The Effect of Cycloheximide on the Interaction between Mitochondrial Respiration and Gluconeogenesis in Guinea Pig and Rat Liver*

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SUMMARY

Cycloheximide (3 to 5 mM) caused a decrease in the rate of gluconeogenesis from pyruvate, lactate, or glycerol in the perfused rat and guinea pig liver. In these experiments, oxygen consumption was reduced by 30% with pyruvate but was unaltered with lactate or glycerol as substrates. The inhibition of gluconeogenesis caused by cycloheximide was rapid in onset (100% of maximal inhibition in 1 to 2 min) and immediately reversible upon cessation of cycloheximide infusion. Perfused livers were freeze-clamped in situ before and during cycloheximide infusion and the mitochondrial NAD: NADH ratio was calculated by a measurement of intermediates of the β-hydroxybutyrate dehydrogenase couplet. There was a marked shift toward reduction of the intramitochondrial pool of nicotinamide coenzymes after cycloheximide infusion. Under these conditions, the hepatic levels of ATP, ADP, and AMP were not significantly altered by cycloheximide in livers of rats perfused with lactate. With pyruvate as substrate, the ratio of ATP:ADP was decreased although no change in AMP levels was observed. Our findings indicate that the metabolic effects of cycloheximide are rapidly reversed, with the inhibitory effect on gluconeogenesis not evident after 10 min of recycling of the perfusate through the liver. Previous studies with cycloheximide have shown this compound to block mitochondrial energy transfer at Site I of the respiratory chain. It is suggested that cycloheximide decreases the rate of hepatic gluconeogenesis by an inhibition of mitochondrial energy transfer at Site I.

Cycloheximide is a potent inhibitor of protein synthesis in mammalian tissues. In the course of our studies with this compound, we noticed that at levels considerably higher (1 to 5 mM) than those required to block protein synthesis in the rat, cycloheximide markedly reduced the rate of P-enolpyruvate formation from malate or α-ketoglutarate by isolated guinea pig liver mitochondria (1, 2). We also reported that cycloheximide blocks mitochondrial energy transfer at Site I of the respiratory chain in a manner analogous to Amytal and at similar concentrations (4 to 8 mM). This effect of cycloheximide was of considerable interest since in previous studies from this laboratory, we had noticed a close association between gluconeogenesis, the rate of P-enolpyruvate formation and the energetic state of the cell (5, 0). The present work indicates that cycloheximide causes a marked, but readily reversible inhibition of gluconeogenesis from a variety of substrates in both the perfused rat and guinea pig liver.

EXPERIMENTAL PROCEDURE

Materials—Adenylate kinase (EC 2.7.4.3), β-hydroxybutyrate dehydrogenase (EC 1.1.1.30), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), hexokinase (EC 2.7.1.1.), lactate dehydrogenase (EC 1.1.1.27), pyruvate kinase (EC 2.7.1.40), phosphoenolpyruvate, NAD+, NADH, and NADP+, were obtained from Boehringer Mannheim. Alumunin (Fraction V) was obtained from Pentex. Pyruvate and β-hydroxybutyrate were purchased from Calbiochem. Cycloheximide, rotenone, lactate, and octanoate were obtained from Sigma. Fermocenzyme 952 DM was provided by Fermecolab. A purified standard of cycloheximide was a generous gift from Dr. G. S. Fonken of the Upjohn Corp., Kalamazoo, Mich.

Liver Perfusion—The technique for liver perfusion employed in these studies was the hemoglobin-free perfusion as described by Scholz et al. (7, 8). Male Sprague-Dawley rats were fasted 24 hours and guinea pigs of the Hartley strain fasted 48 hours prior to the experiment. The animals were anesthetized with sodium pentobarbital (Nembutal) injected intraperitoneally (50 mg per kg). The liver was perfused through the portal vein and the perfusate collected via the vena cava with the apparatus designed by Scholz et al. (7, 8). The isolation of the liver was completed within 5 min. The critical period between the incision of the portal vein and its cannulation was less than 5 s.

Livers were perfused by either the nonrecycling method in which the perfusion medium was not returned to the oxygenator, or the recycling procedure in which the venous effluent was re-
turned to the oxygenator. The nonrecycling method had several important advantages. It was ideally suited for kinetic studies since it allowed continuous sampling of perfusion medium. Since the medium passed through the liver only once, the concentrations of substrate could be maintained at lower, and presumably more physiological concentrations throughout the perfusion. However, the large volumes of perfusion medium used in the nonrecycling method precluded the routine use of bovine serum albumin. This could have been important when fatty acids were included in the perfusate. In preliminary experiments with this system both rat and guinea pig livers were perfused for 120 min with Krebs-Ringer bicarbonate buffer, containing 2 mM lactate and 0.4 mM octanoate, with or without 3% defatted bovine serum albumin. The rates of oxygen consumption, lactate utilization, and glucose production during this period were identical with and without albumin. Furthermore, a comparison of the recycling and nonrecycling methods with 10 mM lactate as a substrate, indicated no significant differences in gluconeogenesis between the two procedures. The rates of bile formation, and the wet weight to dry weight ratio were also the same with both methods. An additional advantage of the nonrecycling perfusion is the potential for the determination of the instantaneous rate of glucose formation without subtracting the preformed glucose produced by the recycling of the small perfusate volume.

Determination of Metabolites—The left lobe of the liver was freeze-clamped during the perfusion and the metabolites extracted by procedures described in detail previously (5). The concentration of metabolites in tissue extracts and in the liver perfusate was expressed as micromoles per g of liver, wet weight. Automated determinations of glucose were carried out with an AutoAnalyzer (Technicon) using the glucose-oxidase peroxidase system (9). The concentration of acetacetate and β-hydroxybutyrate (10), ATP (11), ADP and AMP (12) were determined enzymatically.

RESULTS

The nonrecycling perfusion system was used to measure alterations in hepatic glucose formation with varying concentrations of cycloheximide. Gluconeogenesis from pyruvate (2 mM) by isolated rat livers was progressively inhibited with increasing concentrations of cycloheximide, such that a concentration of 10 mM cycloheximide caused a 92% inhibition in glucose formation (Fig. 1). When the infusion of cycloheximide was stopped, the rate of glucose formation returned to levels approximately 60% of those noted before the infusion. Oxygen consumption also decreased, with a partial return to control levels after cycloheximide infusion was stopped.

The kinetic data of this cycloheximide-induced reduction in glucose formation are shown in Fig. 2. At a cycloheximide concentration of 3.5 mM, the rate of glucose formation from 2 mM pyruvate was maximally inhibited after only 1 min. This was completely reversed within 1 min after the termination of the cycloheximide infusion. In contrast to the results with 10 mM cycloheximide infusion, at lower levels (3.5 mM) the effect of this inhibitor on gluconeogenesis from pyruvate was completely reversible. Such a remarkably rapid and completely reversible inhibition of a process as complex as hepatic gluconeogenesis suggested that cycloheximide was active at an important site of the regulation of the pathway, presumably at, or close to, one of the rate-limiting steps.

When guinea pig livers were perfused with pyruvate under similar conditions (Fig. 3), oxygen consumption and gluconeogenesis were progressively inhibited with increasing concentrations of cycloheximide. However this inhibition was much less marked (50 to 80%) than with rat livers (compare Figs. 1 and 3). After cessation of cycloheximide infusion, glucose production was restored to 80% of the control levels.

Gluconeogenesis from substrates other than pyruvate was also susceptible to cycloheximide inhibition. As noted in Fig. 4, glucose synthesis from lactate in guinea pig livers was substantially decreased although oxygen consumption remained unaffected. Complete restoration of gluconeogenesis from lactate upon withdrawal of cycloheximide was similar to that with pyruvate as substrate (compare with Fig. 3). As shown in Table I, the decreases in gluconeogenesis and in oxygen consumption were less marked when rat livers were perfused with lactate rather than pyruvate. Taken together, these data indicate that the inhibition of gluconeogenesis by cycloheximide was independent of the substrate used, and was essentially reversed after withdrawal of the inhibitor. Furthermore, inhibition of oxygen consumption was noted only when pyruvate was the hepatic substrate. No significant effect was observed with oxygen consumption from lactate, despite the significant decrease in net glucose formation.

Since pyruvate and lactate differ primarily in the relative degree of oxidation, gluconeogenesis from another relatively reduced substrate was evaluated. In both rat (Fig. 5) and guinea pig liver (Fig. 6) perfused with 2 mM glycerol, increasing concentrations of cycloheximide produced a pattern of inhibition similar to that observed with lactate. This indicated that the inhibition of glucose formation occurred even with substrates which were not necessarily metabolized within the mitochondria,

![Fig. 1. The effect of cycloheximide infusion on gluconeogenesis and oxygen consumption from pyruvate by the isolated, perfused rat liver. Livers from 24-hour fasted rat were perfused with Krebs-Ringer bicarbonate buffer, containing 2 mM pyruvate, by the nonrecycling perfusion procedure explained in detail under "Experimental Procedure." After 15 min of pyruvate infusion, increasing concentrations of cycloheximide were introduced by a constant infusion pump. The bars indicate the standard error of the mean for four animals.](http://www.jbc.org/)
The effect of cycloheximide on gluconeogenesis and oxygen consumption from pyruvate by the isolated, perfused rat liver. Livers from 24-hour fasted rats were perfused with 2 mM pyruvate by the nonrecycling perfusion procedure. After 15 min of perfusion with pyruvate, 3.5 mM cycloheximide was introduced by a constant infusion pump. The effect of cycloheximide on glucose synthesis is expressed as a percentage of its maximal effect. The infusion of cycloheximide was stopped at the time indicated in the figure. The bars indicate the standard error of the mean for three animals.

**FIG. 3 (center).** The effect of cycloheximide infusion on gluconeogenesis and oxygen consumption from pyruvate by the isolated, perfused rat liver. Livers from 24-hour fasted rats were perfused with 2 mM lactate or pyruvate followed by 5 mM cycloheximide. The livers were freeze-clamped 15 min after substrate infusion or after 6 min of cycloheximide addition. ATP, ADP, and AMP were determined enzymatically on the neutralized tissue extracts. Other details are given under "Experimental Procedure." Values are the means ± standard error for five animals.

<table>
<thead>
<tr>
<th></th>
<th>Lactate</th>
<th>Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oxygen</td>
<td>2.42±0.15</td>
<td>2.36±0.15</td>
</tr>
<tr>
<td>consumption</td>
<td>μmoles/min/g liver</td>
<td>μmoles/min/g liver</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.48±0.044</td>
<td>0.27±0.016</td>
</tr>
<tr>
<td>formation</td>
<td>μmoles/g liver</td>
<td>μmoles/g liver</td>
</tr>
<tr>
<td>ATP</td>
<td>2.47±0.141</td>
<td>2.28±0.126</td>
</tr>
<tr>
<td>ADP</td>
<td>0.93±0.058</td>
<td>0.91±0.060</td>
</tr>
<tr>
<td>AMP</td>
<td>0.23±0.033</td>
<td>0.24±0.052</td>
</tr>
</tbody>
</table>

such as glycerol. The reversibility of the effects of cycloheximide may result from either a rapid hepatic inactivation of the inhibitor, or from a nonspecific clearance of the compound produced by the nonrecycling perfusion system. This point was tested in recycling perfusion experiments with rat liver synthesizing glucose from lactate (Fig. 7). In these studies the perfusate was recycled back to the oxygenator so that the duration of the inhibition due to a single addition of 3.5 mM cycloheximide could be measured. Glucose formation was rapidly decreased without alterations in oxygen consumption but the effect was not sustained for more than 10 min. After this time, the rate of gluconeogenesis returned to control rates suggesting that cycloheximide was rapidly inactivated by the liver.

Previous studies on the action of cycloheximide on both guinea pig and rat liver mitochondria indicated that this compound blocked energy transfer at the first site of oxidative phosphorylation in the respiratory chain (1, 2). This effect, at Site I, was obtained at cycloheximide concentrations similar to those which, in this study, were associated with an inhibition of gluconeogenesis in isolated, perfused livers. Since the rapid action of cycloheximide on hepatic glucose synthesis suggested that its action was not due to a diminished rate of protein synthesis, some other mechanism such as the previously observed effect on mitochondrial energy transfer seemed probable. An inhibition of oxidative phosphorylation at Site I in the perfused liver should result in an accumulation of NADH within the mitochondria and consequently a decrease in the intramitochondrial oxidation-reduction potential. Such an effect was observed in the experiments described in Figs. 8 and 9. Cycloheximide infused into rat livers metabolizing 2 mM lactate and 0.3 mM octanoate, caused an inhibition of glucose synthesis and, at the same time, reduced the acetoacetate to β-hydroxybutyrate ratio measured in the perfusate (Fig. 8). When the infusion was terminated, both the rate of gluconeogenesis and the acetoacetate to β-hydroxybutyrate ratio increase. A similar effect of cycloheximide on both glucose synthesis from lactate and on the ratio of acetoacetate to β-hydroxybutyrate was also noted in the per-
Siiling et al. (13) and with observations from our laboratory (14, perfused guinea pig liver, agreeing with the previous report by during cycloheximide infusion in order to determine the intra-animals.

octanoate itself caused a marked drop in gluconeogenesis in the perfused guinea pig liver. Livers from 48-hour fasted animals were perfused with 2 mM glycerol and cycloheximide as described in Fig. 1. The bars indicate the standard error of the mean for five animals.

The effect of cycloheximide on oxygen consumption and gluconeogenesis from glycerol by the isolated, perfused guinea pig liver. Livers from 48-hour fasted animals were perfused with 2 mM glycerol and cycloheximide as described in Fig. 1. The bars indicate the standard error of the mean for three animals.

If the action of cycloheximide on the perfused liver was pri-

marily localized to energy transfer at Site I, as observed with isolated liver mitochondria (1, 2), then an increased availability of reducing equivalents might account, in some way, for the variable substrate-dependent sensitivity of hepatic gluconeogenesis and oxygen consumption to infused cycloheximide. If the action of cycloheximide on the perfused liver was primarily localized to energy transfer at Site I and gluconeogenesis. As shown in Table III, increasing concentrations of rotenone and cycloheximide on oxygen consumption and gluconeogenesis.
FIG. 8 (left). The combined effect of octanoate and cycloheximide on oxygen consumption, gluconeogenesis, and ketone body formation from lactate by the isolated, perfused rat liver. Livers from 24-hour fasted animals were perfused with 2 mM lactate and with 0.3 mM sodium octanoate and 5 mM cycloheximide at the times indicated in the figure. All other conditions are as outlined in Fig. 1. The bars indicate standard error of the mean for four animals.

FIG. 9 (center). The combined effect of octanoate and cycloheximide on oxygen consumption, gluconeogenesis, and ketone body formation from lactate by the isolated, perfused guinea pig liver. Livers from 48-hour fasted animals were perfused with 2 mM lactate and with 0.3 mM sodium octanoate and 5 mM cycloheximide at the times indicated in the figure. All other conditions are as outlined in Fig. 1. The bars indicate standard error of the mean for three animals.

FIG. 10 (right). The effect of cycloheximide infusion on gluconeogenesis and oxygen consumption from pyruvate and β-hydroxybutyrate by the isolated, perfused rat liver. Livers from 24-hour fasted rats were perfused with 2 mM pyruvate, 8 mM β-hydroxybutyrate, and 5 mM cycloheximide at the times indicated in the figure. All other conditions are as outlined in Fig. 1. The bars indicate the standard error of the mean for five animals.

In the previous paper we reported that cycloheximide, at concentrations (3 to 5 mM) considerably higher than those required to inhibit protein synthesis in rat, blocked energy transfer at Site 1 of the respiratory chain of liver mitochondria isolated from rat liver (17). The effects of rotenone on both glucose formation and oxygen consumption were calculated by subtracting the rate of these processes noted with ethanol alone from that found with both rotenone and ethanol. The inhibition of gluconeogenesis and oxygen consumption by rotenone is striking and occurs at concentrations of rotenone three orders of magnitude lower than that noted with cycloheximide (compare Table III and Fig. 1).

TABLE II

Influence of cycloheximide on concentration of intermediates of β-hydroxybutyrate dehydrogenase equilibrium in freeze-clamped rat liver

<table>
<thead>
<tr>
<th>β-Hydroxybutyrate</th>
<th>Acetoacetate</th>
<th>NAD+/NADH</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmole/g liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before cycloheximide</td>
<td>0.131 ± 0.011</td>
<td>1.50 ± 0.001</td>
</tr>
<tr>
<td>With cycloheximide</td>
<td>0.163 ± 0.002</td>
<td>1.20 ± 0.001</td>
</tr>
</tbody>
</table>

served in guinea pig liver (17). The effects of rotenone on both glucose formation and oxygen consumption were calculated by subtracting the rate of these processes noted with ethanol alone from that found with both rotenone and ethanol. The inhibition of gluconeogenesis and oxygen consumption by rotenone is striking and occurs at concentrations of rotenone three orders of magnitude lower than that noted with cycloheximide (compare Table III and Fig. 1).

TABLE III

Inhibitory effect of rotenone on gluconeogenesis from pyruvate by perfused rat liver

<table>
<thead>
<tr>
<th>Rotenone concentration</th>
<th>Glucose formation</th>
<th>Oxygen consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>0.025</td>
<td>39.2 ± 5.7</td>
<td>11.4 ± 1.9</td>
</tr>
<tr>
<td>0.1</td>
<td>39.9 ± 8.8</td>
<td>12.0 ± 2.6</td>
</tr>
<tr>
<td>0.5</td>
<td>60.2 ± 1.9</td>
<td>15.0 ± 2.2</td>
</tr>
<tr>
<td>1.0</td>
<td>68.0 ± 5.4</td>
<td>20.1 ± 2.0</td>
</tr>
<tr>
<td>2.0</td>
<td>74.5 ± 5.0</td>
<td>30.1 ± 2.3</td>
</tr>
<tr>
<td>5.0</td>
<td>92.2 ± 2.3</td>
<td>35.1 ± 2.8</td>
</tr>
</tbody>
</table>

DISCUSSION

In the previous paper we reported that cycloheximide, at concentrations (3 to 5 mM) considerably higher than those required to inhibit protein synthesis in rat, blocked energy transfer at Site 1 of the respiratory chain of liver mitochondria isolated from rat liver (17). The effects of rotenone on both glucose formation and oxygen consumption were calculated by subtracting the rate of these processes noted with ethanol alone from that found with both rotenone and ethanol. The inhibition of gluconeogenesis and oxygen consumption by rotenone is striking and occurs at concentrations of rotenone three orders of magnitude lower than that noted with cycloheximide (compare Table III and Fig. 1).
both rat and guinea pig. The specificity of this effect at Site I was shown to be analogous to the action of Amytal (18). Furthermore, cycloheximide addition to mitochondria isolated from guinea pig liver markedly reduced the rate of P-enolpyruvate formation from either malate or α-ketoglutarate as substrates. In these experiments (1, 2) cycloheximide also lowered the rate of oxidative phosphorylation.

Our interest in the metabolic role of mitochondrially generated P-enolpyruvate for hepatic gluconeogenesis in these species containing substantial activities of intramitochondrial P-enolpyruvate carboxylase prompted us to determine the effects of cycloheximide infusion in the isolated, perfused liver. As noted in Figs. 1, 3, and 4, and Table I, cycloheximide caused a remarkably rapid, yet reversible inhibition of glucose synthesis from lactate or pyruvate in perfused livers from both the rat and guinea pig. Furthermore, the rapid onset of this inhibition (Fig. 2), and its nearly complete reversibility within 1 to 2 min suggests that cycloheximide, even at the relatively high levels used (3.5 µm), is not acting only as an inhibitor of protein synthesis nor is its action merely a nonspecific toxic effect. The rapidity of the effect of cycloheximide rules out a primary interaction between gluconeogenesis and protein synthesis, despite the fact that at the concentrations of inhibitor used in this study, the synthesis of hepatic protein would be almost completely blocked. In support of this hypothesis, the infusion of both actinomycin D and puromycin were without effect on the rate of gluconeogenesis and oxygen consumption from pyruvate by perfused rat liver.

**Effect of Cycloheximide on Oxidation-Reduction Potential**—The results of this study on the action of cycloheximide on gluconeogenesis in the perfused liver do not provide a completely unequivocal mechanism for its action. However, the experimental data are consistent with the metabolic consequences of an inhibitory effect of cycloheximide on energy transfer at Site I of the respiratory chain. An inhibition of gluconeogenesis may then be attributed either to the direct reduction in the rate of oxidation of mitochondrial reducing equivalents, or to a decreased rate of oxidative phosphorylation. Cycloheximide infusion increased the intramitochondrial levels of NADH as calculated from the intermediates of the β-hydroxybutyrate dehydrogenase system in freeze-clamped livers perfused with this inhibitor (Table II). Cycloheximide also caused a similar shift toward reduction in both rat and guinea pig livers metabolizing lactate and octanoate, as determined by the rates of β-hydroxybutyrate and acetoacetate production in the perfusate (Figs. 8 and 9). However, the rate of over-all glucose production appears more sensitive to inhibition at any given concentration of cycloheximide, than does oxygen consumption. Also the relative degree of oxidation of the substrate used affects the sensitivity of both oxygen consumption and gluconeogenesis to inhibition by cycloheximide. These observations may be attributed to a specific inhibition at Site I. Such an inhibition may also account for the decreased rate of oxygen consumption by the liver. Studies with rat and guinea pig liver mitochondria have shown that cycloheximide reduces oxygen consumption with all NAD⁺-linked substrates in the isolated organells (1, 2). However, in the perfused liver system employed in this study we noted that cycloheximide substantially inhibited oxygen consumption from pyruvate but not from lactate or glycerol. The principal difference between lactate and pyruvate as substrates for hepatic glucose synthesis is the mandatory formation of NADH in the cytosol catalyzed by lactate dehydrogenase. A similar cytosolic dehydrogenase step is required for gluconeogenesis from glycerol. Since both lactate and glycerol appear able to maintain oxygen consumption and oxidative phosphorylation during the cycloheximide infusion, some mechanism must exist for the utilization of these cytosolic reducing equivalents by the electron transport chain. Such a mechanism may be the α-glycerophosphate shuttle, which can transport reducing equivalents from the cytosol to the mitochondria in the form of glycerol phosphate. This intermediate can be reoxidized within the mitochondria to glyceroldehyde 3-phosphate thereby generating reduced flavin coenzyme. These reducing equivalents may then enter the electron transport chain at Site II, effecttively bypassing a cycloheximide-induced block in electron transport at Site I. It is therefore possible that cytosolic reducing equivalents generated from lactate or glycerol may be utilized by the electron transport chain, maintaining oxygen consumption and oxidative phosphorylation. Despite the attractiveness of this mechanism, a number of studies have suggested that the activity of α-glycerophosphate dehydrogenase in liver is considerably less than that noted in skeletal muscle (19). It is also possible that cytosolic reducing equivalents enter the mitochondria by some other mechanism, such as the Bösted cycle (20) and are directly converted to NADH in the mitochondrial matrix.

Regardless of the intracellular site of NADH generation, be it cytosolic with lactate and glycerol, or mitochondrial with β-hydroxybutyrate, increased concentrations of this reduced coenzyme are in some way capable of partially relieving the inhibition of gluconeogenesis caused by a block at Site I caused by cycloheximide. This is illustrated by the fact that the infusion of β-hydroxybutyrate together with pyruvate reverses the cycloheximide-induced inhibition of oxygen consumption observed with pyruvate alone (compare Fig. 10 and Fig. 1). Also, cycloheximide more effectively inhibits gluconeogenesis from pyruvate than from lactate, glycerol, or pyruvate plus β-hydroxybutyrate. It therefore seems clear that increasing the production of reducing equivalents in the cytosol or in the mitochondria can partially overcome, rather than enhance the inhibition of gluconeogenesis caused by cycloheximide.

**Effect of Cycloheximide on ATP Formation**—It is possible that a decreased rate of ATP formation or a diminished availability of high energy intermediates required for a variety of mitochondrial processes (21) may be responsible for this effect. Studies with mitochondria from rat and guinea pig liver have shown that cycloheximide inhibits energy coupling at Site I with a number of NAD⁺-dehydrogenase-linked substrates such as malate and α-ketoglutarate (1, 2). This resulted in a 30% decline in the rate of oxidative phosphorylation with these substrates. The concentrations of cycloheximide used in the present study were identical with the concentrations found to inhibit Site I in isolated mitochondrial preparations. It is therefore probable that if cycloheximide produces the same effect in the intact liver, an inhibition of gluconeogenesis may be attributed to a reduction in the rate of oxidative phosphorylation. As noted in Table I, adenine nucleotide balance in perfused rat liver metabolizing pyruvate was markedly altered by cycloheximide infusion. The ratio of ATP:ADP declined from 2.0 to 1.4 in the absence of any alteration in the total pool of hepatic adenine nucleotides. This is associated with a 70% decrease in the rate of gluconeogenesis by the perfused liver. Cycloheximide had less of an effect on adenine nucleotide balance in livers perfused with lactate and was correspondingly less effective in inhibiting gluconeogenesis. It therefore seems likely that an inhibition of gluconeogenesis caused by a block at Site I of the respiratory chain is mainly due to a decrease in the mitochondrial availability of ATP, or of intramitochondrial high energy intermediates.
The infusion of fatty acids has been shown to cause a reduction in gluconeogenesis from lactate in isolated perfused guinea pig liver (13-15) while an opposite effect has been observed in rat liver (22-24). In the present study we have again noted a reduction in glucose synthesis from lactate in guinea pig liver when octanoate was added. Factors which decrease the intramitochondrial NADH/ NADP+ ratio, such as the infusion of octanoate or β-hydroxybutyrate (Fig. 10) caused a marked stimulation of gluconeogenesis by isolated rat liver but inhibited gluconeogenesis in guinea pig liver (14, 15). These differences, when considered with the work of Sölting et al. (13), suggest that the regulation of glucose synthesis in rat and guinea pig livers is markedly different. However, cycloheximide, which increases the NADH levels in the mitochondria of both species (Figs. 8 and 9), inhibits gluconeogenesis similarly in both species. This implies that the effect of cycloheximide on the oxidation-reduction state of the mitochondria is related secondarily to a more direct mechanism, such as the observed reduction in hepatic ATP levels. The importance of ATP formation in the regulation of the hepatic oxidation-reduction state, and its relationship to gluconeogenesis have also been pointed out by Stubbs et al. (25).

In several studies with isolated mitochondria from both guinea pig and rabbit liver, it was observed that uncoupling of oxidative phosphorylation was a necessary requisite for maximal rates of P-enolpyruvate formation (26), and the suggestion was made that a physiological uncoupling process is necessary for mitochondrial P-enolpyruvate formation (26), and the suggestion was made that an energy coupling at this site plays a particularly important role in regulating the overall gluconeogenic pathway. The exact mechanism by which phenethylbiguanide inhibits hepatic glucose synthesis is not clear, there are similarities between its effect and that of cycloheximide. Since both compounds appear to act specifically at Site I of the respiratory chain, it is probable that energy coupling at this site plays a particularly important role in regulating the overall gluconeogenic pathway.

Acknowledgments—We acknowledge the skillful technical assistance of Mrs. Emilia Siojo and Mr. Edward H. Goodman, Jr., and also thank Doctors Leon Salaganicoff and Ifeanyi Arinze for their help and suggestions during the course of this study. We are especially indebted to Dr. Roland Scholz for design and construction of the liver perfusion apparatus used in this work and for his advice in the techniques of liver perfusion.

REFERENCES


34. KRUGER, F. A., SKILLMAN, T. C., HAMWI, G. J., GRUBBS, R. C., and DANFORTH, N. (1960) *Diabetes* 9, 17


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