over, control extracts of muscle or testis were without effect.

We postulate that the hormone from the corpus cardiacum acts at perhaps two points in the pathway of carbohydrate metabolism. First, it increases the rate of glycogen degradation and, second, it diverts the mobilized hexose residues from the glycolytic pathway and into the blood as trehalose. As a consequence, the endogenous metabolism of the fat body shifts toward increased utilization of lipid. The increased metabolic rate reflected in an increased QO_2 should provide greater amounts of adenosine triphosphate for regeneration of the uridine triphosphate utilized in the endergonic process of trehalose synthesis. Thus, the lower rate of conversion of glucose to lipid in fat body treated with hormone (Table 1) may be due to inhibition of the conversion of phosphorylated hexoses to acetate and to increased utilization of lipid.

Where may the hormone act in addition to increasing the amount of active phosphorylase? We believe that the most probable activities are (i) inhibition of glycolysis at phosphofructokinase and (ii) activation of trehalose-6-phosphate synthetase. The regulation of carbohydrate oxidation by phosphofructokinase should be considered, since it may be a rate-limiting step in mammals (*12*) as well as in insects (*13*). This enzyme in rat heart appears to be easily inhibited by a number of metabolites (*12*) and is activated by serotonin and adenosine-3′,5′-monophosphate in *Fasciola* (*14*). The regulation of trehalose synthesis by trehalose-6-phosphate synthetase may occur by interconversion between active and inactive forms of the enzyme, such as is known to occur for phosphorylase and for glycogen synthetase (*15*). Hormonal control of either enzyme could cause the shifts in metabolic pattern which the data indicate.

Trehalose itself inhibits the trehalose-6-phosphate synthetase, but an increased concentration of glucose-6-phosphate relieves this inhibition (*16*). The corpus cardiacum hormone must upset this homeostatic state in favor of a rate of synthesis of trehalose greater than that expected from simple changes in chemical equilibria. This supposition is supported by experiments in vivo (*8*) and in vitro (*9*) that show that trehalose is synthesized by the fat body and released into the blood at a much greater rate in the presence of corpus cardiacum hormone. Whether this hormone is a product of the corpora cardiaca or is synthesized elsewhere and stored in the corpora cardiaca is still a matter of conjecture. However, it has been firmly established that the corpus cardiacum of *L. maderae* does possess the morphological characteristics of an endocrine gland (*17*).

In situations of stress (*18*) or hyperactivity, relatively large amounts of trehalose are made available to other tissues such as muscle. Under these conditions the central nervous system may regulate the release of hormone from the corpus cardiacum, and thereby cause an increased rate of trehalose synthesis in the fat body and release into the blood. This view is in accord with proposals by others (*1, 7, 8*).

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6 July 1965

Glycerol Metabolism in the Human Liver: Inhibition by Ethanol

Abstract. *Glycerol is metabolized predominantly in the liver, the first step presumably being phosphorylation to α-glycerophosphate. When ethanol is present in the blood the rate of glycerol uptake by the splanchnic organs is reduced to about one-third of the control value. At the same time glycerophosphate accumulates in the liver. Hepatic blood flow and oxygen consumption are not influenced by the combined infusion of glycerol and ethanol. The phenomenon may be connected with the increased concentration of the reduced form of diphosphopyridine nucleotide present in the liver during ethanol metabolism.*

When ethanol is oxidized in the normal human liver, more than half the oxygen consumption of this organ is required for the transformation of ethanol to acetate, which is released into the venous blood (*1*). Major changes in the metabolic processes of the liver must therefore take place during alcoholemia, especially an inhibition of the tricarboxylic acid cycle. However, other changes are also observed, for example, in the metabolism of galactose (*2*) and fructose (*3*).

Lundsgaard (*4*) recorded the concentration of glycerol in the blood after oral ingestion of glycerol by human subjects. He observed a marked reduction in the slope of the curve when ethanol was given together with glycerol. This observation could be explained by the increased release of glycerol from peripheral tissues or by the decreased rate of its removal from the circulation by the liver and possibly other organs.

We have now studied the metabolism of glycerol in livers of patients who were considered metabolically normal. Glycerol was administered as a continuous infusion for about 50 minutes (first period). Three samples of arterial blood and hepatic venous blood were collected during the last half of the period, when the concentration had be-

come constant (about 1.3 mM). The glycerol infusion was continued at the same rate and ethanol was added to the infusion during a second period of 50 minutes, during the latter half of which three sets of blood samples were again taken. The concentration of ethanol in the blood was about 3 mM, that is, far below that necessary to cause intoxication, but sufficient to ensure elimination at a maximum rate. The procedures for the determination of glycerol, ethanol, acetate, pyruvate, lactate, glucose, oxygen, and carbon dioxide have been described (*3*). Hepatic blood flow was estimated by the indocyanine green–infusion method.

Table 1 shows a consistent reduction in the uptake of glycerol in the splanchnic area while ethanol was being administered to about 30 percent of the uptake in the control period. Three experiments in which ethanol was infused alone in the first period, and then ethanol plus glycerol in the second period, showed no significant change in the rate of ethanol metabolism or in the output of acetate from the liver.

Lactate consumption by the liver (average, 0.3 mmole/min in the control periods) was not changed by infusion of glycerol alone, but glycerol and ethanol together caused a change to lactate output, as ethanol alone does. The ratios of the concentrations of lactate to the concentrations of pyruvate in the hepatic venous blood were not significantly increased during glycerol metabolism, while the administration of ethanol alone or together with glycerol caused a fourfold increase in the ratio which is believed to mirror the ratio of NADH to NAD (*5*) in the liver cytoplasm (*6*).

Experiments in which glycerol and ethanol were infused together during a 2-hour period suggest that the inhibitory effect on the uptake of glycerol increases in the course of the experiment. The glycerol uptake (averaged from three experiments) was 0.48 mmole/min at the beginning and 0.28 mmole/min at the end of the test period.

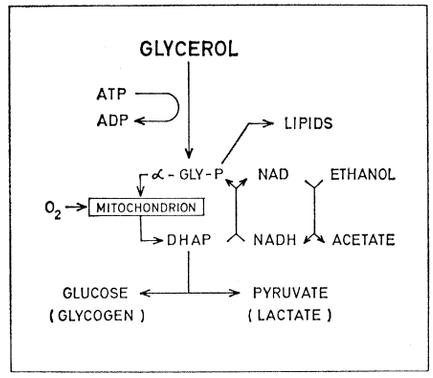

Fig. 1. Possible utilization of glycerol by the liver. ADP, adenosine diphosphate; ATP, adenosine triphosphate; DHAP, dihydroxyacetone phosphate; α-Gly-P, alpha glycerophosphate; NAD, diphosphopyridine nucleotide; and NADH, diphosphopyridine nucleotide, reduced form.

The hepatic blood flow showed no significant changes. The oxygen uptake in the splanchnic area was independent of the presence of glycerol in the blood. The output of glucose in the splanchnic area did not reveal consistent changes, but the concentration of glucose in arterial blood was increased during treatment with glycerol alone, whereas the simultaneous presence of ethanol seems to prevent this rise.

The possibility that the effect of ethanol on glycerol metabolism might be explained through a competition for alcohol dehydrogenase can be ruled out, as glycerol is oxidized only at a negligible rate by liver alcohol dehydrogenase. The first stage in glycerol metabolism is probably phosphorylation by means of glycerokinase to α-glycerophosphate. In some way this process must be inhibited in the presence of ethanol. A connection might be sought in the marked increase in the concentration of NADH, which is a most striking change produced by ethanol in liver tissue. Oxidation of glycerophosphate by the mitochondrial α-glycerophosphate dehydrogenase is one of the pathways open to glycerophosphate.

The dihydroxyacetone phosphate formed in this reaction may be transformed to glycogen (or glucose) or to pyruvate (and lactate), or it may be reduced again to glycerophosphate by means of the cytoplasmic NAD-requiring glycerophosphate dehydrogenase. This last process would be favored by the increased concentration of NADH found during ethanol combustion. In this way a considerable concentration of glycerophosphate might be built up (Fig. 1) if the possibility of removing dihydroxyacetone phosphate by other routes is limited. Some glycerophosphate may be utilized for formation of lipids (triglycerides and phospholipids), but this pathway can probably account for only a small fraction of the glycerol metabolized.

Experiments with rat-liver slices (*7*) have shown an even larger inhibition of glycerol metabolism by ethanol than that observed in vivo. In these experiments an increase in the concentration of glycerophosphate to 5 or 6 mM, or about ten times the control value, was observed in the presence of ethanol and glycerol. This accumulation of glycerophosphate may be the cause of the inhibitory effect of ethanol on glycerol metabolism, either through a direct inhibition of the kinase or through a decreased ratio of ATP to ADP (*5*), which is known to influence the activity of this enzyme (*8*).

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Table 1. Alteration in the consumption of glycerol in the liver as a result of treatment with ethanol. Values given are averages of six experiments; figures in parentheses are ranges of the values.

Infusion	Hepatic blood flow (liter/min)	Splanchnic oxygen consumption (mmole/min)	Splanchnic glycerol consumption (mmole/min)
		First period	
Glycerol	1.61 (1.03 to 2.01)	3.04 (2.06 to 3.61)	0.87 (0.45 to 1.29)
		Second period	
Glycerol + ethanol	1.65 (1.16 to 2.18)	2.82 (1.98 to 3.33)	0.25 (0.11 to 0.41)

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