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# Interactions between Glycolysis and Mixed Function Oxidation: Studies with 7-Ethoxycoumarin in Perfused Livers from β-Naphthoflavone-Treated Rats

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**SUMMARY**

Interaction between glycolysis and mitochondrial oxidations to supply reducing equivalents at high rates for mixed function oxidation was evaluated in the perfused liver after treatment of rats with β-naphthoflavone. Livers from fasted β-naphthoflavone-treated rats were employed because rates of 7-ethoxycoumarin *O*-deethylation were constant (16 μmol/g/hr) for at least 1 hr of perfusion. Preinfusion with KCN, an inhibitor of oxidative phosphorylation, caused the rate of 7-ethoxycoumarin *O*-deethylation to decline by 60% over 30 min of perfusion. The decline in rates of mixed function oxidation in the intact liver was not due to a direct effect of KCN on cytochrome P-450, inasmuch as cyanide did not diminish rates of 7-ethoxycoumarin *O*-deethylation by isolated microsomes. Cyanide rapidly decreased hepatic oxygen uptake by 70% and increased rates of glycolysis (lactate plus pyruvate production) from less than 10 to over 60 μmol/g/hr. Rates of glycolysis and mixed function oxidation subsequently declined in parallel during infusion of KCN. Infusion of ethanol (20 mM), a known inhibitor of glycolysis, decreased the stimulation of glycolysis caused by KCN to 20 μmol/g/hr and lowered maximal rates of 7-hydroxycoumarin production to about 6 μmol/g/hr. Both mixed function oxidation and glycolysis also declined in parallel over 30 min of perfusion in the presence of ethanol and KCN. When cyanide infusion was terminated, rates of oxygen uptake returned rapidly to basal values; however, rates of mixed function oxidation remained low. In contrast, infusion of ethanol in the absence of cyanide had no effect on rates of mixed function oxidation. Infusion of glucose (30 mM) or pyruvate (1 mM) after KCN restored maximal rates of mixed function oxidation in parallel with increases in rates of glycolysis. In contrast to results obtained in livers from fasted rats, cyanide and ethanol had little effect on 7-ethoxycoumarin *O*-deethylation in livers from fed rats. Taken together, these results argue strongly that rates of mixed function oxidation in the intact livers of fasted rats are sustained by reducing equivalents derived from mitochondrial oxidations. Glycolysis can supply substrates needed for the transport of reducing equivalents from the mitochondria into the cytosol for mixed function oxidation. Because glycogen reserves are minimal in the fasted state, rates of glycolysis and mixed function oxidation declined in parallel during the infusion of cyanide, because reducing equivalents derived from mitochondria are not available.

Identifying sources of reducing equivalents for the metabolism of 7-ethoxycoumarin in periportal and pericentral regions of the liver lobule, following induction of the monooxygenase pathway with either phenobarbital or β-naphthoflavone, has been a major focus of our laboratory (1, 2). These studies indicated that rates of NADPH supplied by the pentose cycle and mitochondrial oxidations for mixed function oxidation were similar after treatment with phenobarbital (1). In contrast, about 70% of the reducing equivalents required for monooxygenation were derived from the mitochondria in livers from rats treated with β-naphthoflavone (2). These studies suggested that inducing agents affect pathways associated with the formation of NADPH as well as increase components of the mixed function oxidase system. Because NADPH supply is rate limiting for high rates of mixed function oxidation in the intact liver (1, 2), the effect of inducing agents on pathways that generate NADPH may also have profound effects on mixed function oxidation. Reducing equivalents formed via mitochondrial oxidation may have a unique role in the oxidation of 7-ethoxycoumarin in livers from animals treated with the carcinogen 3-methylcholanthrene or its analog, β-naphthoflavone. Both of these agents increase significantly the concentration of one cytochrome P-450 isozyme, P-450-MC[2] (3). This isozyme possesses high catalytic activity toward polycyclic aromatic hydrocarbons such as benzo(*a*)pyrene and 7-ethoxycoumarin (4). Under a wide variety of conditions, rates of 7-hydroxycoumarin production were high and constant for long periods of time in perfused rat livers from fed and fasted β-naphthofla-

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[2] Cytochrome P-450-MC is used to denote the hemoproteins classified as P-450 I.A.1. in the standard nomenclature [D. W. Nebert, *et al. DNA* 6:1-11 (1987)]

vone-treated rats (2). When mitochondrial function was inhibited with cyanide, rates of 7-ethoxycoumarin *O*-deethylation were not sustained in perfused livers from fasted rats, in which carbohydrate reserves were minimal. A similar decline in rates of oxidation of another substrate, *p*-nitroanisole, was observed during prolonged perfusion in the absence of KCN (5). Conway *et al.* (6) also observed that rates of mixed function oxidation reached maximal values 6 min after infusion of *p*-nitroanisole and then declined by about 50% during the next 30 min of perfusion in livers from fed C57BL/6J mice. Treatment of rats with β-naphthoflavone or 3-methylcholanthrene not only increased rates of *p*-nitroanisole *O*-demethylation about 3-fold, but it also prevented the decline in rate completely. Comparison of *Ah* locus-responsive and -nonresponsive mice indicated that the ability to maintain high rates of mixed function oxidation was due to enhanced turnover of NADPH and was linked to the *Ah* locus (6). Fasting prevented the effect of these inducing agents on NADPH turnover in C57BL/6J mice, as indicated by a decline in the rate of *p*-nitroanisole metabolism, an effect that was reversed by the infusion of glucose.

Substrate shuttle pathways involving carbohydrate intermediates are necessary to transport reducing equivalents from the mitochondria into the cytosol to support monooxygenation (7, 8). One mechanism whereby glucose could influence rates of mixed function oxidation, particularly when mitochondrial oxidations are rate limiting for the supply of NADPH, is to generate substrate shuttle intermediates. The purpose of this study was to determine whether glycolysis is involved in the generation of intermediates needed for the movement of reducing equivalents from the mitochondria to the cytosol to support monooxygenation in the intact cell.

## Materials and Methods

**Animals and liver perfusion.** Female Sprague-Dawley rats (250–350 g) were given intraperitoneal injections of β-naphthoflavone (30 mg/kg) dissolved in corn oil, once daily for 3 days (2, 6). In some experiments, rats were fasted for 24 hr before use or were injected with 6-aminonicotinamide (70 mg/kg, intraperitoneally) 4 hr before perfusion.

Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°) saturated with an oxygen/carbon dioxide mixture (95:5) using a nonrecirculating system, as described previously (5). The fluid was pumped into the liver via a cannula placed in the portal vein and flowed past a Teflon-shielded, Clark-type, oxygen electrode before being discarded. Rates of oxygen uptake were calculated from the influent minus effluent oxygen concentration difference, the flow rate, and the liver wet weight. A stock solution of 7-ethoxycoumarin was dissolved in *N,N*-dimethylformamide and was added to Krebs-Henseleit bicarbonate buffer to give a final concentration of 3.2 mM *N,N*-dimethylformamide and 250 μM 7-ethoxycoumarin when infused into the liver. *N,N*-Dimethylformamide had no effect on mixed function oxidation. Potassium cyanide was dissolved in 0.5% albumin (Fraction V/Sigma Chemical Co., St. Louis, MO) and was infused into the liver at a final concentration of 2 mM. Ethanol was diluted with buffer and was infused into the liver at a final concentration of 20 mM.

**Determination of conjugates of 7-hydroxycoumarin.** During infusion of 7-ethoxycoumarin, samples of effluent perfusate were collected every 2 min. Glucuronide and sulfate conjugates of 7-hydroxycoumarin were determined by measuring 7-hydroxycoumarin formed after incubation of 1.0-ml samples of perfusate with 0.5 ml of 180 mM potassium phosphate buffer, pH 7.4, containing 250 units of purified β-glucuronidase and 25 units of sulfatase activity (Sigma), respectively, for 1.5 hr at room temperature. This procedure hydrolyzed over 95% of glucuronide and sulfate conjugates of 7-hydroxycoumarin. The concentration of 7-hydroxycoumarin was measured fluorometrically (366 to 450 nm) using an Eppendorf fluorometer. Rates of 7-hydroxycoumarin production were calculated from the sum of free and conjugated 7-hydroxycoumarin produced, the flow rate, and the liver wet weight.

**Metabolite measurements.** Samples of effluent perfusate were collected at timed intervals (2–4 min) for determination of glucose, lactate, and pyruvate by standard enzymatic procedures (9). Rates of

TABLE 1

**Effect of potassium cyanide and ethanol on 7-ethoxycoumarin *O*-deethylation and lactate production in perfused livers from fasted β-naphthoflavone-treated rats**

Potassium cyanide (2 mM) and ethanol (20 mM) were infused into livers from fasted β-naphthoflavone-treated rats as depicted in Figs. 2 and 5. Maximal values of 7-hydroxycoumarin formation and lactate production were measured 6 or 30 min after 7-ethoxycoumarin infusion. Data represent means ± standard error from four livers in each group.

| Addition | 7-Ethoxycoumarin *O*-Deethylation | | Lactate Production | |
|---|---|---|---|---|
| | Maximal rate | Rate after 30 min | Maximal rate | Rate after 30 min |
| | μmol/g/hr | | | |
| None | 16.6 ± 0.6 | 16.2 ± 0.5 | 8.0 ± 0.5 | 8.3 ± 0.7 |
| Ethanol | 15.9 ± 0.8 | 15.6 ± 0.4 | 3.5 ± 1.0 | 3.2 ± 1.1 |
| KCN | 13.0 ± 1.1 | 5.1 ± 0.5[a] | 62.5 ± 6.7[b] | 24.5 ± 4.0 |
| Ethanol + KCN | 6.2 ± 0.9[a] | 2.2 ± 0.5[a] | 20.8 ± 2.2[b] | 10.9 ± 1.0 |

[a] $p < 0.001$ as compared with no addition.
[b] $p < 0.01$ as compared with no addition.

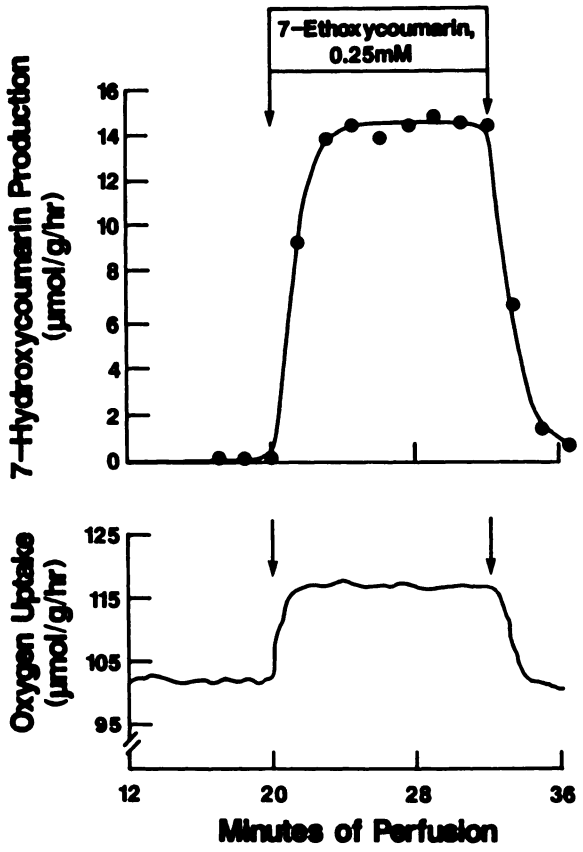


**Fig. 1.** Kinetics of 7-hydroxycoumarin production in the presence of potassium cyanide. 7-Ethoxycoumarin (0.25 mM) was infused into the liver of a fasted β-naphthoflavone-treated rat at the times indicated by the *horizontal bars* and *vertical arrows*. Rates of 7-hydroxycoumarin production (*upper*) and oxygen uptake (*lower*) were calculated as described in Materials and Methods. A typical experiment is shown.

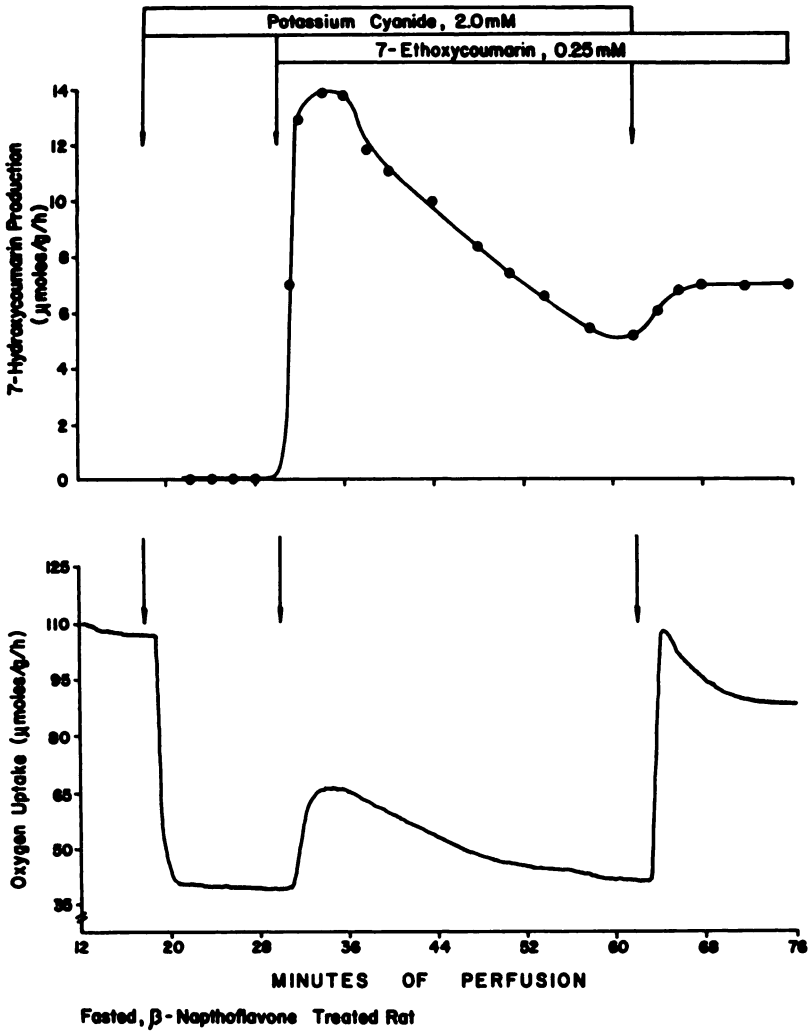


**Fig. 2.** Kinetics of 7-hydroxycoumarin production in the presence of potassium cyanide. Conditions are as in Fig. 1.

glycolysis were determined from the concentration of pyruvate plus lactate in the effluent perfusate, the flow rate, and the liver wet weight. In livers from fasted rats, rates of lactate production alone served as an index for rates of glycolysis because pyruvate formation was at the limits of detection (< 1.0 μmol/g/hr).

## Results

**Kinetics of 7-ethoxycoumarin *O*-deethylation and glycolysis in livers from β-naphthoflavone-treated rats.** Following the infusion of 7-ethoxycoumarin, 7-hydroxycoumarin was produced at maximal rates of about 16 μmol/g/hr, which remained constant for at least 30 min in livers from fasted, β-naphthoflavone-treated rats (Table 1; Fig. 1). When cyanide (2 mM), an inhibitor of oxidative phosphorylation, was infused before 7-ethoxycoumarin, maximal rates of mixed function oxidation (13 μmol/g/hr) were not diminished significantly but declined rapidly to values around 5 μmol/g/hr over subsequent 30 min of perfusion (Fig. 2; Table 1). Under these conditions, KCN decreased rates of oxygen uptake from 110 to 40 μmol/g/hr (Fig. 2). Subsequent infusion of 7-ethoxycoumarin increased oxygen uptake transiently to 65 μmol/g/hr for 6 min; values then declined in parallel with rates of 7-hydroxycoumarin production (Fig. 2). When cyanide infusion was terminated, oxygen uptake returned rapidly to basal values, but rates of 7-hydroxycoumarin production remained depressed (Fig. 2).

Because endogenous glycogen stores are virtually absent in livers from fasted rats (10), basal rates of lactate (<5 μmol/g/hr) and glucose (<20 μmol/g/hr) production were also very low (Fig. 3) compared with livers from fed rats. Infusion of cyanide increased lactate and glucose production to 60 and 30 μmol/g/hr, respectively. Addition of 7-ethoxycoumarin increased lactate production further, to values around 80 μmol/g/hr; however, this increase was transient and lasted only about 4 min (Fig. 3). Rates of lactate production subsequently declined in parallel with rates of monooxygenation (Fig. 3; Table 1). Glucose production declined precipitously to basal levels after a lag time of about 4 min. Thus, a good correlation ($r = 0.87$) between rates of glycolysis and 7-hydroxycoumarin production was observed during cyanide infusion (Fig. 4). After cyanide infusion was terminated, lactate production decreased further, to rates around 20 μmol/g/hr (Fig. 3).

When glycolysis was inhibited by ethanol (11), the increase in lactate production due to KCN was diminished by over 80% (Fig. 5; Table 1). Under these conditions, maximal rates of 7-hydroxycoumarin production were less than half (6 μmol/g/hr) those observed in the absence of ethanol. As with cyanide alone, mixed function oxidase activity and lactate production declined in parallel over the next 30 min of perfusion (Fig. 5; Table 1). After 30 min, low rates (around 1 μmol/g/hr) were not increased when ethanol and KCN infusions were termi-

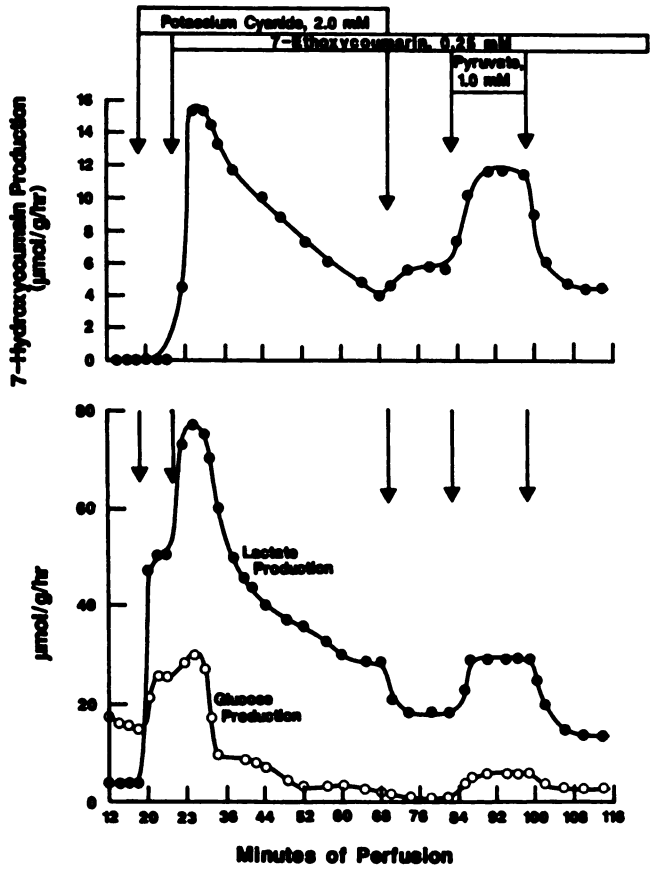


**Fig. 3.** Reversal of cyanide-mediated inhibition of 7-hydroxycoumarin production by pyruvate. Potassium cyanide (2.0 mM), 7-ethoxycoumarin (0.25 mM), and pyruvate (1.0 mM) were infused into the liver of a fasted β-naphthoflavone-treated rat at the times indicated by the *horizontal bars* and *vertical arrows*. Rates of 7-hydroxycoumarin (*upper*), lactate (●), and glucose (○) production (*lower*) were determined as described in Materials and Methods. A typical experiment is shown.

nated. If ethanol was infused in the absence of KCN, maximal rates of 7-hydroxycoumarin production were not diminished. Moreover, rates were sustained for at least 30 min under these conditions, even though rates of glycolysis were nearly undetectable (<5 μmol/g/hr; Table 1).

Livers from fed rats are characterized by high rates of glycolysis (e.g., 140 μmol/g/hr) from endogenous glycogen. In contrast to results obtained with livers from fasted rats, cyanide and ethanol decreased rates of 7-hydroxycoumarin production only slightly in the fed state (Table 2). It is noteworthy that rates of 7-hydroxycoumarin production and glycolysis remained high in livers from fed rats for at least 30 min of perfusion in the presence of cyanide and ethanol.

**Effect of pyruvate and glucose on 7-ethoxycoumarin metabolism.** Infusion of pyruvate (1.0 mM) after cyanide infusion was terminated restored rates of 7-hydroxycoumarin production in livers from fasted rats nearly to maximal values (Fig. 3). Pyruvate also increased rates of lactate production slightly to 25 μmol/g/hr but only increased glucose production slightly (Fig. 3). When pyruvate infusion was terminated, both mixed function oxidation and lactate production decreased to near levels observed after termination of KCN (Fig. 3). Pyruvate addition in the presence of KCN did not alter rates of 7-hydroxycoumarin production (data not shown). Addition of glucose after cyanide infusion was terminated also rapidly restored maximal rates of monooxygenation (Fig. 6). Because glucose provides substrate for both the pentose cycle and glycolysis, experiments were performed in livers from fasted rats (Fig. 6) treated with 6-aminonicotinamide, which is a potent inhibitor of the pentose cycle (12–14). The time course for increases in 7-hydroxycoumarin production also paralleled lactate production ($r = 0.95$) in livers from 6-aminonicotinamide-treated rats.

## Discussion

The results from this study indicate that glycolysis can influence rates of mixed function oxidation independent of pentose cycle activity and oxidative phosphorylation. Inhibition of 7-ethoxycoumarin metabolism by cyanide in livers from fasted rats is not due to a direct effect by cyanide on 7-ethoxycoumarin *O*-deethylation, because mixed function oxidase activity in livers from fed rats was decreased only slightly (15%) by cyanide (Table 2). In addition, cyanide did not affect 7-ethoxycoumarin *O*-deethylation in microsomes isolated from either fed or fasted rats (1, 2). Finally, maximal rates of 7-hydroxycoumarin production were restored by infusion of pyruvate or glucose (Fig. 3), indicating that cytochrome P-450 was not inhibited or destroyed during the course of cyanide infusion.

Because the inhibition observed with cyanide cannot be explained by a direct action on cytochrome P-450, it is concluded that cyanide inhibits 7-ethoxycoumarin *O*-deethylation in the intact liver by inhibiting the supply of reducing equivalents. NADPH utilized for mixed function oxidation may be generated by either cytosolic or mitochondrial reactions. Inhibition of 7-hydroxycoumarin production by cyanide is not linked to an effect on NADPH generation by the pentose cycle. NADPH can be generated in the cytosol by the enzymes of the pentose cycle, glucose-6-phosphate and 6-phosphogluconate dehydrogenase. In studies reported above, 7-ethoxycoumarin *O*-deethylation was inhibited only after pentose cycle activity had already been reduced by greater than 90% (12) by fasting. Moreover, previous studies (12) demonstrated that the infusion of cyanide does not block the increase in NADPH generation by the pentose cycle during the metabolism of *p*-nitroanisole in livers from fasted rats. The effect of cyanide on mixed function oxidation appears to involve the supply of reducing equivalents via the mitochondria, where the majority of NADPH is concentrated (15, 16).

A major enzyme for NADPH generation is the energy-dependent transhydrogenase, which utilizes NADH to reduce $NADP^+$ (16). Because the inner mitochondrial membrane is impermeable to pyridine nucleotides (7), substrate shuttles are required to move reducing equivalents from the mitochondrial space into the cytosol (16, 17). Two shuttle pathways involved in the transfer of hydrogen from mitochondria to cytosol are the isocitrate shuttle (15) and the malate shuttle (17). The isocitrate shuttle utilizes the enzyme isocitrate dehydrogenase, which transfers hydrogen from NADPH to isocitrate by reductive carboxylation of α-ketoglutarate. Isocitrate crosses the mitochondrial membrane and regenerates NADPH in the cytosol via isocitrate dehydrogenase (Fig. 7). Cytosolic NADPH can also be generated by a malate shuttle via carboxylation of pyruvate to oxalacetate, reduction to malate, egress of malate into the cytosol, and production of NADPH via cytosolic malic enzyme. Inhibition of the mitochondrial electron transport

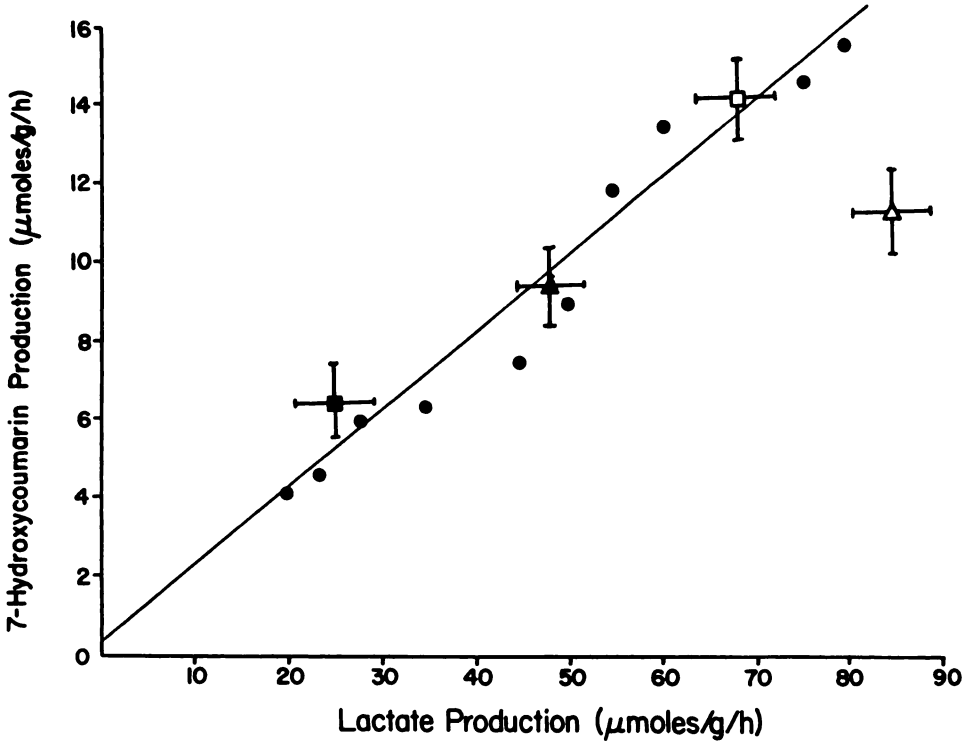


**Fig. 4.** Correlation between rates of 7-hydroxycoumarin production and lactate production in the perfused liver. Values are from experiments typified by Fig. 3. A good correlation between glycolysis and mixed function oxidation ($r$ = 0.98) was observed. In three additional experiments similar to that depicted in Fig. 3, correlation coefficients ranged from 0.90 to 0.98. The average means ± standard error for peak rates of 7-hydroxycoumarin production and rates of glycolysis during cyanide infusion in livers from fasted (*squares*) and 6-aminonicotinamide-treated rats (*triangles*) in the presence and absence of ethanol (*closed* versus *open symbols*) were plotted against the rates of 7-hydroxycoumarin and lactate production depicted in Fig. 3. When rates of glycolysis and mixed function oxidation were compared from all data in the figure, the correlation coefficient was 0.87.

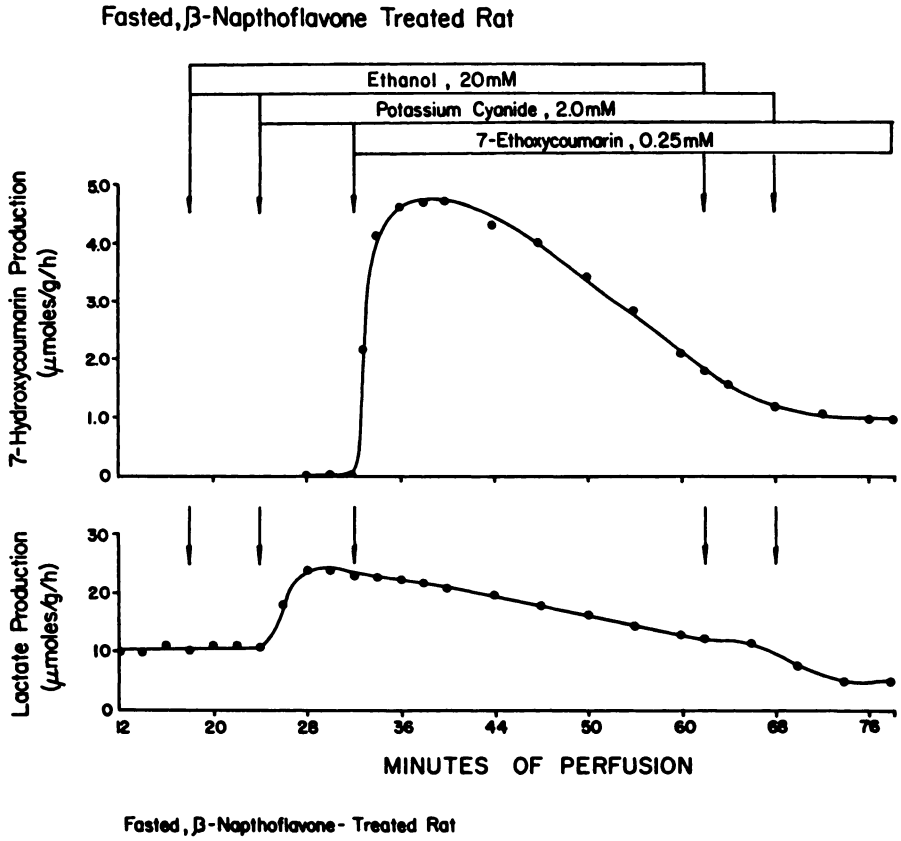


**Fig. 5.** Effect of ethanol on 7-hydroxycoumarin production from 7-ethoxycoumarin in the presence of potassium cyanide. Ethanol (20 mM), potassium cyanide (2 mM), and 7-ethoxycoumarin (0.25 mM) were infused into the liver of a fasted, β-naphthoflavone-treated rat at the times indicated by the *horizontal bars* and *vertical arrows*. Rates of 7-hydroxycoumarin production (*upper*) and lactate production (*lower*) were calculated as described in Materials and Methods.

chain by cyanide should inhibit energy-dependent transfer of NADPH across the mitochondrial membrane and, thus, decrease rates of mixed function oxidation. Indeed, rates of 7-ethoxycoumarin metabolism were diminished by about 60% by preinfusion of cyanide. However, nearly 30 min of perfusion with cyanide were required for this effect to occur; maximal initial rates were not affected.

One possible explanation for the delay in inhibition by cyanide may be related to activation of glycogenolysis, which may occur as a result of impaired ATP synthesis by the respiratory chain (18). Glycolysis could provide ATP for the energy-dependent transhydrogenase and provide substrates needed for the malate shuttle. In the presence of cyanide, the amount of NADH in the mitochondria would also increase (19). Collec-

TABLE 2

**Effect of potassium cyanide and ethanol on 7-ethoxycoumarin O-deethylation and lactate production in perfused livers from fed β-naphthoflavone-treated rats**

Potassium cyanide (2.0 mM) was infused into livers from fed β-naphthoflavone-treated rats 16 min after infusion of 7-ethoxycoumarin. Ethanol (20 mM) infusion was initiated 12 min after cyanide. Values represent maximal rates of 7-hydroxycoumarin formation and lactate plus pyruvate production. Similar values were observed after 30 min of perfusion. Data represent means ± standard error from four livers in each group.

| Treatment | Addition | 7-Ethoxycoumarin O-Deethylation | Lactate + Pyruvate Production |
|---|---|---|---|
| | | μmol/g/hr | |
| None | None | 18.5 ± 1.7 | 139.8 ± 17.5 |
| None | KCN | 15.8 ± 1.6* | 223.0 ± 13.2* |
| None | KCN + Ethanol | 14.2 ± 1.6* | 154.4 ± 20.3 |

*$p < 0.01$ as compared with no addition.

tively, these actions could lead to the generation of NADPH and the transfer of reducing equivalents from the mitochondria into the cytosol (Fig. 7). Because glycogen reserves in livers from fasted rats are minimal, these effects would only be transient and rates of monooxygenation would not be maintained in the presence of cyanide. Several independent experiments from this study support this hypothesis. First, a good correlation was observed between rates of glycolysis and monooxygenation under a variety of conditions in the presence of cyanide (Figs. 3, 5, and 6). Secondly, infusion of ethanol, which inhibits the glyceraldehyde-3-phosphate dehydrogenase reaction in the glycolytic pathway (8) (Fig. 7) markedly reduced both the cyanide-mediated increase in glycolysis and maximal rates of 7-hydroxycoumarin production (Fig. 5; Table 1). In contrast, rates of 7-ethoxycoumarin metabolism were largely unaffected by either cyanide or ethanol in livers from glycogen-rich fed rats (Table 2). Finally, rates of *p*-nitroanisole *O*-deethylation in perfused livers from fed, phenobarbital-treated rats reached maximal values 6 min after *p*-nitroanisole infusion but declined steadily over the next 30 min (5). The decline in *p*-nitrophenol production correlated with rates of glycolysis. Parallel decreases in rates of mixed function oxidation and glycolysis occurred in the absence of cyanide, possibly due to uncoupling of oxidative phosphorylation by *p*-nitrophenol (20). Taken together, these data provide strong evidence that glycolysis can maintain high rates of mixed function oxidation when oxidative phosphorylation is impaired.

Activation of glycolysis by cyanide could supply reducing equivalents for mixed function oxidation either by the generation of glycolytic ATP for the transhydrogenase reaction or the malate shuttle or by providing substrate for movement of reducing equivalents via shuttle pathways (Fig. 7). The possibility that glycolysis provides substrate for the shuttle pathways is supported by the finding that the infusion of carboxyatractyloside (30 μM), an inhibitor of ATP translocation from the cytosol to the mitochondrial (21), did not affect rates of 7-ethoxycoumarin metabolism in the perfused liver (data not shown). Furthermore, the fact that rates of oxygen uptake recovered rapidly to basal levels after removal of cyanide indicates that the mitochondria were not damaged irreversibly by perfusion with cyanide, yet maximal rates of 7-hydroxycoumarin production were not restored. However, rates were restored by infusion of pyruvate, which supplies substrate for the malate shuttle. Similar results were obtained when glucose was infused under conditions where the pentose cycle was inhibited by 6-aminonicotinamide treatment (12) (Fig. 6). Infusion of glucose increases rates of glycolysis and supplies three-carbon intermediates needed for NADPH transport (Fig. 7). These studies indicate that cyanide can activate glycogenolysis and glycolysis, thereby supplying pyruvate needed for substrate shuttle pathways, which in turn provide reducing equivalents

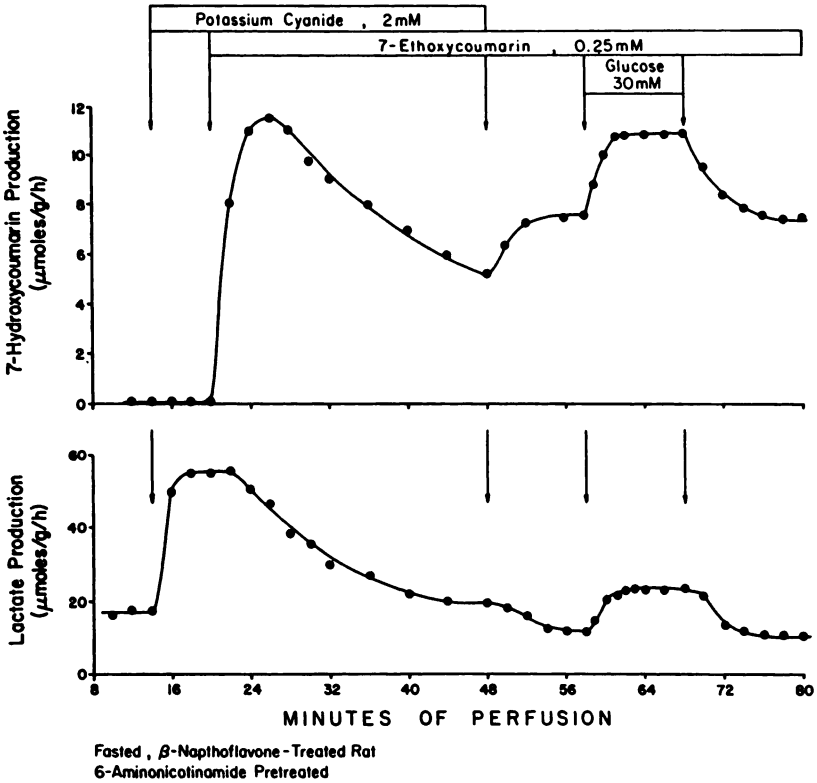


**Fig. 6.** Effect of glucose on the cyanide-mediated inhibition of 7-hydroxycoumarin production from 7-ethoxycoumarin. Potassium cyanide (2 mM), 7-ethoxycoumarin (0.25 mM), and glucose (30 mM) were infused, at times indicated by the *horizontal bars* and *vertical arrows*, into the liver of a fasted β-naphthoflavone-treated rat. The rat was treated with 6-aminonicotinamide, as described in Materials and Methods. Other conditions are as described in Fig. 2. A typical experiment is shown.

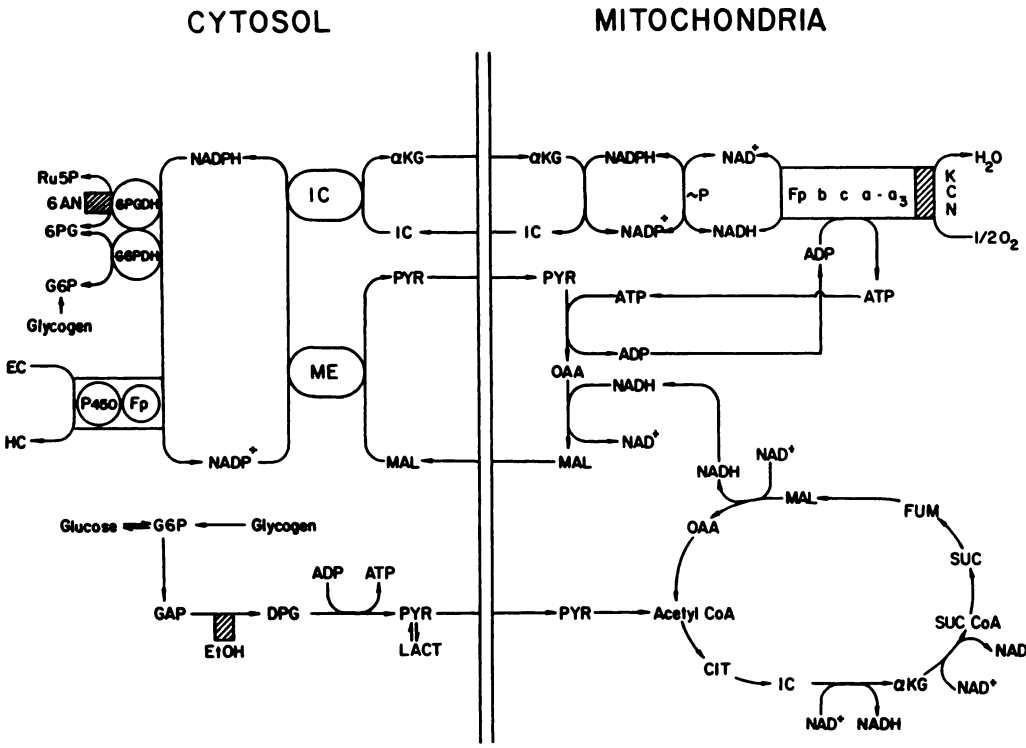


**Fig. 7.** Scheme depicting the interaction between glycolysis and NADPH-generating pathways. Inhibition of the respiratory chain with potassium cyanide (KCN) causes a decrease in mitochondrial ATP generation and activation of glycogenolysis, which produces glucose-6-phosphate (*G6P*) via glycolysis. Glycolytic ATP can provide energy for the energy-requiring transhydrogenase (*~P*) or the malic enzyme (*ME*) shuttle, which transfers reducing equivalents from the mitochondria into the cytosol. *GAP*, glyceraldehyde-3-phosphate; *IC*, isocitrate; *αKg*, α-ketoglutarate; *PYR*, pyruvate; *MAL*, malate; *OAA*, oxalacetate; *CIT*, citrate, *SUC Coa*, succinyl CoA; *SUC*, succinate, *FUM*, fumarate; *EtOH*, ethanol; *6PG*, 6-phosphogluconate; *Ru5P*, ribulose-5-phosphate; *G6PDH*, glucose-6-phosphate dehydrogenase; *6PGDH*, 6-phosphogluconate dehydrogenase; *6AN*, 6-aminonicotinamide; *LACT*, lactate; EC, 7-ethoxycoumarin; HC, 7-hydroxycoumarin; FP, flavoprotein.

for mixed function oxidation when mitochondrial function is impaired. The role of the malate shuttle in this process is supported by the observation that 7-ethoxycoumarin infusion increased hepatic malate concentration greatly (2). Because glycogen reserves are minimal in the fasted state, maximal rates of mixed function oxidation cannot be sustained. Collectively, these data indicate that glycolysis is crucial for maintenance of mixed function oxidation dependent on reducing equivalents derived from mitochondria.

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