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Serum clearance and urinary excretion of pteroylmonoglutamic acid in gestating and lactating dairy cows

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The present experiment was undertaken to verify if the demand of tissues for pteroylglutamic (folic) acid, evaluated by serum clearance and urinary excretion of folates, is different between multiparous dairy cows in late gestation (five gestating dry cows, 52·6 (SD 8·4) d before parturition), and in early lactation (four lactating non-gestating cows, 18·0 (SD 5·9) d after parturition). On day 1 the cows received one intravenous (i.v.) injection of 50 μg pteroylmonoglutamic acid/kg body weight (BW). Blood samples were taken at 0, 5, 15, 30 min, 1, 2, 4, 8 and 24 h after the i.v. injection. On day 3 the cows received one intramuscular (i.m.) injection of 0·3 mg pteroylmonoglutamic acid/kg BW. Blood and urine samples, as well as urine volume, were taken at 0, 1, 2, 4, 8, 12, 24, 36 and 48 h after the i.m. injection. On days 5, 6 and 7 a daily i.m. injection of 0·5 mg pteroylmonoglutamic acid/kg BW was given in an attempt to saturate tissues with folates. Then the cows received one i.v. (day 8) and one i.m. (day 10) injection of pteroylmonoglutamic acid according to the same procedures described previously for days 1 and 3. On day 12 plasma volume was determined. Before tissue saturation, serum clearance of folates during the 24 h following an i.v. injection was similar for gestating and lactating cows but after tissue saturation serum clearance was slower for lactating than gestating cows (stage × saturation, $P = 0·04$). The percentage of folates excreted in urine was not affected by the physiological stage ($P \geqslant 0·6$) or the level of tissue saturation ($P \geqslant 0·5$). In conclusion, serum clearance and urinary excretion of pteroylmonoglutamic acid seem to support the hypothesis that, in multiparous cows, although there are no deficiency symptoms, tissue demand for folic acid is high, especially during gestation.

Pteroylmonoglutamic acid: Dairy cows: Gestation: Lactation

In the rat, maternal and fetal utilization as well as fetal storage of folates increase dramatically the demand for pteroylglutamic (folic) acid during gestation (Potier de Courcy & Terroine, 1979; McNulty *et al.* 1993). In the human too, the demand for folic acid is highly increased during gestation and lactation (Bailey, 1990).

The ruminant animal is generally considered to be independent of an exogenous supply of folates; synthesis by rumen micro-organisms makes their inclusion in the diet unnecessary (Agricultural Research Council, 1980; National Research Council (NRC), 1988). Although minor changes in concentrations of folates in rumen contents are linked to the nature of the diet, they are not sufficient to modify the concentration of serum folates (Girard *et al.* 1994). However, in multiparous dairy cows total serum folates decrease by 40% from 2 months postpartum, around mating, to the next parturition, giving an indication that in dairy cows too the demand for folic acid could be increased during gestation (Girard *et al.* 1989). Moreover, long-term administration of a parenteral supplement of pteroylmonoglutamic acid increases milk production by 14% during the last half of the lactation curve and the proportion of milk protein by over 10% during the last half of the lactation curve and the first 6 weeks after parturition in multiparous cows

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(Girard *et al.* 1995). According to these results it seems that in multiparous dairy cows the requirements for folic acid are not completely met by the diet and synthesis by rumen microflora. Moreover, the demand for folic acid seems to be greater during gestation than lactation, although in multiparous cows these two physiological stages are concomitant most of the time.

In an attempt to determine if the tissue demand for folic acid is different between gestating and lactating dairy cows, serum clearance and urinary excretion of folates were studied in multiparous dairy cows during late gestation and early lactation.

MATERIALS AND METHODS

Animals

Nine multiparous cows of the Agriculture Canada Research Station dairy herd, five gestating dry cows approximately 2 months before calving and four lactating non-gestating cows during the first month postpartum, were used in this experiment (Table 1). The cows were kept in a tie-stall barn under 16 h of light (06.00 to 22.00 hours). Lactating cows were milked twice daily at 06.00 and 18.00 hours. They were fed on a total mixed ration prepared to meet NRC requirements (NRC, 1988) based on lactational performance of cows and physiological stage. Each cow also received 2 kg grass hay/d. The animals were cared for according to the recommended code of practice (Agriculture Canada, 1990).

Sampling and treatments

On day 0 each cow was implanted with a jugular catheter. The injections were always given after the morning milking and before feed distribution although Tremblay *et al.* (1991) found no nycterohemeral variation of serum concentrations of folates in gestating dry cows and a variation less than 1 ng/ml in lactating non-gestating cows. Amounts of pteroylmonoglutamic acid injected were calculated per kg body weight (BW), based on the initial weight taken 4 d before day 0. On day 1 the cows received one intravenous (i.v.) injection of 50 μg pteroylmonoglutamic acid (ICN Biochemicals, Cleveland, OH, USA)/kg BW, prepared as a solution of 10 mg/ml (pH 8·5). Blood samples were taken at 0, 5, 15, 30 min, 1, 2, 4, 8 and 24 h after the i.v. injection. On day 3 the cows received one intramuscular (i.m.) injection of 0·3 mg pteroylmonoglutamic acid/kg BW, prepared as a solution of 40 mg/ml. Blood samples were taken at 0, 1, 2, 4, 8, 12, 24, 36 and 48 h after the i.m. injection. Urine was collected with a rubber tube of 8 cm diameter kept close to the cow's vulva by four light chains tied to two pieces of rubber grid glued under the vulva and on the rump. The upper part of the rubber tube was fixed in place under the pressure of a small piece of flexible metal to avoid faeces contamination. Urine was collected in a stainless steel container. Total volume of urine was measured and samples were collected at the same times as blood samples.

On days 5, 6 and 7 a daily i.m. injection of 0·5 mg pteroylmonoglutamic acid/kg BW was given in an attempt to produce tissue saturation in folates. After these 3 d the cows received one i.v. (day 8) and one i.m. (day 10) injection of pteroylmonoglutamic acid according to the same procedures as described previously for days 1 and 3.

On the last day of sampling, plasma volume was determined according to the procedure described by Girard *et al.* (1989); packed cell volume was also measured.

Folate determination

The procedure used for serum storage and folate determination was as described by Girard *et al.* (1989). In the present experiment, inter-assay coefficient of variation for serum folates was 4·2 (SD 2·9)%.

Table 1. *Body weight, time before or after parturition and parity number of dairy cows used in the experiment*

Cow no.	Parity number	Initial weight (kg)	Time before (−) or after (+) parturition (d)
Gestating cows			
1	3	512	−58
2	3	590	−58
3	3	672	−53
4	3	825	−38
5	2	596	−56
Mean	2·8	639	−53
SD	0·4	118	8·4
Lactating cows			
6	3	549	+25
8	3	559	+11
9	3	513	+20
10	3	607	+16
Mean	3	557	+18
SD	0	31	5·9

At sampling time, urine was placed in tubes with ascorbic acid (0·05 ml of a solution of 20 g ascorbic acid/l per ml urine) and kept frozen at −20°. Urine samples were diluted with a known quantity of saline (9 g NaCl/l); this quantity varied according to the folate concentration of the sample. A further dilution 1:1 with bovine serum albumin (70 g/l) was carried out and urinary folates (200 μl of this last solution) were measured in duplicate by radioassay with a commercial kit used for human serum (Quantaphase Folate; Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON, Canada). Recovery of pteroylmonoglutamic acid (ICN Biochemicals) added to cow urine was 104·1 (SEM 1·4)% (*n* 42). The inter-assay coefficient of variation was 4·0 (SD 2·8)%.

Statistical analysis

A change-over design was used in the present experiment to minimize the number of animals involved while maintaining reasonable sensitivity of the experiment. Moreover, this design is particularly effective in reducing experimental error if the major source of random variation is the subjects themselves (Gill, 1978). Data from one lactating cow were removed from the study due to health problems. Responses of serum folates to the i.v. injection of pteroylmonoglutamic acid were measured by the area under the curve calculated using the trapezoidal summation method after excluding the area under the basal level before injection (Abramovitz & Slegun, 1972). All the variables, areas under the curve of serum folates after i.v. injection of pteroylmonoglutamic acid as well as following i.m. injection, total serum folates and cumulative quantity of urinary folates reported for each sampling time as a percentage of the quantity injected, were analysed as a split-plot design with the physiological stage (dry gestating cows *v.* lactating non-gestating cows) as the main factor and the level of tissue saturation (before or after the daily administration of pteroylmonoglutamic acid) as the second factor. Analysis according to a split-plot design was necessary because the second factor, level of tissue saturation, was not randomized (Gill, 1978). The following model was used: $Y_{ijk} = \mu + P_i + C_j(P_i) + S_k + PS_{ik} + \epsilon_{ijk}$, where Y_{ijk} indicates the dependent variables, μ is the overall mean, P_i is the physiological stage, S_k is the level of tissue saturation in pteroylmonoglutamic acid, $C_j(P_i)$, cow inside physiological stage, is the error a and ϵ_{ijk} is the residual error. A protected least significant difference (LSD) was used to test for significant differences among means when the interaction PS_{ik} reached a level of significance of 95% (Snedecor & Cochran, 1971).

RESULTS AND DISCUSSION

Plasma volume and packed cell volume

Mean plasma volume and packed cell volume were 30·7 (SD 5·5) litres and 0·31 (SD 0·02) respectively for gestating cows and 35·1 (SD 5·6) litres and 0·29 (SD 0·01) respectively for lactating cows.

These measurements were critical for a reliable interpretation of the results in the present experiment. For example, before the tissue saturation, 1 h after the i.m. injection of pteroylmonoglutamic acid, serum concentration of folates of lactating cows (461·3 (SD 144·4) ng/ml) was lower than that of gestating cows (528·8 (SD 30·9) ng/ml). However, when changes in blood and plasma volumes induced by gestation and lactation were taken into account, corresponding total serum folates were similar for the two physiological stages, 16·1 (SD 2·2) mg for gestating cows and 16·3 (SD 5·6) mg for lactating cows. Therefore, changes in serum concentration of folates must be evaluated with caution when comparing cows at different stages of gestation and lactation. Moreover, the analysis of the folate responses as total quantity rather than concentrations of folates could also explain the discrepancy between the present experiment and the results previously reported for dairy cows by Girard *et al.* (1989) in which i.m. injections of pteroylmonoglutamic acid increased serum concentrations of folates of gestating cows but failed to increase significantly those of lactating cows. Consequently, in the present experiment, serum folates are always reported as total serum folates rather than as concentrations.

Serum clearance of folates after one intravenous injection of pteroylmonoglutamic acid

Serum clearance rates of folates by gestating and lactating cows were similar before tissue saturation, but after tissue saturation serum clearance was slower for lactating than gestating cows (Table 2 and Fig. 1; interaction stage × saturation, $P = 0{\cdot}04$). In fact, in gestating cows, serum clearance was not modified by the saturation injections whereas in lactating cows it was slowed after saturation injections. Consequently, it seems that in lactating non-gestating cows one daily i.m. injection of 0·5 mg pteroylmonoglutamic acid/kg BW given during 3 d was sufficient to increase the level of tissue repletion in folates and to decrease the tissue demand for circulating folates. However, the same dose given to dry gestating cows was not sufficient to modify significantly either the tissue repletion or the tissue demand for circulating folates.

The response of serum folates to the saturation injections observed before the i.v. injection gives another indication that the saturation injections were not sufficient to increase tissue repletion in folates in gestating cows. Before the i.v. injections of pteroylmonoglutamic acid, total serum folates were, before and after saturation injections respectively, 0·49 (SD 0·24) and 0·58 (SD 0·12) mg for gestating cows and 0·50 (SD 0·15) and 0·88 (SD 0·14) mg for lactating cows. The response of total serum folates to the saturation injections was different according to the physiological stage (interaction stage × saturation, $P = 0{\cdot}04$). Before the saturation injections, total serum folates of gestating and lactating cows were similar but after the saturation injections, total serum folates were 56% higher in lactating than gestating cows. Total serum folates of lactating cows were increased by 76% after the saturation injections whereas those of gestating cows did not vary significantly.

Serum clearance of folates after one i.v. injection of pteroylmonoglutamic acid followed

Table 2. *Areas under the curve of serum folates (mg.h) of dairy cows after one i.v. injection of pteroylmonoglutamic acid according to the physiological stage and the level of tissue saturation in folates**

(Mean values and standard deviations)

	Gestating cows (n 5)		Lactating cows (n 4)	
	Mean	SD	Mean	SD
Before tissue saturation	21·1	5·3	23·5	4·3
After tissue saturation	24·7	7·5	31·2	4·8

* Interaction physiological stage × level of tissue saturation, $P = 0{\cdot}04$.

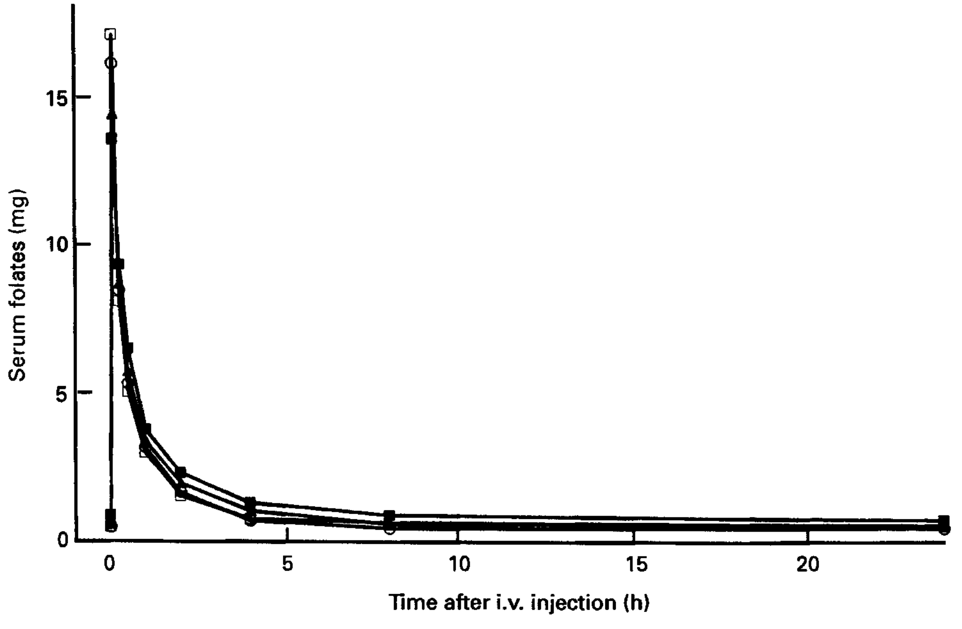

Fig. 1. Total quantity of serum folates during the 24 h following one i.v. injection of pteroylmonoglutamic acid to gestating dairy cows (n 5), before (—○—) and after (—▲—) tissue saturation and to lactating dairy cows (n 4), before (—□—) and after (—■—) tissue saturation with pteroylmonoglutamic acid. Values are arithmetic means. At 0, 5, 15 and 30 min and 1, 2, 4, 8 and 24 h after the i.v. injection, SD values were, for gestating cows, 0·2, 6·0, 2·6, 1·6, 0·9, 0·4, 0·2, 0·1 and 0·1 mg before tissue saturation and 0·1, 2·9, 2·2, 1·8, 0·9, 0·7, 0·4, 0·2 and 0·1 mg after tissue saturation and for lactating cows, 0·2, 3·4, 0·6, 0·5, 0·4, 0·3, 0·2, 0·1 and 0·3 mg before tissue saturation and 0·1, 2·0, 2·2, 1·4, 1·0, 0·6, 0·2, 0·2 and 0·1 mg after tissue saturation. For details of procedures, see pp. 858–859.

a pattern similar to that observed in humans where an initial rapid disappearance phase of 10–15 min was followed by a second slower phase of disappearance, injected pteroylmonoglutamic acid being completely eliminated from plasma 2 h after the injection (da Costa *et al.* 1979). However, Shojania & Hornady (1970) observed, in adult humans, the disappearance of 36·8% of the intravenously injected pteroylmonoglutamic acid 5 min after the injection. In the present experiment, 5 min after the i.v. injection of pteroylmonoglutamic acid, 37–50% of the dose of pteroylmonoglutamic acid injected had already disappeared from the serum. Surprisingly, 5 min after the i.v. injection of

Table 3. *Folates recovered (% of the injected dose) in the serum of dairy cows after one i.m. injection of pteroylmonoglutamic acid according to the physiological stage and the level of tissue saturation**

(Mean values and standard deviations)

Time after i.m. injection (h)	Gestating cows (n 5)				Lactating cows (n 4)			
	Before saturation		After saturation		Before saturation		After saturation	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1†	8·80	1·65	7·85	1·11	10·67	2·58	11·65	2·25
2	7·03	1·55	7·04	1·94	7·10	2·11	7·91	1·74
4	3·12	0·86	4·38	1·71	3·76	2·06	3·73	0·66
8	1·26	0·67	1·50	0·73	1·02	0·44	1·30	0·66
12	0·40	1·01	0·83	0·48	0·49	0·16	0·72	0·17
24†	0·23	0·04	0·33	0·08	0·44	0·12	0·44	0·06
36†	0·23	0·03	0·25	0·03	0·55	0·16	0·45	0·05
48†	0·31	0·20	0·30	0·04	0·46	0·12	0·53	0·08

* For details of procedures, see pp. 858–859.
† Effect of physiological stage, $P \leqslant 0{\cdot}04$.

pteroylmonoglutamic acid the highest values of serum folates were observed in cows before tissue saturation, whatever the physiological stage (Fig. 1; $P = 0{\cdot}06$); mean serum folates were 16·5 (SD 4·7) and 14·1 (SD 2·4) mg for cows before and after saturation injections. As previously observed by Girard *et al.* (1989), the extent of the immediate response seems to be inversely related to the level of serum folates before the injection. However, thereafter and until the end of the studied period the situation was reversed and the highest values of total serum folates were observed in cows after saturation injections. Whatever the physiological stage or the level of tissue saturation, 90–95% of the injected quantity of pteroylmonoglutamic acid had already disappeared from the serum 2 h after the i.v. injection and 98% had disappeared 24 h after the injection.

Serum clearance of total folates after one i.v. injection seems to indicate that tissues of lactating and gestating cows are not fully repleted in folates, the demand being greater for gestating cows. A more efficient incorporation of plasma folates by tissues would contribute to a greater body retention of folates and to a more rapid clearance of exogenous folates from the plasma in folate-deficient subjects after a load with pteroylmonoglutamic acid (Eisenga *et al.* 1992). However, it is not possible to rule out the possibility that the accelerated serum clearance in gestating cows could be related to an augmentation of urinary excretion of folates as observed in pregnant women (Landon & Hytten, 1971).

Urinary excretion of folates after one intramuscular injection of pteroylmonoglutamic acid

One hour after the i.m. injection of pteroylmonoglutamic acid, less folate was recovered in the serum of gestating than lactating cows; only 8·3 (SD 1·4)% of the amount of injected pteroylmonoglutamic acid was found in the serum of gestating cows compared with 11·2 (SD 2·3)% for lactating cows (Table 3, $P = 0{\cdot}04$). The percentage of folates appearing in serum after the injection of pteroylmonoglutamic acid was not modified by the saturation injections ($P = 0{\cdot}99$). The percentage of the injected dose recovered in serum did not vary from 24 to 48 h after the i.m. injection and it was very low, approximately 0·3% for gestating cows and 0·5% for lactating cows ($P \leqslant 0{\cdot}02$); there was no effect of the level of tissue saturation ($P > 0{\cdot}2$).

Table 4. *Folates recovered (% of the injected dose) in urine of dairy cows after one i.m. injection of pteroylmonoglutamic acid according to the physiological stage and the level of tissue saturation**

(Mean values and standard deviations)

	Gestating cows (*n* 5)				Lactating cows (*n* 4)			
	Before saturation		After saturation		Before saturation		After saturation	
Time after i.m. injection (h)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8	37·2	18·1	30·4	2·9	32·1	5·5	41·2	7·2
12	42·3	19·2	35·5	4·7	35·8	6·6	46·2	6·9
24	43·7	19·1	40·0	6·5	37·8	7·3	48·8	6·5
36	44·3	19·2	41·1	6·6	38·7	7·5	49·9	6·2
48	44·6	19·2	41·9	6·8	39·6	7·8	50·9	5·9

* For details of procedures, see pp. 858–859.

Recovery of injected pteroylmonoglutamic acid in urine during the first 4 h after the i.m. injection is not reported here because for some cows there was no urine collected during this period. The percentage of folates excreted in urine was not affected by the physiological stage ($P > 0{\cdot}6$) or the level of tissue saturation ($P > 0{\cdot}5$), the overall means being 35·1 (SD 10·6), 39·8 (SD 11·3), 42·5 (SD 11·4), 43·4 (SD 11·5) and 44·2 (SD 11·5)% at 8, 12, 24, 36 and 48 h after the i.m. injection (Table 4). A similar absence of effect of pteroylmonoglutamic acid administration on the urinary and faecal exogenous folate excretion during the 24 h post-injection was observed in rats fed on a diet normal or deficient in folic acid in spite of a more rapid plasma clearance of folates in deficient animals (Eisenga *et al.* 1992).

Urine is the major excretion route for folates after the administration of a supplement of pteroylmonoglutamic acid, faecal excretion accounting for only 20–30% of total excretion in an 8 d period (Bhandari & Gregory, 1992). For humans, catabolites of folates are not a major excretory product following folate supplementation (Kownacki-Brown *et al.* 1993). In the present experiment the percentage of folates excreted in urine was greater than those reported in humans after an oral dose of pteroylmonoglutamic acid where values varied from 6–7% of the daily ingested dose in 24 h (Gregory *et al.* 1990) to 25% during the first 8 h after ingestion of the supplement (Tamura & Stockstad, 1973), but it was similar to values of 31% of the injected dose observed 6 h after an i.m. injection (Shojania & Hornady, 1970). Therefore, in the present experiment, as urinary excretion was not modified by the physiological stage and the level of tissue saturation, it seems that the greater disappearance of serum folates in the gestating cows reflected an increased uptake by the tissues. This observation supports the hypothesis that in dairy cows, as in other species, the tissue demand for folates is increased during gestation. In the rat there is accumulation of folates in fetuses during the last half of the gestation, more than 40% of the ingested dose by the end of the gestation (Potier de Courcy & Terroine, 1979). In the sow, also, there are more folates in blastocysts at day 15 than day 12 of gestation (Matte *et al.* 1994). Moreover, catabolism of folates increases during gestation in rats and reflects folate requirement for DNA synthesis, both of fetal and placental tissues (McNulty *et al.* 1993).

Furthermore, results of the present experiment are in accordance with production data after long-term administration of a parenteral supplement of pteroylmonoglutamic acid to dairy cows from 45 d of gestation to 6 weeks after parturition, the supplement of pteroylmonoglutamic acid having more effect during the last half of the lactation curve when cows were also in gestation than after parturition (Girard *et al.* 1995). Supplementary pteroylmonoglutamic acid increases placental and colostral transfer of folates to the calf. It also increases milk production by 14% during the last half of the lactation curve and increases the percentage of protein in milk of multiparous cows during the last half of the lactation curve and the first 6 weeks after parturition.

In conclusion, serum clearance and urinary excretion of folates seem to support the hypothesis that in multiparous cows, although there are no deficiency symptoms, requirements of folic acid are not completely fulfilled by the diet and synthesis by rumen microflora, especially during gestation.

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