Ethanol-induced Hypoglycemia

II. Mechanism of Suppression of Hepatic Gluconeogenesis

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SUMMARY

The postulation that the increased $NADH_2$/NAD ratio generated in the liver cell during ethanol metabolism causes the suppression of hepatic gluconeogenesis has been tested in several ways in twenty-eight fasted glycogen-depleted dogs in whom hepatic gluconeogenesis was inhibited by infusions of ethanol. First, it was shown that fructose, a non-NAD-dependent precursor of glucose, produced a rapid restoration of hepatic glucose output during ethanol-induced suppression of hepatic gluconeogenesis. Second, in contrast, the infusion of glutamate and α-ketoglutarate, both NAD-dependent precursors of glucose, failed to augment the depressed rate of hepatic gluconeogenesis induced by ethanol. Finally, the administration of methylene blue, a redox dye which oxidizes $NADH_2$ to NAD, not only prevented the expected fall in hepatic glucose output when infused simultaneously with ethanol, but also produced a rapid restoration of hepatic glucose output previously depressed by ethanol administration in fasting dogs. These data are consonant with the thesis that the increased $NADH_2$/NAD ratio, which characterizes ethanol oxidation by the liver cell, causes a partial block at several points in the gluconeogenic pathway and is responsible for the ethanol-induced suppression of hepatic gluconeogenesis. DIABETES *16*:252-58, April, 1967.

Since its original description over twenty-five years ago,[1] at least 101 cases of ethanol-induced hypoglycemic coma have been documented. These reports on eighty-nine adults and twelve children appeared in twenty-three different publications originating from nine countries.[2] The majority of the 101 cases were reported from the United States (thirty-six subjects), Brazil (twenty-nine subjects), and the Republic of South Africa (twenty-three subjects). The dearth of reports from England, France, Greece and Sweden, and the absence of reports from other European countries cannot be judged as evidence that this syndrome is a rare complication of the deliberate intake of alcohol in adults or the accidental ingestion of ethanolic beverages in children. Where physicians have been acquainted with its manifold neurological manifestations and have been alerted to its occurrence, large numbers of cases have been recognized in short periods of time. Within a ten-month period, twenty-three cases were found in South Africa by Neame and Joubert.[3] A similar experience was recorded from Brazil by Bottura et al.[4] and Neves et al.[5] In our emergency ward twelve to eighteen cases are recognized each year by house physicians familiar with the hypoglycemic potential of ethanol in fasted or glycogen depleted patients. It is important that prompt treatment be instituted, for permanent neurological deficits and even death may follow prolonged and profound hypoglycemia; indeed, the mortality rate for adults is 11 per cent and for children 25 per cent.[2] The clinical manifestations, the setting in which ethanol-induced hypoglycemia occurs and the mortality figures recently have been reviewed in detail by Madison.[2] It must be emphasized that ethanol-induced hypoglycemia can occur in children, adolescents and adults in the absence of either a history of chronic alcoholism or evidence of liver disease and malnutrition.

Previous studies in fasted dogs have shown that ethanol induces hypoglycemia entirely as a consequence of a marked decrease in hepatic glucose output.[6,7] Although those studies indicated the manner in which the hypoglycemia was produced, they did not identify the precise biochemical lesion within the hepatic cell responsible for the decreased gluconeogenesis.

On the basis of substantial in vitro[8-10] and in vivo[6,7,11] experimental evidence it has been postulated that the increased $NADH_2$/NAD ratio (reduced/oxidized nicotinamide adenine dinucleotide) generated in the liver cell[12-14] during oxidation of ethanol to acetaldehyde and then to acetate is causally linked with suppression of hepatic gluconeogenesis. The present studies were designed to test this hypothesis in three ways: first, to quantitate the effect on hepatic glucose output during ethanol inhibition of hepatic gluconeogenesis of fructose, a non-NAD-dependent precursor of glucose; second, to determine the effect of the administration of

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the NAD-dependent precursors of glucose, α-ketoglutarate and glutamate, on the output of glucose by the liver during ethanol-induced suppression of hepatic gluconeogenesis in glycogen-depleted dogs fasted for forty-eight to seventy-two hours; third, to ascertain the effect of infusion of methylene blue, a redox dye capable of rapidly oxidizing $NADH_2$ to NAD, on hepatic glucose output during ethanol-induced inhibition of hepatic gluconeogenesis.

METHODS AND PROCEDURE

All twenty-eight studies were performed in healthy mongrel dogs with chronic end-to-side portacaval shunts. This preparation was chosen since it separates the liver from the extrahepatic splanchnic bed and thereby permits quantitation of hepatic rather than splanchnic glucose balance.[15] Complete recovery after the operative procedure was assured by waiting at least one month after the second stage of the two-stage shunt operation before performing these studies. All dogs were fasted for two to three days prior to each study to be certain that hepatic glucose output was being derived exclusively from gluconeogenesis. Hepatic venous blood was obtained from a cardiac catheter passed under fluoroscopic control deep in an hepatic vein. Arterial blood samples were collected via an indwelling Cournand needle placed in a femoral artery. Hepatic blood flow was estimated by the clearance and extraction method of Bradley et al.[16] using I-131-labeled rose bengal as the extractable substance.[17] Hepatic glucose output, the product of hepatic venous-arterial glucose concentration difference and hepatic blood flow, was determined at ten-minute intervals both during the thirty-minute control period and for the ensuing two hours during which time ethanol (2.5 to 5 per cent in 0.6 per cent saline) was infused into a hindlimb vein at a rate of 0.5 mM. per minute.

Hepatic vein and arterial glucose concentrations were determined in triplicate by the copper-iodometric method of Somogyi.[18] In the six studies where fructose was infused, the o-toluidine technic[19] for determining blood glucose was used since preliminary studies in our laboratory showed that the addition of fructose, in final concentrations up to 150 mg. per 100 ml., to control blood samples, did not influence blood glucose levels. Blood ethanol levels were determined by the microdiffusion technic of Newman and Newman.[20] The procedures, technics and methods used in this laboratory have been described in detail in previous publications.[15,21]

Five types of studies were performed. In all twenty-eight experiments, after a thirty-minute control period, ethanol was infused at a rate of 30 mM. per hour for two hours. In four groups of six studies each during the last sixty minutes of ethanol infusion either fructose (60 mM. per hour), α-ketoglutarate (100 mM. per hour), glutamate (90 mM. per hour) or methylene blue (5 to 8 mg. per kg.) were simultaneously administered and the effect on hepatic glucose output quantitated. In the fifth group of four studies, after the usual control period methylene blue was administered with ethanol to determine whether this would prevent or alter the anticipated ethanol-suppression of hepatic gluconeogenesis.

RESULTS

In each of the twenty-four studies in which ethanol was administered alone for the first hour, the infusion of ethanol in fasted dogs produced a prompt and sustained decrease in hepatic glucose output. These findings are consonant with previous reports from this laboratory on the consistent effect of ethanol on hepatic glucose output in dogs depleted of liver glycogen by fasting and depending on gluconeogenesis for maintenance of hepatic glucose output.[6,7,11] In the present studies the infusion of small amounts of ethanol which produced blood ethanol levels between 10 and 20 mg. per cent resulted in a 58 per cent fall in mean hepatic glucose output from 50 to 21 mg. per minute (figures 1 to 4). This depression in hepatic gluconeogenesis is not related to any unique sensitivity of dogs with portacaval shunts since a 78 per cent depression in splanchnic glucose output has been reported from this laboratory in fasted nonshunted mongrel dogs during ethanol infusion.[7]

In all six experiments the infusion of fructose during ethanol-induced suppression of hepatic glucose output resulted in a significant increase in hepatic glucose output to or greater than the control value (figure 1), indicating that the gluconeogenic enzymatic pathway from fructose-1,6-diphosphatase to glucose is not inhibited by ethanol. Ethanol infusion alone resulted in a decline in hepatic glucose output from 51 to 20 mg. per minute. Despite the continued administration of ethanol, fructose infusion produced a 170 per cent increase in hepatic glucose output to 54 mg. per minute (figure 1).

In contrast, the infusion of either α-ketoglutarate or glutamate, both NAD-dependent precursors of glucose, failed in twelve studies to augment the output of glu-

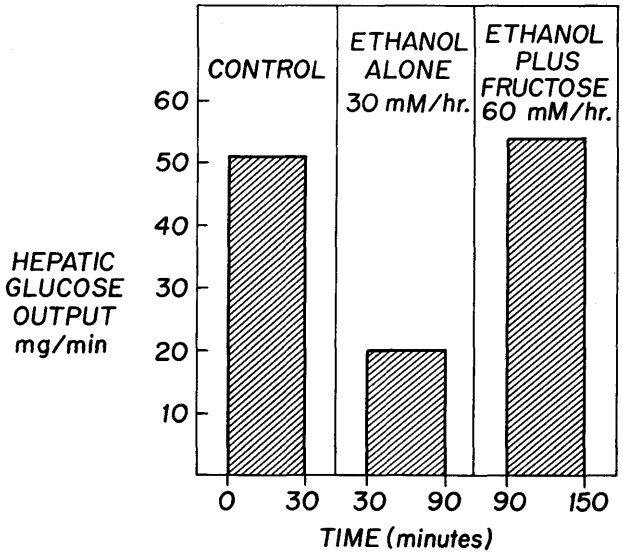

FIG. 1. Effect of fructose, a non-NAD-dependent precursor of glucose, on hepatic glucose output during ethanol-induced suppression of hepatic gluconeogenesis in fasted dogs (mean changes in six studies).

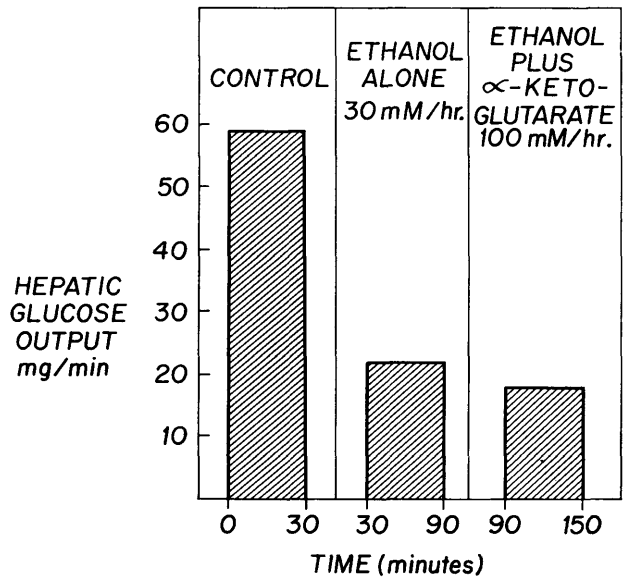

FIG. 2. Effect of α-ketoglutarate, a NAD-dependent precursor of glucose, on hepatic glucose output during ethanol-induced suppression of hepatic gluconeogenesis in fasted dogs (mean changes in six studies).

cose by the liver when hepatic glucose output was suppressed by ethanol (figures 2 and 3). In both groups of studies, hepatic glucose output continued to fall during substrate infusion.

The administration of methylene blue in six studies consistently produced a rapid restoration of hepatic glucose output to control values when infused during

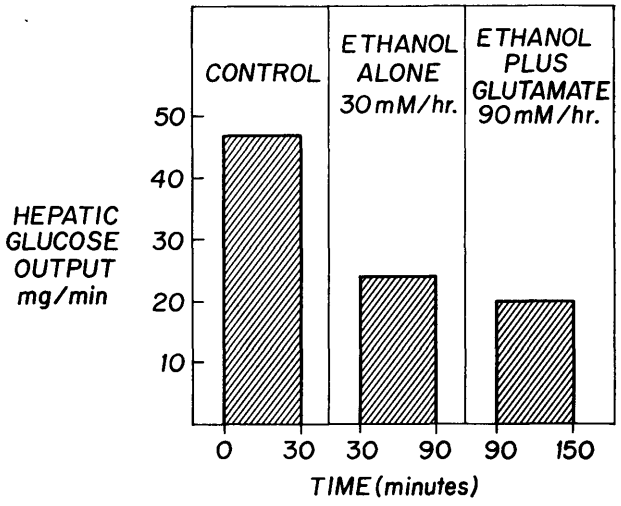

FIG. 3. Effect of glutamate, a NAD-dependent precursor of glucose, on hepatic glucose output during ethanol-induced suppression of hepatic gluconeogenesis in fasted dogs (mean changes in six studies).

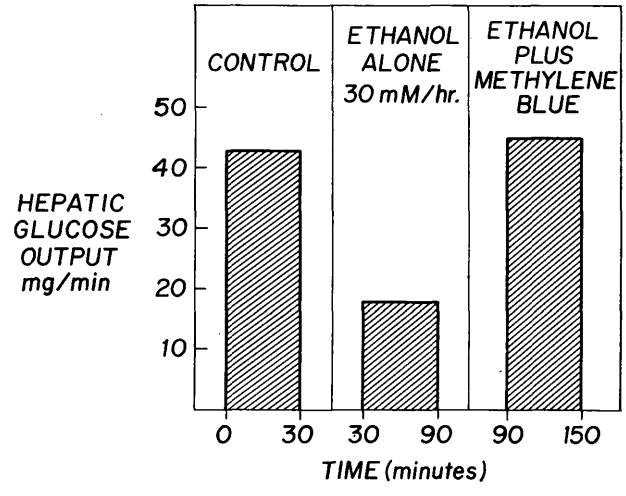

FIG. 4. Effect of methylene blue, a redox dye capable of oxidizing $NADH_2$ to NAD and thereby decreasing the elevated $NADH_2$/NAD ratio generated in the liver cell during ethanol oxidation, on hepatic glucose output during ethanol-induced suppression of hepatic gluconeogenesis in fasted dogs (mean changes in six studies).

ethanol-induced inhibition of hepatic gluconeogenesis (figure 4). Mean hepatic glucose output which had decreased from 43 mg. per minute in the control period to 18 mg. per minute during ethanol infusion rose promptly, within minutes, to 45 mg. per minute when methylene blue was infused. Moreover, the simultaneous administration of methylene blue and ethanol prevented the anticipated decline in hepatic glucose output invariably produced in dogs fasted two to three days (figure 5).

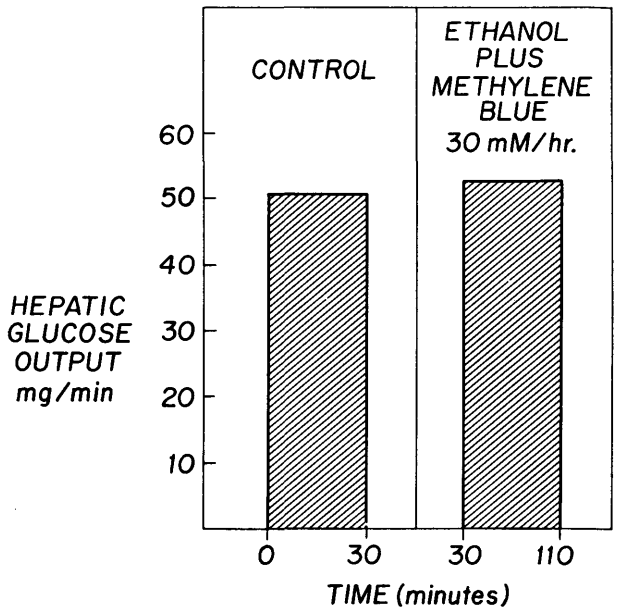

FIG. 5. Effect of simultaneous infusion of methylene blue and ethanol on hepatic glucose output in fasted glycogen-depleted dogs (mean changes in four studies). The anticipated decrease in hepatic gluconeogenesis following ethanol administration was prevented by methylene blue.

DISCUSSION

The data from the present studies not only afford further evidence for an ethanol-induced suppression of hepatic gluconeogenesis but in addition they also support the hypothesis that the increased $NADH_2$/NAD ratio generated in the hepatic cell by the oxidation of ethanol blocks gluconeogenesis. Before examining these data, evidence for an ethanol-induced inhibition of gluconeogenesis and for an altered $NADH_2$/NAD ratio in liver cells during ethanol oxidation will be discussed.

The studies of Field et al.[8] and Freinkel and associates[9,10] provide direct and convincing in vitro evidence that ethanol suppresses hepatic gluconeogenesis. A significant decrease in the generation of glucose-C-14 from alanine-C-14 and a simultaneous reciprocal rise in lactic acid-C-14 by rat, rabbit and human liver slices exposed to ethanol has been reported.[9,10] Moreover, other data indicate that ethanol perfusion of the isolated glycogen-depleted rat liver results in a 50 per cent decrease in hepatic new glucose production.[8] Evidence that ethanol also produces a suppression of hepatic gluconeogenesis in vivo was reported by Lochner and Madison[6,11] and Lochner et al.[7]; their studies showed that in the glycogen-depleted, fasted dog whose hepatic glucose output originated via gluconeogenesis from lactate, amino acids and glycerol, the infusion of ethanol produced a prompt 68 per cent decrease in hepatic glucose output.

The oxidation of ethanol to acetaldehyde and acetate, respectively, by alcohol and acetaldehyde dehydrogenase is accompanied by the simultaneous reduction of NAD to $NADH_2$. This $NADH_2$ produced in the cytoplasmic portion of the liver cell may be oxidized to NAD both by extra- and intramitochondrial reactions.[22] The reduction of acetoacetate, dihydroxyacetone phosphate, 1,3-diphosphoglycerate and pyruvate, respectively, to β-hydroxybutyrate, L-α-glycerophosphate, glyceraldehyde-3-phosphate and lactate result in the simultaneous reoxidation of $NADH_2$ to NAD. Moreover, β-hydroxybutyrate and L-α-glycerophosphate act as shuttles delivering reducing equivalents generated in the extramitochondrial part of the cell to the intramitochondrial flavoprotein cytochrome system,[22] the major route for the reoxidation of extramitochondrial $NADH_2$. In spite of these many routes for reoxidation of $NADH_2$, the $NADH_2$/NAD ratio within the hepatic cell increases two- to fourfold during the oxidation of ethanol.[12,13,23] During starvation, total hepatic NAD plus $NADH_2$ decreases and the $NADH_2$/NAD ratio rises.[13,14] Moreover, the $NADH_2$/NAD ratio increases to a greater degree in starved, compared to fed, animals following ethanol administration.[13] This increase in the $NADH_2$/NAD ratio during ethanol metabolism is also reflected by changes in the reduced and oxidized form of several metabolites whose redox state follows changes in the $NADH_2$/NAD ratio within the liver cell. An increase in the β-hydroxybutyrate/acetoacetate and lactate/pyruvate ratios in hepatic venous effluent blood following ethanol administration has been reported.[24-26] These in vivo and in vitro data leave little doubt that ethanol oxidation within the liver results in a diminished availability of NAD and an excess of $NADH_2$; the former may result in a decreased catalysis of certain important reactions necessary for gluconeogenesis and the latter may drive reactions in a reductive direction and thereby diminish the absolute amount of substrate available for biosynthesis of glucose.

It is not our intent to discuss the feedback and enzymatic control of gluconeogenesis[27] or the factors which determine the change from glycolysis to gluconeogenesis but rather to deal with only those factors which suddenly might diminish a given rate of gluconeogenesis during fasting. Only those points in the pathways of gluconeogenesis which may be blocked partially by excessive generation of $NADH_2$ during ethanol

metabolism will be stressed. Gluconeogenesis depends on the operation of specific enzymes requiring additional energy sources.[27] These are numbered in figure 6 within large arrows and include: (1) pyruvate carboxylase which, by CO_2 fixation, converts pyruvate to oxaloacetate; (2) phosphoenolpyruvate carboxykinase which converts oxaloacetate to phosphoenolpyruvate; (3) fructose-1,6-diphosphatase which hydrolyzes fructose-1,6-diphosphate to fructose-6-phosphate; and finally, (4) glucose-6-phosphatase which hydrolyzes glucose-6-phosphate to free glucose.

The data from the present studies indicate that the pathway from fructose-1,6-diphosphate to glucose (figure 6) is not blocked during ethanol-induced suppression of hepatic gluconeogenesis, since fructose produced a rapid increase in hepatic glucose output (figure 1). An increased production of glucose from fructose by the isolated rat liver perfused with ethanol also has been reported.[8] The studies of Tygstrup et al.[26] indicate

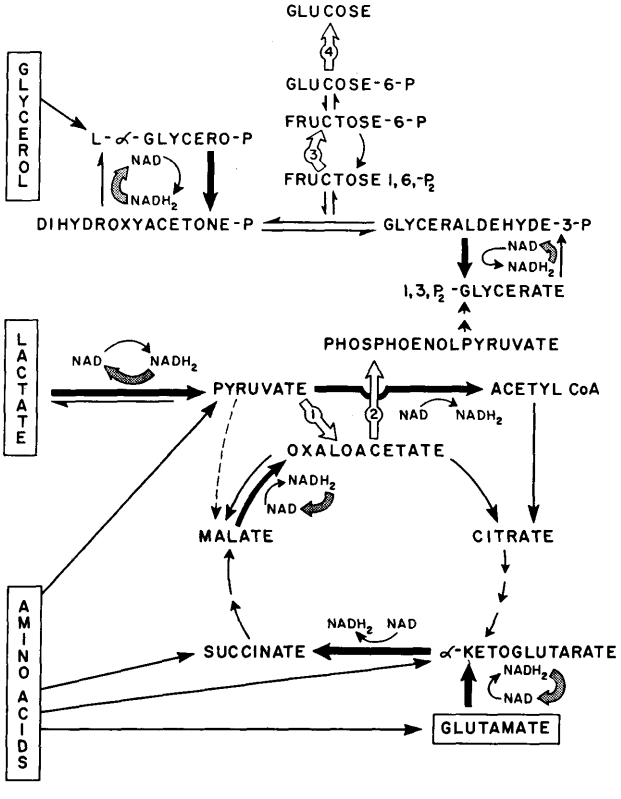

FIG. 6. Pathways of hepatic gluconeogenesis during starvation. The specific enzymes upon which gluconeogenesis is dependent are numbered within the large arrows. The NAD-dependent points in the pathway of gluconeogenesis are shown by the bold solid arrows. The direction of reactions in the presence of an elevated $NADH_2$/NAD ratio is depicted by the stippled curved arrows. See text for details.

that during ethanol metabolism fructose after phosphorylation to fructose-1-P is converted to dihydroxyacetone and glyceraldehyde. The former is available for conversion to fructose-1,-6,P_2 and, subsequently, to glucose; the glyceraldehyde by reduction to glycerol oxidizes $NADH_2$ to NAD. Moreover, some fructose is reduced to sorbitol, again oxidizing $NADH_2$ to NAD. Presumably, by furnishing available triose for conversion to glucose and by decreasing the elevated $NADH_2$/NAD ratio, fructose administration results in augmented hepatic gluconeogenesis. Indeed, during ethanol administration, when the pathways of other precursors for conversion to glucose apparently are blocked, more glucose is produced from fructose than when fructose is administered without ethanol.[26]

During prolonged fasting, the gluconeogenic precursors of glucose include lactate, amino acids and glycerol. These precursors of glucose are shown in rectangles on the left side of figure 6. Amino acids utilized for gluconeogenesis first must be converted either to pyruvate or to intermediates in the citric acid cycle. The points in the pathway of gluconeogenesis that are NAD-dependent are shown by the bold, solid arrows in figure 6. The direction of the reactions in the presence of an increased $NADH_2$/NAD ratio is shown by the stippled, curved arrows. The present data indicate (figures 2 and 3) that the conversion of glutamate and α-ketoglutarate to glucose is diminished during ethanol administration. The infusion of large amounts of α-ketoglutarate (100 mM. per hour) and glutamate (90 mM. per hour) failed to augment hepatic gluconeogenesis previously suppressed by ethanol. The generation of phosphoenolpyruvate via pyruvate and oxaloacetate is essential for maintaining a steady rate of gluconeogenesis during starvation. The data from the present studies (figures 2 and 3) suggest that conversion of citric cycle intermediates to oxaloacetate is dampened during ethanol metabolism. Moreover, the diminished oxidation to C-14-O_2 of acetate-C-14 and palmitate-C-14,[23,28] of alanine-C-14[9,10] and of glucose and pyruvate-C-14[8] plus the decrease in the absolute amount of CO_2 formed during ethanol perfusion of the isolated rat liver[29] are further evidence of depressed citric cycle activity. The failure of α-ketoglutarate (figure 2) to augment hepatic gluconeogenesis during ethanol infusion is very likely the consequence of a reduction in the oxidation of α-ketoglutarate to succinate, and of malate to oxaloacetate in the presence of a diminished availability of NAD (figure 6). Moreover, in addition to depressed citric cycle activity, the

conversion of glutamate to glucose is further depressed (figure 3) by the inhibition of glutamic dehydrogenase by an increased $NADH_2/NAD$ ratio.[30]

The increased $NADH_2/NAD$ ratio also would be expected to decrease the availability of pyruvate for conversion to oxaloacetate and then to phosphoenolpyruvate since the altered ratio produces a reduction of pyruvate to lactate (figure 6). The absolute amount of lactate leaving the liver during ethanol metabolism has been shown to be increased[24,25] and lactate accumulation in the incubation media during ethanol metabolism by liver slices has been reported.[9,29] Since more lactate leaves the liver than is delivered to it, lactate cannot be counted as a precursor for glucose during ethanol metabolism.

Glycerol (figure 6) is another precursor for glucose.[31] The decreased hepatic utilization of glycerol during ethanol metabolism[32,33] may also contribute to the ethanol-induced suppression of hepatic gluconeogenesis. The decreased conversion of glycerol to glucose and the accumulation of L-α-glycerophosphate during ethanol oxidation[32-34] indicate that glycerol metabolism is slowed at the L-α-glycerophosphate point in the pathway of gluconeogenesis (figure 6) by the increased $NADH_2/NAD$ ratio generated by ethanol.

It would be anticipated that if the increased $NADH_2/NAD$ ratio resulting from ethanol oxidation was causally linked to the decreased rate of gluconeogenesis, then an experimentally induced decrease in this ratio should result in the restoration of gluconeogenesis. The present studies show just such an effect. When methylene blue, a redox dye capable of oxidizing $NADH_2$ to NAD, was infused during ethanol-induced suppression of gluconeogenesis, a rapid and sustained increase in hepatic new glucose production occurred (figure 4). Moreover, the simultaneous administration of methylene blue and ethanol prevented the anticipated reduction in hepatic new glucose formation (figure 5).

Although the reductive biosynthesis of glyceraldehyde-3-P from 1,3-diphosphoglycerate may be stimulated by the increased $NADH_2/NAD$ ratio (figure 6) and, in this manner, tend to augment gluconeogenesis, it is likely that the glyceraldehyde-3-P is diverted to L-α-glycerophosphate since it has been shown that during ethanol metabolism L-α-glycerophosphate accumulates in the liver[32-34]

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