Oxidative Metabolic Pattern of Normal and Ketotic Cow Liver Incubated With 1-C^{14}-Labeled Aliphatic Acids.* (24666)

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It is well accepted that the ruminant derives the major portion of its energy from volatile fatty acids (VFA) produced in the rumen as a result of fermentation of ingested feed by rumen microflora. By and large, the VFA produced in the rumen are absorbed as such and reach the liver directly through the portal system. The major VFA concerned are acetic, propionic, butyric and valeric acids, listed in their normal descending order of production and absorption. In addition, formic acid is also an important metabolite for ruminants since we showed it was produced in rather marked amounts by perfused goat livers(4). If one assumes that the ruminant liver is very similar in its behavior, in a given set of conditions, to the liver of the monogastric animal, then the major metabolic dissimilarities between these animals must arise from differences in substrates available to livers through the portal blood (glucose for monagastrics and VFA for ruminants) and in any enzymatic and endocrine adaptation to these differences. In addition it is commonly accepted that liver function is disturbed(5,6) when ruminants, particularly dairy cattle, exhibit clinical ketosis, a primary metabolic disorder or syndrome believed associated with stress(7). For above reasons, studies were initiated concerning oxidative metabolic pathways of various VFA by normal and ketotic ruminant liver slices.

Methods. The methods used including chromatographic - autoradiographic technic were described by Katz and Chaikoff(3) and Brown *et al.*(1). Independent incubations of liver slices (0.5 g) were made in duplicate with the following 1-C^{14} labeled acids: formic, acetic, propionic, butyric and valeric. For each incubation, 25 μM of substrate containing 25 μc of C^{14} was employed. The flasks were incubated 2 hours at 39 and 37°C for cow and rat liver respectively. The relative position of non-volatile compounds separated by 2-dimensional chromatography is shown in Fig. 1.

Results. In all incubations the volatile fraction contained the largest percentage (83-98%) of recovered C^{14}. Since this fraction represents non-utilized substrate, it can be assumed that large excesses of substrate were present during all incubations. In addition, no significant lipogenesis was observed during incubations.

Formate-C^{14}. Formate carbon was incorporated to greatest extent into glucose, followed by what appear to be phosphate esters (Table I). A marked decrease occurred in $C^{14}O_2$ production and in incorporation of formate into non-volatile compounds in ketotic cow liver slices. Compared to the nor-

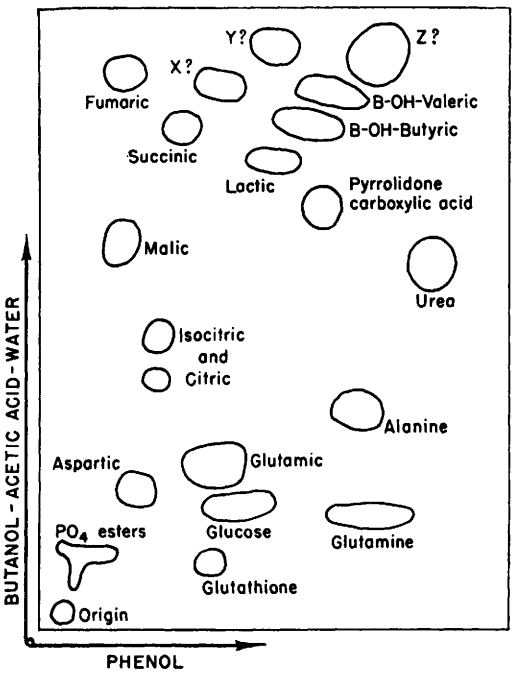

FIG. 1. Diagrammatic representation of location and identity of radioactive spots observed on various autoradiograms.

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TABLE I. Percentage Incorporation of Isotope from Formate-C^{14} into Chromatographic Nonvolatile Compounds and Various Major Fractions by Liver Slices.

	Liver sources: "Normal" cow*		Ketotic cow A		Ketotic cow B		Rat	
	Flask		Flask		Flask		Flask	
	I	II	I	II	I	II	I	II
Glucose	55.3	55.2	82.4	83.3	31.8	35.2	59.6	62.9
β-Hydroxybutyrate	3.7	2.2	.0	.0	5.8	7.0	Tr	.0
β-Hydroxyvalerate	.0	.0	.0	.0	8.3	4.9	.0	.0
Malate	4.6	2.7	.0	.0	Tr	Tr	3.8	3.2
Aspartate	.0	.0	.0	.0	21.7	11.1	.0	4.9
Glutamate + glutamine	7.2	7.1	.0	.0	9.4	7.7	3.0	2.9
Isocitrate + citrate	1.3	2.5	.0	.0	Tr	Tr	.0	.0
Lactate	3.5	3.8	.0	.0	4.5	4.9	4.2	3.6
Alanine	.0	.0	.0	.0	.0	.0	2.9	.0
Succinate	2.2	1.8	.0	.0	.0	.0	Tr	.0
Fumarate	Tr	Tr	.0	.0	.0	.0	.0	.0
PO_4 esters	8.1	10.4	Tr	Tr	6.4	16.1	13.0	11.3
Urea	4.9	3.6	.0	.0	Tr	5.9	5.5	7.0
Glutathione	.0	.0	.0	.0	.0	.0	3.4	.0
Unidentified	2.3	3.1	.0	.0	.0	.0	.0	.0
Origin	5.5	6.9	Tr	Tr	Tr	Tr	Tr	4.2
Total chromatogram radioactivity (cpm)	2,308	1,699	158	140	668	603	1,405	1,263
Major fractions	% of recovered C^{14}							
Respired CO_2	1.9	2.0	.5	.4	2.2	1.4	1.0	1.2
Nonvolatile fraction	2.4	3.5	3.3	1.3	2.4	5.2	1.1	1.2
Volatile "	95.7	94.5	96.2	98.3	95.4	93.4	97.9	97.6
Total recovered C^{14} (cpm × 10^6)	14.0	14.0	4.0	3.8	3.5	3.6	15.0	16.0

* Liver sample obtained by biopsy. Tr = Trace.

TABLE II. Percentage Incorporation of Isotope from Acetate-1-C^{14} into Chromatographic Nonvolatile Compounds and Various Major Fractions by Liver Slices.

	Liver sources: "Normal" cow-S*		Ketotic cow A		Ketotic cow B		Rat	
Chromatographic compounds	Flask		Flask		Flask		Flask	
	I	II	I	II	I	II	I	II
Glucose	4.8	4.2	18.7	16.9	11.0	11.3	6.0	5.0
β-hydroxybutyrate	48.0	48.5	12.5	6.1	31.6	32.9	50.1	53.1
β-hydroxyvalerate	.0	.0	5.6	12.4	.0	Tr	.0	.0
Malate	7.5	6.3	7.7	7.5	6.8	7.0	5.3	6.2
Aspartate	7.5	9.5	9.7	7.4	11.2	12.8	3.5	3.7
Glutamate + glutamine	15.2	16.9	12.0	21.8	11.8	14.0	13.9	15.6
Isocitrate + citrate	10.5	11.0	19.7	16.9	13.8	9.9	8.3	6.2
Lactate	4.2	Tr	Tr	Tr	2.5	Tr	Tr	Tr
Alanine	.0	.0	.0	.0	.0	.0	"	"
Succinate	.0	Tr	Tr	Tr	3.6	Tr	2.0	1.6
Fumarate	.0	.0	"	"	Tr	"	Tr	Tr
PO_4 esters	.0	.0	5.0	"	.0	"	"	"
Urea	.0	Tr	.0	.0	.0	.0	2.9	2.7
Glutathione	.0	.0	.0	.0	.0	.0	Tr	Tr
Pyrrolidone carboxylic acid	.0	.0	Tr	Tr	2.5	2.9	.0	.0
Origin	Tr	.0	"	"	3.1	2.1	Tr	Tr
Total chromatogram radioactivity (cpm)	720	874	875	1,106	1,452	1,594	2,198	2,780
Major fractions	% of recovered C^{14}							
Respired CO_2	.4	.3	.1	.1	.1	.1	.1	.2
Nonvolatile fraction	1.7	2.1	1.4	1.5	2.1	2.7	2.5	3.2
Volatile "	97.9	97.6	98.5	98.4	97.8	97.2	97.4	96.6
Total recovered C^{14} (cpm × 10^6)	4.8	4.9	5.8	5.7	5.4	5.1	5.9	6.1

* Liver sample obtained from abbatoir.

TABLE III. Percentage Incorporation of Isotope from Butyrate-1-C^{14} into Chromatographic Nonvolatile Compounds and Various Major Fractions by Liver Slices.

	Liver sources:								
	"Normal" cow-S*		"Normal" cow†		Ketotic cow A		Ketotic cow B		Rat
Chromatographic compounds	Flask I	Flask II	Flask I	Flask II	Flask I	Flask II	Flask I	Flask II	I
Glucose	10.1	9.8	.0	Tr	.0	Tr	Tr	Tr	.0
β-hydroxybutyrate	74.3	74.8	77.0	76.5	57.1	36.9	47.0	48.6	77.4
β-hydroxyvalerate	.0	.0	1.9	2.6	5.2	9.4	4.3	4.7	1.6
Malate	2.8	2.8	2.2	1.6	2.2	2.3	3.4	4.0	2.0
Aspartate	3.3	4.7	2.9	3.3	8.5	11.7	13.0	12.4	1.0
Glutamate + glutamine	4.9	5.5	11.7	12.4	21.9	29.7	21.7	21.7	10.1
Isocitrate + citrate	.0	.0	Tr	1.1	Tr	Tr	Tr	Tr	Tr
Lactate	Tr	1.4	"	Tr	"	"	3.3	3.5	"
Alanine	"	.0	.0	.0	.0	.0	Tr	.0	"
Succinate	"	Tr	Tr	Tr	Tr	Tr	"	Tr	"
Fumarate	.0	.0	"	"	"	.0	"	"	"
X ?	.0	.0	"	"	"	Tr	1.3	"	"
Y ?	.0	.0	1.2	1.1	.0	"	.0	.0	.0
Urea	.0	.0	.0	.0	.0	"	Tr	Tr	2.2
Glutathione	.0	.0	.0	.0	.0	.0	.0	.0	Tr
Pyrrolidone carboxylic acid	.0	.0	.0	.0	.0	Tr	Tr	Tr	"
Origin	Tr	.0	Tr	Tr	Tr	"	"	"	"
Total chromatogram radioactivity (cpm)	2,049	3,324	6,193	5,905	1,717	1,281	3,347	2,604	4,639
Major fractions					% of recovered C^{14}				
Respired CO_2	.4	.6	.5	.5	.3	.4	.7	.6	.4
Nonvolatile fraction	3.9	4.9	13.6	13.4	3.9	3.6	6.3	6.0	8.8
Volatile "	95.7	94.5	85.9	86.0	95.8	96.3	93.0	93.4	90.7
Total recovered C^{14} (cpm × 10^6)	3.7	3.8	3.1	3.0	3.3	3.2	3.1	3.3	3.5

* Liver sample obtained from abbatoir. † Liver sample obtained by biopsy.

mal cow, the ketotic cow B liver slives incorporated a smaller % of formate into glucose and a larger % into aspartate, glutamine, β-hydroxybutyric acid (BHBA) and β-hydroxyvaleric acid (BHVA). Of the 2 ketotic cows, cow B exhibited the most marked hypoglycemia and ketonuria. The different distribution pattern observed in the case of ketotic cow A may have been due to extremely low incorporation of C^{14} (159 cpm) into the non-volatile components.

Acetate-C^{14}. Total incorporation of acetate label into the non-volatile components was low (Table II). This agrees with results by McCarthy *et al.*(4) which showed that labeled acetate passes through the perfused goat liver with only slight utilization. The data are similar to those observed for rat liver by Katz and Chaikoff(3) and mouse liver by Brown *et al.*(1), except for a lesser amount of label in glucose. The principal difference between normal and ketotic cows was in the higher incorporation into glucose and the lower incorporation into BHBA of labeled acetate by ketotic cow liver slices. The increased incorporation of acetate label into glucose by the ketotic liver slices is of interest in connection with the observation that hypoglycemia in ketotic cows can be ameliorated by oral administration of acetate (7).

Butyrate-1-C^{14}. Results from rat liver slice incubations with butyrate are in close agreement with those of normal cow liver slices (Table III). No marked differences in labeled CO_2 production were noted for the various liver slices. On the average, normal cow liver incorporated about twice as much butyrate into non-volatile compounds as ketotic cow liver. BHBA and the glutamate-glutamine group accounted for the bulk of the label from butyrate with lesser amounts occurring in aspartate, malate and BHVA. Very slight incorporation into C_3 intermediates was observed. The major differences between normal and ketotic cow liver were in incorporation of isotope into non-volatile compounds. Expressed as percentage incor-

poration, ketotic liver, compared to normal, produced (a) one third less BHBA, (b) twice the relative amount of glutamate-glutamine and BHVA, and (c) 3 times the relative amount of aspartate. Only in the case of the abbatoir cow were significant amounts of label from butyrate found in glucose. This may have been a physiological response to fasting since this animal had been fasted more than 24 hours. Comparing the results obtained with butyrate with those of acetate would suggest that degradation of butyrate into 2 acetate units is far from the complete story concerning metabolism of butyrate by bovine liver slices.

Propionate. Malate, aspartate and lactate contained most of the label from propionate with lesser amounts in BHVA, BHBA and succinate (Table IV). The unidentified compounds designated as X? and Y? (Fig. 1) also contained an appreciable portion of the label. Lower incorporation of label into malate was obtained with ketotic than with normal liver slices. A high incorporation of label was observed in the succinate and X and Y compounds of ketotic cow A.

Valerate-1-C^{14}. The most striking observation with this substrate was its wide distribution into non-volatile intermediates and its marked utilization by cow liver slices (Table V). BHVA and BHBA contained most of the label from valerate except for the ketotic cows in which the label in BHBA was low. It is of considerable interest to note (Table II) that incorporation of acetate label into BHBA by ketotic cow liver tissue was also low. The incorporation of valerate label into malate and glutamate (plus glutamine) was also lower for the ketotic cows. Perhaps the major difference between normal and ketotic liver with this substrate was its large incorporation into an unidentified compound, designated Z (Fig. 1), with negligible incorporation into this compound by normal cow liver slices. The spotting of this substance on the radiogram encompassed the greatest total area, suggesting a large turnover. A slight incorporation of labeled propionate into this substance by ketotic liver slices will be noted in Table IV. Identification of this compound may provide an important clue to the etiology of bovine ketosis. As previously stated, ketotic liver slice incubations resulted in exceptionally marked decreases in oxidation and incorporation of formate. This altered formate metabolism must be an important part of any metabolic explanation concerning boving ketosis.

It should be noted that the ketotic liver slices resulted in increased incorporation of formate, acetate and butyrate into aspartate. An increased production of glutamate was also observed for ketotic liver slices incubated with labeled butyrate. This increased production of aspartate and glutamate may be related to faulty gluconeogenesis in ketotic cow liver slices. This is of interest in relation to the reports of Shaw(7) concerning the

TABLE IV. Percentage Incorporation of Isotope from Propionate-1-C^{14} into Chromatographic Nonvolatile Compounds and Various Major Fractions by Liver Slices.

Chromatographic compounds	Liver sources: "Normal" cow-S* Flask I	Ketotic cow A Flask I	Ketotic cow A Flask II	Ketotic cow B Flask I	Ketotic cow B Flask II
Glucose	1.0	.0	Tr	Tr	Tr
β-hydroxybutyrate	Tr	3.4	3.1	2.6	3.3
β-hydroxyvalerate	1.6	4.3	2.4	5.8	2.1
Malate	34.0	9.7	6.4	16.8	17.5
Aspartate	25.9	24.0	18.8	35.6	35.3
Glutamate + glutamine	2.0	1.6	1.6	2.1	2.0
Isocitrate + citrate	.0	Tr	1.3	4.8	2.5
Lactate	6.1	9.4	5.1	8.7	8.1
Alanine	1.1	Tr	Tr	3.1	1.9
Succinate	3.0	9.2	11.2	3.6	4.3
Fumarate	5.1	2.0	Tr	2.8	2.7
X ?	7.6	17.6	23.3	5.5	6.1
Y ?	10.4	16.4	21.5	6.1	6.5
Z ?	.0	.0	Tr	.0	5.0
PO_4 esters	.0	.0	.0	Tr	Tr
Urea	Tr	Tr	Tr	1.3	1.3
Glutathione	"	.0	"	.0	Tr
Origin	"	Tr	"	Tr	"
Total chromatogram radioactivity (cpm)	3,317	2,937	2,241	8,454	7,308
Major fractions		% of recovered C^{14}			
Respired CO_2	.3	.1	.1	.2	.2
Nonvolatile fraction	3.3	1.7	1.4	4.0	3.2
Volatile fraction	96.4	98.2	98.5	95.8	96.6
Total recovered C^{14} (cpm × 10^6)	4.6	13.0	13.0	13.0	13.0

* Liver sample obtained from abbatoir.

TABLE V. Percentage Incorporation of Isotope from Valerate-1-C^{14} into Chromatographic Non-volatile Compounds and Various Major Fractions by Liver Slices.

Chromatographic compounds	Liver sources: "Normal" cow* Flask I	"Normal" cow* Flask II	Ketotic cow A Flask I	Ketotic cow A Flask II	Ketotic cow B Flask I	Ketotic cow B Flask II
Glucose	Tr	Tr	Tr	Tr	.5	.7
β-hydroxybutyrate	27.5	23.9	4.1	2.7	5.5	7.0
β-hydroxyvalerate	43.8	46.9	57.8	52.8	48.7	42.7
Malate	3.6	3.1	.8	1.1	1.4	1.6
Aspartate	4.3	3.9	2.1	4.4	4.0	4.7
Glutamate + glutamine	9.8	10.0	4.0	5.9	6.0	5.5
Isocitrate + citrate	1.8	2.7	2.3	3.4	2.6	2.2
Lactate	1.1	1.1	.8	Tr	1.9	1.7
Alanine	.0	Tr	Tr	.0	Tr	Tr
Succinate	1.0	1.0	1.6	5.0	1.2	"
Fumarate	.7	.8	.0	.0	Tr	"
X ?	1.1	1.1	3.7	10.2	2.7	1.2
Y ?	3.0	2.7	.0	3.4	2.5	3.2
Z ?	Tr	Tr	18.7	6.7	19.5	25.7
PO_4 esters	"	.6	Tr	Tr	Tr	Tr
Urea	.7	.5	2.1	1.8	1.3	1.1
Pyrrolidone carboxylic acid	.6	.7	1.1	1.4	1.2	.9
Origin	Tr	.5	Tr	Tr	Tr	Tr
Total chromatogram radioactivity (cpm)	12,179	10,718	9,195	5,474	9,545	10,178
Major fractions			% of recovered C^{14}			
Respired CO_2	.5	.5	.4	.4	.4	.4
Nonvolatile fraction	5.6	5.0	3.7	3.4	3.8	4.0
Volatile "	93.9	94.5	95.9	96.2	95.8	95.6
Total recovered C^{14} (cpm × 10^6)	13.0	14.0	13.0	13.0	13.0	13.0

* Liver sample obtained by biopsy.

effective treatment of ketosis by administration of ACTH and glucocorticoids.

The overall decreased metabolic activity *in vivo* studies by Robertson *et al.*(6) and Peeters *et al.*(5).

Conclusions. 1) The oxidative metabolic patterns of normal and ketotic cow liver studies with 1-C^{14}-labeled C_1 through C_5 aliphatic acids, were determined using a chromatographic-autoradiographic technic. 2) The label from each metabolite had its own distinctive distribution pattern as evidenced by autoradiograms. 3) Normal cow liver slices incorporated carboxyl-labeled carbons of the various aliphatic acids, to the greatest extent, into non-volatile compounds as follows: formate, into glucose and what appear to be phosphate esters; acetate, into β-hydroxybutyrate, glutamate, citrate and small amounts of glucose and lactate; butyrate, into β-hydroxybutyrate and glutamate-glutamine with little incorporation into C_3 intermediates; propionate, into malate, aspartate and lactate; valerate, into β-hydroxybutyrate and β-hydroxyvalerate with a very wide distribution into the other intermediates chromatographically obtainable. 4) Ketotic cow liver slices exhibited a marked reduction in utilization of formate and an altered distribution in labeling of intermediates when incubated with various labeled aliphatic acids.

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