GLUCOSE METABOLISM IN THE LACTATING DAIRY COW*

by

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Mature ruminants differ from non-ruminants in the pathway by which they acquire blood glucose and the level at which this metabolite is maintained in the blood. No appreciable quantities of glucose are assimilated by the ruminant through the gastro-intestinal tract[1,2] as the carbohydrates in the diet are rapidly broken down by the micro-organisms of the rumen to volatile fatty acids and absorbed as such into the blood-stream[3,4]. The role of primary blood glucose precursor in the ruminant has been ascribed to propionate which is known to be converted to glucose by the liver[5,6].

The low blood sugar levels in the cow, compared with the higher levels in dogs, rats and man[7,8] might therefore be due to the cow's peculiar digestive system or may reflect the lesser quantitative importance of glucose as a metabolite in her body. This latter view is supported by the observation that glucose may be partially replaced by acetate for energy metabolism in the ruminant[9,10]. Other comparative studies have shown that mammary gland slices of non-ruminants use glucose preferentially as a substrate for the synthesis of fatty acids; ruminant slices, however, utilize acetate more readily for the same synthesis[11,12].

We have studied in some detail, the quantitative aspects of carbohydrate metabolism in the cow's body, her body glucose pool and the relative importance of glucose for milk synthesis, energy metabolism and interconversion into other metabolites. The data of this paper show that per kg of body weight, the cow possesses a smaller glucose pool than non-ruminants and that of the available glucose in her body, a smaller fraction is used for energy metabolism than has been reported for the rat [13] or dog[14].

METHODS

A lactating Jersey dairy cow, 6 years old, weighing 457 kg, and producing 10 kg of milk daily, was injected with 2.9 mc of glucose uniformly labeled with ^{14}C (glucose U-^{14}C) prepared by photosynthetic techniques from $^{14}CO_2$[15]. The method of injecting the tracer into the left jugular vein through plastic tubing, has been described previously[16]. The measurement of the ^{14}C activity in the cow's respired air has also been described[17].

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During the course of the two day trial, eleven 100–125 ml samples of blood were withdrawn through plastic tubing. The tubing was inserted into the right jugular vein in advance of the trial. The blood samples represent less than 5 % of the cow's total blood volume. Blood and plasma glucose levels were determined by the method of FOLIN AND MALMROS[18]. The plasma volume was measured with Evans Blue[19] and a reading for the hematocrit value obtained after the blood was spun in a centrifuge at 2000 *g* for 30 minutes.

Plasma glucose was isolated as the phenylglucosazone. The method used was that described by SEARLE AND CHAIKOFF[20] with the following modifications: (1) After deionization and concentration of the glucose-containing solution the phenylglucosazones were prepared by adding 0.1 ml of liquid phenylhydrazine and 0.15 ml of glacial acetic acid to every 10 mg of glucose (estimated) in solution. The highest yields of osazones are reported as occurring under these conditions[21]. After washing with water and ether (2) the phenylglucosazones were redissolved in absolute ethyl alcohol and separated out by the addition of 4 volumes of water.

Decomposition point, carbon, hydrogen, and nitrogen analyses as well as microscopic appearance indicated an almost pure product. The method of phenylglucosazone preparation was tested by preparing a sample of the osazone from glucose U-^{14}C of known specific activity. The calculated specific activity from the phenylglucosazone fell only 2 % below the known specific activity of the starting material.

The milk samples were collected, fractionated and counted as described before[22]. All glucosazone samples were converted to CO_2 by dry combustion in a Sargent micro-combustion apparatus. The CO_2 was collected in NaOH and precipitated with $BaCl_2$ as previously described[17]. The $BaCO_3$ planchets were counted in a flowgas-type Geiger-Mueller tube. Samples were counted for periods of sufficient duration, to reduce the standard error of counting to less than 1 %.

RESULTS AND DISCUSSION

Rate components of plasma glucose curve. Following the intravenous injection of glucose U-^{14}C, the decreasing specific activity of the plasma glucose was measured over a period of 36 hours. The curve π in Fig. 1. represents these results as plotted on a semilog scale. If we assume that this plot is the resultant of first-order or quasi first-order reactions, the curve can be resolved into three straight line components π_a π_b and π_c, by the method of JONES[23]. Corresponding regression coefficients (k), half times ($t\frac{1}{2}$), and turnover times (t_t) may be calculated from the slope of these lines (Table I.).

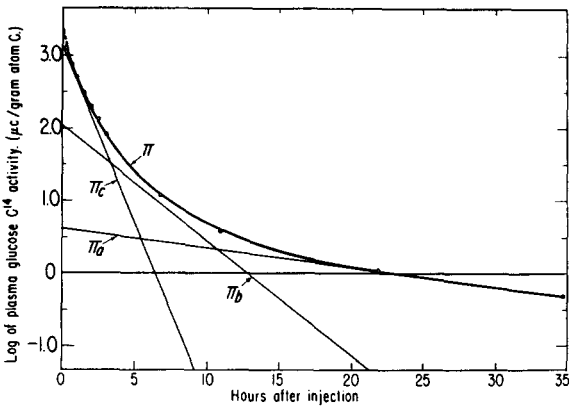

Fig. 1. Changes of the specific activity in the plasma glucose with time, after the intravenous injection of glucose U-^{14}C into cow. The semilog plot of the specific activity curve π is resolved into 3 first order components according to the method of JONES[23].

The fastest and best defined component of the curve π_c, is represented by six experimental points over a period from 30 to 180 minutes after the isotope injection. The best straight line through these points, determined by the method of least squares has a mean deviation of 1.3%. The first experimentally determined point (20 minutes) falls well above the line π_c. This may indicate that complete mixing of the labeled plasma glucose with the more extensive body glucose pool was not reached at 20 minutes after the injection of labeled glucose. A similar phenomenon has been reported in dogs[20].

The term "body glucose pool" is used in this paper as defined by FELLER *et al.*[13]. Biokinetically such a pool may be defined as consisting of all organic material within

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TABLE I

AN ANALYSIS OF THE PLASMA GLUCOSE SPECIFIC ACTIVITY CURVE

Component of curve	Data from graph: Log $\frac{a_0}{a_t}$	Data from graph: t minutes	Regression coefficient k	Half time $t\frac{1}{2}$ minutes	Turnover time t_t: hours	Turnover time t_t: minutes
π_a	0.595	1350	0.001014	680	16	28
π_b	2.05	765	0.006163	112	2	42
π_c	3.140	385	0.0188	36.7		53.2

a_0 = specific activity of plasma glucose at time zero
a_t = specific activity of plasma glucose at time t

$$k = \text{Log}\frac{a_0}{a_t} \times \frac{2.3}{t},\quad t\tfrac{1}{2} = \frac{0.69}{k} \text{ and } t_t = 1/k$$

the cow, whose carbon atoms are in sufficiently rapid exchange with those of the plasma glucose that, after a short initial mixing period and subject to the limitations imposed by the accuracy of the measuring equipment, they appear to behave as a metabolic entity with the plasma glucose.

Body glucose pool and transfer rate. The slope of π_c represent the turnover rate of the body glucose pool. The apparent size of this pool, which is assumed to be constant, has been derived by extrapolating π_c to zero time and treating the data as a simple isotope dilution problem.

$$\text{Body glucose pool (in grams of glucose)} = \frac{\text{injected dose } (\mu c)}{\text{Specific activity at zero time (in } \mu c \text{ per gram glucose)}}.$$

Injected dose = 2870 μc. The specific activity at zero time has been calculated from Fig. 1 as being 1380 μc per gram atom C = 46 μc per gram of glucose. The body glucose pool therefore equals 2870/46 = 62.4 grams. The transfer rate of glucose out of this pool may be calculated from the relationship:

$$\text{Transfer rate } (r) = \frac{\text{Pool size in grams } (P)}{\text{turnover time in minutes } (t_t)} = 1.17 \text{ grams per min} = 70.4 \text{ grams}$$

per hour. It is possible, that the tracer was injected and mixed initially with a portion or compartment of the body glucose pool which is more immediately available for milk synthesis and oxidation, than the glucose of the body glucose pool as a whole. This would result in ^{14}C leaving the system for the first few minutes of the trial, at a rate which is greater than indicated by the slope of the extrapolated line for the body glucose pool (Fig. 1). Under such conditions the size of the body glucose pool and consequently the transfer rate would have been overestimated and the results above would represent maximum values.

The cow's plasma volume was 20.3 liters, her average blood hematocrit was 36% and the glucose levels in the whole blood and blood plasma amounted to 49 mg % and 60 mg % respectively. These results correspond to a blood glucose content of 15.5 grams, of which 12.2 grams are contained in the plasma. Plasma glucose thus appears to be in rapid exchange with carbon compounds forming a pool which represents four times as much glucose as is contained in the plasma. The ratio of the volumes of plasma to interstitial fluid has been given as 3:1[24] and 4.6:1[25]. Our result for the ratio of the cow's plasma glucose: body glucose pool (less plasma glucose) is 4.2:1. This result is consistent with the concept that the largest portion of the body glucose pool is located in the extracellular fluids of the body of the cow.

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Glucose as a contributor to energy metabolism. The distribution of the injected glucose U-^{14}C in the milk products has been reported[26]. After 48 hours virtually all of the ^{14}C had left the cow's body; 56.9% *via* the main organic milk constituents and 40.3% *via* the respired CO_2. Carbon-14 elimination *via* the urine, feces, rumen gases, citrate and other organic trace substances in milk, should account for most of the remaining 2.8% of the injected dose.

We have calculated from our results, the extent to which glucose contributed to the energy metabolism of the cow as a whole. The glucose oxidation quotient q_E expresses the amounts of carbon from glucose to total carbon in the respired CO_2 formed during a given time interval. The longer the time interval the smaller the error introduced by the delays involved in the removal of glucose carbon from the blood and its subsequent elimination as CO_2 in the respired air.

Algebraically q_E is derived in a manner similar to that used by KLEIBER for "q" the "carbonate fixation quotient"[27]. Assuming that no significant error is introduced by ignoring the very small amount of $^{14}CO_2$ fixed or eliminated by the cow *via* alternate pathways (*e.g.* milk, urine, rumen gases, *etc.*)[22], the ^{14}C appearing in the respired air should about equal the amount of $^{14}CO_2$ produced from glucose oxidation.

q_E may thus be written as g/m which $= \dfrac{\int_{t_0}^{t_x} \varrho \mathrm{d}t}{\int_{t_0}^{t_x} \pi \mathrm{d}t}$

where g = rate of glucose oxidation to CO_2 (moles of CO_2/hour)
m = mean overall CO_2 production (moles of CO_2/hour)
μ = specific activity of respired air (μc/gram atom C)
μ = specific activity of plasma glucose (μc/gram atom C)
t = time in hours

The integration of $\int_0^{35\,h} \varrho \mathrm{d}t$ is shown in Table II and that of $\int_0^{35\,h} \pi \mathrm{d}t$ in Table III. From 11 hours after the injection of glucose U-^{14}C, q_E attains a value which changes little with time. At 35 hours after the injection, when most of the injected ^{14}C had left

TABLE II

AN INTEGRATION OF THE SPECIFIC ACTIVITY OF THE RESPIRED $^{14}CO_2$ WITH TIME, AFTER THE INTRAVENOUS INJECTION OF GLUCOSE U-^{14}C INTO COW

Time from injection *Minutes*	*Average specific activity (ϱ) of respired CO_2 during time interval* *μc/gram atom C*	$\varrho \Delta t \times 10^{-3}$	$\int_0^t \varrho \mathrm{d}t \times 10^{-3}$
0			
	48.5	2.910	
60			2.910
	41.6	2.496	
120			5.406
	27.9	1.674	
180			7.080
	14.6	3.504	
420			10.584
	3.3	0.841	
675			11.425
	1.1	0.709	
1320			12.134
	0.4	0.294	
2055			12.430

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TABLE III

AN INTEGRATION OF THE SPECIFIC ACTIVITY OF PLASMA GLUCOSE WITH TIME AFTER THE INTRAVENOUS INJECTION OF GLUCOSE U-^{14}C INTO COW

Time from injection	*Average specific activity (π) of plasma glucose during time interval*		$\pi \Delta t$		$\int_0^t \pi dt$	
Minutes	*μc gram atom C*					
(1)	(2a)	(2b)	(3a)	(3b)	(4a)*	(4b)**
0						
	1618*	3250**	32.36	65.0	32.36	65.0
20						
	1007.2		20.11		58.47	85.14
40						
	639.7		12.79		65.26	97.93
60						
	401.7		12.05		77.31	109.98
90						
	249.1		7.72		85.03	117.71
121						
	166.8		5.34		90.37	123.04
153						
	107.5		3.23		93.60	126.27
183						
	46.5		10.42		104.07	136.69
407						
	7.9		1.98		106.00	138.66
657						
	2.5		1.63		107.63	140.29
1309						
	0.76		0.58		108.20	140.86
2073						

* The zero time specific activity of plasma glucose and the average level of glucose U-^{14}C in the plasma for the first 20 minutes of the trial is based upon the assumption that equilibration of the plasma glucose with total glucose in the extracellular fluid of the cow, proceeds at a rate, similar to that involved in the complete mixing of the tracer in the plasma pool. The value used here for π_0, is the extrapolated value of π_c at zero time. (See Fig. 1.)

** The zero time specific activity and average level for the first 20 minutes in the plasma glucose, is based upon the assumption that equilibration of plasma glucose with glucose in the interstitial spaces is *slow* compared to the mixing rate of glucose in the plasma pool. The value used here for π_c is based upon the injected dose, diluted by the plasma glucose pool, whose size is known.

the cow's body, q_E (as formulated above) was found to be within the limits* of 0.088 and 0.115. This means that only 8.8 to 11.5% of the total CO_2 in the cow's respired air originated from glucose oxidation. (The cow's respiration rate was 123 liters CO_2 per hour, measured at S.T.P.) Thus the cow depended upon body glucose for only about 10% of her energy metabolism.

Comparative data. It is of physiological interest to compare the relative size and performance of the body glucose pool of the cow, with corresponding data in non-ruminants. Table IV compares our results for the lactating cow with similar data obtained by FELLER *et al.*[13,14] for normal non-lactating dogs and rats. In evaluating these comparative data, it should be noted that the dogs[14] unlike our cow, were in a post-absorptive nutritional state at the time of experimentation. With reference to the rat

* The limits represent the difference of two extreme assumptions in calculating the average specific activity of plasma glucose for the first 20 minutes after the intravenous injection of glucose U-^{14}C. These assumptions are specified in the footnote of Table III.

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TABLE IV

A COMPARISON OF THE BODY GLUCOSE POOL CHARACTERISTICS IN RATS, DOGS AND THE COW

Animal	*Body weight* W	*Body glucose pool* P	*Relative pool size* P/W	*Turnover time* t_t	*Transfer rate* r	*Relative transfer rate* $r/W^{3/4}$ *	*Body glucose oxidized to* CO_2	*Respired* CO_2 *from glucose oxidation*
	kg	*grams*	–	*hours*	*grams/hour*	—	*per cent*	*per cent*
Rats[13]	0.205	0.26	1.27	1.23	0.212	0.7	67[a]	45
Dog A[b, c]	6.85	2.95	0.43	1.35	2.3	0.5	93	60
Dog B[b 14]	6.5	4.3	0.66	1.7	2.5	0.6	78	53
Cow[d]	457.2	62.4	0.136	0.89	70.4	0.7	40.3	8.8 to 11.5

[a] This result is in general agreement with results obtained by several other workers making similar trials with rats[29,30].

[b] Post absorptive state. The results are in overall agreement with data from a similar trial reported by another worker[20].

[c] Average of two trials.

[d] Lactating.

* $W^{3/4}$ is an expression of the Metabolic Body Size[32].

data[13] it should be noted that FELLER's mathematical treatment of the results is open to criticism[28]. However, several of the values which FELLER *et al.* obtained for normal rats, have been substantially confirmed by other workers[29,30]. Subject to the afore mentioned limitations, a comparison of the data for dogs, rats, and cow, and certain conclusions on the basis of these comparisons appear feasible.

As the data in Table IV demonstrate, the body glucose pool per kg of body weight is lower in the lactating cow than in non-lactating dogs and rats. However, the transfer rate of glucose (in grams per hour) out of the body pool, expressed as a function of metabolic body size ($W^3/_4$)[32], is almost the same for the three species. One explanation for this similarity may be found in the differing experimental conditions under which each species was tested. Thus in our cow, lactation may have been accompanied by an increase transfer rate of glucose, in order to meet the greater demand for blood sugar in milk synthesis (especially lactose). Starvation on the other hand, may have depressed the relative transfer rate of glucose in the bodies of the fasted dogs.

The differences in the utilization of glucose for energy metabolism by the three species are striking. While in the rats and dogs 45% or more of the respired CO_2 originated from glucose, only about 10% of the CO_2 in the respired air of the cow originated from this source. Since 40 %of the glucose was converted to CO_2, *even if all of the glucose* which passed out of the cow's body glucose pool *had been oxidized to* CO_2, *no more than 25% of the cow's respired* CO_2 *could have originated from glucose oxidation.* Lactation *per se* cannot, therefore, be responsible for the differences observed in Table IV between the cow on the one hand and rats and dogs on the other. Our results, as well as results obtained from experiments with tissue slices[11,12] support GARNER's recent statement[31] that the low blood sugar levels found in ruminants were accompanied by a reduced ability of ruminant tissue to utilize glucose.

Our data confirm the belief that in the energy metabolism of the cow glucose metabolism is of secondary importance and that some other substance, presumably acetate[10] plays a more important role.

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SUMMARY

1. A lactating Jersey dairy cow was injected intravenously with glucose uniformly labeled with ^{14}C. The rate at which the injected tracer left the blood stream and the amount and speed with which it appeared in the milk and expired air was measured.

2. Our results indicate that in the cow, the metabolic body glucose pool (62 g or less) was about five times as large as the plasma glucose content (12 g).

3. The turnover time for the body glucose pool was less than one hour and the transfer rate of glucose out of this pool greater than 1 g per minute.

4. The lactating cow derived only about 10% of her exhaled CO_2 from pathways involving the oxidation of body glucose to CO_2.

5. In the energy metabolism of the lactating cow, glucose is relatively less important than it is in the energy metabolism of dogs and rats.

RÉSUMÉ

1. Du glucose uniformément marqué par ^{14}C a été injecté par voie intraveineuse à une vache laitière Jersey en lactation. La vitesse à laquelle le traceur injecté quitte la circulation sanguine et la quantité et la vitesse avec laquelle il apparaît dans le lait et dans l'air expiré ont été déterminées.

2. Les résultats indiquent que chez la vache le pool du glucose métabolique du corps (62 g au plus) est environ cinq fois plus important que la teneur en glucose du plasma (12 g).

3. Le temps nécessaire au renouvellement du pool du glucose du corps est inférieur à une heure et la vitesse de transfert du glucose à partir de ce pool est supérieure à 1 g par minute.

4. Chez la vache en lactation, 10% seulement environ du CO_2 expiré dérive de mécanismes mettant en jeu l'oxydation du glucose du corps en CO_2.

5. Dans le métabolisme énergétique de la vache en lactation, le glucose est relativement moins important qu'il ne l'est dans celui du chien ou du rat.

ZUSAMMENFASSUNG

1. Einer säugenden Jersey-Milchkuh wurde intravenös gleichmässig mit ^{14}C markierte Glukose verabreicht. Die Geschwindigkeit, mit welcher die injizierte markierte Substanz den Blutkreislauf verliess, sowie die Menge und Geschwindigkeit der in der Milch und in der ausgeatmeten Luft auftretenden Radioaktivität wurden gemessen.

2. Unsere Ergebnisse zeigen, dass in der Kuh die metabolischen Gesamtglukosereserven (62 g oder weniger) ungefähr fünfmal grösser sind, als die Plasmaglukose (12 g).

3. Die Umsatzzeit für die Glukosereserven des Körpers war kleiner als eine Stunde, und die Übertragungsgeschwindigkeit der Glukose aus diesem Pool grösser als ein Gramm pro Minute.

4. Die säugende Kuh bezog nur ungefähr 10% des ausgeatmeten CO_2 von metabolischen Bahnen, welche Oxydation von Glukose zu CO_2 mit sich bringen.

5. Im Energiemetabolismus der säugenden Kuh ist Glukose verhältnismässig unwichtiger als im Energiemetabolismus der Hunde und Ratten.

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