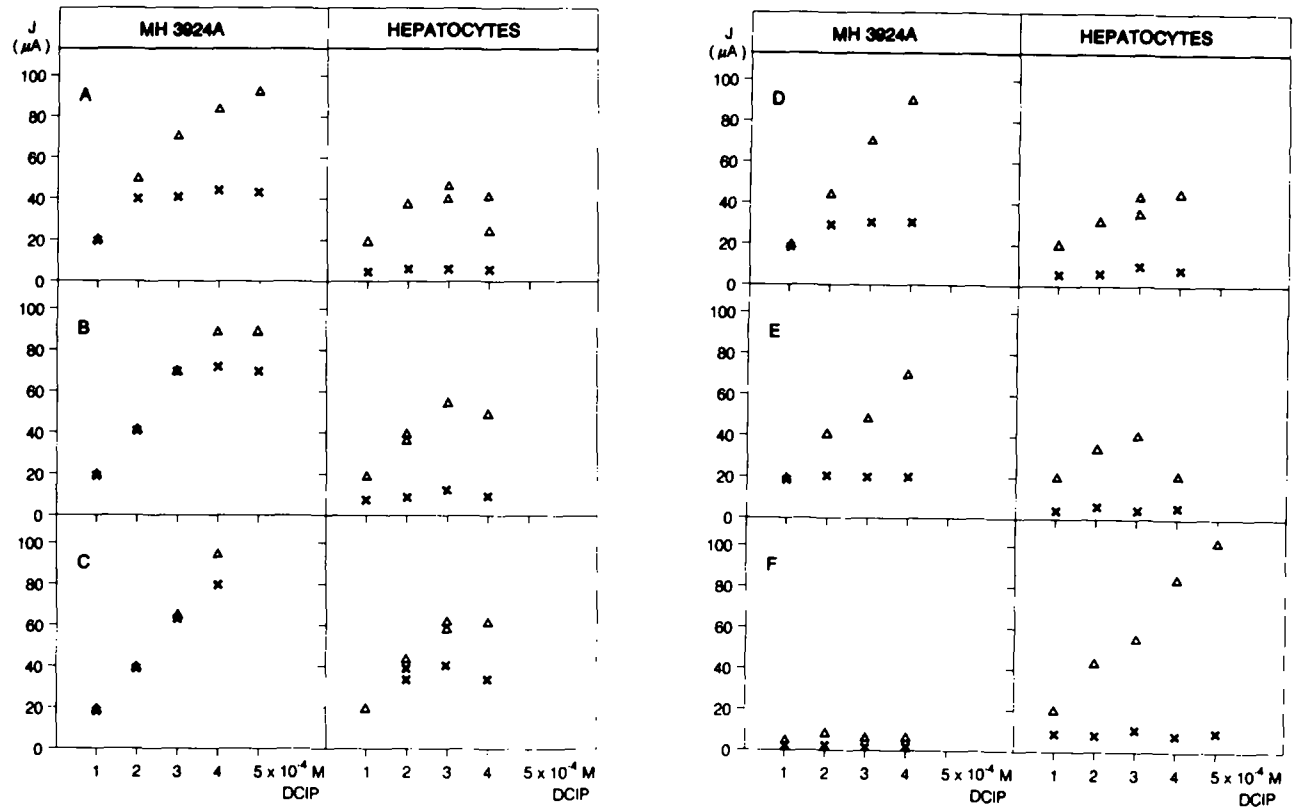

Fig. 1. Anodic currents indicating the final concentration of reduced DCIP as a function of the concentration of DCIP added, obtained after the reaction of the dye with Morris hepatoma 3924A cells and normal isolated hepatocytes. Cells were incubated under anaerobic (Δ) and aerobic (×) conditions in the presence of 0.02 M glucose (A); 0.02 M glucose and $5 \cdot 10^{-3}$ M azide (B); 0.02 M glucose and 10^{-3} M KCN (C); 0.02 M glucose and 10^{-5} M oligomycin (D); 0.02 M glucose and 10^{-4} M iodoacetate (E); and 0.02 M succinate (F).

Transformed cells were more affected by azide than normal cells.

Oligomycin, an inhibitor of mitochondrial ATPase, yielded a slight increase in DCIP-reduction in hepatocytes but a decrease in MH 3924A cells under aerobic conditions. In the absence of oxygen the cells were not affected by this substance in their total reducing capacity for DCIP. Finally, iodoacetate (Fig. 1E), which besides other effects is an inhibitor of glycolysis, yielded an inhibition of DCIP reduction in the tumour cells. Hepatocytes were not, or only slightly, affected. In contrast to KCN and azide, iodoacetate required a preincubation of 2–3 min with the cells to exhibit its inhibitory effect. This is reasonable, since iodoacetate

TABLE I

Pseudo-first-order rate constants k (s^{-1}) of DCIP reduction by isolated hepatocytes under anaerobic conditions

Hepatocytes ($2.5 \cdot 10^6$ cells in 6.5 ml) were incubated with no inhibitor (n.I.) or with inhibitor present at the concentrations indicated and with glucose (0.02 mol/l) as substrate. DCIP concentration was varied as described. *k* values ±S.D. were calculated from the initial phase of the time-course of DCIP reduction [3]. Data were obtained from individual cell preparations.

Inhibitor (mol/l)	*k* (s^{-1})		
	DCIP, $2 \cdot 10^{-4}$ mol/l	DCIP, $3 \cdot 10^{-4}$ mol/l	DICP, $4 \cdot 10^{-4}$ mol/l
n.I.	$7.5 \cdot 10^{-3} \pm 3.7 \cdot 10^{-4}$	$5.8 \cdot 10^{-3} \pm 4.8 \cdot 10^{-3}$	$4.2 \cdot 10^{-4} \pm 0.5 \cdot 10^{-4}$
KCN(10^{-3})	$9.2 \cdot 10^{-3} \pm 0.8 \cdot 10^{-3}$	$4.5 \cdot 10^{-3} \pm 0.5 \cdot 10^{-3}$	$1.2 \cdot 10^{-3} \pm 1.4 \cdot 10^{-5}$
n.I.	$7.2 \cdot 10^{-3} \pm 3.9 \cdot 10^{-3}$	$9.8 \cdot 10^{-4} \pm 6.1 \cdot 10^{-4}$	$4.2 \cdot 10^{-4} \pm 0.5 \cdot 10^{-4}$
Azide($5 \cdot 10^{-3}$)	$3.8 \cdot 10^{-3} \pm 3.5 \cdot 10^{-4}$	$3.0 \cdot 10^{-3} \pm 3.9 \cdot 10^{-4}$	$1.4 \cdot 10^{-3} \pm 1.4 \cdot 10^{-4}$
n.I.	$5.3 \cdot 10^{-3} \pm 1.6 \cdot 10^{-4}$	$9.8 \cdot 10^{-4} \pm 6.1 \cdot 10^{-4}$	$4.5 \cdot 10^{-4} \pm 0.2 \cdot 10^{-4}$
Oligomycin(10^{-5})	$9.1 \cdot 10^{-3} \pm 1.5 \cdot 10^{-3}$	$1.5 \cdot 10^{-3} \pm 1.8 \cdot 10^{-4}$	$8.2 \cdot 10^{-4} \pm 1.7 \cdot 10^{-5}$
n.I.	$1.1 \cdot 10^{-2} \pm 1.2 \cdot 10^{-3}$	$9.8 \cdot 10^{-4} \pm 6.1 \cdot 10^{-4}$	$4.5 \cdot 10^{-4} \pm 0.2 \cdot 10^{-4}$
Iodoacetate(10^{-4})	$5.8 \cdot 10^{-3} \pm 3.5 \cdot 10^{-4}$	$1.0 \cdot 10^{-3} \pm 4.0 \cdot 10^{-5}$	$7.4 \cdot 10^{-4} \pm 5.4 \cdot 10^{-5}$

TABLE II

Pseudo-first-order rate constants k (s^{-1}) of DCIP-reduction by Morris hepatoma 3924A cells under anaerobic conditions

MH 3924A cells ($2.5 \cdot 10^6$ cells in 6.5 ml) were incubated with no inhibitor (n.I.) or with inhibitor present at the same concentrations as studied with hepatocytes and with glucose (0.02 mol/l) as substrate. *k*-values ± S.D. were calculated as described for hepatocytes. Data were obtained from individual cell suspensions.

Inhibitor (mol/l)	*k* (s^{-1})		
	DCIP, $2 \cdot 10^{-4}$ mol/l	DCIP, $3 \cdot 10^{-4}$ mol/l	DCIP, $4 \cdot 10^{-4}$ mol/l
n.I.	$4.6 \cdot 10^{-3} \pm 4.3 \cdot 10^{-4}$	$4.6 \cdot 10^{-3} \pm 1.5 \cdot 10^{-3}$	$2.1 \cdot 10^{-3} \pm 3.0 \cdot 10^{-4}$
KCN(10^{-3})	$6.0 \cdot 10^{-3} \pm 6.1 \cdot 10^{-4}$	$3.8 \cdot 10^{-3} \pm 6.2 \cdot 10^{-4}$	$2.4 \cdot 10^{-3} \pm 1.9 \cdot 10^{-4}$
n.I.	$4.5 \cdot 10^{-3} \pm 3.4 \cdot 10^{-4}$	$4.6 \cdot 10^{-3} \pm 1.6 \cdot 10^{-3}$	$1.2 \cdot 10^{-3} \pm 1.1 \cdot 10^{-3}$
Azide($5 \cdot 10^{-3}$)	$5.6 \cdot 10^{-3} \pm 8.0 \cdot 10^{-4}$	$2.5 \cdot 10^{-3} \pm 1.1 \cdot 10^{-3}$	$1.9 \cdot 10^{-3} \pm 0.7 \cdot 10^{-3}$
n.I.	$7.3 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$	$4.2 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$	$1.8 \cdot 10^{-3} \pm 7.5 \cdot 10^{-6}$
Oligomycin(10^{-5})	$9.3 \cdot 10^{-3} \pm 1.2 \cdot 10^{-3}$	$4.7 \cdot 10^{-3} \pm 1.1 \cdot 10^{-3}$	$2.5 \cdot 10^{-3} \pm 0.2 \cdot 10^{-3}$
n.I.	$7.3 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$	$4.2 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$	$1.8 \cdot 10^{-3} \pm 7.1 \cdot 10^{-6}$
Iodoacetate(10^{-4})	$6.5 \cdot 10^{-3} \pm 4.6 \cdot 10^{-4}$	$1.1 \cdot 10^{-3} \pm 2.4 \cdot 10^{-4}$	$2.3 \cdot 10^{-3} \pm 7.5 \cdot 10^{-5}$

reacts with HS groups of proteins (e.g., glyceraldehyde 3-phosphate dehydrogenase), which is a time-dependent process.

When succinate was used as substrate (Fig. 1F) a much higher DCIP reduction was observed than with glucose in normal hepatocytes under anaerobic conditions. In the presence of oxygen the turnover was very low. MH 3924A cells did not consume succinate, either in the presence or in the absence of oxygen. This agrees with data presented in the preceding paper [3].

When the various inhibitors used in the experiments of Fig. 1B–E were studied with succinate instead of glucose as substrate, similar effects were observed in normal hepatocytes (data not shown) MH 3924A cells were not active under these conditions.

The same reactions as shown in Fig. 1 were analyzed with respect to their pseudo-first-order rate constants. *k* values were calculated as described in the preceding paper [3]. Because of the slow but noticeable autoxidation of DCIP in the presence of oxygen, *k* was obtained only from experiments performed under anaerobic conditions. *k* values obtained for reactions in the absence and presence of various inhibitors are listed in Tables I and II for normal hepatocytes and MH 3924A cells, respectively. Because of the variation of *k* values between different cell preparations, individual cell preparations were used for comparison of incubations with and without inhibitors.

As shown earlier [3], *k* values reflect the respective activities of the enzymes involved in DCIP reduction, whereas the maximum amount of $DCIP_{red}$ shown in Fig. 1 reflects the enzymatic reductive capacity of the cells [1]. A comparison of *k* values (Tables I and II) and the maximum $DCIP_{red}$ obtained (Fig. 1) revealed that both quantities changed in parallel. This means that if DCIP was reduced under the influence of various inhibitors to a greater extent, it was usually also reduced more rapidly and vice versa.

The results of the experiments with liver mitochondria are summarized in Table III. These experiments were performed in the presence of $2 \cdot 10^{-4}$ M DCIP, since with higher DCIP concentrations inhibitory effects were observed. Table III shows the maximum amount of $DCIP_{red}$ given as the anodic current measured (I_{anod}). DCIP was reduced by liver mitochondria with various metabolites of the tricarboxylic acid cycle added as substrates. KCN and azide had similar stimulatory effects on mitochondrial DCIP reduction as observed with hepatocytes. Only when succinate was used as substrate, no further increase in $DCIP_{red}$ was observed in the presence of KCN. From the data in the last column of Table III it was concluded that about half of the reduced dye was retained in the mitochondria. I_{anod}

TABLE III

Maximum amount of $DCIP_{red}$ produced by rat liver mitochondria with various substrates and under the influence of inhibitors of the respiratory chain

Mitochondria were incubated under anaerobic conditions with $DCIP_{ox}$ ($2 \cdot 10^{-4}$ mol/l) and substrates in the presence of ADP(10^{-3} mol/l) and inorganic phosphate ($5 \cdot 10^{-3}$ mol/l). KCN and azide were added at the concentrations given. $DCIP_{red}$ concentration is indicated by the anodic current I_{anod} (μA).

Substrate (mol/l)	Inhibitor (mol/l)	I_{anod} (μA)
β-Hydroxybutyrate	–	15
(0.02)	KCN (10^{-3})	22
	Azide ($5 \cdot 10^{-3}$)	21
α-Ketoglutarate	–	9
(0.002)	KCN (10^{-3})	19
	Azide ($5 \cdot 10^{-3}$)	19
Oxalacetate	–	8
(0.004)	KCN (10^{-3})	12
Succinate	–	24
(0.02)	KCN (10^{-3})	24

would rise to 40 μA after complete reduction of $2 \cdot 10^{-4}$ M DCIP that had been released into the solution (see Fig. 1 and Ref. 1). This finding agrees with results from intact cells, where 87% of the dye were found in the solution after reduction in the case of hepatocytes and 97% in the case of MH 3924A cells. Apart from the case of succinate as substrate, ADP and P_i turned out to be essential components for DCIP reduction in mitochondria.

Discussion

It has already been discussed earlier [1] that hepatoma cells exhibit a much higher reducing capacity than hepatocytes under aerobic and anaerobic conditions and that the high DCIP reduction observed in hepatoma cells in the presence of oxygen may mainly be due to the well-known 'aerobic glycolysis' [7]. However, since the activities of the key enzymes of glycolysis as well as of the pentose phosphate pathway were found to be markedly increased in the MH 3924A cells [8] it may be assumed that the high total reducing capacity of the tumour cells is due to both pathways. The NAD(P)H formed in these reactions may be involved in DCIP reduction. The enzymes catalyzing NAD(P)H-DCIP electron transfer include first diaphorases which are known as quinone reductases [9–11] and are localized in the cytosol, and second enzymes of the respiratory chain localized in mitochondria. The interaction of mitochondrial enzymes with DCIP has become evident by our experiments using inhibitors of complex IV and V.

Since NADH-DCIP reductase activity is low in mitochondrial complex I [12,13], DCIP is probably not directly reduced by NADH at the inner mitochondrial membrane. Quinones are, however, readily reduced in a bypass reaction including DT-diaphorase [10], especially if the respiratory chain (complex IV and V) is blocked either by replacing the aerobic atmosphere by nitrogen or by addition of inhibitors of complex IV and V. This was confirmed by our experiments. When KCN or azide were given to cells saturated with oxygen, and thus O_2 reduction and the electron flow were stopped, DCIP as an artificial electron acceptor was reduced (Fig. 1B and C). However, a similar effect of KCN was also observed when cells (hepatocytes as well as hepatoma cells) were kept under nitrogen, where KCN should not exhibit a direct effect on cytochrome oxidase and O_2 reduction. Furthermore, total reductive capacity was increased (Fig. 1), and the reaction was accelerated (Tables I and II) under these conditions as well. As an explanation of this effect it is suggested that DCIP is used as an artificial coupling site. Similar effects have also been described for chloroplasts [9] where, in the photosynthetic membranes artificial electron acceptors like phenylenediamine and benzoquinone have been shown to be reduced by Photosystem II while Photosystem I was blocked by, for example, KCN. O_2 was nevertheless produced and the reaction was still coupled to ATP formation [9]. A similar mechanism is assumed to be involved in the processes discovered in our experiments. KCN and azide prevent cytochrome oxidase from being reduced and thus channel the electron flow towards the artificial electron acceptor DCIP. The finding that the presence of both an N_2-atmosphere and inhibitors resulted in an acceleration of DCIP reduction compared to the effects of N_2 or inhibitors alone may be explained as follows. Under anaerobic conditions traces of oxygen allow a residual electron flow towards cytochrome oxidase. With inhibitors of the latter enzyme, however, this reaction is suppressed. With KCN or azide present under aerobic conditions, on the other hand, autoxidation of the reduced form of DCIP, although a slow process [1], cannot be neglected at higher DCIP concentration. This would explain the maximum effect of KCN and azide in an anaerobic atmosphere.

DCIP as an artificial coupling site could result in ATP synthesis. ATP synthesis is very likely to exist, since mitochondria did not consume β-hydroxybutyrate, α-ketoglutarate or oxaloacetate in the absence of ADP/P_i. Thus, the phosphorylating system seems to be perfectly coupled to electron flow in the presence of DCIP. This is also confirmed by experiments with oligomycin discussed below.

The finding that in contrast to KCN, azide only slightly affected normal hepatocytes is difficult to explain. In particular, the fact that under aerobic conditions DCIP was not reduced to a greater extent in the presence of azide points to another inhibitory action of azide additional to the block of cytochrome oxidase. This additional effect might occur on the level of DCIP reduction. The double action of azide does not seem to exist in tumour cells (Fig. 1).

The effect of oligomycin, an inhibitor of ATP formation, may be explained in analogy to the action of cyanide. ATPase has been shown by Klingenberg and Schollmeyer [14] to be reversibly coupled to the redox chain in mitochondria in the way that an accumulation of ATP drives the redox enzymes into the reduced state. If ATP-formation is blocked by oligomycin it follows that the redox enzymes can also be expected to remain in the reduced state. This would explain the observed acceleration of DCIP reduction by oligomycin (Tables I and II) in normal and transformed cells under anaerobic conditions. Regarding the experiments with inhibitors of complex IV and V, the conclusion seems to be justified that enzymes of the inner mitochondrial membrane interact with the reduction of DCIP. However, DCIP is reduced only if complex IV of the respiratory chain is inhibited. Under physiological conditions, i.e., in an aerobic atmosphere and with no inhibitors present, the flux of electrons from NADH down the total

assembly of complexes of the respiratory chain is apparently sterically and energetically favoured towards the reduction of the artificial electron acceptor. This is in agreement with our findings regarding the almost zero DCIP reduction of normal hepatocytes and mitochondria under aerobic conditions and with glucose and succinate as substrates.

Due to the 'Pasteur effect', glycolytic enzymes seem to play a minor role in cytosolic DCIP-reduction in normal hepatocytes incubated under aerobic conditions. By contrast, MH 3924A cells appear to prefer the electron flux towards the artificial electron acceptor to the main physiological pathway of the transformed cells consisting of lactate formation.

Acknowledgements

We thank Iris Letsch and Anna Hellmann for technical assistance and Brigitte Pétillon for typing the manuscript. The work was supported by the Deutsche Forschungsgemeinschaft.

References

1 Naumann, R., Mayer, D. amd Bannasch, P. (1985) Biochim. Biophys. Acta 847, 90–95.
2 Naumann, R., Mayer, D. and Bannasch, P. (1985) Biochim. Biophys. Acta 847, 96–100.
3 Naumann, R., Mayer, D. Edler, L. and Bannasch, P. (1990) Biochim. Biophys. Acta 1015, 253–257.
4 Seglen, P.O. (1976) in Methods in Cell Biology (Prescott, D.M., ed.), Vol. 13, pp. 28–83, Academic Press, New York.
5 Faulstich, H. Trischmann, H. and Mayer, D. (1983) Exp. Cell Res. 144, 73–82.
6 Heinzel, W. (1960) Verh. Dtsch. Ges. Inn. Med. 66, 636–637.
7 Warburg, O. (1966) in Molekulare Biologie des malignen Wachstums, 17. Colloquium der Gesellschaft für Physiologische Chemie, pp. 1–16, Springer, Berlin.
8 Denis, C. Mayer, D., Trocheris, V., Paris, H. and Murat, J.-C. (1985) Int. J. Biochem. 17, 247–251.
9 Sinha, B.K., Katki, A.G., Batist, G., Cowan, K.N. and Myers, C.E. (1987) Biochemistry 26, 3776–3781.
10 Ernster, L. (1967) Methods Enzymol. 10, 309–317.
11 Schor, N.A. and Morris, H.P. (1977) Cancer Biochem. Biophys. 2, 5–9.
12 Hatefi, Y., Stempel, K.E. and Haustein, W.G. (1969) J. Biol. Chem, 244, 2358–2365.
13 Martonosi, A. (1976) The Enzymes of Biological Membranes, Electron Transport Systems and Receptors, John Wiley & Sons, New York.
14 Klingenberg, M. and Schollmeyer, P. (1961) Biochem. Z. 335, 243–262.