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Utilization of L-glucose and EDTA-chelates as tracers of the distribution volume of glucose in ewes

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SUMMARY - The present experiment was aimed to verify if Cr-EDTA and Co-EDTA are useful as tracers of the glucose distribution volume (GDV) in ewes. Ewes (n=3) fitted with indwelling jugular cannulas and urinary catheters were injected with a single bolus of L-^{3}H-glucose, known to be an excellent tracer of GDV, and doses of Cr- and Co-EDTA. The curves of plasma clearance from tracers, and of urine excretion of tracers were analysed. No difference was found in the rate of plasma clearance from L-^{3}H-glucose, compared with the chelates, and between the doses and asymptotic values calculated by exponential analysis of plasma clearance and urinary excretion. However, GDV, measured by using L-^{3}H-glucose as tracer, tended to be greater ($p<0.12$) than measured by using the EDTA-chelates as tracers. Cr- and Co-EDTA have potential to be useful as tracers of GDV, but additional work with greater numbers of sheep should be carried out before the method is routinely used.

Key words: L-glucose, EDTA-chelates, tracers, glucose distribution volume, ewes.

RESUME - *"Utilisation du L-glucose et des EDTA-chélates comme traceurs du pool de distribution du glucose chez les brebis". L'objectif de cette expérience était de vérifier si les Cr-EDTA et Co-EDTA sont utiles en tant que traceurs du pool de distribution du glucose (GDV) chez les brebis. Les brebis (n=3) munies de canules jugulaires incorporées et de cathéters urinaires, ont reçu une injection d'un bol unique de L-^{3}H-glucose, connu pour être un excellent traceur de GDV, ainsi que des doses de Cr-EDTA et Co-EDTA. On a analysé les courbes "plasma clearance" à partir des traceurs, et de l'excrétion urinaire des traceurs. Aucune différence n'a été trouvée dans le taux de "plasma clearance" à partir du L-^{3}H-glucose, comparé avec les chélates, et entre les doses et les valeurs asymptotiques calculées par une analyse exponentielle du "plasma clearance" et de l'excrétion dans l'urine. Cependant, le GDV, mesuré en utilisant le L-^{3}H-glucose comme traceur, tendait à être plus élevé ($p<0,12$) que lorsque mesuré en utilisant les EDTA-chélates comme traceurs. Les Cr-EDTA et Co-EDTA présentent un certain potentiel d'utilité comme traceurs du GDV, mais des travaux additionnels avec un plus grand nombre d'ovins doivent encore être menés avant d'utiliser la méthode en routine.*

Mots-clés : *L-glucose, EDTA-chélates, traceurs, pool de distribution du glucose, brebis.*

Introduction

In farm situation, the glucose metabolism of animals is not in steady state. Situations of heat or cold stress, feeding and milking impair the steady state condition. Quantifying how an animal changes from one state to another would allow research on metabolism to be more relevant to the problems that are to be solved. The present study was carried out in the frame of a programme aimed at verifying the accuracy of nonsteady state equations for the kinetics of glucose. The kinetics of glucose metabolism can be measured by dilution of radioactive isotopes of glucose administered by single bolus injection (Shipley and Clark, 1972) or primed infusion (for example, Veenhuisen *et al.,* 1987). In the first procedure, the calculation of glucose distribution volume in the body (GDV), identical to extracellular body water, an important step in the quantification of the rates of glucose entry and irreversible loss, is evaluated by extrapolation to t_0 of the dilution curve. The second procedure does not allow its calculation and it assumes GDV constant in all animals (approx. 20% of body volume, Judson and Leng, 1972). In some studies on non-steady state methodologies, glucose distribution

volume was estimated in steady-state and used as a constant in non steady state (Cobelli and Bergman, 1981).

Previous studies have shown that the GDV is not constant in situations of non steady state. It is affected by feeding and thermal stress (Christopherson and Webster, 1972), by some dietary characteristics (Landau *et al.,* 1992) and by ketosis (Kronfeld, 1977). L-glucose is an ideal distribution volume marker for D-glucose, differing from tracer only in stereochemistry. It does not cross cell membranes (Baur and Heldt, 1977) and is removed from plasma by glomerular filtration (Knight *et al.,* 1977). In non steady state studies where only one tracer of GDV is used, total urine collection is necessary, which is tedious and time consuming, and exposes animals to urinary infections, in the case of ewes. Also, if L-^{3}H-glucose is used, no use can be made of D-glucose radioisotopes labelled with ^{3}H, which prevents calculation of glucose recycling (Russell and Young, 1990).

The present study was aimed at assessing the value of chelates of EDTA as tracers of GDV instead of L-^{3}H-glucose, because EDTA-chelates are thought not to cross cell membranes and to be removed from plasma by glomerular filtration.

Materials and methods

Three yearling ewe-lambs fitted with bilateral indwelling jugular cannulas, one for injection and one for sampling, were injected I.V. with 50μCi of L-^{3}H-glucose, and 13.5 mg and 9.8 mg of Cr and Co from EDTA chelates. Cr-EDTA was prepared according to Binnerts *et al.* (1982) and Co-EDTA, as described by Uden *et al.* (1980). Blood was sampled 26 times for 6, i.e., at 5 minutes intervals, during the first 30 minutes, followed by gradually increased intervals of 15 and 30 minutes. Blood was immediately centrifuged and plasma was frozen to -20ºC until thawed for analysis. Urine was recovered at 15 minutes intervals throughout the experiment, synchronously with blood, using a urinary bladder Foley catheter. Blood protein was removed from plasma using an ultrafiltration membrane YMT for micropartition system 1 (Amicon, Danvers, MA). Analyses of Co and Cr in filtrated plasma were carried out, using a Perkin-Elmer 500 graphite furnace, and of Co and Cr in urine and injected solutions, using a Perkin-Elmer 5000 atomic absorption flame spectrophotometer. L-^{3}H-glucose in blood and urine (dpm) was determined using a Beckman LS 1800 liquid scintillation β counter. Amounts cleared from plasma excreted were compared with amounts appeared in urine, by fitting data *vs* time, using PROC NLIN of SAS (1985). Equations used for calculations are shown in Table 1.

Differences between the tracers were assessed by paired-T analysis (GLM, SAS, 1985).

Table 1. Equations used to evaluate the distribution volume of glucose

Tracer concentration in plasma (dpm or ng/ml):	$y(t)=\Sigma A_i e^{-ki\,t}$
Plasma clearance (dose/area, ml min^{-1})	$cl=Dose/\ {}_0\int^{\infty} y\,(t)\,dt$
Dose cleared (dpm or ng)	$C(t)=Ci * {}_0\int^{\infty} y\,(t)\,dt$
Fractional rate of clearance (min^{-1}) (-k of C(t) fitted to the exponential)	$C(t)= P(1- e^{-ki\,t})$
Cumulative recovery of tracer (dpm or ng)	$E(t)=A(1- e^{-ki\,t})$
Fractional rate of urinary appearance (min^{-1})	-k of E(t) equation
Distribution volume of glucose, or Extracellular Body Water (EBW, ml) calculated from y(t)	$EBW=(\Sigma(A_i/k_i^{2})*Dose)/(\Sigma(A_i/k_i)^{2}$

Results and discussion

The plasma concentrations of tracers are shown in Fig. 1 and their cumulative recovery in urine is shown in Fig. 2.

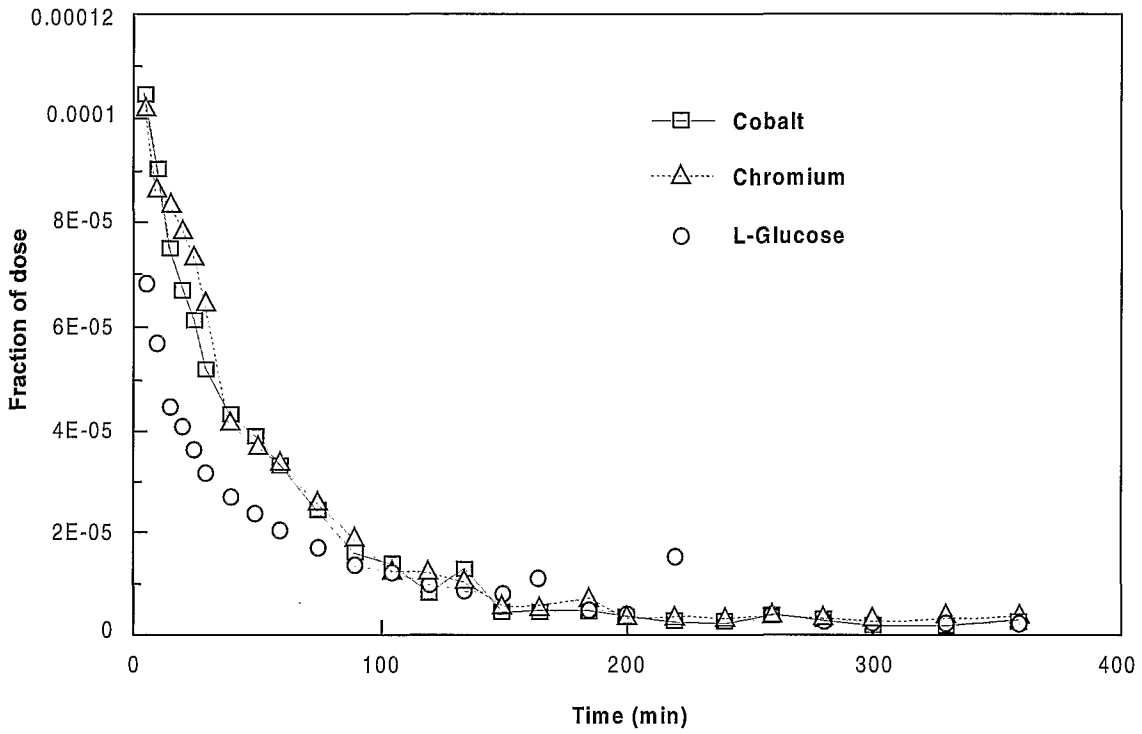

Fig. 1. Plasma concentration of tracers, expressed as fraction of the dose.

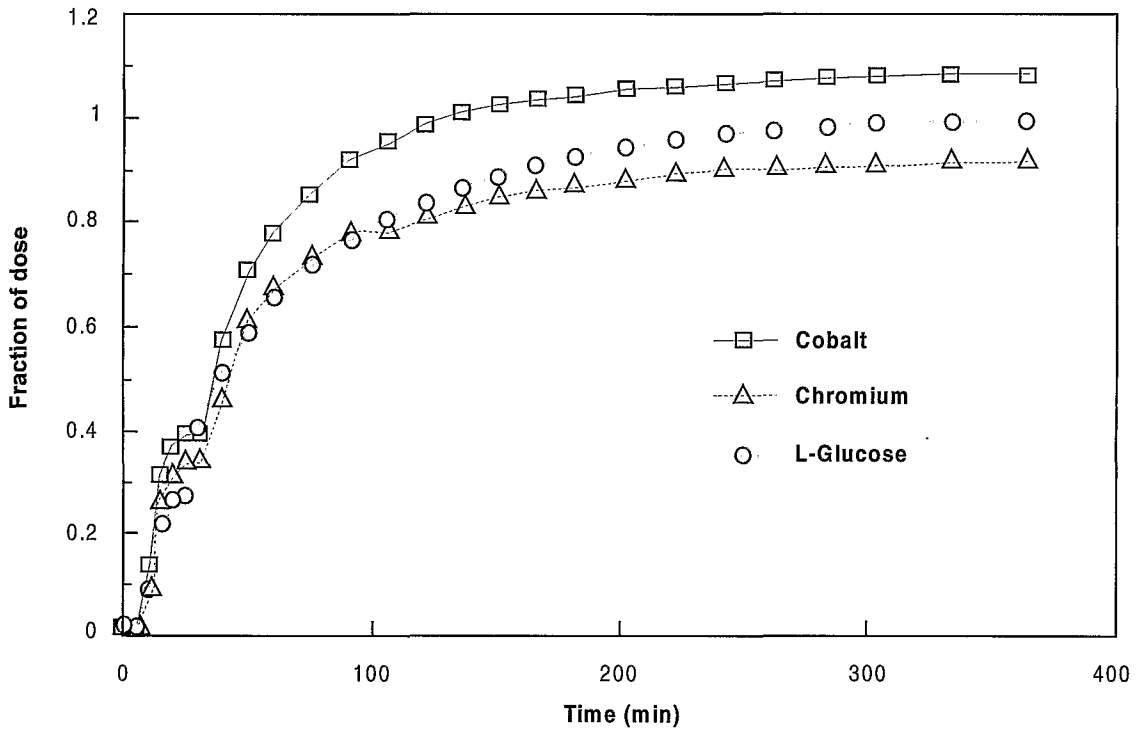

Fig. 2. Cumulative recovery of tracers in urine.

Calculated individual rates of clearance, and glucose distribution volume to BW are shown in Table 2. Individual comparisons of the kinetics of distribution of the extracellular tracers are shown in Table 3, and the statistical evaluation of the chelates as tracers of extracellular volume in ewes is shown in Table 4. A greater ($p<0.05$) percentage of Co than of Cr was excreted, L-^{3}H-glucose being intermediate (106.0, 87.9 and 94.8% of doses, respectively). No difference ($p<0.57$) was found in the rates of clearance from plasma of L-^{3}H-glucose and the chelates (113.8 *vs* 136.8 ml min^{-1}). No difference ($p=0.98$, P-A, Table 4) was found between the doses and the asymptotic values calculated by analysis of exponential curves of plasma clearance and urinary excretion. The slopes of plasma clearance and urinary excretion within individual ewe and tracer were identical, but tended to be greater when measured with L-^{3}H-glucose than with the EDTA chelates ($p=0.13$ and $p=0.21$ for clearance and excretion, respectively). The distribution volume tended to be greater when measured with L-^{3}H-glucose, compared with the Cr- and Co- chelates (28.3%, 20.3% and 22.3% of BW, respectively; $p<0.12$ for L-glucose *vs* chelates, $p=0.31$ for overall tracer effect). In a previous study in humans (Thomaseth *et al.*, 1993), plasma clearance of ^{51}Cr-EDTA after single bolus injection had been found to be identical to glomerular filtration rate (GFR), thus providing an attractive alternative to total urine collection following water loading. The method was found to be sensitive enough to monitor as small as 10% changes in GFR. Because the rates of clearance from plasma are similar to those of L-^{3}H-glucose within individual sheep, and because recovery in urine is almost total, EDTA-chelates seem to have potential as tracers of extra-cellular water in sheep. However, additional work has to insure that their tendency to distribute in a smaller volume than L-^{3}H-glucose, found in the present study, is an artifact caused by the small number of animals on experiment.

Table 2. Body weight (kg), rate of clearance from plasma (ml min^{-1}) and the distribution volume of tracers (l, and % BW)

Sheep	Body weight	Tracer	Rate of clearance	Glucose distribution volume (l)	Glucose distribution volume (% BW)
A	47.2	L-Glucose	111.1	16.41	34.7
A	47.2	Cr-EDTA	183.2	11.55	24.5
A	47.2	Co-EDTA	110.8	13.19	27.9
B	47.2	L-Glucose	126.9	13.32	28.2
B	47.2	Cr-EDTA	242.7	10.54	22.3
B	47.2	Co-EDTA	110.9	9.22	19.5
C	49.9	L-Glucose	103.1	10.53	21.1
C	49.9	Cr-EDTA	65.2	7.05	14.1
C	49.9	Co-EDTA	108.1	9.88	19.4

Table 3. Comparison of the kinetics of the distribution of the extracellular tracers

Sheep	Tracer	Dose (dpm or ng)	Fractional rate constants (min^{-1})		Accounted for (%)
			Plasma disappearance	Urine appearance	
A	L-glucose	105,531,605	0.01265	0.01446	99.0
A	Cr-EDTA	13,600	0.01935	0.02010	93.9
A	Co-EDTA	10,048	0.01899	0.01978	105.4
B	L-glucose	106,820,326	0.01223	0.01574	95.2
B	Cr-EDTA	13,910	0.02301	0.01765	93.6
B	Co-EDTA	9,702	0.01584	0.01772	108.4
C	L-glucose	113,179,973	0.01182	0.01150	90.2
C	Cr-EDTA	13,807	0.00924	0.01382	76.3
C	Co-EDTA	10,276	0.01583	0.01193	106.0

Table 4. Summary of statistical evaluation of L-glucose *vs* EDTA-chelates as tracers of extracellular volume in sheep. P-A is the difference between the doses and the asymptotic values calculated by analysis of exponential curves of plasma clearance and urine excretion

	Distribution volume (% BW)	Fractional rate of plasma clearance (k_c, min^{-1})	Fractional rate of urinary excretion (k_e, min^{-1})	(P-A)/dose
L-glucose	28.0	0.01223	0.00979	0.035
EDTA-chelates	21.3	0.01704	0.01393	0.031
p	0.12	0.13	0.21	0.98

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