Metabolism of Volatile Fatty Acids by the Perfused Goat Liver.* (24419)

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Large quantities of volatile fatty acids (VFA) resulting from microbial activity in the fore-stomach of ruminants reach the liver directly by the portal vein. Experiments with rumen perfusions(1) demonstrated that the proportion of the VFA reaching the liver is very similar to the proportion in which these acids are produced in the rumen. Since the liver is capable of acting on all digestive products, it ultimately determines the nature and quantity of the metabolites presented to the other tissues of the body.

Methods. Livers of goats were perfused through the portal vein with heparinized blood for 1 hour periods by use of pump-oxygenator apparatus similar to one we previously used (2). Mixtures of VFA (C_2 to C_5) in proportions approximating normal absorption from the rumen were added to blood at beginning of each perfusion (Table I). In addition, in each perfusion a different carboxyl-C^{14} labeled acid was added to blood as follows: formate, perfusion 43; acetate, perfusion 44; propionate, perfusion 45; butyrate, perfusion 46. Blood samples were taken as 0, 30 and 60 minutes after initiation of perfusion: initial and final liver samples were also obtained. Blood organic acids were determined by modification of the chromatographic technic of Wiseman and Irvin(3). Blood glucose was measured by method of Somogyi(4). Total acetone bodies were measured by technic of Weichselbaum and Somogyi(5). Liver glycogen was isolated according to procedure of Good *et al.*(6). Three fractions were extracted from liver tissue. A known weight of liver tissue was extracted with 1:1 ethanol-ether (v/v) 3 times in Waring blendor. The supernatant was combined and concentrated under reduced pressure. The ethanol-ether concentrate was then extracted 3 times with a large excess of warm hexane. Phospholipids were precipitated from the hexane extract by addition of acetone. The supernatant contained neutral fat. The remaining ethanol-ether material was extracted with acetone and the residual material from this extraction was taken up in moist ether. A phosphatide fraction was precipitated from the moist ether by addition of acetone and magnesium chloride. The precipitate was again taken up in moist ether and reprecipitated 3 times. The components of this phosphatide fraction were not isolated. A fraction known to contain Coenzyme A was prepared from another sample of liver tissue by the method of Lipmann *et al.* (7). This fraction had the same characteristics as the phosphatide fraction originally prepared, *i.e.*, a yellow, oily material. Also the fraction prepared by the method of Lipmann *et al.* had the same total radioactivity as the one previously prepared. All measurements of radioactivity were made with a windowless flow tube used in the Geiger region. Osazones were prepared for appraisal of activity in blood glucose. Blood acetone bodies were degraded into CO_2 and acetone. The 2,4 dinitrophenylhydrazones of the derived acetone were purified by procedure of Bassette (personal communication). The derived CO_2 was converted to barium carbonate for counting.

Results. In all experiments, flow of blood through the isolated liver attained the estimated *in vivo* rate(8) of 500 ml/minute. The quantitative results for all perfusions are presented in Table I. It will be noted that valerate, butyrate and propionate were removed almost completely from blood and metabolized by the liver. On the other hand, level of blood acetate increased moderately and there was a 2- to 3-fold increase in blood concentration of formate. There was an increase of 60 to 160% in blood acetone bodies. Blood glucose and lactic acid also increased markedly, undoubtedly due to glycolysis, since the increases were accompanied by a decrease in liver glycogen. Production of large quanti-

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TABLE I. Total Quantities of Metabolites in Perfusate and Total Amount of Liver Glycogen at Beginning and End of 4, One-Hour Goat Liver Perfusions.

Metabolites*	Perfusion 43 Initial	Perfusion 43 Final	Perfusion 44 Initial	Perfusion 44 Final	Perfusion 45 Initial	Perfusion 45 Final	Perfusion 46 Initial	Perfusion 46 Final
	(mg)							
Blood								
Glucose	775.6	2661.6	1065.0	5925.0	966.0	3780.0	630.0	3255.0
Acetone bodies†	166.3	275.1	278.4	445.4	256.1	534.5	228.2	579.1
Valeric acid	15.0	.0	8.2	.0	24.2	.0	31.8	.0
Butyric "	32.5	.0	34.8	.0	113.8	.0	37.9	.0
Propionic "	256.9	.0	326.2	5.7	324.2	7.9	238.1	.0
Acetic "	666.9	730.2	913.1	1056.5	880.7	975.8	650.4	715.2
Formic "	124.8	402.3	177.4	612.0	167.5	482.4	107.0	453.6
Lactic "	341.6	938.1	536.9	1508.9	755.5	1323.3	242.4	960.0
Liver								
Glycogen	6870.0	5010.0	3605.0	1442.0	6542.0	4963.0	3910.0	2756.0

* Quantities of valerate, butyrate, propionate and acetate added to the liter of perfusate varied in each perfusion. Initial values reported in the table represent total added except for acetate where values represent quantity added plus amount already present in blood.

† Acetone bodies calculated as β-hydroxybutyrate.

ties of glucose is a characteristic of liver perfusions(9).

The data on distribution of recovered label from the carboxyl-labeled VFA at completion of perfusions are presented in Table II. The label from formate, propionate and butyrate recovered in liver glycogen, blood glucose and lactate accounted for approximately 64, 75, and 83% respectively of the total C^{14} recovered. Only 12% of the recovered label from acetate was found in these compounds, most of the label remaining in the blood acetate.

TABLE II. Distribution of Recovered C^{14} Label following 1-Hr Perfusions of Goat Livers in Which Carboxyl-Labeled Volatile Fatty Acids Were Added to Blood.

Substance isolated	% distribution of label from added metabolite: Formate	Acetate	Propionate	Butyrate
Blood				
Valeric acid	.0	.0	.0	.0
Butyric "	.0	.0	.0	.0
Propionic "	.0	.0	1.6	.6
Acetic "	2.1	75.2	3.6	10.3
Formic "	12.5	4.1	8.3	2.0
Lactic "	5.1	7.4	25.1	49.9
Glucose	1.3	2.4	3.1	1.1
Acetone bodies:				
Derived acetone	.0	.0	.0	.0
Derived carboxyl CO_2	.6	1.5	.6	1.1
Liver				
Glycogen	57.8	2.4	46.5	31.5
Neutral fat	.4	1.9	.7	.5
Phospholipids	5.6	.4	1.1	.2
Nucleotide fraction	15.1	6.0	10.0	4.0
% added C^{14} recovered	61.9	71.6	50.1	90.0

Acetone derived from blood acetone bodies was not labeled and the CO_2 from the carboxyl group of β-hydroxybutyrate in blood was only slightly labeled. Since an increase of 10 to 20 mg% of total acetone bodies occurred in blood, it appears that acetone bodies must have been derived from lipids of liver which were only slightly labeled.

There was little lipogenesis in these 4 perfusions from the labeled substrates. The extent to which the VFA from the rumen may contribute to lipid metabolism has not been reported. The results here show that propionate and butyrate contribute more to carbohydrate metabolism than to lipid metabolism.

There was a fair degree of incorporation of the C^{14} label into the phosphatide fraction. Incorporation was greatest from formate followed by propionate, acetate and butyrate. Isolation of the components of this fraction is necessary before the findings can be properly evaluated.

Discussion. The contribution of large quantities of butyrate to glycogen clarifies the paradoxical situation which existed until this time. Kleiber *et al.*(10) found that in the intact dairy cow there was appreciable labeling of milk lactose and casein from injected C^{14} labeled butyrate. However, butyrate *per*

se apparently contributed little to formation of milk fat. It has generally been believed that within ruminants, butyrate is metabolized as β-hydroxybutyrate(11). In studies at Maryland it was demonstrated with C^{14} labeled β-hydroxybutyrate that this substance is converted into milk fat and protein but not into lactose by the perfused udder(12). With the demonstration here that butyrate is converted to glycogen by the liver the anomalies of these findings are removed.

It is also evident from these studies that butyrate was not metabolized as 2 acetyl groups. Since the known metabolic pathways show acetate and butyrate having a common intermediate, *i.e.*, Acetyl Coenzyme A, the activity distribution for both should have been similar if butyrate had been metabolized in this manner.

The contribution of propionate to carbohydrate metabolism has been demonstrated previously(13,14). The large quantity (75%) of the recovered label remaining in blood acetate indicates that acetate arising from the rumen is not altered appreciably by the liver but is made available as such to the extrahepatic tissues.

Production of formate by the liver explains the origin of formate normally found in ruminant blood. The results of rumen perfusions(1) showed that formate did not arise from the rumen; in fact blood formate was utilized by ruminal tissue. Annison(15) noted earlier that concentration of formate in the portal blood of sheep was no greater than the level in peripheral blood.

The low radioactivity in blood glucose can be explained by the fact that most of the increase in blood glucose occurred very early in the experiment before the labeled substrates were converted appreciably to carbohydrate. The greater total activity in blood lactate than in blood glucose indicates that the lactate was arising in a large part from glycogen in the tissue and not from blood glucose.

Summary. Formate, butyrate and propionate were removed from blood and metabolized by the perfused liver. Butyrate, propionate and formate contributed heavily to carbohydrate metabolism and formation of glycogen. Formate was produced by the liver, and even though size of formate pool increased considerably, there was substantial contribution of this pool to glycogen formation. Acetate levels in blood showed a slight increase during perfusion. The major portion of recovered label from added acetate remained in blood acetate indicating that most of the acetate arising from the rumen passes unaltered through the liver. The bulk of acetone bodies in ruminants apparently arises primarily from hepatic lipid metabolism and not from direct conversion of rumen VFA by the liver.

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