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THE EFFECTS OF PREGNANCY AND PARTURITION ON PHOSPHORUS METABOLITES IN RAT UTERUS STUDIED BY ^{31}P NUCLEAR MAGNETIC RESONANCE

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SUMMARY

1. Concentrations of phosphorus metabolites and intracellular pH have been measured in non-pregnant, late-pregnant and post-partum rat uterus using ^{31}P nuclear magnetic resonance (^{31}P n.m.r.). Intact uterine tissue was superfused with oxygenated de-Jalon solution at 4, 20 or 37 °C while inside the n.m.r. spectrometer.

2. The phosphocreatine concentration [PCr], was higher and the inorganic phosphate concentration [P_i], lower than values determined by chemical analysis of extracts from both pregnant and non-pregnant rat uterus. [PCr] was 1·4-fold greater in late-pregnant than in non-pregnant rat uterus.

3. Following parturition, large changes were observed in [PCr], [P_i] and in an unidentified metabolite in the phosphomonoester (PME) region of the n.m.r. spectrum. The time course of the recovery of these metabolites to prepregnant values was determined. The [PCr] remained below the non-pregnant value for at least 1 week post-partum and the [P_i] was elevated, compared to the non-pregnant value, during this period. More rapid changes were seen in the [PME], which doubled on day 0 post-partum but almost returned to its non-pregnant value on day 1 post-partum.

4. No significant difference was observed between intracellular pH values in late-pregnant and non-pregnant rat uterus; however, there was a large acid shift following parturition. Intracellular pH depended upon the temperature at which the tissue was maintained.

5. The effect of muscular work during parturition was investigated by comparing Caesarian-sectioned uteri with uteri which had undergone normal parturition. Uteri examined 1 day after Caesarian operation showed no differences in metabolite levels from normal, 1 day post-partum uteri.

6. We conclude that concentrations of phosphorus metabolites depend upon the physiological state of the uterus. We suggest that the changes following parturition are not a consequence of the mechanical work performed by the uterus, but must be caused by some other event associated with parturition such as hormonal changes.

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INTRODUCTION

Throughout pregnancy there is enormous growth of the uterus, largely as a result of hypertrophy of the muscular layers (Reynolds, 1949). Pronounced biochemical changes also occur during this period, e.g. increases in concentrations of contractile proteins, glycolytic and oxidative enzymes (Wynn, 1977). During labour the myometrium performs a series of sustained and powerful contractions to expel the young and, following parturition, the hypertrophy and biochemical changes of pregnancy are rapidly reversed in a process known as involution.

Like other muscle types, the myometrium utilizes ATP during each contraction and contains a store of phosphocreatine (PCr) which buffers the supply of ATP. Rather little is known about variations in the concentrations of these energetically important metabolites with the functional state of the myometrium and even less is known about uterine intracellular pH. The [PCr] has been found to be greater in the pregnant uterus than in the non-pregnant uterus, in both humans (Cretius, 1957) and rabbit (Menkes & Csapo, 1952), but in the rat lower values are reported in the pregnant than the non-pregnant uterus (Walaas & Walaas, 1950). While there may be a genuine species difference, methods of extraction and chemical analysis were not reliable at the time these studies were done and the results should be confirmed using more modern techniques. There have been few measurements of uterine phosphorus metabolite concentrations following labour. Likewise little is known concerning the effect uterine metabolite levels might have on the course of labour.

^{31}Phosphorus nuclear magnetic resonance (^{31}P n.m.r.) allows phosphorus metabolites and intracellular pH to be determined in intact tissue (e.g. see Gadian, 1982), and a ^{31}P n.m.r. study of immature uteri has been published (Degani, Shaer, Victor & Kaye, 1984). We have studied mature rat uterus under differing physiological conditions using this technique and have asked the following questions. (i) Do phosphorus metabolite concentrations change as a consequence of pregnancy? (ii) What happens to uterine phosphorus metabolites following parturition and what is the time course of any changes? (iii) How do phosphorus metabolite concentrations measured by ^{31}P n.m.r. in intact isolated uterus compare with those obtained by biochemical analysis of extracts of uterine tissue? (iv) What is the intracellular pH in the uterus and does it change with pregnancy or parturition?

These experiments yielded values for [PCr] which were higher, and values for the inorganic phosphate concentration [P_i] which were lower than those available in the literature. Furthermore, [PCr] was found to be significantly higher in late-pregnant than in non-pregnant uterus. Investigation of the time course of decline in [PCr] and comparison of Caesarian-sectioned uteri which had undergone normal parturition suggest that changes in phosphorus metabolite concentrations may be under hormonal control.

A preliminary account of some of these results has been published (Dawson & Wray, 1983, 1984; Wray & Dawson, 1985).

METHODS

Animals. Sprague–Dawley rats were used. They weighed around 200–250 g and were fed Dixon's diet pellets (GR3-EK) and tap water *ad libitum*. Uteri from pregnant rats were taken from animals on days 21–22 of gestation. (Parturition occurs on day 22.) The day of birth was called 0 post-partum.

Following parturition, mothers were kept in individual cages and suckled their young. In four experiments designed to test the role of uterine activity on the metabolite changes after parturition, all fetal material was removed from the uterus, under ether anaesthesia (Wray, 1982). Incisions were sutured with silk thread and Michel clips. Two of these animals were also given a 10 mg/kg body weight I.P. injection of ritodrine hydrochloride (Yutopar, Duphar labs, Southampton). This is a β-2 sympathomimetic, and was given to ensure relaxation of the uterine smooth muscle. The results from these injected animals were the same as those from the two untreated animals and are therefore not discussed separately. The operations were performed on day 21 of pregnancy and the uteri examined 24 h later. Animals appeared comfortable and healthy after recovery from anaesthesia.

Tissue preparation and maintenance. Under chloroform anaesthesia, the uterus was removed, dissected free of any mesometrium, placental sites or fetuses, and placed in a 15 mm n.m.r. tube. The dissection was done under oxygenated solution at 4 °C. The uteri were superfused (rate = 4 ml min^{-1}) with oxygenated, modified de-Jalon solution while inside the n.m.r. spectrometer (Na, 186 mM; Cl, 161 mM; HCO_3, 31 mM; K, 6 mM; glucose, 3 mM and Ca, 0·3 mM). In some experiments spontaneous contractions were monitored using methods previously described for force measurements during ^{31}P n.m.r. experiments on isolated skeletal muscle (Dawson, Gadian & Wilkie, 1977). The usual temperature was 4 °C but some experiments were performed at 20 and 37 °C. The number of uteri used in each experiment was varied so that around 1·5 g of tissue was used. Thus, uteri from pregnant rats and at 0–2 days post-partum were examined individually, while at other times two to four uteri were pooled.

^{31}P n.m.r. A vertical high-resolution spectrometer (Bruker WM 200, Coventry), operating at a frequency of 81 MHz for phosphorus was used. Spectra were obtained using a pulse duration of 30 μs with a 2 s interval between pulses. The number of pulses averaged to obtain each spectrum varied from 100 to 1000, the usual number being 500. Resonance positions of the spectral peaks were measured relative to that of PCr in parts per million (p.p.m., i.e. 81 Hz = 1 p.p.m.). Line broadening of 12 Hz was used to reduce noise in the spectra, but no other signal-enhancement procedures were applied.

Peak assignment and calculation of metabolite concentrations. Peaks were assigned on the basis of their resonance position and the known presence of particular metabolites in the uterus. Provided that the phosphorus nuclei have time to return to their equilibrium state between radio-frequency pulses, the peak areas are directly proportional to the concentrations of metabolite present. In order to determine the effect of pulse interval, spectra were collected at intervals of 2, 5, 10 and 15 s. The sizes of the signals obtained at 2 s intervals (the pulse interval used routinely) were then corrected with the following 'saturation correction factors': nucleoside triphosphate (NTP), 1·00; PCr, 1·44; P_i, 1·34; phosphomonoesters (PME), 1·75. The intracellular pH was calculated from the resonance position of P_i using the formula:

$$\mathrm{pH} = \mathrm{p}K + \log_{10}\frac{\delta-\delta_1}{\delta_2-\delta},$$

where δ is the observed chemical shift difference between P_i and PCr and δ_1 and δ_2 are the chemical shifts of $H_2PO_4^-$ and HPO_4^{2-} respectively. Values for pK, δ_1 and δ_2 were obtained by titration at 37 and 4 °C of a model solution containing 0·005 M-$MgCl_2$, 0·14 M-KCl and 0·010 M-P_i. Calculated free Mg^{2+} was 3–5 mM, depending on pH, and ionic strength was 0·165–0·170 (A. C. Elliott & M. J. Dawson, in preparation). At 37 °C the pK value was 6·65, δ_1 = 3·14 and δ_2 = 5·61. At 4 °C, pK = 6·71, δ_1 = 3·19 and δ_2 = 5·49.

Statistics. Figures given throughout are mean values of n observations with the S.E. of the mean. Significance of differences was tested using an unpaired t test.

RESULTS

Temperature and tissue variability

We performed experiments on late-pregnant and non-pregnant rat uteri at 4, 20 and 37 °C in order to determine the temperature at which the most reliable measurements of phosphorus metabolite concentrations and intracellular pH could be made. There were no apparent differences between spectra obtained at these

different temperatures except for the expected shifts in the resonance position of the P_i peak (see intracellular pH below) and NTP peaks as the temperature was lowered (at 37, 20 and 4 °C the NTP resonance positions were $-16{\cdot}35 \pm 0{\cdot}01$ p.p.m.; $-16{\cdot}42 \pm 0{\cdot}03$ p.p.m. and $16{\cdot}50 \pm 0{\cdot}02$ p.p.m., in non-pregnant uteri). Mechanical records showed spontaneous contractions when the uteri were maintained at 37 and at 20 °C and contractions could be elicited by electrical stimulation. No contractile activity was observed at 4 °C. Using the superfusion techniques described in the

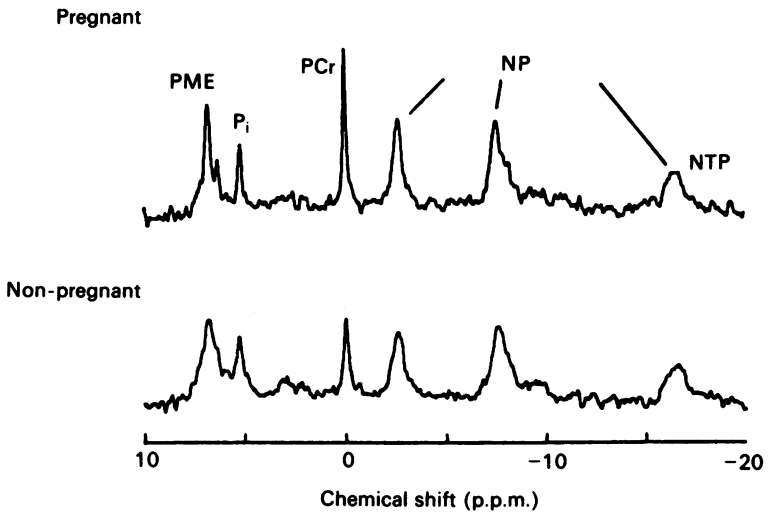

Fig. 1. ^{31}P n.m.r. spectra from pregnant (top) and non-pregnant (bottom) rat uterus at 4 °C. PCr, phosphocreatine; P_i, inorganic phosphate; PME, phosphomonoesters; NP, nucleoside phosphates. The spectra have been normalized to the nucleoside triphosphate peak (NTP) area; p.p.m., parts per million.

Methods, we found that we were able to maintain the uteri in a metabolic steady state, i.e. constant size of phosphorus metabolite peaks and unchanging intracellular pH, for longer periods of time as the temperature was lowered. This greater stability of isolated uteri at low temperature is presumably due to the decreased energy expenditure by the quiescent uterus. Therefore, unless stated otherwise, the results and figures shown are taken from experiments done at 4 °C. Under these experimental conditions the uteri were stable for 12–24 h, as judged by the ^{31}P n.m.r. spectra, visual inspection and tissue weight.

Differences between late-pregnant and non-pregnant rat uteri

Fig. 1 shows typical ^{31}P n.m.r. spectra obtained from late-pregnant and non-pregnant rat uterus. The peaks at $-16{\cdot}5$, $-7{\cdot}5$ and $-2{\cdot}5$ p.p.m. are from nucleoside phosphates (NP). The individual nucleosides resonate so closely to each other that they are indistinguishable in the spectra. Adenosine is the major (75%) nucleotide in the uterus; uridine and guanidine each contributing around 10% (Oliver & Kellie, 1970). The peak at $-16{\cdot}5$ p.p.m. (NTP) is composed solely of triphosphate, the other two peaks contain tri- and diphosphate. Peaks from PCr and P_i can be clearly seen

along with a peak at 6·8 p.p.m., in the PME region of the spectrum. The identity of the PME peak is not known, but evidence suggests that phosphoethanolamine may be the largest contributor to it (see Discussion).

The *Y* axes of the spectra in Fig. 1 have been normalized so that the areas of the NTP peaks are identical. There are no differences in the size of the P_i or PME peaks between spectra from pregnant and non-pregnant rat uteri. In contrast, the PCr peak is clearly greater in the spectrum obtained from the pregnant rat uterus.

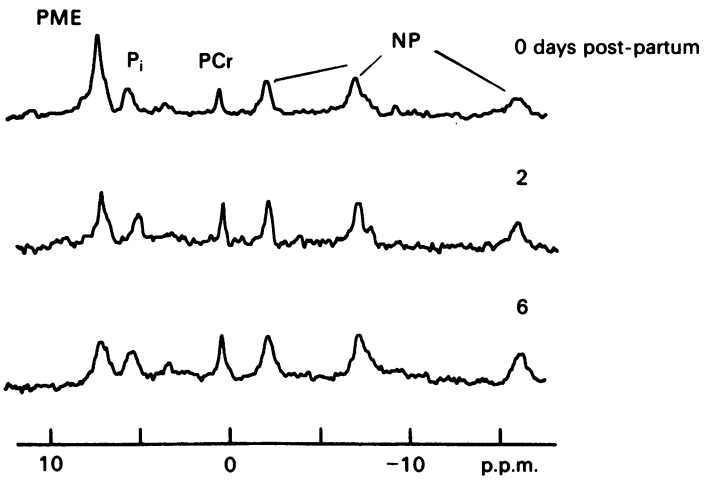

Fig. 2. ^{31}P n.m.r. spectra from 0 (top), 2 (middle) and 6 (bottom) day post-partum rat uterus. Abbreviations as for Fig. 1.

Changes with parturition

Typical ^{31}P n.m.r. spectra from uteri of rats at days 0, 2 and 6 post-partum are shown in Fig. 2. Again, the *Y* axes have been normalized to the NTP peak areas. Clear changes in uterine phosphorus metabolite ratios can be seen to occur following parturition. In particular the PCr peak area falls, and PME and P_i peak areas increase relative to that of NTP. The relative concentrations (relative peak areas after correction for saturation) of PCr, P_i and PME for all experiments are plotted against time post-partum in Fig. 3. The relative [PME] increased dramatically following parturition, almost doubling in size. This was followed by a rapid decrease so that on day 2 post-partum the [PME]/[NTP] was only 20% greater than that in non-pregnant animals.

The ratio of [PCr]/[NTP] was significantly greater in late-pregnant ($1·35 \pm 0·07$, $n = 8$) than non-pregnant animals ($1·05 \pm 0·07$, $n = 9$) ($P < 0·01$). This ratio fell markedly after parturition, reaching $0·89 \pm 0·13$ ($n = 4$) on day 1 post-partum, a value which is below, but not significantly different from, that in non-pregnant animals. The [PCr]/[NTP] ratio showed little change from this low value during the first three weeks after parturition. On day 21 post-partum, the value was still beneath that found in non-pregnant rat uterus ($0·87 \pm 0·14$, $n = 4$ and $1·05 \pm 0·07$, $n = 9$, respectively).

The [P_i]/[NTP] ratio showed changes in the opposite direction, increasing after parturition and then slowly declining during the post-partum period. On day 1 post-partum the [P_i]/[NTP] ratio was almost double the level in late-pregnant rat uteri. Unlike [PCr]/[NTP], the [P_i]/[NTP] ratio was not significantly different in late-pregnant and non-pregnant animals $0{\cdot}60 \pm 0{\cdot}07$ ($n = 8$) and $0{\cdot}50 \pm 0{\cdot}07$ ($n = 9$), respectively.

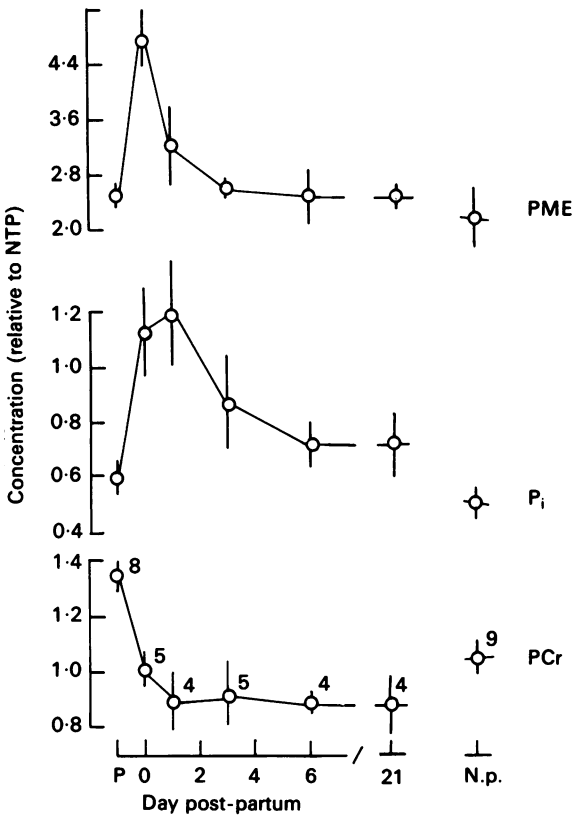

Fig. 3. The post-partum changes in concentration relative to nucleoside triphosphate of phosphomonoesters (PME, top) inorganic phosphate (P_i, middle) and phosphocreatine (PCr, bottom). The number of observations are given next to the points on the PCr graph. Values are means with standard errors indicated by the vertical lines. P, pregnant rat uterus; n.p., non-pregnant rat uterus.

The effect of Caesarian section

To determine what role the uterine contractions in labour played in the changes seen in metabolites after parturition, uteri from normal 1 day post-partum animals were compared to Caesarian-sectioned uteri, 1 day post-operatively. Fig. 4 shows the resulting spectra from one such experiment. It can be seen that the spectrum from the Caesarian-sectioned uterus is very similar to that from the uterus of an animal which had given birth in the normal way. Caesarian section had no apparent effect on any of the spectral peaks. Similar results were found in three other experiments.

Concentration of uterine metabolites

In some experiments absolute metabolite concentrations (mmol l^{-1} uterine volume) were calculated from spectral peak areas. The method used was to obtain the spectral peak area (A, cm^2) when the whole of the volume detected by the spectrometer ('the sensitive volume') is filled with a standard solution of 5 mM-P_i. The fraction (r) of the sensitive volume occupied by the volume of the uterus was determined by

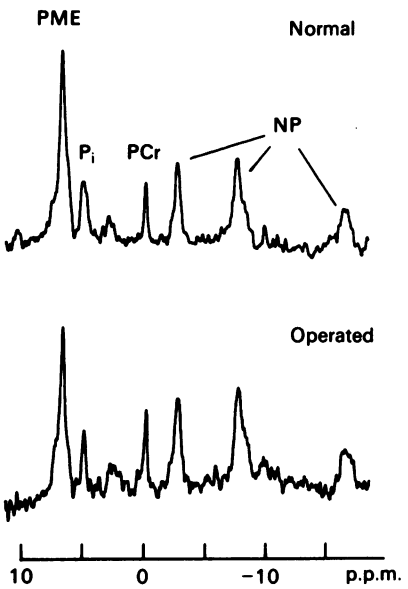

Fig. 4. ^{31}P n.m.r. spectra from 1 day post-partum uterus (top) and 1 day post-Caesarian-sectioned uterus (bottom). Peak identities are as described in Fig. 1. The spectra have been normalized to the NTP peak area.

measuring uterine weight and volume. After correction for signal saturation (see Methods) the peak areas, P in the uterine spectra were converted to concentrations as follows:

$$\text{concentration (mM)} = \frac{P}{r \times A} \times 5.$$

The PCr, NTP, P_i and PME concentrations obtained in this way are given in Table 1. For comparison, this Table also shows data available in the literature which were obtained by chemical analysis of extracts made from rat uterus.

The calculated [NTP] showed little variation with the state of the uterus. As the original spectra, i.e. Figs. 1–4 had been normalized to [NTP], the changes in absolute concentrations of phosphorus metabolites reflected the changes seen in the spectra. Thus, there was significantly ($P < 0.05$) more PCr in the pregnant (4.2 ± 0.5 mM, $n = 5$) than non-pregnant rat uterus (3.0 ± 0.3 mM, $n = 4$). After parturition [PCr] fell to 2.3 ± 0.6 mM. [P_i] rose to 2.5 ± 0.3 mM after parturition from 1.6 ± 0.2 mM in pregnant rat uteri. The [P_i] was not significantly different in uteri from late-pregnant

and non-pregnant animals. NTP showed no significant variation in concentration with the state of the uterus. The [PME] was calculated to be around 6 mM in pregnant and non-pregnant uteri, compared with nearly 10 mM on day 0 post-partum.

Intracellular pH

The mean intracellular pH was determined in uteri from pregnant, non-pregnant and post-partum rats in experiments at 37 and 4 °C. The results are shown in Table 2. There was a marked effect of incubation temperature on pH. The intracellular

TABLE 1. The concentration of metabolites in non-pregnant, pregnant and post-partum rat uterus determined on intact tissue by n.m.r. and by analysis of uterine extracts

Metabolite	Uterus	n	N.m.r. result (mM)	Literature value from chemical extracts (mM)
PCr	Non-pregnant	4	3·0 ± 0·3	1·2 ± 0·4†
	Pregnant	5	4·2 ± 0·5	0·5 ± 0·4*
	Post-partum	4	2·3 ± 0·6	—
P_i	Non-pregnant	4	1·5 ± 0·3	6·0 ± 0·3†
	Pregnant	5	1·6 ± 0·2	5·9 ± 0·9*
	Post-partum	4	2·5 ± 0·3	—
NTP	Non-pregnant	4	2·4 ± 0·4	2·5 ± 0·5†
	Pregnant	5	3·1 ± 0·4	1·1 ± 0·2*
	Post-partum	4	2·3 ± 0·3	—
PME	Non-pregnant	4	6·3 ± 1·1	—
	Pregnant	5	6·6 ± 0·5	—
	Post-partum	4	9·8 ± 1·3	—

* Walaas & Walaas (1950). † Volfin, Clauser & Gautheron (1957).

pH became more alkaline as the temperature was lowered. Temperature of incubation did not, however, affect the relations between pH values obtained in pregnant, non-pregnant and post-partum rat uteri. Table 2 shows that pregnancy had no effect on intracellular pH which was 7·09 ± 0·07 and 7·08 ± 0·03, respectively in non-pregnant and late-pregnant rat uteri maintained at 37 °C. However, parturition caused a large and significant ($P < 0·01$) shift to 6·83 ± 0·11 on the first day post-partum. The results concerning changes in pH with uterine state are the same at 4 °C as at the higher temperature. Therefore the time course of pH change following parturition was determined at an incubation temperature of 4 °C. As shown in Table 2, pH was most acid on the day of parturition and had returned to a level that was not significantly different from that in pregnant or non-pregnant rat uteri by the second to third day post-partum. Caesarian-sectioned uteri had intracellular pHs close to those found in the normal 1 day post-partum uterus (7·18 ± 0·08 and 7·25 ± 0·08, respectively).

Magnesium–NTP binding

The resonance positions of the NTP peaks depend upon how much magnesium is bound to the NTP (e.g. Gadian, 1982). (Mg NTP is the form in which NTP is useful to the cell.) It has been suggested that the amount of free magnesium changes with the functional state of the uterus (Degani *et al.* 1984) and indeed that variations in

free magnesium occur in all mammalian tissue as a result of physiological and pathological changes (Resnick, Gupta & Laragh, 1984). We therefore noted the mean uterine NTP peak position in pregnant, non-pregnant and post-partum animals. As shown in Table 3 there was no significant change in the frequency of resonance for the α, β or γ NP peaks with the functional state of the uterus, at either 4 or 37 °C.

TABLE 2. The intracellular pH of non-pregnant, pregnant and post-partum rat uterus at 4 and 37 °C

	pH	
Uterine preparation	4 °C	37 °C
Non-pregnant	7·49±0·06 (10)	7·09±0·07 (4)
Pregnant	7·47±0·04 (8)	7·08±0·03 (8)
0–1 day post-partum	7·25±0·08 (10)	6·83±0·11 (4)
3 day post-partum	7·47±0·14 (4)	—
6 day post-partum	7·45±0·15 (3)	—

No. of observations (n) in parentheses.

TABLE 3. Nucleoside phosphate resonance positions in pregnant, non-pregnant and post-partum rat uterus at 4 and 37 °C

			Nucleoside phosphate resonance (p.p.m.)		
Temperature	Uterus	n	β peak	α peak	γ peak
4 °C	Pregnant	7	−16·46±0·02	−7·51±0·04	−2·56±0·02
	Non-pregnant	7	−16·50±0·02	−7·51±0·02	−2·56±0·02
	Post-partum	6	−16·47±0·03	−7·53±0·04	−2·59±0·02
37 °C	Pregnant	8	−16·35±0·03	−7·53±0·02	−2·56±0·01
	Non-pregnant	4	−16·35±0·01	−7·54±0·03	−2·51±0·04
	Post-partum	4	−16·38±0·06	−7·56±0·03	−2·60±0·04

DISCUSSION

Marked differences were found in the levels of phosphorus metabolites between pregnant, non-pregnant and post-partum rat uterus studied by ^{31}P n.m.r. Because these experiments were performed on intact, isolated uteri, the results might be different from those that would be obtained if the measurements could be made solely on myometrial tissue in living animals. However, for the reasons discussed below, we believe that our results are reasonable estimates of myometrial metabolite concentrations and their changes with uterine state in living animals. It is not possible to separate the myometrium from the endometrium without breakdown of metabolites (Menkes & Csapo, 1952), and we therefore did not attempt to do so. However, myometrium is the major tissue in the uterus, respresenting 60% of tissue volume and 90% of total cellular volume in non-pregnant animals (Wynn, 1977). Therefore, contributions to the ^{31}P n.m.r. spectra from phosphorus metabolites in endometrial tissue will be negligible. The effects of isolating the uterus, and incubating it in an oxygenated superfusion medium are somewhat more difficult to assess. However, phosphorus metabolite levels were the same in all preparations, regardless of the

incubation temperature, and remained so for many hours which suggests that these levels in the isolated tissue are fairly close to those in the living animal.

At 4 °C, the uterine preparations were stable for at least 12 h permitting repeated measurements to be made. At this low temperature no uterine contraction occurs, a fact which simplifies data accumulation and interpretation. However, it should be noted that studies were also performed at 20 and 37 °C and the conclusions from these studies are identical to those drawn from the 4 °C experiments.

Comparison of metabolite concentrations obtained by ^{31}P n.m.r. with those obtained by chemical analysis

There are clear differences between the estimates of phosphorus metabolite concentrations based upon the ^{31}P n.m.r. spectra obtained in the present study and earlier estimates obtained by chemical analysis of uterine extracts (see Table 1). While [NTP] in the non-pregnant rat uterus is similar when determined by either method, the [PCr] is higher, and [P_i] is lower, when determined by ^{31}P n.m.r. This result is in keeping with the general tendency for [PCr], as determined by ^{31}P n.m.r., either *in situ* in the living animal or in isolated tissues, to be higher than that obtained by conventional chemical methods. Such results have been found in other mammalian tissues (e.g. brain, Tofts & Wray, 1985; skeletal muscle, Dawson, 1982). The most likely explanation for this discrepancy is that, even with the best methods of sampling, freezing and extracting mammalian tissues for chemical analysis, some artifactual hydrolysis of the highly labile PCr occurs (see Dawson & Wilkie, 1984 for a fuller discussion). In the specific case of uterine tissue, examination of the results of Walaas & Walaas (1950) shows that in the seven experiments used to calculate mean [PCr], five showed no PCr at all. This fact argues strongly that artifactual PCr hydrolysis was occurring. The [NTP] in the non-pregnant rat uterus is similar whether determined by ^{31}P n.m.r. or by chemical analysis; the values obtained from non-pregnant uterus are not significantly different from that obtained in pregnant rat uterus by ^{31}P n.m.r. The low [NTP] value obtained by Walaas & Walaas (1950) in *pregnant* rat uterus probably reflects artifactual NTP hydrolysis.

We conclude that the ^{31}P n.m.r. measurements of phosphorus metabolite concentrations in isolated rat uterus are reliable and that discrepancies with earlier measurements are due to known sources of experimental error associated with conventional chemical methods.

Identity and importance of the PME

We are uncertain as to the metabolite or metabolites contributing to the PME peak resonating at 6·8 p.p.m. A peak in this same position has been reported in brain (e.g. Tofts & Wray, 1985) tumours (e.g. Griffiths, Cady, Edwards, McCready, Wilkie & Wiltshaw, 1983) and smooth muscle (rabbit bladder, Krisandra & Kushmerick, 1984; rabbit arteries, Dawson, Spurway & Wray, 1985). Studies of brain extracts have identified this peak as arising from the presence of phosphoethanolamine (e.g. Brenton, Garrod, Krywawych, Reynolds, Batchelard, Cox & Morris, 1985). Although other metabolites resonate in this region of the spectrum, making unequivocal assignment in intact tissues impossible, it is possible that the peak at 6·8 p.p.m. in spectra from uterine tissue may be from phosphoethanolamine. Phosphoethanolamine is present in rat uterus (Awapara, Landua & Fuerst, 1950) and is a product of lipid

catabolism. Thus the increase in size of the PME peak seen after parturition may well be related to the decrease in cell size, and thus membrane area, occurring during uterine involution. The time courses of the changes coincide; the rat uterus halves in size during the first 24–36 h post-partum (Harkness & Harkness, 1954) and the largest changes in the PME peak are seen during this same period.

In one recent ^{31}P n.m.r. study, the remission of a cancerous tumour coincided with a reduction in size of the PME peak (Maris, Evans, McLaughlin, D'Angio, Bolinger, Manos & Chance, 1985). This finding led to the suggestion that changes in the size of the PME peak could be used as a prognostic index, an increase in PME-peak size indicating that the tissue is growing and a decrease in the size of this peak indicating regression. The results of the present study show that changes in PME-peak size cannot serve as a universal index of tissue growth rate: in the uterus, the size of the PME peak *increases* during rapid involution. Attention was first drawn to a possible relation between tissue growth and the presence of a high concentration of phosphoethanolamine by Ferrari & Harkness (1954). Interestingly these authors also caution 'high concentration of this substance appears to be associated with growing tissue, though this is not a universal rule'.

Intracellular pH of the uterus

The small size of smooth muscle cells has meant that few measurements of intracellular pH have been made. We find a value of around 7·08 in non-pregnant and pregnant rat uterus at 37 °C, bathed with an external solution at pH 7·3 and 5% CO_2. Using micro-electrodes in guinea-pig vas deferens, Aickin (1984) measured intracellular pH to be 7·06, with a bathing solution of pH 7·35, temperature 35 °C and 3% CO_2. This value is close to our value for the uterus. N.m.r. studies of arterial smooth muscle give a value of 7·1–7·2 for intracellular pH, at 20 °C and in the absence of CO_2 (Dawson *et al.* 1985). As noted by Aickin (1984) such values show that the H^+ are not in equilibrium across the smooth muscle membrane. Furthermore, the values are similar to those obtained in cardiac and skeletal muscle.

Magnesium–ATP binding

Since magnesium has to be bound to ATP, for the latter to exert its biological activity, we have measured the resonance positions of the NTP peaks which are dependent upon the amount of magnesium bound (e.g. Cohn & Hughes, 1962). We found that the NTP peaks are shifted slightly upfield (i.e. in the direction of Mg unbinding) as compared to frog skeletal muscle (Dawson *et al.* 1977) and that the peak positions are independent of the functional state of the uterus. Degani *et al.* (1984) report a change in the NTP position towards being unbound, in hormonally treated immature rat uteri. It should be emphasized that care has to be taken in assessing and interpreting these peak positions because the signal-to-noise ratio has to be good enough to allow accurate location of the peaks, and because factors other than Mg binding can affect their positions.

Cause of metabolite changes following parturition

There have been no previous studies of phosphorus metabolites following parturition. The changes we found occurring at this time, were marked. The [PCr] fell by a third following birth and remained at this low value throughout the first

3 weeks post-partum. The [P_i] practically doubled following parturition and was still elevated 3 weeks later. In an attempt to relate the changes in metabolites to events occurring around parturition, two possible causes were considered: smooth muscle activity and endocrinological changes, although other causes, e.g. neuronal activity, may exist. The powerful uterine contractions at parturition utilize ATP and PCr will be hydrolysed to maintain ATP levels. The energetic resources of the uterus may well fall as a result of this activity, especially as uterine blood flow may be stopped by the contractions. On the other hand there is also evidence that gestational hormones increase [PCr] in the uterus (Volfin, Clauser & Gautheron, 1957; Degani *et al.* 1984). Immediately after parturition, oestrogen and progesterone fall to extremely low levels (Wynn, 1977) and [PCr] might be expected to decline as a consequence. It was to decide between these two possibilities that the experiments on Caesarian-sectioned animals were undertaken. Caesarian-sectioned uteri undergo far less muscular work than do normal uteri containing fetuses. The results from the Caesarian experiments (Fig. 4), showed that the uteri undergo the same changes in phosphorus metabolites and intracellular pH as do normal uteri. The results therefore would not support the hypothesis that smooth muscle activity is responsible for the metabolic changes observed. Instead they are consistent with the uterine PCr content being under hormonal control.

The level of PCr was found to be higher (4·2 mM) in pregnant than non-pregnant (3·0 mM) rat uterus. This is also consistent with higher levels of gestational hormones stimulating a higher [PCr]. Much other work has showed other uterine constituents, e.g. glycogen, muscle proteins, O_2 consumption to be also influenced by hormonal conditions (see e.g. Wynn, 1977).

In conclusion, we have shown that the concentrations of phosphorus metabolites and intracellular pH are dependent upon the functional state of the uterus. It is suggested that hormonal stimuli are important in controlling these variations.

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