Pergamon

0960-0760(95)00010-0

*J. Steroid Biochem. Molec. Biol.* Vol. 52, No. 6, pp. 587–594, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-0760/95 $9.50 + 0.00

# 31P NMR Study of Phosphorus Containing Metabolites in the Uterus of Hamster: Changes During the Estrous Cycle and the Effect of Hormonal Manipulation

**S. Shivaji,* L. G. Devi, M. B. Ahmad and C. S. Sundaram**

*Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India*

**Changes in the concentrations of phosphorus containing metabolites were monitored by $^{31}$P NMR in the uteri of hamsters during the estrous cycle. Concentrations of phosphocreatine (PCr) and ATP were significantly increased in estrus animals compared to diestrus animals. Concentrations of these metabolites were also increased in immature female hamsters and ovariectomized (OVX) adult hamsters treated with estradiol indicating that estradiol was responsible for this effect. However, the steroid hormones progesterone and testosterone did not increase the concentrations of the phosphorus containing metabolites. Further, immature female hamsters also following treatment with estradiol showed an initial decline in phosphomonoester (PME), PCr, ATP and inorganic phosphate but by 24 h of treatment the concentrations returned to control levels. The NMR study also revealed that the intracellular pH of the hamster uterus was around 7.4 all through the estrous cycle.**

*J. Steroid Biochem. Molec. Biol.*, Vol. 52, No. 6, pp. 587–594, 1995

## INTRODUCTION

In mammals, the uterus during the estrous cycle exhibits cyclic morphological, biochemical and physiological changes such as increase in tissue weight [1–3], accumulation of fluid [4–6] and increase in biosynthetic activity [7]. Apart from an increase in the synthesis of DNA, RNA and proteins under the influence of estradiol, changes in the concentration of high energy metabolites such as phosphocreatine (PCr) and adenosine triphosphate (ATP) have also been observed in the uterus of rat following estradiol administration [8, 9]. But, all these earlier studies were performed using perchloric acid extracts of frozen uteri thus resulting in variations in the reported results due to partial degradation of the labile compounds. However, this difficulty could be overcome by the use of $^{31}$P NMR.

$^{31}$P NMR has been extensively used for the analysis of phosphate containing metabolites in excised tissues and whole animals [10–12] so as to avoid extraction and homogenization methods which are not suitable for the analysis of labile compounds. Using this method, high-energy phosphate metabolites in the uterus of adult rats during pregnancy and parturition [13] and in the uteri of immature rats following estradiol administration [14] have been analyzed. However, as yet, very little is known about the changes in the concentration of high-energy phosphate metabolites during the functional state of the uterus such as during the various phases of the estrous cycle. Since steroid hormones play a direct role in modulating the estrous cycle it would also be important to evaluate the effect of steroid hormones on the concentration of the high-energy phosphate metabolites. Further, even less is known about the intracellular pH of the uterus during the estrous cycle. In an attempt to address the above questions the present paper reports data related to:

(i) Changes in phosphate metabolites in adult hamster uteri during diestrus, proestrus and estrus phases of the estrous cycle.
(ii) Changes in phosphate metabolites in the uteri of adult animals following ovariectomy and subsequent administration of estradiol, progesterone or testosterone.

*Correspondence to S. Shivaji.
Received 7 Oct. 1994; accepted 26 Dec. 1994.

(iii) Effect of estradiol, progesterone and testosterone on the phosphate metabolites of immature hamster uteri.
(iv) Changes in levels of phosphate metabolites in the uteri of immature hamsters following estradiol administration (from 0 to 28 h).
(v) Changes in the intracellular pH of the uteri of adult hamsters during the estrous cycle.

## MATERIALS AND METHODS

*Animals*

Golden hamsters (*Mesocricetus auratus*) were reared at constant temperature ($22 \pm 1°C$) and light (6 a.m. to 6 p.m.) and were provided food and water *ad libitum*. The immature animals were 20 days of age and the mature animals were 6–8 weeks old. Only those mature animals which exhibited an invariant 4-day estrous cycle as judged by regular vaginal smears, were used. Mature female hamsters whose ovaries were surgically removed under the influence of thiopentone (injected i.p.) and which were allowed to recuperate for a period of 3 weeks were used as the ovariectomized (OVX) animals.

*Hormone injections*

Immature female hamsters (20 days of age) and adult OVX animals were injected s.c. for three consecutive days with estradiol (2 μg per 10 g body weight per day), testosterone (200 μg per 10 g body weight per day) or progesterone (200 μg per 10 g body weight per day) as described earlier [15]. Twenty four hours after the last injection the animals were anesthetized by exposure to ether for a brief period and killed by decapitation. The control animals received an equal volume of 1,2-propane diol, the vehicle in which the above steroids were dissolved. In a separate experiment, immature female hamsters were also injected with estradiol and at regular intervals of time (extending from 0 to 24 h) the uterine horns were dissected out and used.

*Preparation of uteri*

The orientation of the uteri in the NMR tubes was essentially similar to that described by Degani *et al.* [14]. Uteri were immediately excised, stripped free of fat and mesentery, rinsed twice in Dulbecco's modified Eagle medium (DMEM) minus inorganic phosphate to clear the tissue free of blood, blotted, weighed and transferred to a 10 mm glass NMR tube. A capillary tube containing the reference solution methylene diphosphoric acid (MDP) was placed inside the NMR tube. This capillary tube was held in position with the help of two perforated teflon plugs, which also helped to fix the position of the uteri such that similar proportions of the reference solution and sample were sensed by the magnetic coil of the NMR spectrometer.

*NMR measurements*

$^{31}P$ NMR measurements were carried out at 5°C on a Bruker AM 300 pulsed FT NMR spectrometer operating at a resonance frequency of 121.5 MHz. FID's (Free induction decay signals) were collected using a radio frequency (RF) pulse width of 6 μs with a spectral width of 8 kHz and 1 K data points. A recycling delay of 1 s was used. Various pulse repetition rates were tried and 1 s was found to be optimum without saturating the signals for the chosen pulse width which was less than 90°. In each experiment, the data was obtained in four consecutive blocks of 1200 scans each. Tissue viability during data acquisition was checked by comparing the spectra of the first and last blocks and they were found to be identical. Addition of 0.25 ml of $D_2O$ to the DMEM medium served as an internal lock. A capillary tube containing 50 mM MDP served as the concentration standard and as an additional chemical shift reference. The 10 mm NMR tube containing the tissue sample was spun at 15 Hz.

The FID's were processed with a line broadening parameter of 20 Hz and later Fourier transformed with 2 K memory size. Chemical shifts of all signals were measured with respect to PCr whose chemical shift was set at 0 ppm. PCr was calibrated with respect to external 8.5% orthophosphoric acid with a chemical shift of −2.49 ppm.

*Intracellular pH measurements*

To measure the intracellular pH of uterine tissue, a calibration curve of pH vs the chemical shift of inorganic phosphate (Pi) (Fig. 1) was constructed by using DMEM containing 10 mM of Pi. This medium was adjusted to different pH values by the addition of HCl/NaOH and the chemical shift of Pi was determined at 5°C. PCr added to the solution served as an internal chemical shift reference.

*Calculation of metabolite concentrations*

The concentrations of the various metabolites in the tissue were estimated by comparison with MDP which was used as the concentration standard. The area of the resonance curves was compared with that of MDP present in the capillary tube. 75 μl of MDP equivalent to 3.75 μmol was within the NMR coil volume and hence the area of the signal was taken to be equal to 3.75 μmol. The area of the MDP curve was further compared with that of standard 5 mM Pi in MEM (under identical experimental conditions) as an additional control against other systematic errors which may arise due to instrument instabilities.

*Statistical analysis*

Statistical analysis was performed using a PC-AT 286 computer. The total data were analyzed by one-

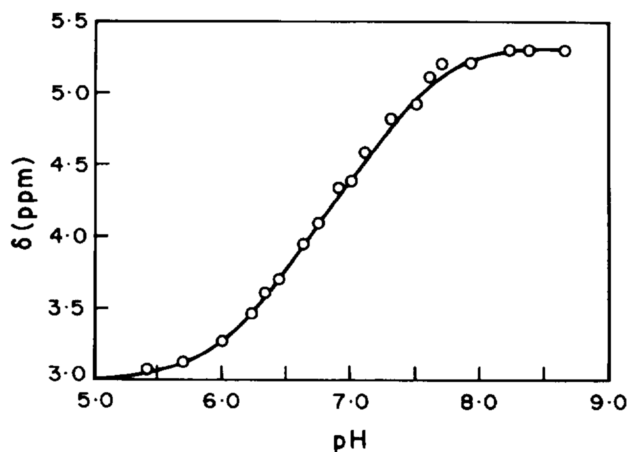


Fig. 1. pH-dependent chemical shift of Pi at 5°C. The medium used was DMEM and it contained 10 mM of Pi.

way analysis of variance and considered significant if $P < 0.05$.

## RESULTS

Preliminary experiments were carried out to determine the stability of the $^{31}P$ NMR spectra of the uteri recorded at various temperatures. The results indicated that the spectra of hamster uteri remained stable when recorded at 5°C for 2 h or more. But, at 25 and 37°C the spectra were stable for about an hour. Hence, in all the experiments described here spectra were recorded at 5°C.

### *$^{32}P$ NMR spectra of uteri from adult hamsters during the estrous cycle*

A typical $^{31}P$ NMR spectra of uteri excised from an adult hamster is shown in Fig. 2. The NMR signals were assigned based on the chemical shifts of the pure compounds at the same pH as the tissue and by the enhancement of the intensity of a particular spectral line following the addition of a standard compound to the sample. The main signals in the spectra were due to sugar phosphate (SP) or phosphomonoester (PME at 6.50 ppm), inorganic phosphate (Pi at 5.00 ppm), glycerophosphocholine (GPC at 2.75 ppm), creatine phosphate (PCr at −0.45 ppm) and the τ, α and β peaks of ATP (at −2.65, −7.50 and −16.50 ppm, respectively).

Figure 3 shows $^{31}P$ NMR spectra of uteri of adult hamsters in various stages of the estrous cycle. The uteri used from the various animals were normalized with respect to a constant weight and all other instrumental conditions so as to facilitate comparison of the spectra and calculation of the concentration of the metabolites. The results (Table 1) indicate that the concentrations of PME and Pi remain similar all through the estrous cycle. But GPC levels were very low and the concentration did not vary significantly during the estrous cycle. PCr levels were similar in the diestrus and proestrus animals but showed a significant increase in the estrus animals. As compared to the above metabolites ATP concentrations which were low in diestrus animals increased significantly in the uteri of estrus hamsters.

### *$^{31}P$ NMR spectra of uteri from OVX hamsters*

The variations in the levels of phosphometabolites observed in the uteri of hamsters during the estrous cycle may reflect variations induced due to changes in the intrinsic hormonal levels of the animals. In order to investigate this aspect $^{31}P$ NMR spectra of the uteri of adult female hamsters following ovariectomy and injection with steroid hormones were studied. The OVX animals showed seven distinct peaks corresponding to PME, Pi, GPC, PCr and the γ, α and β peaks of ATP. OVX animals following treatment with estradiol showed significant increase in the concentrations of PCr and ATP (Table 2). However, such increases in metabolite concentrations were not observed following treatment of OVX animals with either progesterone or testosterone. In fact, progesterone significantly decreased the amount of GPC in the uteri of OVX animals when compared to the respective controls.

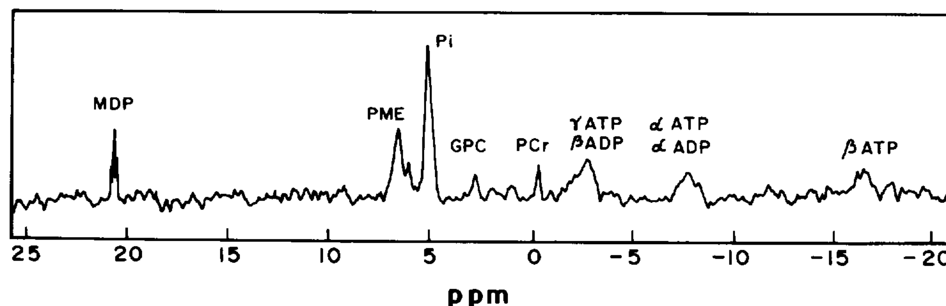


Fig. 2. A typical $^{31}P$ NMR spectrum of an uterus from an adult hamster recorded at 5°C in DMEM. MDP, methylene diphosphoric acid; PME, phosphomonoesters; Pi, inorganic phosphate; GPC, glycerophosphocholine; PCr, phosphocreatine; ADP, adenosine diphosphate; ATP, adenosine triphosphate. The $X$-axis represents the chemical shift (ppm) and the $Y$-axis represents the signal intensity.

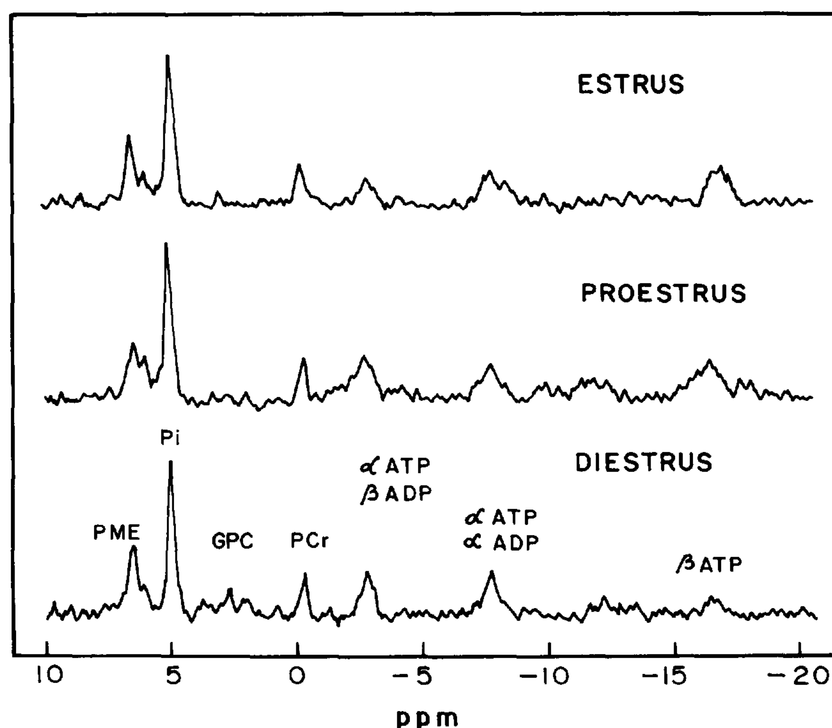


**Fig. 3. $^{31}P$ NMR spectra of uteri of adult hamsters in diestrus, proestrus and estrus phase of the estrous cycle. The spectra were recorded at 5°C in DMEM keeping the wet weight (220 mg) of the uteri constant. For other details refer to Fig. 2.**

Testosterone did not significantly alter the levels of PME, GPC, PCr and ATP in the uteri of OVX animals as compared to the controls. The levels of Pi remained significantly unchanged in OVX animals treated either with estradiol or progesterone. However, OVX animals injected with testosterone showed a decrease in the level of Pi.

*$^{31}P$ NMR spectra of uteri from immature hamsters*

PME, Pi, PCr and ATP were detectable in the $^{31}P$ NMR spectra of uteri of immature hamsters (Fig. 4) but GPC was not clearly visible. Estradiol administration significantly increased the concentration of ATP but did not bring about any significant change in

*Table 1. Concentration of phosphorus containing metabolites in the uteri of adult hamsters during the various phases of the estrous cycle*

| Phase of the estrous cycle | $n$ | Concentration of metabolites (mmol ± SEM/kg wet weight of uterus) | | | | |
|---|---|---|---|---|---|---|
| | | PME | Pi | GPC | PCr | ATP |
| Diestrus | 5 | 7.25 ± 0.7* | 5.95 ± 0.7* | 1.05 ± 0.3* | 2.55 ± 0.5* | 4.12 ± 0.7* |
| Proestrus | 4 | 6.30 ± 0.7* | 5.75 ± 0.7* | 0.95 ± 0.2* | 2.02 ± 0.2* | 5.73 ± 1.0*† |
| Estrus | 6 | 7.18 ± 0.7* | 5.54 ± 0.5* | 1.08 ± 0.3* | 4.23 ± 0.7† | 6.98 ± 1.3† |

Within each vertical row different superscripts (* or †) indicate that the means are significantly different ($P < 0.05$). Spectra were recorded keeping the wet weight of the uteri constant (220 mg) in all the experiments. $n$, represents the number of times the experiment was done.

*Table 2. Concentration of phosphorus containing metabolites in the uteri of adult OVX hamsters following treatment with estradiol, progesterone and testosterone*

| Treatment | $n$ | Concentration of the metabolites (mmol ± SEM/kg wet weight of uterus) | | | | |
|---|---|---|---|---|---|---|
| | | PME | Pi | GPC | PCr | ATP |
| OVX | 4 | 8.49 ± 0.4*† | 4.13 ± 0.5* | 1.70 ± 0.3* | 2.40 ± 0.6* | 6.33 ± 1.0* |
| OVX + estradiol | 4 | 9.82 ± 0.9* | 5.43 ± 0.4* | 1.71 ± 0.2* | 4.13 ± 0.3† | 8.74 ± 0.9† |
| OVX + progesterone | 3 | 7.89 ± 0.7*† | 4.76 ± 0.5* | 1.05 ± 0.2* | 2.02 ± 0.2* | 6.33 ± 1.1* |
| OVX + testosterone | 3 | 7.10 ± 0.6† | 2.03 ± 0.3† | 1.10 ± 0.2† | 2.83 ± 0.7* | 5.14 ± 1.5* |

* Within each vertical row different superscripts (* or †) indicate that the means are significantly different ($P < 0.05$). Spectra were recorded keeping the wet weight of the uteri constant (220 mg) in all the experiments. $n$, represents the number of times the experiment was done.

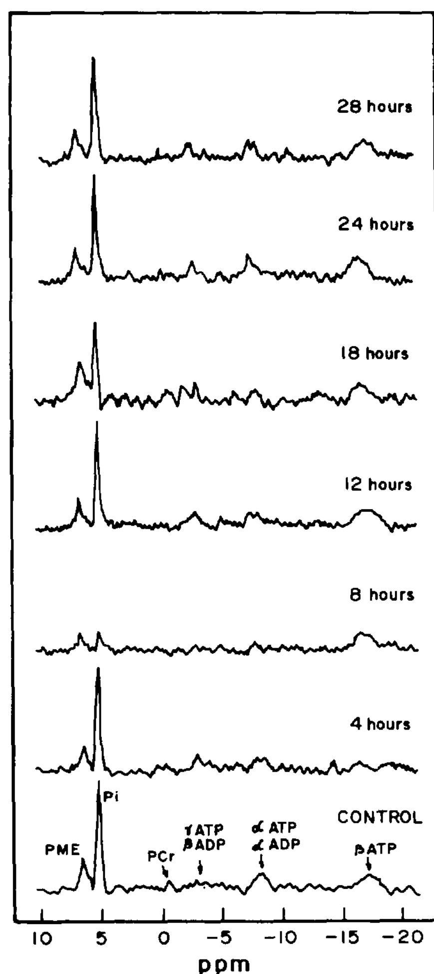


Fig. 4. $^{31}P$ NMR spectra of uteri of immature hamsters at 0 to 28 h after estradiol injection. The spectra were recorded at 5°C in DMEM, keeping the wet weight (180 mg) of the uteri constant. For other details refer to Fig. 2.

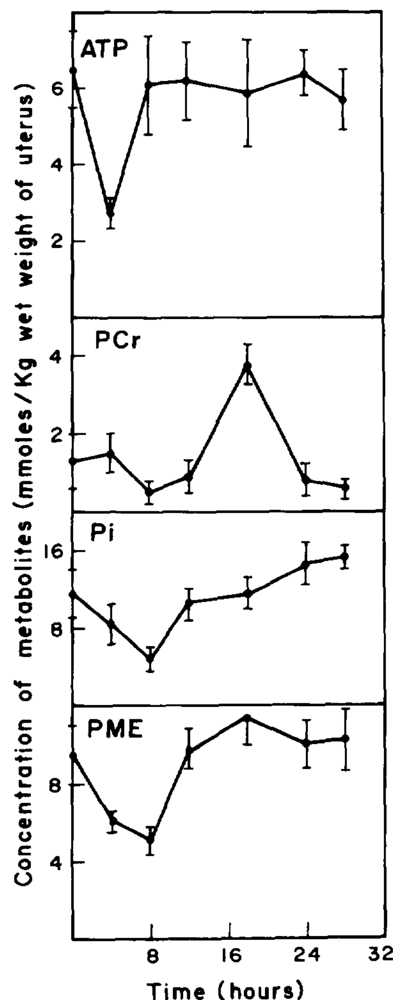


Fig. 5. Changes in the concentrations of PME, Pi, PCr and ATP in the uteri of immature hamsters following injection of estradiol. Calculations on the concentrations of the metabolites is based on spectra which were recorded at 5°C in DMEM keeping the wet weight (180 mg) of the uteri constant.

the concentration of the other metabolites when compared to the untreated immature animals (Table 3). Progesterone and testosterone also did not increase or decrease the concentrations of these metabolites in the uteri of immature hamsters.

In a separate experiment the $^{31}P$ NMR spectra of uteri from immature hamsters at various time points (from 0 to 28 h) after estradiol injection were also recorded (Figs 4 and 5). The results indicated that PME, Pi and PCr concentrations declined upto 8 h and later rose and exceeded control levels by 12 to 18 h (Fig. 5). Subsequently, the concentrations of PME, Pi and PCr remained at levels similar to the untreated immature female hamsters. The concentration of ATP decreased significantly within 4 h following estradiol administration. But, subsequently, by 8 h it reached the

*Table 3. Concentration of phosphorus containing metabolites in the uteri of immature hamsters following treatment with estradiol, progesterone and testosterone*

| Treatment | $n$ | Concentration of the metabolites (mmol ± SEM/kg wet weight of uterus) | | | |
|---|---|---|---|---|---|
| | | PME | Pi | PCr | ATP |
| Immature | 4 | 10.03 ± 1.3*† | 6.50 ± 0.7* | 1.49 ± 0.4* | 4.62 ± 0.5* |
| Immature + estradiol | 5 | 11.98 ± 1.7* | 6.16 ± 0.6* | 2.49 ± 0.8* | 6.89 ± 0.6† |
| Immature + progesterone | 6 | 9.65 ± 1.2*† | 5.37 ± 0.6* | 1.95 ± 0.4* | 4.94 ± 0.9* |
| Immature + testosterone | 7 | 8.11 ± 0.8*† | 6.25 ± 0.6* | 1.88 ± 0.3* | 5.1 ± 0.9* |

*Within each vertical row different superscripts (* or †) indicate that the means are significantly different ($P < 0.05$). Spectra were recorded keeping the wet weight of the uteri constant (180 mg) in all the experiments. $n$, represents the number of times the experiment was done.

control level and remained significantly unchanged until 28 h.

*Intracellular pH*

The pH dependent chemical shift of Pi in DMEM is shown in Fig. 1. The intracellular Pi peak of hamster uteri was identified by the specific enhancement of the peak on addition of Pi to the NMR tube in the presence of the uteri. The chemical shift of Pi in hamster uteri indicates that its internal pH is about 7.4. The intracellular pH of the uterus was independent of the estrous cycle and it was found to be $7.38 \pm 0.02$, $7.40 \pm 0.03$ and $7.37 \pm 0.02$ in the uterus of diestrus, proestrus and estrus animals, respectively.

## DISCUSSION

$^{31}$P NMR spectroscopy has been used effectively to ascertain the phosphorus containing metabolites in the intracellular milieu of a number of cells and organs [9] such as urinary bladder [16], testis [17], smooth muscle, brain and heart [18, 19], liver [20], human eye lens [21], placenta [11], red blood cells [22], algal cells [23], sperm cells [24], sea urchin eggs [25] and embryos of *Xenopus laevis* [26]. In all these organs and cell types the predominant phosphometabolites were identified as sugar phosphates or phosphomonoester (PME), inorganic phosphate (Pi), creatine phosphate (PCr), glycerophosphocholine (GPC) and the $\gamma$, $\alpha$ and $\beta$ phosphate peaks of ATP. Further, it was also consistently observed that the spectral peaks of the various metabolites were broad but distinct when whole tissue, organs or cells were used for NMR. However, whole tissue, organs or cells were preferred for $^{31}$P NMR and not PCA extracts because the latter method was not consistent and was also responsible for the partial degradation of labile compounds like ATP [13, 14]. Hence, in the present study using whole uteri of hamster distinct but broad spectral peaks corresponding to the above phosphometabolites were identified based on their chemical shifts and on comparison with similar NMR data of uteri of rat [11, 13, 14] and rabbit [16]. Recent studies have indicated that the PME signal is a composite peak consisting of phosphoethanolamine (leftmost peak) and phosphocholine (right peak) [12] as visible in Fig. 2 of the present study. The NMR signals were also assigned based on the chemical shifts of the pure compounds recorded under similar conditions as that of the tissue and by specific enhancement studies where increase in the intensity of a particular spectral line was monitored following the addition of a standard compound to the NMR tube containing the tissue. Further, perchloric acid extracts of the uterine horns of hamster also indicated the presence of the above phosphorus containing metabolites (data not shown). Since whole uteri were used in the present investigation, the NMR signals represent metabolites of the whole uteri inclusive of the endometrium and myometrium. Attempts were not made to separate the endometrium and myometrium because such a manipulation would have resulted in the breakdown of metabolites [9]. It is for this reason that earlier studies in rat and rabbit were also carried out with intact whole uteri and preferably at low temperatures [13, 14, 16] as in the present study. The concentrations of PME, GPC, PCr and ATP in the uteri of hamsters are comparable to that reported earlier for the uteri of rat and rabbit [13, 14, 16]. Our own experiments using rat uteri also revealed spectral peaks corresponding to PME, Pi, PCr, GPC and ATP and the concentrations of these metabolites in adult rat uteri (data not shown) were similar to the values reported by Dawson and Wray [13] and Degani *et al.* [14].

The hamster exhibits an invariant 4 day estrous cycle and thus can be used to study biochemical changes during the estrous cycle and to correlate these changes with the existing levels of steroid hormones. The significant increase in PCr and ATP levels in the uteri of estrus animals when compared to the diestrus animals could be attributed to the increased levels of estradiol in the peripheral circulation [27] observed in estrus animals. In contrast, PME, Pi and GPC levels did not exhibit any significant change during the estrous cycle. Such studies on changes in the levels of phosphate containing metabolites in the uteri of mammals during the estrous cycle have not been done earlier thus precluding the possibility of a comparison. The deduction that the increase in PCr and ATP levels during estrus is due to estradiol is supported by the fact that immature rats following estradiol injection also exhibited an increase in these two metabolites [28]. This would also imply an increase in PCr and ATP concentrations in the uteri of OVX animals treated with estradiol. The present study does indeed show such increases and is in agreement with an earlier observation that in the uteri of OVX rats a 90% increase in PCr was observed [28] following estradiol administration. Previous studies have also clearly demonstrated that alterations in levels of phosphometabolites in rats during pregnancy and parturition were under hormonal control [13, 14]. The decrease in the phosphometabolites immediately after estrus and during diestrus may be attributed to an increase in the level of progesterone.

In hamster, as in other mammals, ovulation coincides with estrus and an increased level of progesterone which peaks at this time. Increase in progesterone also results in an abrupt decline in estradiol level [27]. Hence, increase in phosphometabolites may also be due to influence of progesterone. In order to ascertain this effect of progesterone, it would be best to evaluate the levels of phosphometabolites in animals which were under the influence of progesterone alone and in the total absence of estradiol. For this purpose adult OVX animals were injected with progesterone but the results

indicated that progesterone by itself did not increase the concentration of PCr and ATP as observed in estrus or OVX animals treated with estradiol. The male steroid hormone, testosterone also did not increase the level of PCr and ATP in OVX animals when compared to the untreated, control OVX animals although, the testosterone treated animals did show a decrease in Pi levels probably due to reduced utilization of ATP and PCr.

Earlier studies in rats using perchloric acid extracts of frozen uteri and intact frozen uteri had indicated a decrease in ATP concentration within 4 h of estradiol administration [8, 14]. Our NMR experiments with uteri of immature hamsters also indicated a decrease in ATP concentration. In addition, a substantial decrease in PME, Pi and PCr levels was also observed by 8 h. These three metabolites were also demonstrated to decrease in concentration in the uteri of immature rats following estradiol administration [14]. The decrease in ATP is probably a reflection of the increased utilization of ATP needed for the increased rate of synthesis of RNA and phospholipids which occurs 4 h after estradiol administration [29, 30]. The subsequent decrease in PCr after 8 h in hamster (present investigation) and after 6 h in rat [14] is probably due to its conversion to ATP as evidenced by an increased creatine kinase activity during this period [14]. However, after 8 h of estradiol administration, ATP concentration was found to increase to control level and remain significantly unchanged till 32 h. This accumulation of ATP may not be only due to conversion of PCr to ATP because PCr also showed an increase in concentration between 12 to 18 h. In fact, based on similar observations in estradiol treated immature rats Degani *et al.* [14] proposed that certain processes (which are as yet unknown) are initiated 12 to 24 h after estradiol administration to enhance ATP accumulation in the uteri of rats. The PME signal is due to several phosphomonoesters and the decline in concentration observed after 8 h of estradiol administration coincides with the decrease observed in the levels of PCr and Pi. It is difficult to speculate the reason for this decline but it may be worth mentioning that estradiol administration is known to deplete the sugar phosphate pool in the uterus [31].

Very few measurements have been made on the intracellular pH of the uterus. In the hamster, the internal pH of the uteri in the diestrus, proestrus and estrus animals were around 7.4 at 5°C when suspended in a buffer of pH 7.2. Earlier measurements had indicated that the intracellular pH of the rat uterus was 7.08 at 37°C and 7.0 at 18°C. In the former study the tissue was bathed in a buffer of pH 7.3 and in the later in a buffer of pH 7.4 [13, 32]. In both these cases the tissue was maintained in the presence of 5% $CO_2$. Thus it appears that the internal pH of hamster uterus is slightly higher than that reported for the rat uterus.

## CONCLUSIONS

This study demonstrates that as in rat, rabbit and man the main phosphometabolites in hamster uteri are PME, Pi, GPC, PCr and ATP. It also indicates that the concentration of phosphorus containing metabolites in the uteri of adult hamsters varies depending on the phase of the estrous cycle, and that a significant increase in PCr and ATP in the estrus animals is probably due to estradiol. In fact, immature female hamsters and OVX adult hamsters following estradiol administration also exhibited an increase in the concentrations of PCr and ATP, thus confirming the role of estradiol in the induction of synthesis of these metabolites. Besides, progesterone and testosterone did not increase the concentration of the phosphorus containing metabolites. It was also observed that the intracellular pH of the hamster uterus was 7.4 and it did not vary during the estrous cycle.

*Acknowledgements*—Our thanks to Drs R. Nagaraj and M. K. Ray for their comments and useful suggestions. L. Girija Devi was a recipient of a fellowship from the Council for Scientific and Industrial Research, India.

## REFERENCES

1. Edgren R. A. and Calhoun D. W.: Estrogen antagonisms: inhibition of estrone induced uterine growth by testosterone propionate, progesterone and 17-ethyl-19-nortestosterone. *Soc. Exp. Biol. Med. Proc.* **94** (1957) 537–539.
2. Tantayaporn P., Mallikarjuneswara V., DeCarlo S. and Clemetson C.: The effects of estrogen and progesterone on the volume and electrocyte content of the uterine luminal fluid of the rat. *Endocrinology* **95** (1974) 1034–1045.
3. Hall J. L., Stephenson R. B., Mathias C. A. and Brackett B. G.: Hormonal dependence of cyclic patterns in hamster uterine fluid proteins. *Biol. Reprod.* **17** (1977) 738–744.
4. Surani M. A. H.: Qualitative and quantitative examination of the proteins of rat uterine luminal fluid during proestrus and pregnancy and comparison with those of serum. *J. Reprod. Fertil.* **50** (1977) 281–287.
5. Teng C. T., Walker M. P., Bhattacharya S. N., Klapper D. G., D'Augustine R. P. and McLachlan J. A.: Purification and properties of an oestrogen-stimulated mouse uterine glycoprotein (approx. 70 kDa). *Biochem. J.* **240** (1986) 413–422.
6. Murray M. K. and Sower S. A.: Estrogen and progesterone-dependent secretory changes in the uterus of the sheep. *Biol. Reprod.* **47** (1992) 917–924.
7. Kaye A. M., Sheratzky D. and Lindner H. R.: Kinetics of DNA synthesis in immature rat uterus: age dependence and estradiol stimulation. *Biochim. Biophys. Acta,* **261** (1972) 475–486.
8. Oliver J. M. and Kellie A. E.: The effects of oestradiol on the acid-soluble nucleotides of rat uterus. *Biochem. J.* **119** (1970) 187–191.
9. Gadian D. G. and Radda G. K.: NMR studies of tissue metabolites. *A. Rev. Biochem.* **50** (1981) 69–83.
10. Barany M. and Glonek T.: Phosphorus-31 nuclear magnetic resonance of contractal system. *Metab. Enzymol.* **85** (1982) 624–676.
11. Dawson M. J., McFarlane D. K., McFarlin B. L., Noyszewski E. A. and Trupin S. R.: The biochemistry of female reproductive tissues studied by $^{31}$P phosphorus nuclear magnetic resonance spectroscopy: effects of pregnancy, hormonal regulation and disease. *Biol. Reprod.* **38** (1988) 31–38.
12. Dawson M. J. and Raman J.: Uterine Metabolism and energetics. In: *Uterine Function. Molecular and Cellular Aspects* (Edited by M. E. Carsten and J. D. Miller) Plenum Press, NY (1990) pp. 35–70.

13. Dawson M. J. and Wray S.: Changes in metabolites containing phosphorus in rat uterus following parturition. *J. Physiol.* **368** (1985) 19–31.
14. Degani H., Shaer A., Victor T. A. and Kaye A. M.: Estrogen induced changed in high energy phosphate metabolism in rat uterus: $^{31}P$ NMR studies. *Biochemistry* **23** (1984) 2572–2577.
15. Devi L. G., Ahmad M. B. and Shivaji S.: Secretory proteins of the hamster cervix, uterus and oviduct: the effects of estradiol, progesterone and testosterone on the proteins secreted into the medium. *J. Steroid. Biochem. Molec. Biol.* **51** (1994) 107–114.
16. Kushmerick M. J., Dilon P. F., Meyer R. A., Brown T. R., Krisanda J. M. and Lee Sweeney H.: $^{31}P$ NMR spectroscopy, chemical analysis and free $Mg^{2+}$ of rabbit bladder and uterine smooth muscle. *J. Biol. Chem.* **261** (1986) 14,420–14,429.
17. Van der Grond J., Dijkstra G., Roelofsen B. and Mali W. P. T. H.: $^{31}P$ NMR determination of phosphomonoesters in relation to phospholipid biosynthesis in testis of the rat at different ages. *Biochim. Biophys. Acta* **1074** (1991) 189–194.
18. Ackerman J. J. H., Grove T. H., Wong G. G., Gadian D. G. and Radda G. K.: Mapping of metabolites in whole animals by $^{31}P$ NMR using surface coils. *Nature* **283** (1980) 167–170.
19. Grove T. H., Ackerman J. J. H., Radda G. K. and Bore P. J.: Analysis of rat heart *in vivo* by phosphorus nuclear magnetic resonance. *Proc. Natn. Acad. Sci. U.S.A.* **77** (1980) 299–302.
20. Murphy E. J., Brindle K. M., Rorison C. J., Dixon R. M., Rajagopalan B. and Radda G. K.: Changes in phosphatidyl ethanolamine metabolism in regenerating rat liver as measured by $^{31}P$ NMR. *Biochim. Biophys. Acta* **1135** (1992) 27–34.
21. Merchant T. E., Lass J. H., Meneses P., Greiner J. V. and Glonek T.: The effects of age on phosphatic metabolites of the human crystalline lens. *Exp. Eye Res.* **52** (1991) 641–646.
22. Moon R. B. and Richards J. H.: Determination of intracellular pH by $^{31}P$ magnetic resonance. *J. Biol. Chem.* **248** (1973) 7276–7278.
23. Bental M., Pick U., Avron M. and Degani H.: Polyphosphate metabolism in the alga *Dunalcella salina* studied by $^{31}P$ NMR. *Biochim. Biophys. Acta* **1092** (1991) 21–28.
24. Phadke R. S., Srivastava S. and Govil G.: Magnetic resonance methods for studying intact spermatozoa. *J. Biosci.* **15** (1990) 125–134.
25. Winkler M. W., Matson G. B., Hershey J. W. B. and Bradbury M. E.: $^{31}P$ NMR study of the activation of the sea urchin egg. *Exp. Cell Res.* **139** (1982) 217–222.
26. Colman A. and Gadian D. G.: $^{31}P$ nuclear magnetic resonance studies on the developing embryos of *Xenopus laevis*. *Eur. J. Biochem.* **61** (1976) 387–396.
27. Saidapur S. K. and Greenwald G. S.: Peripheral blood and ovarian levels of sex steroids in the cyclic hamster. *Biol. Reprod.* **18** (1978) 401–408.
28. Volfin P., Clauser H. and Gautheron D.: Influence of estradiol and progesterone injections on the acid-soluble phosphate fractions of the rat uterus. *Biochim. Biophys. Acta* **24** (1957) 137–140.
29. Gorski J. and Nicolette J.: Early estrogen effects on newly synthesised RNA and phospholipid subcellular fractions of rat uteri. *Arch. Biochem. Biophys.* **103** (1963) 418–423.
30. Aizawa H. and Mueller G. C.: The effect of *in vivo* and *in vitro* of estrogens on lipid synthesis in the rat uterus. *J. Biol. Chem.* **236** (1961) 381 –386.
31. Smith D. E. and Gorski J.: Estrogen control of uterine glucose metabolism: an analysis based on the transport and phosphorylation of 2 deoxyglucose. *J. Biol. Chem.* **243** (1968) 4169–4174.
32. Dawson M. J. and Wray S.: $^{31}P$ nuclear magnetic resonance (NMR) studies of isolated rat uterus. *J. Physiol.* **366** (1983) 19–20.