SUMMARY

Five lactating dairy cows were injected intravenously with acetate-1-^{14}C or acetate-2-^{14}C. Eight amino acids, recovered from casein collected at 3, 10, 22, and 34 hours after acetate-^{14}C injection, were assayed for carbon-14.

Carbon from acetate was transferred most efficiently to glutamic and aspartic acids and in lesser amounts to alanine, serine, glycine, proline, and arginine. Lysine did not contain significant amounts of ^{14}C.

The labeling of amino acids from acetate-^{14}C differed markedly from that previously observed for glucose-^{14}C. Carbon from uniformly labeled glucose was transferred most efficiently to alanine and serine and in smaller amounts to glutamic and aspartic acids, glycine, proline, and arginine.

The specific activities of alanine, serine, and lactose were quite similar after acetate-^{14}C injection suggesting a close relationship between the precursors of the three carbon amino acids and lactose.

The distribution of ^{14}C among the amino acids formed by the intact cow was consistent with results that would be expected if the TCA cycle and the glycolytic pathway were the pathway for transfer of carbon from acetate to amino acids of casein.

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THE TRICARBOXYLIC ACID CYCLE AS A PATHWAY FOR TRANSFER OF CARBON FROM ACETATE TO AMINO ACIDS IN THE INTACT COW*

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INTRODUCTION

The role of acetate as a precursor of amino acids of casein was discussed in a preceding publication[1]. Carbon from acetate was transferred most directly to glutamic acid and with greater dilution to aspartic acid, serine, and alanine. The level of specific activity among the amino acids was consistent with a pathway for transfer of carbon from acetate via the Tricarboxylic Acid (TCA) Cycle and the Embden-Meyerhof glycolytic scheme.

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For additional information on the intermediate pathway from acetate, several amino acids were degraded stepwise and individual carbon atoms assayed separately for carbon-14. This method reveals the intramolecular pattern of labeling which is a particularly reliable index of metabolic pathways since it is independent of dilution by unlabeled amino acids from exogenous sources. Under some conditions, however, results obtained by degradation are difficult to interpret, particularly when several alternate pathways are involved. In systems where a single pathway predominates and when intermediates are constantly removed from participation in metabolic reactions, as, for example, during milk synthesis, results obtained by degradation methods are less ambiguous.

The results reported in the present paper correspond in each case with results that would be expected if the TCA cycle were the major pathway for transfer of carbon from acetate to amino acids in the intact cow.

METHODS

The data concerning experimental animals were presented in the preceding publication[1]. Methods used in the separation of amino acids from casein[1] and for ^{14}C assay of samples[2] have been described.

Amino acids were decarboxylated with ninhydrin according to the method of VAN SLYKE[3]. When aspartic acid was decarboxylated, a gas scrubbing bottle containing 50 ml of 0.3% $KMnO_4$ in 1 N H_2SO_4 was inserted in the reaction system between the ninhydrin reaction vessel and the CO_2-absorber. This modification eliminated variable results which presumably arose when volatile aldehydes produced by the ninhydrin reaction with aspartic acid entered the NaOH–carbon dioxide absorber, and polymerized and contaminated the $BaCO_3$ used for carbon-14 assay of carboxyl carbons.

The same reaction system was used for decarboxylating alanine with ninhydrin. A gas scrubbing bottle, containing 50 ml of 1 N H_2SO_4 and 1.9 g $KMnO_4$, trapped the acetaldehyde produced from the C-2 and C-3 carbons of alanine. After standing at room temperature for ½ hour the excess $KMnO_4$ was destroyed with dilute $FeSO_4$ and acetic acid was recovered from the resulting solution by steam distillation. The acetic acid was degraded by the Schmidt reaction[4] to recover separately carbon atoms 2 and 3 of alanine.

Serine was degraded as described by SAKAMI[5]. Glutamic acid was degraded according to the method of MOSBACH, PHARES AND CARSON[6] as modified by KOEPPE AND HILL[7]. This method is very satisfactory and permits separate recovery of each carbon atom of the molecule.

RESULTS AND DISCUSSION

^{14}C in aspartic and glutamic acid carboxyl carbons

When acetate-^{14}C enters the Tricarboxylic Acid (TCA) Cycle the ^{14}C becomes distributed intramolecularly in the cycle intermediates in a definite pattern. The ^{14}C distribution can be predicted for any TCA cycle intermediate under the following conditions: (a) citric acid behaves asymetrically as OGSTON proposed[8] (b) succinic and/or fumaric acid behave as symmetrical molecules, (c) no extraneous carbon enters the cycle (such as pyruvate via CO_2 fixation), (d) the specific activity of the condensing acetate remains constant or the rate of turnover of carbon in the TCA cycle is very rapid compared to the rate of decrease of specific activity of the condensing acetate. The latter condition permits calculation of specific activities of cycle intermediates relative to that of acetate entering the cycle. Under these conditions the ^{14}C distribution in any given intermediate of the TCA cycle depends on the number of "turns" the cycle has made since the acetate entered until, after several "turns", the system reaches equilibrium and the isotope pattern becomes fixed.

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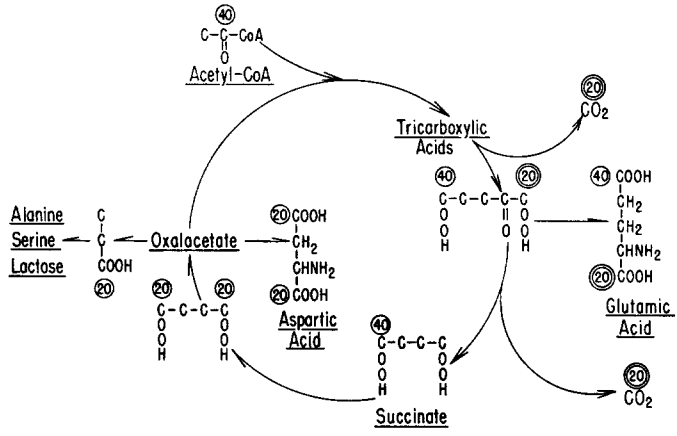

Fig. 1. Abbreviated schematic diagram of TCA cycle. Specific activities of carbon atoms are indicated by the numbers inside circles. Single and double circles designate specific ^{14}C activity of a carbon atom on its first and second "turn" through the cycle, respectively. Acetyl-CoA labeled with 40 units ^{14}C in the carboxyl carbon gives rise to α-ketoglutarate with ^{14}C (40 units) exclusively on the γ-carboxyl group during its first "trip" through the cycle. The ^{14}C passes on to the dicarboxylic acids where, in succinate, it becomes randomized between the carboxyl carbons so that each has 20 units of ^{14}C. During the second "turn" of the cycle another 40 units of ^{14}C enters with acetate and α-ketoglutarate is now formed with ^{14}C in two positions; 40 units ^{14}C are located in the γ-carboxyl and 20 units ^{14}C in the α-carboxyl carbon. The ^{14}C that entered with the first acetate-^{14}C molecule is eliminated as CO_2 during the second "turn" of the cycle, as illustrated. At this point isotope equilibrium has been attained in the cycle and the ^{14}C distribution in intermediates undergoes no further change.

When acetate-1-^{14}C enters the TCA cycle, the intermediates become labeled as indicated in Fig. 1. The glutamic and aspartic acid arising from transamination of α-ketoglutarate and oxalacetate, respectively, would theoretically have the ^{14}C distribution shown in Fig. 2. The glutamic acid formed from acetate-1-^{14}C via the TCA cycle would have 33.3% of its ^{14}C located in the C-1 carboxyl carbon; aspartic acid formed along the same pathway would have 100% of its ^{14}C in the two carboxyl carbon atoms C-(1 + 4).

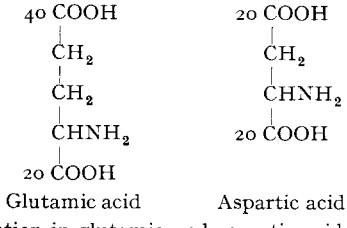

Fig. 2. Theoretical ^{14}C distribution in glutamic and aspartic acids arising from TCA cycle intermediates during metabolism of acetate containing 40 units ^{14}C on the carboxyl carbon.

The same method can be used to establish the theoretical ^{14}C distribution in glutamic and aspartic acids when acetate-2-^{14}C is introduced into the TCA cycle. In this case, equilibrium is not reached until several "turns" of the cycle, but theoretically the isotope finally approaches the distribution shown in Fig. 3.

According to this distribution glutamic acid, formed from α-ketoglutarate, would have 14.3% of its ^{14}C in the C-1 carbon and aspartic acid, formed from oxalacetate, 33.3% of its ^{14}C in the two carboxyl carbon atoms C-(1 + 4).

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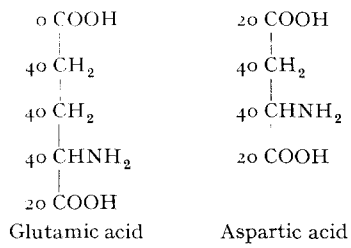

Glutamic acid Aspartic acid

Fig. 3. Theoretical ^{14}C distribution in glutamic and aspartic acids arising from TCA cycle intermediates during metabolism of acetate containing 40 units ^{14}C on the methyl carbon after isotopic equilibrium has been established.

The results obtained by decarboxylation of glutamic and aspartic acids recovered from casein correspond closely with these theoretical distributions. Table I lists the specific activities of carboxyl carbon for glutamic acids (C-1) and aspartic acids C-(1 + 4) recovered from individual casein samples. The specific activities for aspartic and glutamic acids were listed in the preceding paper (see Tables II and III)[1]. These data were used to calculate the per cent ^{14}C in carboxyl carbons which appear in columns 5 and 6 of Table I for each sample.

TABLE I

CARBON-14 IN CARBOXYL CARBON OF GLUTAMIC AND ASPARTIC ACIDS AFTER INJECTION OF ACETATE-1-^{14}C AND ACETATE-2-^{14}C

Trial	*Hours after injection*	*Carboxyl specific activity*		*% ^{14}C in carboxyl*		*Theoretical % ^{14}C for TCA cycle pathway**	
		C-1 Glutamic	*C-1 + 4 Aspartic*	*C-1 Glutamic*	*C-1 + 4 Aspartic*	*C-1 Glutamic*	*C-1 + 4 Aspartic*
				Acetate-1-^{14}C			
Acetate III	3	3.98	2.39	31.2	91.2		
	10	2.35	1.70	36.4	105		
	22	0.718	0.414	36.8	94.2		
	34	0.284	0.143	33.4	95.4		
Acetate VI	3	5.88	3.80	31.2	91		
	10	1.45	1.00	32.8	100		
	22	0.279	0.237	32.3	101		
	34	0.132	0.111	29.1	95.7		
Acetate VII	3	4.70	3.79	29.5	99.2		
	10	3.88	2.88	32.3	95.4		
	22	2.87	2.15	30.9	94.3		
	34	0.698	0.535	29.8	99.4		
		Calculated mean		32.2	96.8	33.3	100
				Acetate-2-^{14}C			
Acetate II	3	2.02	1.34	12.8	32.7		
	10	1.73	1.08	13.9	32.5		
	22	0.762	0.495	15.6	33.9		
	34	0.318	0.196	14.9	39.2		
Acetate X	3	1.18	0.922	10.0	33.4		
	10	1.63	1.18	15.5	40.1		
	22	0.726	0.439	15.1	37.8		
	34	0.301	0.182	14.9	42.0		
		Calculated mean		14.2	36.4	14.4	33.3

* See text for discussion of method for calculating theoretical per cent ^{14}C.

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The calculated mean value for 12 casein samples collected from three different cows injected with acetate-1-^{14}C was, for per cent ^{14}C of glutamic acid in C-1, 32.2% (theoretical, 33.3%) and, for per cent ^{14}C of aspartic acid in C-(1 + 4), 96.8% (theoretical, 100%).

The calculated mean value for 8 casein samples collected from two different cows injected with acetate-2-^{14}C was, for per cent ^{14}C of glutamic acid in C-1, 14.2% (theoretical 14.4%), and, for per cent ^{14}C of aspartic acid in C-(1 + 4), 36.4% (theoretical, 33.3%).

The close agreement between observed and theoretical results provides strong support for the conclusion that the TCA cycle is the major pathway for transfer of carbon from acetate to glutamic and aspartic acids of casein.

^{14}C pattern in glutamic acid

The results obtained by complete degradation of two glutamic acid samples are given in Table II. According to the pathway via the TCA cycle one would expect to find 1/3 of the ^{14}C of glutamic acid in the α-carboxyl carbon and 2/3 in the γ-carboxyl carbon. The results listed in Table II agree with this distribution. Similar results have been observed in glutamic acid isolated from rat liver protein[7] and from protein of yeast[9] in studies with acetate-1-^{14}C.

TABLE II

DISTRIBUTION OF ^{14}C IN GLUTAMIC ACID AFTER INJECTION OF ACETATE-1-^{14}C AND ACETATE-2-^{14}C

Carbon atom	*Acetate-1-^{14}C*		*Acetate-2-^{14}C*	
	*λs**	*% distribution*	*λs**	*% distribution*
Total molecule	2.55	100	2.36	100
COOH	3.98	31.2	1.18	10
$CHNH_2$			2.45	20.8
CH_2	0.04	0.3	2.80	23.7
CH_2			4.66	39.5
COOH	8.4	65.9	0.922	7.8

The glutamic acid was recovered from the first casein sample collected 3 hours after isotope injection for both acetate-1-^{14}C (Trial III) and acetate-2-^{14}C (Trial X).

* λs designates specific activity in terms of microcuries ^{14}C/gram atom carbon per microcurie ^{14}C injected/kg body wt.

The ^{14}C distribution in the glutamic acid recovered from casein 3 hours after injecting acetate-2-^{14}C (Table II) differs somewhat from the theoretical distribution based on the TCA cycle pathway (see Fig. 3). This result might occur if glutamic acid were synthesized before isotope equilibrium had become established in the cycle. During the first "turn" of the cycle ^{14}C would appear only in the C-4 carbon atom of glutamic acid (or α-ketoglutarate). On subsequent "turns" the ^{14}C would be distributed equally to carbon atoms C-2 and C-3 and, in lesser amounts, to C-1*. ^{14}C would

* C-2 and C-3 would have specific activities 50% as great as that on C-4 initially and would approach the specific activity of C-4 at equilibrium. The specific activity of C-1 would approach 50% of the specific activity of C-2.

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not appear in the C-5 position as a result of cycling within the TCA cycle but could be accounted for by the sequence shown in Fig. 4. The acetate formed along this devious route would contain ^{14}C on both carbon atoms and, upon re-entering the TCA cycle by condensation with oxalacetate, would lead to the formation of glutamic acid with ^{14}C in the C-5 position.

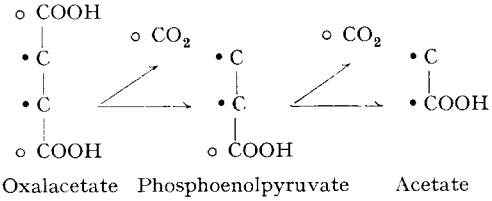

Fig. 4. The ^{14}C distribution is shown for oxalacetate formed during metabolism of acetate-2-^{14}C in the TCA cycle. Decarboxylation produces first phosphoenolpyruvate and then acetate with indicated ^{14}C distribution. The symbol (•) represents a greater specific activity than (o).

The ^{14}C distribution observed in glutamic acid after injection of acetate-2-^{14}C might also result from a dilution by 4-carbon units entering the TCA cycle according to equations (1) or (2), even after isotope equilibrium had become

$$\text{Pyruvate} + CO_2 \rightarrow \begin{cases} \text{oxalacetate} \\ \text{malate} \end{cases} \quad (1)$$

$$\text{Propionate} + CO_2 \rightarrow \text{succinate} \quad (2)$$

established. The entry of unlabeled 4-carbon units into the TCA cycle would result in lower ^{14}C levels in carbon atoms C-1, C-2, and C-3 and a higher ^{14}C level in C-4 relative to the theoretical distribution shown in Fig. 3.

If the TCA cycle is to function as a source of intermediates for biosynthesis carbon must enter at some position other than the acetyl-CoA oxalacetate condensation. Direct measurements have shown that the level of TCA cycle intermediates in aminal cells is very low[10]. Thus it would seem that these intermediates could not serve as reservoirs of carbon, and in the normal turnover of acetate in the cycle, there is not a net gain of carbon. Since propionate is a major nutrient for ruminants, it is possible that the succinate reaction involving CO_2 fixation (equation 2, above) might be a source of TCA cycle intermediates. This reaction has not been definitely established for animal tissues but it could account for the ^{14}C distribution observed for glutamic acid after injection of acetate-2-^{14}C.

An indication whether C-4 units were entering the TCA cycle can be obtained by comparing the observed ratio of specific activities for aspartic/glutamic acids with those predicted for the TCA cycle pathway. Unlabeled 4-carbon units entering the TCA cycle (according to equation 1 or 2) would reduce this ratio below the theoretical level.

When acetate containing 40 units ^{14}C on the carboxyl carbon enters the TCA cycle, glutamic and aspartic acids are produced with specific activities of 12 and 10 respectively (see Fig. 2). Under the same conditions acetate-2-^{14}C gives rise to glutamic and aspartic acids with specific activities of 28 and 30, respectively (see Fig. 3). The theoretical ratios of specific activities, aspartic/glutamic acids, would be 0.833 and 1.07 for acetate-1-^{14}C and acetate-2-^{14}C, respectively.

To make this ratio an index for the pathway of synthesis it is necessary first to

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correct the amino acid specific activities for dilution with unlabeled amino acids from body pools or exogenous sources. This correction can be based on the specific activities of carboxyl carbons. Figs. 2 and 3 shows that the specific activity of glutamic acid C-1 should equal that of aspartic acid C-(1 + 4) either after acetate-1-^{14}C or acetate-2-^{14}C An adjustment Factor (F), [F = specific activity glutamic C-1/specific activity aspartic C-(1 + 4)] can be calculated, which will correct for the relative diluti onby unlabeled amino acids of aspartic and glutamic acids formed via the TCA cycle according to the following formula: (F) × (aspartic acid)/(glutamic acid). The calculated values of (F) are listed in Table III for each sample.

The corrected ratios of specific activities [(F) × (aspartic acid)/(glutamic acid)] have been calculated for each sample and are listed in Table III. The mean value for this ratio after acetate-1-^{14}C was 0.827 (theoretical, 0.833) and after acetate-2-^{14}C was 0.97 (theoretical, 1.07).

TABLE III

RATIO OF SPECIFIC ACTIVITIES $\frac{\text{ASPARTIC ACID}}{\text{GLUTAMIC ACID}}$ AFTER INJECTION OF ACETATE-1-^{14}C AND ACETATE-2-^{14}C

Trial	*Hours after injection*	*(F)**	*λs(aspartic) · F*	$\frac{\lambda s(\text{aspartic}) \cdot F}{\lambda s(\text{glutamic})}$	*Theoretical ratio* $\frac{\text{aspartic}}{\text{glutamic}}$
		Acetate-1-^{14}C			
Acetate III	3	1.66	2.18	0.80	
	10	1.38	1.12	0.87	
	22	1.73	0.38	0.98	
	34	1.98	0.149	0.876	
Acetate VI	3	1.55	3.27	0.858	
	10	1.45	0.725	0.82	
	22	1.18	0.137	0.79	
	34	1.19	0.069	0.76	
Acetate VII	3	1.24	2.37	0.74	
	10	1.35	2.03	0.847	
	22	1.33	1.52	0.817	
	34	1.30	0.35	0.750	
			Calculated mean	0.827	0.833
		Acetate-2-^{14}C			
Acetate II	3	1.51	3.09	0.978	
	10	1.60	2.66	1.07	
	22	1.54	1.124	1.147	
	34	1.62	0.406	0.952	
Acetate X	3	1.28	1.77	0.748	
	10	1.43	2.10	1.00	
	22	1.65	0.931	0.968	
	34	1.65	0.359	0.887	
			Calculated mean	0.97	1.07

* See text for explanation of factor (F) which adjusts for dilution of amino acids formed via the TCA cycle by exogenous amino acids.

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The observed ratios are almost equal to, but slightly below, the theoretical ratios for both acetate-1-^{14}C and acetate-2-^{14}C. This result indicates that, if dilution of TCA cycle intermediates occurs at the four carbon level, it is relatively slow compared to the rate of "turnover" of the cycle.

In addition, it is possible that dilution occurs elsewhere in the TCA cycle that would not be revealed by studies with acetate-^{14}C. For example, citric acid might be supplied to the mammary gland by the blood. Milk contains about 100 mg citric acid per 100 ml which suggests either excess production of citric acid in the mammary gland or supply from the blood in excess of that needed for biosynthesis.

^{14}C distribution in alanine and serine

The results obtained by stepwise degradation of serine and alanine are listed in Table IV. After acetate-1-^{14}C, alanine and serine contained ^{14}C predominately in the C-1 carbon position which would be expected for the pathway via the TCA cycle (see Fig. 1). Trace amounts of ^{14}C were also detected in carbon atoms C-2 and C-3 of serine and presumably result from minor pathways. Similar ^{14}C distributions were obtained for alanine and serine, from rat liver protein[11].

When acetate-2-^{14}C is metabolized via the TCA cycle, oxalacetate becomes labeled primarily on the center carbon atoms. The three-carbon precursors of serine and alanine, presumably arising by decarboxylation of oxalacetate, would have a greater specific activity on carbon atoms C-2 and C-3 than on C-1 (see Fig. 4). The results from degradation of alanine and serine, listed in Table IV, agree with this distribution. The equal specific activity on carbon atoms C-2 and C-3 indicate a symmetrical intermediate on the pathway from acetate. This intermediate could be succinate and/or fumarate of the TCA cycle.

TABLE IV

^{14}C-DISTRIBUTION IN SERINE AND ALANINE FROM ACETATE-1-^{14}C AND ACETATE-2-^{14}C

Trial	*Hours after injection*	*Serine λs* (Carbon atom No.)***				*Alanine λs* (Carbon atom No.)***			
		ΣC	*C-1*	*C-2*	*C-3*	*ΣC*	*C-1*	*C-2*	*C-3*
				Acetate-1-^{14}C					
Acetate III	3	0.56	1.50	0.04	0.06	0.50	1.31	—	—***
	10	0.60	1.36	0.13	0.09	0.57	1.36	—	—
Acetate VII	3	0.562	1.52	0.04	0.06	0.574	1.54	—	—
	10	0.658	1.56	0.05	0.06	0.582	1.61	—	—
				Acetate-2-^{14}C					
Acetate II	3	1.01	0.59	1.15	1.33	1.12	0.67	1.33§	1.36
Acetate X	3	1.50	0.68	1.94	2.0		—	—	—
	10	1.50	1.07	1.88	1.71	1.68	1.27	1.67	1.68
	22	0.78	0.49	0.71	0.78		—	—	—
	34	0.31	0.21	0.36	0.36		—	—	—

* λs designates specific activity. See footnote to Table II.

** ΣC designates specific activity of total molecule. C-1 and C-2 designate specific activity of carboxyl carbon and amino carbon, respectively. 3 (ΣC) should equal the sum of (C-1 + C-2 + C-3).

*** — indicates values were not determined.

§ The carbonate was lost on this sample so value was calculated by difference.

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One can calculate the theoretical relationship between the specific activity of oxalacetate and a three-carbon precursor, phosphopyruvate*, of alanine, serine, and lactose for transfer of acetate-^{14}C via the TCA cycle (see Fig. 1). When oxalacetate labeled from acetate-1-^{14}C is decarboxylated only 50% of the isotope is transferred to the three-carbon intermediate, phosphopyruvate, the other 50% go to CO_2. In the case of oxalacetate labeled from acetate-2-^{14}C, 83%** of the isotope would be transferred to phosphopyruvate. Thus one would predict for the TCA cycle pathway that the ratio of specific activities of serine, alanine, or lactose to that of aspartic acid (representing oxalacetate) would be 83%/50% = 1.67 times as great for acetate-2-^{14}C as for acetate-1-^{14}C.

The mean specific activities of the amino acids and lactose during the 34 hour experimental period are listed in Table V. The ratios of the mean specific activities of serine/aspartic acid and of alanine/aspartic acid were 1.66 times as great after acetate-2-^{14}C as after acetate-1-^{14}C (theoretical = 1.67). In the case of lactose the observed ratio was 1.49. The close agreement of these observed ratios, 1.49 to 1.66, with the theoretical ratio, 1.67, supports the conclusion that the TCA cycle provides three-carbon precursors for alanine, serine, and lactose.

TABLE V

MEAN SPECIFIC ACTIVITY OF AMINO ACIDS AND LACTOSE AFTER INJECTION OF ACETATE-1-^{14}C AND ACETATE-2-^{14}C

The mean specific activities were calculated for each amino acid for the 34-hour experimental period according to the following method: $\bar{\lambda}s = 1/t \Sigma \lambda s \cdot \Delta t$, where λs was the average specific activity of the amino acid at each time period for the three trials with acetate-1-^{14}C or for the two trials with acetate-2-^{14}C.

Amino acid	$\bar{\lambda}s$		$\bar{\lambda}s$ *relative to aspartic acid*		*Ratio of* $\bar{\lambda}s$ *relative to aspartic acid*
	1*	2*	1*	2*	2/1
Aspartic acid	0.571	0.784	1.0	1.0	
Serine	0.304	0.692	0.532	0.883	1.66
Alanine	0.291	0.664	0.510	0.847	1.66
Lactose	0.321	0.656	0.562	0.838	1.49
Glycine	0.292	0.407	0.512	0.519	0.987

* 1 designates data from acetate-1-^{14}C trials; 2 designates data from acetate-2-^{14}C trials.

Similar treatment of results obtained with glycine indicates that it too is formed as a result of transfer of carbon from acetate via the TCA cycle. According to the scheme shown in Fig. 5, 50% of the isotope from oxalacetate would be lost to CO_2 and the C_1 unit during formation of, first a three-carbon intermediate (serine?), and then the two-carbon compound (glycine) after either acetate-1-^{14}C or acetate-2-^{14}C. Glycine formed along this pathway, in contrast to alanine and serine, would have approximately the

* For convenience of discussion the three-carbon precursor formed by decarboxylation of oxalacetate will be assumed to be phosphopyruvate. As discussed elsewhere[1] it appears that there are three different precursors for serine, alanine, and lactose but all three precursors are derived from phosphopyruvate during their formation from C of acetate.

** Reference to Fig. 3 shows the isotope distribution in oxalacetate (aspartic acid) at equilibrium when acetate-2-^{14}C is metabolized. Decarboxylation of this oxalacetate results in transfer of 100/120 units ^{14}C = 83% of the isotope to phosphopyruvate.

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same mean specific activity relative to aspartic acid for both types of labeled acetate since 50% of the ^{14}C is lost in each case. The observed ratio of 0.987 (see Table V) is essentially the same as the theoretical value, 1.0, and indicates that the TCA cycle is the pathway from acetate to glycine of casein.

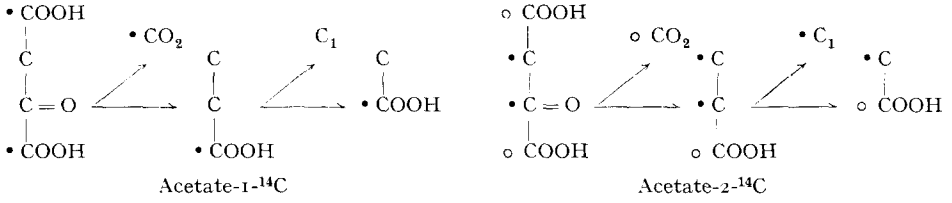

Fig. 5. The distribution of isotope in oxalacetate and in the three-carbon and two-carbon compounds derived therefrom is shown after labeling by acetate-1-^{14}C and acetate-2-^{14}C. The symbol (•) indicates labeling with high specific activity and (o) indicates labeling with low specific activity. (o) will represent a specific activity of zero on the first turn of the TCA cycle after acetate-2-^{14}C enters; the specific activity (o) will increase through continuous cycling to 50% of that on the center carbon atoms (•) at equilibrium. C_1 designates the one carbon unit that is presumably formed when serine is converted to glycine.

Our results on the distribution of ^{14}C in amino acids and on the relative specific activity among the amino acids provide strong support for the conclusion that the TCA cycle supplies intermediates for the synthesis of some amino acids of casein and of lactose.

Dual role of TCA cycle in cow

KREBS has proposed that the TCA cycle has two different functions which include supplying intermediates for biosynthesis and serving as terminal pathway for oxidation of metabolites[12]. KREBS, GURIN AND EGGLESTON[14] concluded from their studies with yeast that, in this organism, the TCA cycle functions primarily as a source of intermediates for biosynthesis.

There is considerable evidence that the TCA cycle functions as the terminal pathway for oxidation of metabolites in animal tissues. Our previous studies on transfer of carbon from acetate to CO_2 in the cow were consistent with this mechanism. With acetate-1-^{14}C the respiratory CO_2 reached a maximum specific activity of 31 units at about 9 minutes after isotope injection. With acetate-2-^{14}C, on the other hand, a maximum specific activity of 15.4 units was reached in the respired CO_2 at about 18 minutes after isotope injection[13]. According to the TCA cycle pathway 100% of the carboxyl carbon from acetate would be eliminated as CO_2 after 1½ "turns" of the cycle while only 50% of the methyl carbon would be eliminated as CO_2 after 3½ "turns". Thus, if acetate were oxidized via the TCA cycle, one would expect the observed results, that is, a higher specific activity at an earlier time after acetate-1-^{14}C than after acetate-2-^{14}C.

The distribution of carbon-14 in the carboxyl carbons of glutamic and aspartic acids recovered from casein indicated that approximate isotope equilibrium had been reached in the TCA cycle at the time the amino acids were synthesized. Thus, it would appear, especially in the case of acetate-2-^{14}C, that many "turns" of the TCA cycle occur per amino acid molecule synthesized. Under these conditions a large amount of CO_2 would arise from acetate carbon while isotope equilibrium was becoming estab-

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lished. It seems reasonable, therefore, to conclude that CO_2 and biosynthetic intermediates arise from the same TCA cycle.

Our results on the metabolism of acetate-^{14}C presented in this and the preceding paper[1] provide strong support for the conclusion that the TCA cycle performs a dual function in the intact cow; in addition to its katabolic role, the TCA cycle supplies intermediates for the synthesis of organic constituents of milk.

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SUMMARY

Results are presented on the ^{14}C distribution revealed by the decarboxylation and complete degradation of amino acids recovered from casein after injecting intact cows with acetate-1-^{14}C and acetate-2-^{14}C.

The labeling pattern in the amino acids provides strong evidence for the theory that the Tricarboxylic Acid Cycle is the pathway for transfer of carbon from acetate to amino acids of casein and to lactose.

Our studies with acetate-^{14}C support the idea that the Tricarboxylic Acid Cycle functions as a pathway of terminal oxidation and also supplies intermediates for the synthesis of milk constituents.

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