formed and the organism is released in its infectious and protected form.

The reasons for the differences in these results from those described previously are not clear. The MP suspensions used by Allen (3) were prepared from organisms grown in chick embryos, and were of different ages than those used in the above experiments. It seems to us that a more likely explanation may lie in the use of trypsin in the purification of MP organisms. We have found that trypsin treatment of RB suspensions causes a reduction in optical density of 60% within 4 min, and we have used this procedure in the preparation of cell envelopes of RB (8). The use of trypsin may very well remove cytochrome C reductase activity from RB preparation, and the large forms in the upper bands of potassium tartrate gradients may be RB membranes and not intact organisms. The number of RB in the sediment of such gradients, even though a small portion of total organisms, may well account for most of the enzyme activity of the pellet.

Summary. Purified suspensions of both mature infectious elementary bodies and intermediate reproductive reticulate bodies of meningopneumonitis organisms were tested for NADH-cytochrome C reductase activity. Both intact and disrupted suspensions of reticulate bodies showed a relatively high enzyme activity. Intact elementary body preparations showed very low activity, but a 12-fold increase occurred when the organisms were disrupted by sonic treatment. The results indicate difference in envelope or membrane permeability at different developmental stages.

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The Effect of Ethanol on the Concentration of Gluconeogenic Intermediates in Rat Liver (33329)

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The mechanism of ethanol-induced hypoglycemia has not been completely defined. In experimental animals ethanol decreases hepatic glucose production (1–3) and peripheral glucose utilization (3). Ethanol hypoglycemia seems to result from an inhibition of hepatic gluconeogenesis. It has been postulated that this inhibition of hepatic glucose production is related to the increased hepatic NADH concentration (1–5) which is associated with ethanol metabolism (6–8). A high hepatic NADH to NAD ratio could remove substrate from the gluconeogenic pathway by shifting the steady state concentration of the substrates involved in the following reactions:

$$\text{Pyruvate} + \text{NADH} \leftrightharpoons \text{Lactate} + \text{NAD}, \quad (1)$$

$$\text{Dihydroxacetone-P} + \text{NADH} \leftrightharpoons \alpha\text{-Glycerophosphate} + \text{NAD}, \quad (2)$$

$$\text{Oxaloacetate} + \text{NADH} \leftrightharpoons \text{Malate} + \text{NAD}, \quad (3)$$

towards lactate, α-glycerophosphate (3–5) and malate, respectively. This idea is supported by the fact that the administration of ethanol increases the blood lactate concentration (9–13), *in vitro* lactate production by

TABLE I. The Effect of Ethanol on the Hepatic Concentration of Intermediates Involved in Oxidation–Reduction Reactions and Aspartate and Glutamate.[a]

Intermediate	Feeding		p[b]
	Water	Ethanol	
Dihydroxyacetone-P	23.6 ± 4.94 (9)	26.2 ± 2.11 (8)	NS[c]
α-Glycerophosphate	438 ± 44.2 (10)	975 ± 81.7 (9)	<0.001
Malate	202 ± 17.2 (10)	591 ± 49.5 (10)	<0.001
Aspartate	674 ± 60.4 (10)	432 ± 65.0 (9)	<0.001
Glutamate	1057 ± 33.0 (10)	1916 ±176.0 (10)	<0.001
Pyruvate	61.7 ± 4.31 (10)	77.5 ± 9.14 (9)	NS
Lactate	1465 ±160 (10)	1671 ±200 (9)	NS

[a] Concentrations are mμmoles/g of wet wt. liver; mean ± SE; value in parentheses indicates number of livers analyzed.

[b] p value for difference between means.

[c] NS = not significant at 0.05 level.

liver (2, 14), the hepatic α-glycerophosphate concentration (14, 16), and decreases the conversion of ^{14}C-labeled amino acids to glucose. Ethanol also decreases the hepatic uptake of glycerol (17–19) which seems to be related to its effect on reaction (2). Since a high NADH concentration dissociates glutamic dehydrogenase (E.C. 1.4.1.2) (20), ethanol could block the conversion of glutamate to glucose, and interfere with the flow of amino acids into gluconeogenic pathways by decreasing the activity of the transamination reactions. Thus, there is evidence that ethanol interferes with hepatic glucose production by shunting intermediates away from the gluconeogenic pathway and by blocking the entry of substrate into this pathway. On the basis of currently available data it is not possible to estimate the relative quantitative importance of these different mechanisms. It is also not possible to exclude an ethanol inhibition of the reversal of glycolysis. In order to determine the specific gluconeogenic reactions affected by ethanol *in vivo*, the relative importance of each of these interactions for ethanol-induced hypoglycemia, and to exclude ethanol inhibition of the reversal of glycolysis, the effect of ethanol on the hepatic concentration of gluconeogenic intermediates has been investigated.

Methods. Enzymes, substrates, and cofactors were purchased from Calbiochem, Sigma Chemical Co., or Boehringer-Manneim Company. Female Sprague-Dawley rats weighing approximately 200 g were used in all experiments.

Animals were fasted for 48 hr prior to the feeding of ethanol. During this time they were allowed water. Each rat was then fed 0.8 g of ethanol (33% solution in water, v/v) by stomach tube. Control rats received 3 ml of water by tube. Four hr after feeding, the rats were anesthetized with ether, the abdomen was opened, and a portion of liver was removed by clamping between aluminum blocks cooled to the temperature of liquid N_2. The tissue was powdered at the temperature of liquid N_2 in a percussion mortar, deproteinized by addition to ice-cold 6% (v/v) $HClO_4$, and centrifuged at 5000 rpm. The precipitate was washed once in 3% (v/v) $HClO_4$, and the combined extracts adjusted to pH 4–5 with KOH. All intermediates were assayed by standard methods outlined in Ref. (21) using a Gilford model 2000 recording spectrophotometer.

Results. Ethanol administration led to changes in the concentration of several gluconeogenic intermediates involved in oxidation–reduction reactions (Table I). Although the concentration of dihydroxyacetonephosphate was the same in ethanol and control livers, the hepatic concentration of α-glycerophosphate was increased 2-fold by ethanol administration. The hepatic concentration of malate was also increased in the ethanol fed rats. Since the hepatic concentration of oxaloacetate in both control and

TABLE II. The Effect of Ethanol on the Hepatic Concentration of Gluconeogenic Intermediates between Phosphoenolpyruvate and Glucose-6-P.[a]

Intermediate	Feeding		p[b]
	Water	Ethanol	
Phosphoenolpyruvate	57.2 ± 2.68 (10)	42.8 ± 7.15 (9)	NS[c]
2-Phosphoglycerate	16.6 ± 1.75 (6)	5.99 ± 1.31 (8)	<0.001
3-Phosphoglycerate	135 ± 8.34 (10)	45.6 ± 7.00 (9)	<0.001
Fructose-1,6-P	18.7 ± 1.64 (10)	13.3 ± 2.05 (9)	<0.05
Fructose-6-P	30.6 ± 3.00 (10)	14.9 ± 2.05 (9)	<0.001
Glucose-6-P	126 ±12.6 (10)	43.2 ±11.9 (9)	<0.001

[a] Concentrations are mμmoles/g of wet wt. liver; mean ± SE; value in parentheses indicates number of livers analyzed.

[b] p value for difference between means.

[c] NS = not significant at 0.05 level.

ethanol fed groups was too low to measure accurately, we cannot calculate the change in the malate to oxaloacetate ratio induced by ethanol feeding. However, the lower aspartate concentration in ethanol fed rats (Table I) suggests that ethanol shunted oxaloacetate to malate. In contrast to the findings for α-glycerophosphate and malate, ethanol feeding had no effect on the hepatic concentration of pyruvate or lactate. Thus ethanol feeding did not affect the steady state concentrations of all hepatic oxidation–reduction couples.

The hepatic concentration of glutamate was increased in the ethanol fed animals as compared to the controls (Table I), which is consistent with the *in vitro* NADH interference with glutamic dehydrogenase activity (20).

The concentrations of gluconeogenic intermediates between phosphoenolpyruvate and 3-phosphoglycerate were decreased in ethanol fed rats as compared to the controls (Table II), though the difference between ethanol and control animals was not statistically significant for the phosphoenolpyruvate concentration. The concentrations of gluconeogenic intermediates beyond the triosephosphate level were all significantly lower in the livers from ethanol fed as compared to control rats (Table II).

Discussion. The data in Tables I and II indicate that the increase in NADH concentration associated with ethanol oxidation (6–8) blocked glutamic dehydrogenase, shunted oxaloacetate to malate, and dihydroxyacetone-phosphate to α-glycerophosphate. However, ethanol did not increase the hepatic lactate concentration. Measurements in man have failed to demonstrate that ethanol consistently increases the hepatic vein lactate concentration in comparison to arterial lactate (10, 12, 13). In fact, ethanol decreases hepatic lactate production from fructose (13). Ethanol-induced increases in the blood lactate concentration in intact animals seem to reflect decreased hepatic uptake of lactate rather than increased hepatic lactate production.

The data for the concentration of gluconeogenic intermediates is consistent with the postulate that ethanol decreases hepatic glucose production by shunting substrates away from the gluconeogenic pathways. However, in comparison to control livers, the accumulation of malate after ethanol feeding was 0.4 μmoles/g of liver, of α-glycerophosphate 0.5 μmoles/g of liver and of lactate 0.2 μmoles/g of liver. An estimate of the amount of α-glycerophosphate utilized for the synthesis of plasma and hepatic triglycerides and phospholipids after ethanol feeding is 26 μmoles/g of liver/4 hr (25). The total amount of carbon removed from the gluconeogenic pathways after ethanol feeding in the present experiments was approximately 20 μmoles of carbon/g of liver/hr. The amount of carbon shunted away from gluconeogenesis is small in comparison to the amount of glucose synthesized by the rat liver (26–28). If ethanol shunted similar amounts of substrate

away from gluconeogenesis in the human or dog liver, the shunting mechanism could not account for the observed reductions in hepatic glucose synthesis. Thus, the present data indicate that the ethanol-induced shunting of substrate away from gluconeogenic pathways is not quantitatively important for the effect of ethanol on glucose production by the intact liver.

Since ethanol decreases the hepatic oxidation of fatty acids to CO_2 (2, 7, 22), it could lower the mitochondrial concentration of acetyl-CoA. The conversion of pyruvate to phosphoenolpyruvate via the intermediate formation of oxaloacetate is dependent on the mitochondrial acetyl-CoA concentration because this intermediate is an allosteric activator of pyruvate carboxylase (E.C.6.4.1.1) (23). Thus, ethanol could theoretically interfere with the metabolism of pyruvate to phosphoenolpyruvate. Although the ethanol inhibition of fatty acid oxidation seems to be related to a block in the metabolism of acetyl-CoA to CO_2 rather than of fatty acids to acetyl-CoA (24), there are no data available to indicate what effect ethanol has on the mitochondrial acetyl-CoA concentration.

Lactate, glycerol, and amino acids (26, 28, 29–31), are important sources of carbon for hepatic glucose production. Ethanol decreases hepatic glycerol uptake in both man and the rat (17–19). In man a positive value for the difference in arterial lactate and hepatic vein lactate concentration becomes slightly negative after ethanol administration (13) suggesting that ethanol blocks hepatic lactate uptake. As a result of its effect on glutamic dehydrogenase, the production of glucose from glutamate and amino acids other than glutamate might also be decreased by ethanol. The data of Fields *et al.* (1) demonstrating that ethanol decreases hepatic urea production is consistent with this type of effect. These considerations suggest that ethanol inhibition of hepatic glucose production is due primarily to an inhibition of the entry of gluconeogenic substrates into the gluconeogenic pathways.

Summary. Ethanol administration to fasted rats increased the hepatic concentration of glutamate, malate, and α-glycerophosphate. The concentration of lactate was unchanged. The concentrations of asparate, 2-phosphoglycerate, 3-phosphoglycerate, fructose-1-6-P, fructose-6-P, and glucose-6-P were decreased by ethanol. Although these data are consistent with the hypothesis that ethanol decreases hepatic glucose production as a result of shunting substrates to lactate and α-glycerophosphate, the amount of α-glycerophosphate, lactate, and malate which accumulated after ethanol feeding was small in comparison to reported estimates of glucose production by the rat liver. It is concluded that the primary mechanism for ethanol-induced inhibition of hepatic glucose production is that ethanol blocks the entry of intermediates into the gluconeogenic pathways.

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Direct Coombs' Test Reactivity after Cephalothin or Cephaloridine in Man and Monkey (33330)

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The development of positive direct Coombs' reactions (DCR) in humans during administration of drugs has attracted increasing attention in recent years. This association can be of practical importance since in some instances the Coombs' reactivity may also be associated with hemolytic anemia; notable examples have included methyldopa and penicillin (1–3). Preliminary studies indicate that 40–70% of patients given cephalothin may develop positive DCR (4,5). This unusually high incidence emphasizes the need to define possible mechanisms of red blood cell alteration or sensitization, and to establish any association with red cell damage that might influence their survival. Although structurally similar to cephalothin (6), cephaloridine has been reported to have caused a positive DCR in only one patient (7).

The present study was conducted to determine (1) the incidence of positive DCR, (2) relationships to serum antibiotic concentrations, and (3) possible associated red blood cell abnormalities in patients and rhesus monkeys treated with cephalothin or cephaloridine.

Materials and Methods. During the period January through June, 1967, 143 patients at University Hospital were observed during cephalothin therapy. Serial DCR were performed by washing 2% suspensions of red blood cells three times in normal saline in 10 × 75-mm tubes, decanting the supernatant fluid; 2 drops of Coombs' anti-human serum (Pfizer) were added and the contents centrifuged for 15 sec in a Clay-Adams serofuge. DCR were interpreted as 4+ to 1+ depending upon the degree of macroscopic agglutination; microscopic agglutination was recorded as ± and considered insignificant. Serial hemoglobin (cyanmet-hemoglobin) and hematocrit (micromethod) determinations were obtained twice weekly during cephalothin treatment (8).

The comparative effect *in vitro* on DCR of human and monkey erythrocytes exposed to graded concentrations of cephalothin or