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

by

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INTRODUCTION

Recent publications from our laboratory have shown that glucose plays a relatively minor role as a source of energy for the cow[1] and that in milk synthesis it is used mainly for lactose formation[2]. The distribution of isotope from glucose among the milk fractions, 80% in lactose, 12% in milk fat, and 4% in casein, showed that only a small part of the glucose pool ultimately appeared in milk protein and indicated that glucose was not an important precursor for milk protein in the cow. Glucose, however, may be an important precursor for some of the constituent amino acids of the casein molecule. To investigate this possibility, amino acids were isolated from casein samples taken at intervals up to 34 hours after administering uniformly labeled glucose. The specific activity of these amino acids was measured and compared to the specific activity of plasma glucose over the same time interval.

The present paper presents the results of this investigation and our estimated value for the quantitative importance of glucose as a precursor of several amino acids. These results indicate that glucose is an important precursor of alanine and serine and that smaller but significant amounts of glucose carbon appear in glutamic and aspartic acids and glycine.

METHODS

2.7 millicuries of uniformly labeled glucose were injected intravenously into a lactating dairy cow. Milk samples were collected at 3, 11, 22 and 34 hours following the injection and purified casein was prepared from the milk according to methods previously described[3]. A 5 g sample of purified casein, taken from each time period, was hydrolyzed with 6 *N* HCl for 18 hours and then treated to remove humin, phenylalanine, and tyrosine[4]. The excess acid was removed by repeated distillation to dryness. The remaining amino acids in the hydrolysate were dissolved in a small volume of water, added to a cation exchange column and eluted with HCl as described in a previous publication[3]. Additional columns were used to separate serine and threonine from one another and from small amounts of contaminating aspartic and glutamic acids. Fourteen amino acids were recovered from each casein sample as white crystalline preparations and were checked for purity by paper chromatography.

A sample of each amino acid was combusted in a micro-combustion furnace and the resulting

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carbon dioxide collected in a gas scrubber bottle containing 60 ml of 0.25 *N* NaOH. The dilute base was prepared as needed from a saturated solution of NaOH using CO_2-free water. Sufficient quantities of amino acid were combusted, in each case, to provide carbonate for two planchets of "infinite-thickness" for carbon-14 assay.

Preparation of planchets

The basic solution containing the trapped carbonate was divided equally into two small Erlenmeyer flasks, covered, and heated over a steam bath for about 10 minutes. A solution of 0.25 *M* $BaCl_2$ and 5 *M* NH_4Cl was added to the hot carbonate solution slowly and with constant agitation until there was a 3 to 6-fold excess of barium ion over carbonate ion and a 20% excess of ammonium ion over NaOH.

The solution was allowed to cool to room temperature and the $BaCO_3$ was then filtered onto small tarred filter paper discs. A commercial precipitation apparatus with a cross-sectional area of 2.83 cm^2 was used to prepare the planchets. After the first filtration the $BaCO_3$ pellet was resuspended in 95% alcohol within the precipitation apparatus. The alcohol was filtered off and the process repeated one or more times until the resulting $BaCO_3$ planchet had a smooth uniform surface free of cracks, pits, and broken edges. The filter paper disc together with the $BaCO_3$ pellet was transferred to a stainless steel cup and dried under an infra-red lamp for 30 minutes before counting. The planchets remained under a heat lamp at all times until the counting was completed except when the planchet was in the counter. The above method of planchet preparation is essentially a combination of procedures described in the literature[5,6].

Sample counting

The $BaCO_3$ planchets were counted in a windowless flow gas geiger counter for at least two 10 minute periods and for sufficient time to reduce the probable error of counting to less than 2%. For the essential amino acid samples, which had very low count rates, the probable error was generally larger.

After the count rate had been determined the planchets were weighed to confirm the presence of 50 mg or more of $BaCO_3$ ("infinitely thick"). The net count rate was then converted to absolute units (microcuries ^{14}C per gram atom of carbon) using a factor established in our counting system with a National Bureau of Standards Carbon-14 Beta Ray Standard*. Our final results are expressed as microcuries carbon-14 per gram atom carbon per unit injected dose (*i.e.* microcurie or millicurie of ^{14}C-injected per kilogram body weight). In this experiment a 457 kg cow was injected with 2.87 millicuries of uniformly labeled glucose giving a factor of 6.28 microcuries ^{14}C/kilogram body weight

RESULTS AND DISCUSSION

The specific activities of the amino acids are listed in Table I for each of the four time periods after injection of labeled glucose. Based on their specific activities, the amino acids divide into two groups, the first group containing the non-essential amino acids which have high specific activities and the second group containing the essential amino acids all with low specific activities. These results with labeled glucose are, in general, the same as those observed after administering ^{14}C-labeled carbonate, acetate, propionate, and butyrate[3] and are further proof that only the non-essential amino acids are synthesized in significant quantities by tissues of the cow.

The exceptions to the amino acid groupings in Table I are arginine with relatively low specific activities and methionine with relatively high specific activities for their respective groups. These discrepancies will be discussed later.

The isotope levels in the non-essential amino acids are greatest in the first sample, collected three hours after the glucose injection, and decrease rapidly in succeeding samples. These results reflect the rapid turnover of glucose in the cow and would be expected considering the calculated turnover time for the cow's plasma glucose pool which was found to be less than one hour[1]. It is apparent that the biochemical transformations associated with the catabolism of glucose, transamination of metabolic

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TABLE I

SPECIFIC ACTIVITY* OF AMINO ACIDS FROM CASEIN AT DIFFERENT TIMES AFTER INTRAVENOUS INJECTION OF UNIFORMLY-LABELED GLUCOSE

Amino acid	*Time after injection of glucose*			
	3 h	*11 h*	*22 h*	*34 h*
Alanine	14.20**	4.06	0.69	0.24
Serine	13.86	2.90	0.47	0.21
Glutamic acid	5.76	1.44	0.28	0.07
Aspartic acid	5.12	1.28	0.25	0.07
Glycine	3.04	0.98	***	0.18
Proline	0.73	0.20	0.05	0.04
Arginine	0.10	0.12	0.04	0.02
Methionine	0.10	0.11	0.05	—
Lysine	0.01	0.03	0.03	—
Tyrosine	0.01	0.01	0.01	—
Leucine-*iso*Leucine	0.01	0.01	0.01	—
Threonine	0.03	0.04	—	—
Valine	0.00	0.01	0.01	—
Histidine	0.00	0.00	0.00	—

* Specific activity $= \frac{\text{microcuries } {}^{14}C/\text{gram atom carbon}}{\text{microcuries injected/kilogram body weight.}}$

** Specific activity of 14.20 $\frac{\mu c\ {}^{14}C/\text{gram atom carbon}}{\mu c \text{ injected/kilogram body weight}}$ = 3134 net counts per minute on infinitely thick $BaCO_3$ planchets in our counting system.

*** Sample lost.

intermediates and incorporation of the resulting amino acids into protein are very rapid in the cow.

The three carbon amino acids, alanine and serine, have the highest specific activity in each sample throughout the experimental period. This result indicates that some intermediate of glucose metabolism is converted to alanine and serine more rapidly than to any of the other amino acids. The identity of this intermediate must await further investigation but one possibility would be pyruvate since it is a common product of glucose metabolism by various pathways. Pyruvate, by transamination, would be converted directly to alanine. The enzyme serine dehydrase which is active in animal tissues[7] catalyzes the conversion of serine to pyruvate, however, the reversibility of this reaction has not yet been demonstrated. Such a mechanism would explain our observed results but it is also possible that another metabolite is the immediate precursor of serine.

Pyruvate may also enter the tricarboxylic acid cycle by any of the following pathways:

1. Pyruvate + CO_2 ⟶ { Oxalacetate[8] / Malate[9] }

2. Pyruvate $\xrightarrow[\text{COA·SH}]{-CO_2}$ COA·S-Acetyl $\xrightarrow[\text{Oxalacetate}]{}$ Citrate[10] ⟶ α-ketoglutarate

Subsequent transamination of the cycle intermediates, oxalacetate and α-ketoglutarate, would result in the appearance of isotope in aspartic and glutamic acids. The data in

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Table I shows that these acidic amino acids contain appreciable quantities of isotope though at much lower levels than the three carbon amino acids.

The biochemical relationship between serine and glycine and the non-nitrogen source of these amino acids in the intact animal have been the subject of many recent investigations. SHEMIN[11] demonstrated that serine was converted to glycine and that the β-carbon of serine was split off during the conversion. The reverse reaction, involving the conversion of glycine to serine, has been demonstrated in rats by GREENBERG's laboratory[12,13]. The interconversion of serine and glycine was very rapid as indicated by the approximately equal isotope concentration in these two amino acids isolated from rat liver protein after administering ^{14}C-labeled glycine[12]. Thus the interrelation between glycine and serine has been well established but the non-nitrogen source of these non-essential amino acids is still undecided.

CHAO, DELWICHE AND GREENBERG[14] studied the role of several compounds as precursors of glycine in the rat. They concluded that glycine was derived mainly from serine, threonine and dietary glycine and that carbohydrate did not furnish appreciable carbon for glycine synthesis. Other laboratories, however, have obtained results indicating that carbohydrate may be a precursor of serine and/or glycine. SONNE, BUCHANAN AND DELLUVA[15] administered labeled lactate to pigeons and, from the isotope distribution in excreted uric acid, postulated that the lactate was converted to pyruvate and this, in turn, to serine and glycine. ANKER[16] studied the biosynthesis of glycine in rats using labeled pyruvate and found that carbon atoms 1 and 2 of pyruvate were transferred to carbon atoms 1 and 2 of glycine, respectively. He postulated that serine was an intermediate in this transformation. KRUEGER[17], on the other hand, has reported that non-isotopic pyruvate administered to rats leads to significant increases of liver glycine but not serine. Ketone bodies, however, increased liver serine levels significantly and thus KRUEGER concluded that serine arises primarily from products of fat metabolism while glycine arises from carbohydrates.

The data listed in Table I show clearly that, in the cow, serine is more closely related to carbohydrate metabolism than is glycine and in general they agree with the results obtained by SONNE *et al.*[15], and ANKER[16]. Our results suggest some mechanism such as:

3. Glucose $\longrightarrow$ (X) $\longrightarrow$ Serine $\longrightarrow$ Glycine + C_1

The one carbon unit formed in the conversion of serine to glycine would be available for transfer to the methyl group of methionine and would, in turn, account for the relatively high ^{14}C level which we observed in this essential amino acid.

To gather additional information on the nature of the intermediate (X) in reaction 3, above, serine samples from each time period were degraded stepwise. The specific activities of the individual carbon atoms, recorded in Table II, show that the isotopic carbon is approximately uniformly distributed in the serine molecule. These results suggest that the intermediate (X) is most likely a three carbon compound derived directly from glucose. Based on our present knowledge of intermediate pathways, pyruvate, among the glycolytic products, is the most probable precursor of alanine but there are other three carbon intermediates that might function as the precursor of serine including phospho-enol pyruvate or phospho-glycerate.

STAFFORD, MAGALDI AND VENNESLAND[18] have isolated an enzyme from plant material that reversibly reduces hydroxypyruvate to glycerate. CAMMARATA AND COHEN[19]

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TABLE II

DISTRIBUTION OF ^{14}C IN SERINE MOLECULE AFTER INJECTING UNIFORMLY LABELED GLUCOSE

Specific activity

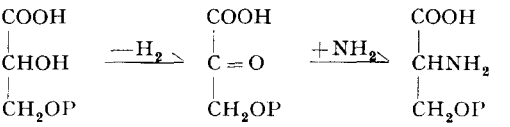
Time after injection	*Serine C-1*	*Carbon C-2*	*Atom* C-3*	*$\bar{M}$***	*C****
3 h	14.2	13.7	13.5	13.8	13.86
10 h	2.61	2.62	2.56	2.6	2.90
22 h	0.49	0.43	0.42	0.45	0.47
34 h	0.19	0.21	0.23	0.21	0.21

* C-1 refers to the carboxyl carbon, C-2 the amino carbon, and C-3 hydroxyl carbon.

** $\bar{M}$ = mean specific activity of the carbon atoms individually determined by stepwise degradation.

*** C = specific activity of whole molecule determined by combustion of serine.

have demonstrated that serine can be transaminated and, while the resulting product is unknown, hydroxypyruvate* would seem a reasonable possibility. Thus a possible pathway for the transfer of glucose carbon to serine is *via* glycerate. If a similar mechanism exists for the phosphorylated intermediate, 3-phosphoglycerate, then the following pathway would seem to be plausible:



Phosphoserine, rather than serine, would be the product formed. The fact that phosphoserine is the principle form in which serine and phosphorus are found in casein[21]** lends credence to the postulated mechanism.

The possibility has not been excluded that glucose can be metabolised *via* a shunt pathway in the cow, for example, the hexosemonophosphate shunt[23]. Under these conditions, additional intermediates would be formed that might serve as precursors of serine.

To estimate the quantitative importance of glucose as a precursor of the amino acids, the specific activity of plasma glucose was measured at intervals during the 34 hour experimental period. These data were used to calculate the mean specific activity of plasma glucose (π_s) during each of the four sample periods***. The ratio of the specific activity of each amino acid (λ_s) to that of plasma glucose summated for the 34 hour period: $\frac{\sum_0^t \lambda_S \cdot \Delta t}{\int_0^t \pi_S \, dt}$ provides an estimate of the quantitative importance of glucose as a precursor of each amino acid. The general method for this calculation has been discussed more fully in another paper[24]. The accuracy of our results is limited by the validity of our assumption that the mean specific activity of plasma glucose reflects

* Hydroxypyruvic acid has been found in animal tissue[20].

** Another phosphoprotein, phosphovitin of egg yolk, has been shown to contain approximately stoichiometric amounts of serine and phosphorus[22].

*** The results of these calculations are listed in the preceeding paper[2] in the third column of Table VI under the sub-heading Trial I.

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the mean specific activity of glucose at the site of amino acid formation. If this assumption is incorrect our calculated values will be in error but the relative values between the various amino acids should be approximately correct.

Our calculated results for each of the amino acids are recorded in the first column of figures in Table III. These calculations indicate that approximately 1/4 of the carbon in the alanine and serine of milk casein arose from carbon in plasma glucose; about 10% of the carbon in glutamic and aspartic acids and 7% of that in glycine had a similar origin. The remaining amino acids were not derived from glucose carbon to any appreciable extent.

We conclude from these calculations that, in the cow, glucose is an important precursor for the three carbon amino acids, alanine and serine, and is of significant but lesser importance in the synthesis of the acidic amino acids and glycine.

TABLE III

PERCENT OF AMINO ACID CARBON DERIVED FROM PLASMA GLUCOSE AND PERCENT INDIRECTLY TRANSFERRED *via* PLASMA CARBONATE POOL

Amino acid	*Percent amino acid carbon from plasma glucose*	
	Total	*Percent of total transferred via plasma carbonate*
Alanine	26.2	2.7
Serine	22.8	2.2
Glutamic acid	10.2	4.3
Aspartic acid	9.1	6.7
Glycine	7*	8.0
Proline	1.5	8.7
Arginine	0.6	62
Methionine	0.53	26
Lysine	0.16	71
Tyrosine	0.08	68
Leucine-*iso*Leucine	0.05	87
Valine	0.04	100**
Histidine	0.01	67

* An estimated specific activity of 0.32 was assigned to the glycine that was lost from the 22 hour casein sample to permit calculation of this figure.

** A value of 130% was actually obtained in the calculation of this result. The isotope levels in the essential amino acids were low and the counting errors correspondingly high leading to inaccuracies in the calculated values which probably accounts for the high result obtained.

The difference in the calculated values for serine and glycine do not indicate a rapid carbon exchange between these two amino acids in the cow such as GOLDSWORTHY, WINNICK, AND GREENBERG observed in rats[12]. Further investigation will be necessary to decide if the difference between the cow and rat, in this respect, is due to species variation or if it results because phosphoserine, unlike serine, does not exchange carbon with glycine extensively. Assuming that all glucose carbon in glycine was transferred *via* serine (or phosphoserine) our calculations indicate that about 30% of the glycine arose from serine. This figure represents a maximum value since other pathways exist for transfer of glucose carbon to glycine (*e.g.* CO_2 fixation).

Some of the isotope from glucose appeared as carbonate and earlier work has

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demonstrated that carbonate can be transferred to all of the amino acids[25]. It is of interest to distinguish between the glucose carbon transferred *via* plasma carbonate and that following a more direct pathway. The general method of this calculation has been described previously[26]; it requires the following data: (1) the fixed carbon level, (q), for each amino acid, (2) the mean specific activity of the plasma carbonate pool during the experimental period $\left(\int_{t_1}^{t_2} \varrho s \cdot dt\right)$, and (3) the total ^{14}C content in each amino acid $\left(\sum_{t_1}^{t_2} \lambda s \cdot \Delta t\right)$. The quotient: (1) $\times$ (2)/(3) represents the fractional part of glucose carbon transferred to the amino acid *via* plasma carbonate. This quotient, calculated for each amino acid, is listed in the second column of figures in Table III.

Only a small part of the glucose carbon that appeared in the non-essential amino acids was transferred *via* the plasma carbonate pool. Arginine was an exception to this generalization since almost 2/3 of the glucose carbon was transferred *via* the carbonate pool. This result probably reflects formation of arginine during urea synthesis by the Kreb's-Henseleit cycle. Degradation of arginine from the 3 hour casein sample gave results in line with this explanation in that 80% of the isotope was found in the C-6 (guanidyl) carbon atom.

The indirect pathway (*via* plasma carbonate) plays a more important role in the formation of the essential amino acids in which case it appears that 2/3 or more of the glucose carbon is transferred *via* the plasma carbonate pool. This greater importance of the indirect pathway corroborates our previous observation that carbonate was a better precursor of the essential amino acids than acetate, propionate or butyrate[3]. In the case of methionine the transfer of glucose carbon as C_1 units makes the indirect pathway relatively less important and accounts for the relatively low value of 26% *via* plasma carbonate observed for this essential amino acid.

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SUMMARY

A lactating dairy cow was injected intravenously with 2.9 millicuries of uniformly ^{14}C-labeled glucose. The specific activity was determined for plasma glucose and for amino acids of casein during the subsequent 34 hours and these data were used to evaluate glucose as a precursor of amino acids.

Glucose carbon was transferred most rapidly and in largest amount to the three carbon amino acids, alanine and serine. Our calculations indicate that 25% of alanine and serine, 10% of aspartic and glutamic acids, and 7% of glycine was derived from plasma glucose. Proposed pathways for the transfer of carbon from glucose to the amino acids are discussed.

The amount of glucose carbon transferred to the amino acids *via* the plasma carbonate pool was evaluated. This indirect pathway, *via* carbonate, was of minor importance for the non-essential amino acids but was the major pathway for transfer of carbon to the essential amino acids.

RÉSUMÉ

Nous avons étudié le rôle du glucose du plasma dans la synthèse des acides aminés de la caséine grâce à une injection intraveineuse à une vache normale en lactation, de glucose uniformément marqué par ^{14}C.

La caséine fut extraite des laits obtenus 3, 10, 22, et 34 heures après l'injection. Les acides aminés furent ensuite isolés de ces différents échantillons de caséine.

L'activité spécifique du ^{14}C de ces acides aminés comparée à l'activité correspondante du glucose du plasma indiquait que la plupart du carbone transféré du glucose à la caséine se trouvait dans

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l'alanine et la sérine. Un quart du carbone de ces deux acides aminés provenait du glucose du plasma et ce glucose était également la source de 10% du carbone dans l'acide aspartique et de 7% du carbone dans la glycine.

Nous discutons des voies de transfert suivies par le carbone en possant du glucose plasmatique aux acides aminés.

Nous avons calculé qu'une fraction très petite du carbone transféré du glucose aux acides aminés non essentiels passait par le CO_2 plasmatique, et que, par contre, la plupart de ce transport du carbone aux acides aminés essentiels passait par le "pool" du carbonate plasmatique.

ZUSAMMENFASSUNG

Glukose, gleichmässig mit ^{14}C signiert, wurde in die Blutbahn einer Kuh injiziert um die Rolle der Plasmaglukose für die Synthese der Aminosäuren im Kasein zu studieren.

Aus 4 Milchproben, gewonnen während einer Periode von 34 Stunden nach der Injektion, wurde das Kasein präpariert und daraus wurden die Aminosäuren isoliert.

Die spezifische ^{14}C-Aktivität in diesen Aminosäuren wurde verglichen mit der zugehörigen spezifischen Aktivität in der Plasmaglukose. Aus diesem Vergleich konnten wir folgern, dass der Hauptteil des Kohlenstoffs, der aus der Plasmaglukose ins Kasein übergeht, in Alanin und Serin erscheint. 25% des Kohlenstoffs in diesen beiden Aminosäuren stammen aus der Plasmaglukose, die ausserdem 10% des Kohlenstoffs der Asparaginsäure und 7% des Kohlenstoffs des Glykokolls liefert.

Hypothesen über die Wege des Kohlenstofftransports aus der Glukose in die Aminosäuren werden erörtert.

Wir haben berechnet welcher Anteil dieses Kohlenstofftransportes *via* Plasmakohlensäure geht. Der Weg über Kohlensäure war unbedeutend für diejenigen Aminosäuren, welche in den tierischen Geweben aufgebaut werden; aber der Hauptteil des Kohlenstoffs, der von der Plasmaglukose die andern, essentiellen, Aminosäuren erreicht geht über Kohlensäure.

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