ACETATE AS A PRECURSOR OF AMINO ACIDS OF CASEIN IN THE INTACT DAIRY COW*

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INTRODUCTION

Acetate is normally produced in large amounts by the rumen microflora and is an important metabolite for ruminants[1]. Its role as a precursor of the major organic constituents of milk has been investigated in the intact cow[2]. In these studies it was shown that carbon from acetate-^{14}C was transferred mainly to milk fat but 1% to 2% of the injected carbon-14 was recovered in casein during the first 33 hours after injection.

Preliminary studies with ^{14}C-labeled short chain fatty acids indicated that in the tissues of the cow, carbon from acetate was transferred in significant amounts only to the non-essential** amino acids in casein[3]. The present paper reports on a more extensive study of the relationship between acetate and amino acids in the intact cow. Amino acids were recovered from twenty casein samples collected from five cows at intervals after injection of acetate labeled with ^{14}C on either carbon atom. In each case it was found that carbon from acetate was transferred most efficiently to glutamic acid. Glucose, which was studied earlier[4], was a better precursor of serine and alanine. The differences between glucose and acetate as precursors of amino acids are discussed.

METHODS

Twenty casein samples were prepared from milk collected at intervals after injecting five normal, lactating cows with a single dose of 4 to 5 millicuries of acetate-^{14}C. The methods for these procedures have been described previously[5]. Data concerning the cows used for these studies are listed in Table I.

The casein samples were hydrolyzed and amino acids separated on ion-exchange columns of Dowex-50 and IR4-B[5]. The amino acids isolated from the columns were converted to their free base form with propylene oxide and then crystallized from solution according to methods described previously[5]. Our procedure for recovery of alanine was modified when we observed that

TABLE I

DATA ON EXPERIMENTAL ANIMALS USED IN ACETATE TRIALS

Trial No.	*Cow No.*	*Breed*	*Cow's wt kg*	*Millicuries injected*	*Compound injected*
AC III	905	Jersey	414	4.7	Acetate-1-^{14}C
AC VI	962	Jersey	499	5.5	Acetate-1-^{14}C
AC VII	1099	Holstein	700	4.0	Acetate-1-^{14}C
AC II	805	Jersey	500	5.0	Acetate-2-^{14}C
AC X	947	Jersey	465	3.86	Acetate-2-^{14}C

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** Glutamic and aspartic acids, alanine, serine, glycine, proline, and arginine.

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occasionally alanine recovered directly from the Dowex-50 column, used for the initial hydrolysate fractionation, was contaminated with small amounts of glycine even after crystallization. The contaminating glycine was eliminated by adding the alanine to a Dowex-50 column and eluting with ammonium formate buffer (pH = 3.2) according to the method of HIRS, MOORE AND STEIN[6]. Most of the buffer could be removed from the recovered alanine by sublimation; the last traces of buffer were removed by adding the alanine sample to a small Dowex-50 column and eluting with 1.3 *N* HCl.

The method for combustion of amino acids, planchet preparation and carbon-14 assay were described in an earlier publication[4].

RESULTS AND DISCUSSIONS

The specific activities of the amino acids are expressed in terms of the relative injected dosages, that is, microcuries per gram atom carbon/microcuries injected per kilogram body weight. Specific activity expressed in terms of the relative injected dose has the advantage that results obtained with animals of different body size which receive different amounts of carbon-14 are more directly comparable.

The specific activities of amino acids recovered from casein after intravenous injection of acetate-1-^{14}C and acetate-2-^{14}C are listed in Tables II and III, respectively. In each trial the carbon from acetate was recovered in significant quantities only in the seven non-essential amino acids, glutamic and aspartic acids, alanine, serine, glycine, proline and arginine.

Lysine was the only essential amino acid recovered in this study since previous work has indicated that essential amino acids are not formed by tissues of the cow[3,4]. Using lysine as a representative of the essential amino acids, these results with acetate ^{14}C corroborate our earlier conclusion[3] that only the non-essential amino acids are formed in appreciable quantities by the tissues of the cow.

Glutamic and aspartic acids

Glutamic acid had the greatest specific activity among the amino acids for each casein sample after injection of both acetate-1-^{14}C and acetate-2-^{14}C. The specific activity of aspartic acid was smaller than that of glutamic but was generally greater than that of any of the other amino acids. From these results it may be concluded that the carbon from acetate is transferred most efficiently, that is with least dilution, to glutamic and aspartic acids, indicating that, among the amino acids, the most direct pathway from acetate is to the dicarboxylic amino acids. One explanation for these results is the transfer of acetate carbon to amino acids via the Tricarboxylic Acid Cycle (TCA) as shown in Fig. 1.

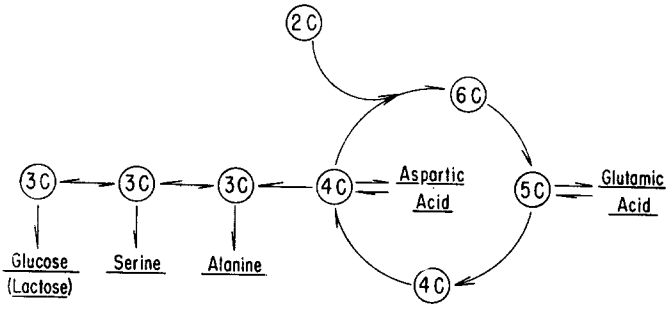

Fig. 1. Abbreviated schematic diagram of the TCA cycle. This diagram shows the transfer of carbon-14 from acetate (2C) via the TCA cycle to α-ketoglutarate (5C) and, after transamination, to glutamate; from (5C) the isotope passes to four carbon intermediates of the cycle (4C) and to aspartic acid; or by decarboxylation of a (4C) dicarboxylic acid to produce three carbon (3C) intermediates which serve as precursors of alanine, serine, and glucose (lactose).

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TABLE II

SPECIFIC ACTIVITY OF LACTOSE AND AMINO ACIDS FROM CASEIN AFTER INTRAVENOUS INJECTION OF ACETATE-1-^{14}C

Trial	*Hours after injection*	*Glutamic acid*	*Aspartic acid*	*Alanine*	*Serine*	*Lactose*	*Glycine*	*Proline*	*Arginine*	*Lysine*
Acetate III										
	3	2.55*	1.31	0.50	0.56	0.56	0.45	0.46	—	0.00
	10	1.29	0.81	0.57	0.60	0.68	0.68	0.31	0.42	0.01
	22	0.39	0.22	0.19	0.18	0.24	0.26	0.12	0.15	0.008
	34	0.17	0.075	0.073	0.078	0.09	0.11	0.055	0.058	0.005
Acetate VI										
	3	3.77	2.09	0.78	—	0.90	0.33	—	0.60	0.01
	10	0.886	0.500	0.396	0.318	0.44	0.356	0.341	0.226	0.06
	22	0.173	0.116	0.101	0.095	0.08	0.136	0.095	0.104	0.05
	34	0.091	0.058	0.049	0.059	0.04	0.077	—	0.055	0.03
Acetate VII										
	3	3.19	1.91	0.574	0.562	0.56	0.474	0.45	0.423	0.01**
	10	2.40	1.51	0.582	0.658	0.67	0.518	0.48	0.43	0.005
	22	1.86	1.14	0.51	0.55	0.56	0.46	0.37	0.37	0.01
	34	0.469	0.269	0.184	0.214	0.17	0.224	0.144	0.16	0.04

* Specific activity of 2.55 $\frac{\mu c/\text{gram atom carbon}}{\mu c \text{ injected/kg body wt}}$ = 1020 net c.p.m. in our counting system with $BaCO_3$ of "infinite thickness".

** Leucine collected instead of lysine.

TABLE III

SPECIFIC ACTIVITY OF LACTOSE AND AMINO ACIDS FROM CASEIN AFTER INTRAVENOUS INJECTION OF ACETATE-2-^{14}C

Trial	*Hours after injection*	*Glutamic acid*	*Aspartic acid*	*Alanine*	*Serine*	*Lactose*	*Glycine*	*Proline*	*Arginine*	*Lysine*
Acetate II										
	3	3.16	2.05	1.12	1.01	1.03	0.457	1.04	0.49	0.00
	10	2.49	1.66	1.09	1.06	1.09	0.548	0.88	0.46	0.01
	22	0.98	0.73	0.55	—	0.43	0.34	—	—	—
	34	0.426	0.25	0.22	0.23	0.20	0.18	0.17	0.14	0.02
Acetate X										
	3	2.36	1.38	0.89	1.50	1.30	0.506	0.360	0.263	0.00
	10	2.10	1.47	1.68	1.50	1.66	1.00	0.427	0.446	0.00
	22	0.962	0.563	0.651	0.775	0.698	0.572	0.215	0.222	0.00
	34	0.405	0.217	0.225	0.308	0.214	0.069	0.083	0.114	0.007

Serine, alanine and lactose

The specific activities of alanine and serine were approximately equal for each casein sample and were very close to the specific activities of lactose recovered from the same milk sample. These results suggest that, in the intact cow, carbon from acetate either passes to a common precursor for alanine, serine, and lactose, or, more probably, passes to the three precursors of these compounds with almost no change of specific activity (see Fig. 1). The latter possibility would indicate that there are no large pools of intermediates on the pathway between precursors of alanine, serine, and lactose or that there is rapid isotope equilibrium between the precursor pools.

Several studies have demonstrated a relationship between glycine and serine in animal tissues[7,8,9]. Previous studies with the intact cow have indicated that carbon from glucose was primarily transferred along a pathway that may involve serine as a precursor of glycine[4] and ARNSTEIN has reached a similar conclusion from studies with rats[10]. Our results obtained with acetate-^{14}C (Tables II and III) support the same pathway. The specific activity of serine always exceeded that of glycine initially (at 3 hours) and then decreased relative to glycine at later time periods. These changes of specific activities would be expected if serine were the precursor of glycine but would not occur if glycine were the precursor of serine.

Proline and arginine

There is evidence that glutamic acid, proline, and arginine are interconvertible in animal tissues[11,12]. The most reasonable explanation for the data in Tables II and III would involve a pathway for the transfer of acetate carbon to glutamic acid and then to proline and arginine. If this were the major pathway involved then one would expect the ratio of specific activities for these amino acids to remain constant. The ratios of mean specific acitivites for glutamic acid/proline have been calculated from the data in Tables II and III to be 4.2, 5.0, and 5.0 for trials III, VII, and X, respectively. Similar calculations of the glutamic acid/arginine ratio were 3.7, 5.1, and 4.8 for trials VI, VII, and X, respectively. Since there was not a large variation between these ratios for the amino acids from different cows, our results suggest a relationship between glutamic acid, arginine, and proline in the intact cow. These ratios indicate that a maximum of about 20 to 25% of proline and arginine in casein may arise from glutamic acid.

Acetate vs. *glucose as precursor of amino acids*

The relative specific activities of the amino acids after injecting acetate-^{14}C differed markedly from that previously observed[4] when glucose-^{14}C was injected into cows. These differences are brought out by the curves in Fig. 2 and 3 which show the change of specific activity of glutamic acid and serine as a function of time after injecting glucose-^{14}C or acetate-^{14}C.

The specific activity of serine (and alanine*) was 2 to 3 times as great as that of glutamic (and aspartic*) acid when glucose-^{14}C was injected. These results indicate that glucose is a more direct precursor for the three carbon amino acids than for the dicarboxylic amino acids. When acetate was injected, on the other hand, (see Fig. 3)

* The specific activities for these amino acids were very nearly the same so that the curve for one represents the change of specific activity for both.

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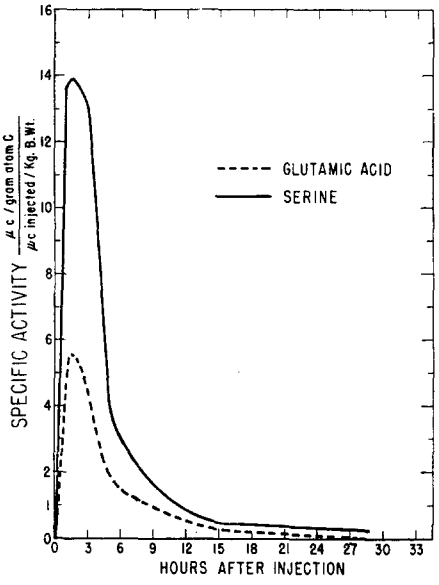

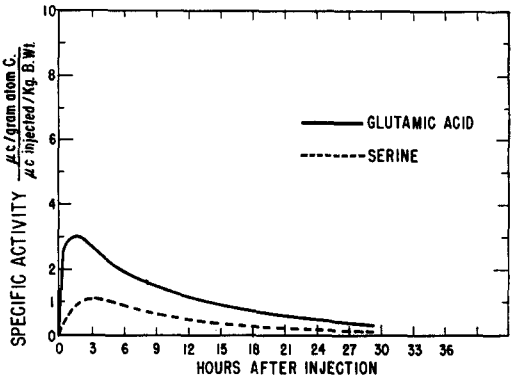

Fig. 3. ^{14}C in glutamic acid and serine after intravenous injection of acetate-^{14}C. The specific activities are plotted as the mean result for all of the acetate-1-^{14}C and acetate-2-^{14}C trials. The specific activity of serine alanine and lactose were approximately equal so that the curve for serine represents the change of specific activity for all three compounds.

Fig. 2. ^{14}C in glutamic acid and serine after intravenous injection of uniformly labeled glucose. The curve for serine also represents the change of specific activity for alanine since these amino acids had approximately the same specific activities throughout the experimental period. The curve for glutamic acid represents the change of specific activity in aspartic acid, as well, for the same reason.

the reverse relationship was observed; the specific acitivity of glutamic acid was 2 to 3 times as great as that of serine (alanine and lactose). These results may be interpreted as indicating that the precursor of glutamic acid is "closer" to the "acetate pool" than the precursors of the three carbon amino acids (see Fig. 1). This relationship suggests that, quantitatively, acetate is a more important precursor for glutamic acid than for the three carbon amino acids.

The fact that the specific activity curves for serine and glutamic acid maintain their same relative position throughout the experimental period for both glucose-^{14}C and acetate-^{14}C suggests that there is not extensive mixing of carbon between the "acetate" and "glucose" pools. Otherwise one would expect the curves would coincide or even cross over at later intervals.

For each amino acid, except arginine, the specific activity per unit injected dose was greater after glucose than after acetate in the initial sample collected three hours after injection. This result could be explained by the postulate that in the cow the acetate pool is larger than the glucose pool. This condition would result in a greater dilution of the injected acetate-^{14}C and correspondingly lower specific activities in the amino acids. The specific activity of the amino acids at later time intervals was reduced to between 1/5 and 1/10 for acetate while for glucose the final specific activity was 1/70 to 1/80 of the initial value. These results indicate that the smaller glucose pool is turning over more rapidly than the larger acetate pool.

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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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