Endogenous purine and pyrimidine derivative excretion in pregnant sows

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The present experiment was carried out to study the endogenous losses of purine and pyrimidine derivatives from pregnant sows. Three pregnant and three non-pregnant Large White × Landrace sows were fed on a purine-free diet composed of starch, glucose, sucrose and vegetable oil, with casein as the protein source. The experiment began, for the six animals, after diagnosis of pregnancy and was divided into six 12 d periods. Urine was collected during the first 3 d of each experimental period by means of a urethral catheter for determination of allantoin, uric acid, xanthine, hypoxanthine and pseudouridine concentrations. In the absence of dietary nucleic acids (NA), allantoin and, as a consequence, excretion of total purine derivatives (PD) decreased significantly to a constant value (128·3 (SE 7·07) μmol/kg metabolic live weight ($W^{0.75}$) per d), an amount assumed to represent endogenous excretion. Excretion of uric acid (38·7 (SE 2·15) μmol/kg $W^{0.75}$ per d), hypoxanthine (21·0 (SE 2·58) μmol/kg $W^{0.75}$ per d) and xanthine (11·2 (SE 0·83) μmol/kg $W^{0.75}$ per d) were not affected by the experimental treatment, although there was a significant decrease in hypoxanthine excretion in pregnant sows (from 25·5 to 5·2 μmol/kg $W^{0.75}$ per d) compared with non-pregnant sows (from 26·7 to 44·8 μmol/kg $W^{0.75}$ per d). Creatinine excretion was not affected by pregnancy and was used as an internal urinary marker. Purine excretion, either expressed as μmol/kg $W^{0.75}$ per d or as the ratio PD:creatinine, was not affected by experimental treatment, although an apparent increase in pseudouridine excretion, a modified unsalvageable catabolite of RNA-pyrimidine, was found in late pregnancy (3·6 *v.* 5·2 mol/100 mol creatinine in non-pregnant sows compared with pregnant sows at 102 d collection).

Purine derivatives: Pseudouridine: Pregnancy: Sow

Metabolism of nucleic acids (NA) involves a substrate cycle whereby nucleotides are degraded continuously to nucleosides and free bases which are subsequently salvaged for re-synthesis of new nucleotides (Murray, 1971). However, this NA turnover is not fully efficient, generating, as a consequence, endogenous losses which constitute an important fraction of the total urinary excretion of purine derivatives (PD). These endogenous losses, measured as urinary PD excretion when there is no exogenous NA supply, have been measured in single-stomached animals fed on purine-free diets (Greife, 1980; Chen *et al.* 1990*b*), in preruminants maintained on a purine-free liquid diet (Lindberg, 1991) and in ruminants, either by avoiding rumen fermentation (Ørskov *et al.* 1979; Chen *et al.* 1990*b*) or by substituting duodenal flow for a purine-free solution (Balcells *et al.* 1991).

In previous reports, changes in nutritional status, i.e. protein (Fujihara *et al.* 1987) or energy supply (Giesecke *et al.* 1984; Lindberg & Jacobsson, 1990), had little effect on endogenous excretion of PD. However, there is a lack of information about the variations in endogenous losses in relation to different physiological states, and how these variations must be taken into account in order to establish the relationship between duodenal supply and urinary excretion of PD. On the other hand, pseudouridine is one of the most

important modified transfer RNA (tRNA) and ribosomal RNA (rRNA) nucleosides which, after RNA-chain degradation, cannot be salvaged and is obligately excreted in urine. Urinary excretion of pseudouridine has been suggested as a RNA-turnover marker (Schöch *et al.* 1982) and, further, as a marker of protein synthesis if it is assumed both processes are linked (Sander *et al.* 1986*a*).

In the present study endogenous excretion of PD and pseudouridine were measured in non-pregnant sows and pregnant sows throughout pregnancy. A preliminary report of some of the results has been made by Martin *et al.* (1993).

MATERIALS AND METHODS

Animals and feeding

Six Large White × Landrace sows with an initial parity of four farrowings were used. Three of them (210 (SD 15) kg live weight) were mated and, after a positive pregnancy diagnosis (30 d after mating), these and the remaining three non-pregnant sows (220 (SD 7·2) kg live weight) were fed on a semi-synthetic NA-free diet (3 kg fresh matter/d) containing (relative proportions) maize starch (0·27), glucose (0·27), sucrose (0·20), vegetable oil (0·04), casein (0·08), dicalcium phosphate (0·03), straw (ground at 3 mm) (0·10), methionine + threonine (0·003) and a vitamin–mineral mixture (0·007). Vitamins and minerals were included at levels recommended by the Agricultural Research Council (1981).

The experimental diet analysed from weekly samples contained 920 g DM kg with the following composition (g/kg DM): organic matter 957, protein (N × 6·25) 80·4, diethyl ether extract 58, crude fibre 56. The diet contained 19 mg adenine and 25 mg guanine/kg DM and 14·63 MJ estimated metabolizable energy/kg DM calculated on an as-fed basis. Animals were fed twice daily (08.30 and 15.30 hours) and kept in individual-stall-housing with free access to drinking water.

Experimental design

The experiment began after diagnosis of pregnancy, with a gradual substitution of a commercial diet by the experimental diet over 4 d. Urinary collection started 8 d later for three consecutive days using a Foley catheter (Folatex; 28 mm, 10 ml balloon) inserted into the bladder. The same collection procedure was repeated every 12 d until parturition, giving a total of six collection periods. All animals were weighed twice, in the pre-experimental period and at the beginning of the experiment, while non-pregnant sows were also weighed at the end of the experiment.

Sample preparation and chemical analyses

Daily total urine excretion was collected over 1 M-H_2SO_4 (final pH < 3). Urine weight and specific gravity were recorded and four subsamples (0·3 % of total) were stored immediately at −20° until analysed. Urinary allantoin, uric acid, hypoxanthine and xanthine were analysed by reversed-phase HPLC, using two Spherisorb C-18 ODS-5 (4·6 × 250 mm) columns, according to the technique described by Balcells *et al.* (1992). Pseudouridine from urine, and adenine and guanine from feed samples were also analysed using the technique described previously. Before analysis, feed samples (250 mg) were hydrolysed for 1 h at 100° with 6 ml 1·7 M-perchloric acid–0·1 mM-Allopurinol followed by immediate neutralization with 4 M-KOH. Retention times for pseudouridine in urine samples and adenine and guanine in feed samples were 11·0, 13·5 and 18·0 min respectively, with the three compounds showing a similar chromatographic behaviour to that described in the original technique. The average recovery of standards added to urine was in the order of 90 % and the day-

to-day variability was < 10%. Pseudouridine was not detected in feed samples. Creatinine was analysed using a Technicon RA-500 with a picric acid reaction (Technicon Instruments Co. Inc. 1989).

Calculation and statistical analysis

Data were subjected to a factorial analysis of variance, with animals within physiological stages as the main plot and collection period as the subplot (Steel & Torrie, 1980). An orthogonal set of contrasts over collection time was used to compare treatment means. All calculations were made using the BMDP (1990) package.

RESULTS

Animals remained in good health throughout the experiment, consuming all the experimental diet. However, one of the pregnant sows aborted (54 d after mating) and the experimental procedure was subsequently repeated with the same animal. Mean period of gestation was 116 (SE 0·7) d, and litter sizes were nine, fourteen and twelve piglets respectively for each sow. Mean birth weight, recorded 3 h post-farrowing when piglets were clean and dry, was 1·6 (SE 0·06) kg. The weights of the sows remained constant during the gradual substitution of the commercial diet, and in non-pregnant sows throughout the experimental period. All variables were related to initial body weight.

Purine-derivative excretion

Daily excretion for individual PD and the statistical significance of treatment effects are shown in Table 1. Allantoin excretion decreased significantly following dietary exclusion of purines, from previous values of 401 (SE 40·7) μmol/kg metabolic live weight ($W^{0·75}$) per d to a mean level of 128·3 (SE 7·07) μmol/kg $W^{0·75}$ per d. However, the purine-free diet did not affect the urinary excretion of allantoin precursors, with means of 38·7 (SE 2·15), 11·2 (SE 0·83) and 21·0 (SE 2·58) μmol/kg $W^{0·75}$ per d for uric acid, xanthine and hypoxanthine respectively. The mean excretion of total endogenous PD was 199·2 (SE 9·23) μmol/kg $W^{0·75}$ per d with relative amounts of allantoin, uric acid, xanthine and hypoxanthine of 64 (SE 1·3), 19 (SE 0·8), 6 (SE 0·3) and 11 (SE 1·3)%.

There were no differences in total endogenous PD excretion between pregnant sows (197·5 (SE 13·77) μmol/kg $W^{0·75}$ per d) and non-pregnant sows (200·9 (SE 12·68) μmol/kg $W^{0·75}$ per d), reflecting the constant excretion levels of the main PD (allantoin, uric acid), although hypoxanthine excretion, as salvageable PD, decreased significantly ($P < 0·005$) through pregnancy (from 25·5 to 5·2 μmol/kg $W^{0·75}$ per d).

Significant variations ($P = 0·05$) in PD excretion (particularly in allantoin ($P = 0·05$), uric acid ($P = 0·03$) and xanthine ($P = 0·02$)) during the experimental period were detected which were independent of the physiological status. In order to avoid errors associated with possible urinary losses through the catheter urinary excretion was corrected using creatinine as an internal marker. Mean amounts of PD relative to creatinine excretion (PD:creatinine) are shown in Fig. 1. This method of expression reduced residual variation (coefficient of variation (CV) 27·8, 22·5, 37·3 and 49·0% for daily excretion, compared with 19·9, 19·2, 27·8 and 48·5%, for the ratios of allantoin, uric acid, xanthine and hypoxanthine to creatinine respectively). When corrected for creatinine excretion the variation in PD excretion in relation to the experimental period remained significant ($P = 0·05$).

As stated previously, allantoin:creatinine, uric acid:creatinine and xanthine:creatinine values were not modified by pregnancy (constant mean values were 12·3, 3·7 and 1·0 mol/100 mol creatinine respectively), whereas hypoxanthine:creatinine showed a different pattern ($P < 0·005$), decreasing in pregnant animals. Orthogonal contrasts showed

Table 1. *Daily urinary excretion (μmol/kg metabolic live weight ($W^{0.75}$) per d) of allantoin, uric acid, xanthine and hypoxanthine, throughout the experimental period, in pregnant (P) and non-pregnant (NP) sows fed on a semi-synthetic nucleic acid-free diet**

(Mean values for three animals)

		Period of pregnancy (d)						Phase†		Period‡		Phase × period	
		42	54	66	78	90	102	SE (4 df)	Statistical significance of treatment effects	SE (20 df)	Statistical significance of treatment effects: $P =$	SE (20 df)	Statistical significance of treatment effects: $P =$
Purine derivatives	**NP**	138·5	166·7	**204·0**	226·7	212·9	256·8	**17·66**	**NS**	**19·07**	**0·05**	**26·97**	**NS**
	P	170·6	178·4	255·3	182·5	193·5	204·4						
Allantoin	**NP**	78·4	108·7	141·7	146·5	125·7	145·6	15·99	NS	14·55	0·05	20·58	NS
	P	107·8	110·9	166·1	126·5	130·3	151·7						
Uric acid	**NP**	28·3	39·1	45·4	43·8	49·8	51·1	5·19	NS	3·63	0·03	5·13	NS
	P	28·4	30·9	46·0	30·2	35·8	35·1						
Xanthine	**NP**	5·1	6·1	9·1	11·4	13·4	15·20	1·10	NS	2·24	0·02	1·71	NS
	P	8·9	9·0	15·7	11·9	16·2	12·5						
Hypoxanthine	**NP**	26·7	12·7	7·7	24·9	24·0	44·8	5·48	NS	4·20	NS	5·94	0·0009
	P	25·5	27·7	27·6	14·0	11·2	5·2						

NS, not significant ($P > 0·1$).

* For details of procedures, see pp. 376–377. † Physiological state (P *v.* NP). ‡ 12 d collection periods.

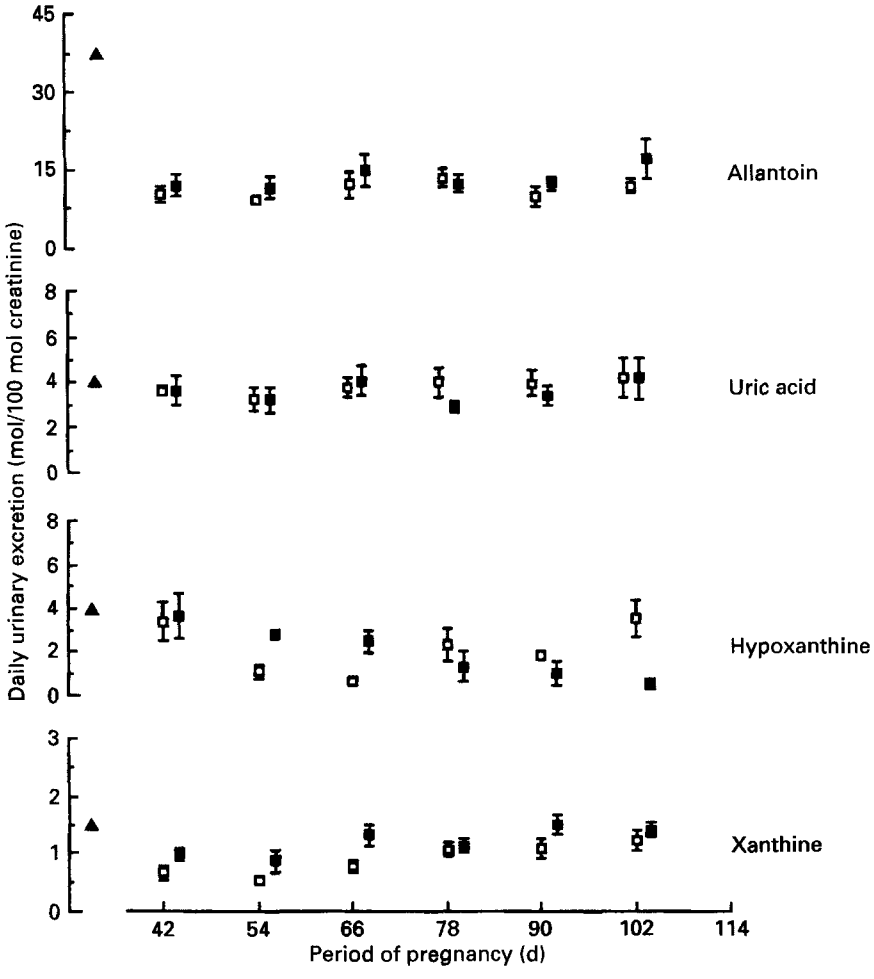

Fig. 1. Daily urinary excretion (mol/100 mol creatinine) of allantoin, uric acid, xanthine and hypoxanthine throughout the experimental period, and their pre-experimental levels (▲), in pregnant (■) and non-pregnant (□) sows fed on a semi-synthetic nucleic acid-free diet. Values are means and standard deviations represented by vertical bars for three animals. For details of procedures, see pp. 376–377.

a lower ratio in the last two collection periods (90–102 d collection compared with 42–90 d collection ($P = 0{\cdot}009$), and 90–102 d collection compared with 42–78 d collection ($P = 0{\cdot}014$)).

Pseudouridine

Mean excretion of pseudouridine, as a non-salvageable pyrimidine derivative, is presented in Table 2, expressed as μmol/kg $W^{0{\cdot}75}$ per d or as pseudouridine:creatinine (mol/100 mol creatinine). Fig. 2 shows the changes in pseudouridine:creatinine throughout the experimental period compared with that of total PD:creatinine.

The absence of dietary NA stimulated a decline in pseudouridine excretion followed by a further recovery until the 66 d collection to reach a mean value of 36·0 (SE 2·04) μmol/kg $W^{0{\cdot}75}$ in the experimental period without differences between physiological status. However, when expressed as mol/100 mol, creatinine showed a tendency to

Table 2. *Daily urinary excretion (μmol/kg metabolic live weight ($W^{0.75}$) and mol/100 mol creatinine) of pseudouridine, throughout the experimental period, in pregnant (P) and non-pregnant (NP) sows fed on a semi-synthetic nucleic acid-free diet**

(Mean values for three animals)

Urinary excretion of pseudouridine		Period of pregnancy (d) 42	54	66	78	90	102	Phase† SE (4 df)	Phase† Statistical significance of treatment effects	Period‡ SE (20 df)	Period‡ Statistical significance of treatment effects: $P =$	Phase × period SE (20 df)	Phase × period Statistical significance of treatment effects: $P =$
μmol/kg $W^{0.75}$	NP	15·4	30·4	42·9	39·7	42·0	44·5	2·22	NS	3·78	0·0005	5·35	NS
	P	21·6	30·1	42·8	33·7	43·5	45·3						
mol/100 mol creatinine	NP	2·0	2·6	3·6	3·6	3·3	3·6	0·38	NS	0·24	0·0001	0·35	0·097
	P	2·5	3·1	3·7	3·2	4·1	5·2						

NS, not significant ($P > 0·1$).

* For details of procedures, see pp. 376–377. † Physiological state (P *v.* NP). ‡ 12 d collection periods.

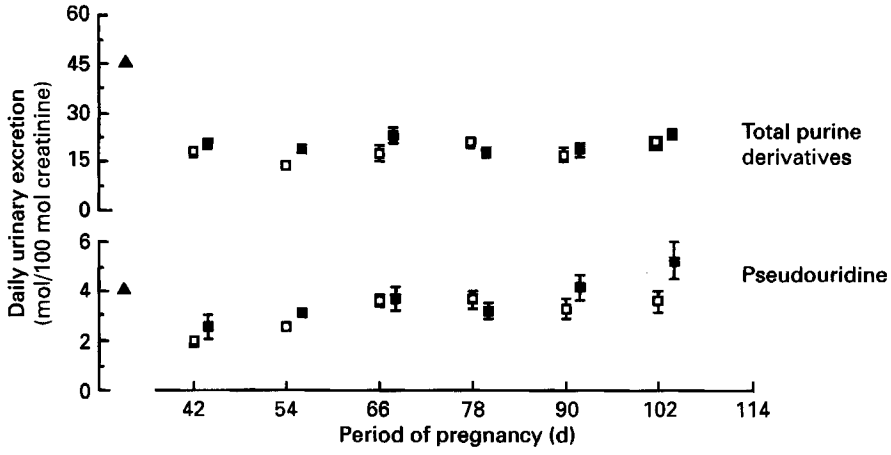

Fig. 2. Daily urinary excretion of total purine derivatives and pseudouridine (mol/100 mol creatinine) throughout the experimental period, and their pre-experimental levels (▲), in pregnant (■) and non-pregnant (□) sows fed on a semi-synthetic nucleic acid-free diet. Values are means and standard deviation represented by vertical bars for three animals. For details of procedures, see pp. 376–377.

continue onwards with pregnant animals whereas non-pregnant animals maintained a constant value as shown. This effect was apparent ($P = 0{\cdot}097$) when values were related to creatinine excretion and also when the last period (90–120 d) was compared with previous collection periods ($P = 0{\cdot}086$).

DISCUSSION

Many recent studies have investigated the origin and significance of PD and pyrimidine derivatives excreted in urine, usually by measuring the endogenous fraction after avoiding the flow of dietary NA to the duodenum. The same procedure was followed in the present study, maintaining the animals on a NA-free diet during pregnancy, to compare endogenous losses of pregnant and non-pregnant sows.

In order to avoid differences in feeding level, assuming a certain presence of NA in the diet, which could mask variations in urinary excretion of purine and pyrimidine derivatives, both groups of animals were fed on the same amount of diet. The fact that non-pregnant animals kept a constant body weight throughout the experiment suggested a possible restriction on pregnant animals; however, the effect of moderate variations in energy and protein supply on endogenous excretion seems to be negligible (Giesecke *et al.* 1984; Fujihara *et al.* 1987; Lindberg & Jacobsson, 1990).

Endogenous excretion of purine derivatives

Dietary absence of NA was followed by a rapid, significant decrease in allantoin and, consequently, total PD excretion; the latter reached a basal level of 199·2 (SE 9·23) μmol/kg $W^{0{\cdot}75}$ per d which is considered the endogenous fraction of the total urinary excretion. Uric acid, hypoxanthine and xanthine excretion remained unaffected by the experimental treatment. The consistency in the rate of urinary excretion of these allantoin precursors is in agreement with findings from previous studies on ruminants (Fujihara *et al.* 1988; Balcells *et al.* 1991, 1993), although a progressive increase in allantoin precursors, mainly uric acid, in response to duodenal purine input, have been reported for both single-stomached animals (Roth & Kirchgessner, 1979) and ruminant animals (Chen

et al. 1990*a*). Although uric acid precipitation in urine samples cannot be fully excluded we could not find any differences when PD and pyrimidine derivatives were analysed in fresh samples or after collection and storage (−20°) below pH 3.

Exogenous PD coming from absorbed diet precursors are carried by the portal vein to the liver, which constitutes the main site of degradation to allantoin due to the high activities in this organ of xanthine oxidase (*EC* 1.1.3.22) and uricase (*EC* 1.7.3.3; al-Khalidi & Chaglassian, 1965; Chen *et al.* 1990*b*), key enzymes in purine catabolism. Thus, it can be expected that an increase in duodenal PD absorption would be recovered as urinary allantoin. Although extrahepatic cells contain trace amounts of xanthine oxidase and uricase (al-Khalidi & Chaglassian, 1965), they may degrade a low but significant amount of endogenous NA, allowing disposal of salvageable catabolites by the kidneys without being transported to the liver. This might explain the constant fraction of endogenous allantoin precursors in the physiological urine.

The endogenous excretion of PD observed in the present study agreed well with the values reported by Chen *et al.* (1990*b*) in growing pigs fed on a similar diet (166 μmol/kg $W^{0.75}$ per d), or those of 164, 168, 190 and 202 μmol/kg $W^{0.75}$ per d reported by Fujihara *et al.* (1987), Chen *et al.* (1990*b*), Balcells *et al.* (1991) and Giesecke *et al.* (1984) respectively in sheep. The higher values observed in steers (443–468 μmol/kg $W^{0.75}$ per d; Fujihara *et al.* (1987)) and cows (514 μmol/kg $W^{0.75}$ per d; Chen *et al.* (1990*b*)) reflect the wide tissue distribution of xanthine oxidase activity (al-Khalidi & Chaglassian, 1965). Uric acid excretion in humans, as the endproduct of purine metabolism, was 123·8 μmol/kg $W^{0.75}$ per d (Folin *et al.* 1924) or 2·87 mmol/d (Zöllner, 1982) but Greife (1980) reported a much higher value for allantoin plus uric acid excretion in rats (130 μmol/d or 969 μmol/kg $W^{0.75}$ per d) probably due to the higher rates of body protein turnover in this species (Waterlow, 1984).

Earlier work by Condon *et al.* (1970) showed that sheep receiving a standard supply of exogenous purines incorporated into their NA labelled purines infused duodenally in preference to labelled glycine, the main precursor of the '*de novo*' pathway. Later studies confirmed the significance of the salvage process on the metabolism of PD in sheep (Smith *et al.* 1974; Razzaque *et al.* 1981), and models developed recently to estimate duodenal flow of PD in ruminants (Chen *et al.* 1990*a*; Balcells *et al.* 1991) suggest that endogenous losses could be replaced by '*de novo*' synthesis as the basis to the absence of response in PD excretion at the low levels of exogenous purine input. This assumption, however, needs to be demonstrated experimentally given that a simple purine-pool depletion cannot be excluded.

From our results the pattern of endogenous losses during the experimental period seems to preclude a depletion of the organic purine pool, suggesting constant replacement of the endogenous losses by '*de novo*' synthesis. It should be pointed out that purine analysis of the experimental diet showed a negligible contribution by the exogenous purine supply, i.e. a maximum of 16·5 μmol/kg $W^{0.75}$ per d, assuming total digestion of dietary straw purine.

Endogenous synthesis balanced the purine pool in non-pregnant sows and, in addition, was able to supply enough substrate to allow the growth of the gravid uterus. However, the probable increase in metabolic activity and purine synthesis during pregnancy was not reflected in the endogenous excretion level (Fig. 2). In contrast to our results, in a study on the effect of a continuously-altered N balance on the endogenous PD excretion using intragastric-fed animals, Chen (1989) reported a significantly higher endogenous excretion in pregnant ewes than in non-pregnant ewes.

There is a significant increase in N retention during late pregnancy (Faulkner, 1983) and probably also in the NA pool. That would lead to an increase in endogenous losses, assuming the rates of NA turnover are similar in all tissues. This increase might have been

masked by the high residual variation recorded in the present experiment. However, if pregnancy brings about an improvement in the efficiency of tissue NA turnover, it would explain the lack of response to changes in the physiological state. The significant decrease in urinary hypoxanthine excretion, as major salvageable PD (Hitchings, 1978) in late pregnancy, is in accordance with this suggestion.

On the other hand, only urinary PD excretion was measured and the possibility of alternative routes of purine loss during pregnancy might be considered. From the commencement of foetal renal function, amniotic fluid acts as a waste pool (Pitkin, 1974; Seeds, 1974) in which the differential rates of foetal excretion and maternal absorption produce a storage of several catabolites like creatinine, uric acid (Free & Free, 1974) and possibly all urinary PD and pyrimidine derivatives. Storage of these compounds in amniotic fluid may be a factor affecting the relationship between purine turnover and urinary losses during pregnancy.

Endogenous pseudouridine excretion

We have been searching for a non-invasive method to study RNA turnover, in order to investigate the significance of the variations in this process on urinary excretion of PD and pyrimidine derivatives. Pseudouridine is a modified pyrimidine-derivative constituent of t-RNA (2–9 residues/molecule) and r-RNA (252 residues/molecule; Sander *et al.* 1986*b*). Like every modified nucleoside pseudouridine is not incorporated into RNA (Borek *et al.* 1977; Gehrke *et al.* 1979), being excreted in urine during RNA turnover without possible further metabolism (Weissman *et al.* 1962). If it is assumed that the RNA chain contains less than 1 % of total nucleosides as pseudouridine (Weissman *et al.* 1962), the high urinary excretion recorded (36·0 (SE 2·04) μmol/kg $W^{0.75}$ per d), about 20% of total purine excretion, confirms the organic inability to re-utilize this compound and, hence, its potential value as a RNA-turnover marker. The extent of pseudouridine losses (36·0 μmol/kg $W^{0.75}$ per d or 3·4 mol/100 mol creatinine) is consistent with observations reported by Weissman *et al.* (1962) in mice (28·4 μmol/kg $W^{0.75}$ per d), and Puchala *et al.* (1993) in cows (20·5 μmol/kg $W^{0.75}$ per d) and sheep (20·3 μmol/kg $W^{0.75}$ per d), although these values were consistently higher than those reported for children (15·2 μmol/kg $W^{0.75}$ per d; Schöch *et al.* 1982) or adults (7–10·4 μmol/kg $W^{0.75}$ per d; Borek & Kerr, 1972).

The fact that in the present experiment animals were fed on a NA-free diet could explain the differences between our findings and those recorded in normally-fed animals. However, Puchala *et al.* (1993) demonstrated that urinary pseudouridine excretion is not affected by duodenal NA supply. Nevertheless, it is difficult to explain the wide variation between studies, assuming that for mammals both pseudouridine distribution and turnover rate are similar (Khan *et al.* 1978). However, significant differences associated with body development have been reported in the literature (Trisch *et al.* 1979; Sander *et al.* 1986*a*; Puchala *et al.* 1993) and this may help to explain the variation discussed previously.

The authors are not familiar with any findings on urinary excretion of modified purine or pyrimidine residues in pregnant animals, but the trend towards increased pseudouridine excretion in pregnant *v.* non-pregnant sows ($P = 0{\cdot}097$, Table 2) seems to reflect a higher rate of protein synthesis and NA turnover in late pregnancy, although further studies are needed to elucidate this possibility.

Conclusion

It is concluded that pregnancy did not affect endogenous PD excretion in sows. Thus, equations developed previously for non-pregnant animals (Balcells *et al.* 1991) to estimate the duodenal flow of purines from measurements of urinary PD excretion would not appear invalid when extended for use with pregnant animals.

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