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A Possible Role for 5-Phosphoribosyl 1-Pyrophosphate in the Stimulation of Uterine Purine Nucleotide Synthesis in Response to Oestradiol-17β

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1. It has been reported that the rate of purine nucleotide synthesis *de novo* in the immature rat uterus is doubled at 6h after administration of oestradiol-17β. The present work confirms an increased incorporation of glycine and adenine into uterine nucleotides between 2 and 6h after hormone treatment and investigates the mechanism of this response. 2. Activation of regulatory enzymes is unlikely to promote increased nucleotide synthesis: the activities of 5-phosphoribosyl 1-pyrophosphate amidotransferase (EC 2.4.2.14) and adenine phosphoribosyltransferase (EC 2.4.2.7) are the same in uterine extracts from control and oestrogen-treated rats. 3. Therefore it was proposed that oestradiol might promote an increased supply of a rate-limiting substrate. The low oestrogen-sensitive rate of AMP synthesis from adenine and endogenous 5-phosphoribosyl 1-pyrophosphate in the intact uterus compared with the high, oestrogen-insensitive rate in uterine extracts supplemented with 5-phosphoribosyl 1-pyrophosphate is evidence that the supply of 5-phosphoribosyl 1-pyrophosphate limits purine nucleotide formation and may increase after hormone treatment. This proposal is supported by the decrease in AMP synthesis in the whole tissue in the presence of guanine and 7-amino-3-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (formycin). These compounds do not inhibit adenine uptake or adenine phosphoribosyltransferase activity, but they both decrease the availability of 5-phosphoribosyl 1-pyrophosphate, the former by promoting its utilization by hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8) and the latter by inhibiting its synthesis from ribose 5-phosphate and ATP by ribose 5-phosphate pyrophosphokinase (EC 2.7.6.1). 4. It is unlikely that the increased availability of 5-phosphoribosyl 1-pyrophosphate results from hormonal stimulation of ribose 5-phosphate formation. Methylene Blue and phenazine methosulphate both increase ribose 5-phosphate without altering the supply of 5-phosphoribosyl 1-pyrophosphate. 5. The activity of ribose 5-phosphate pyrophosphokinase is low in uterine extracts and increases rapidly in response to oestradiol. Therefore the hormonal activation of the routes of purine nucleotide synthesis both *de novo* and from preformed precursors may be due, at least in part, to an increased availability of the common rate-limiting substrate 5-phosphoribosyl 1-pyrophosphate, mediated by activation of ribose 5-phosphate pyrophosphokinase.

There are two alternative pathways of purine nucleotide synthesis in most mammalian cells: synthesis *de novo* from non-purine precursors (5-phosphoribosyl 1-pyrophosphate, glutamine, glycine, formate and aspartate) and synthesis from preformed bases and 5-phosphoribosyl 1-pyrophosphate by adenine phosphoribosyltransferase (EC 2.4.2.7) and hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8). These pathways have been described in detail by Hartman (1970). Some controversy exists as to whether these synthetic routes are controlled by enzyme activities or by the supply of essential substrates. It has been proposed that the synthesis of purines *de novo* may be regulated by the amount and catalytic activity of 5-phosphoribosyl 1-pyrophosphate amidotransferase (EC 2.4.2.14); this is the first obligate enzyme of the multienzyme pathway and it is subject to strict feedback control by purine nucleotides (Henderson & Khoo, 1965*c*; Hill & Bennett, 1969; Murray, 1971). Recent evidence suggests that the availability of 5-phosphoribosyl 1-pyrophosphate, a substrate for both routes of purine nucleotide synthesis as well as for synthesis of the ribonucleotides of orotic acid, nicotinic acid, nicotinamide, imidazolylacetic acid, quinolinic acid and histamine (for references see Henderson & Khoo, 1965*a*), may also

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influence the rate of purine nucleotide formation. For example, competition between the two purine nucleotide-synthetic pathways and the route of pyrimidine nucleotide synthesis for 5-phosphoribosyl 1-pyrophosphate for has been demonstrated in a range of mammalian cells and tissues (Rajalakshmi & Handschumacher, 1968; Marko *et al.*, 1969; Kelley *et al.*, 1970), and Henderson & Khoo (1965*a*,*b*) have shown that nucleotide synthesis from purine bases is limited by the availability of 5-phosphoribosyl 1-pyrophosphate in cells of the Ehrlich ascites carcinoma. Further, mutant cell lines exist, which are characterized by elevated amounts of 5-phosphoribosyl 1-pyrophosphate, accompanied by a large increase in purine synthesis *de novo* (Rosenbloom *et al.*, 1968), and Fausto (1969) has shown that an increased supply of 5-phosphoribosyl 1-pyrophosphate accompanies the increased synthesis of pyrimidine nucleotides in regenerating rat liver.

The present work examines the control of purine nucleotide synthesis in the immature rat uterus. Since nucleotide synthesis is increased in this tissue during the first 6h after administration of oestradiol (Jervell *et al.*, 1958), it was proposed that regulatory factors might be identified from differences in the activities of enzymes and/or in the supply of substrates between uteri from control and hormone-treated animals.

Experimental

Oestradiol [5μg in 0.1ml of 20% (v/v) ethanol in 0.9% NaCl] or vehicle alone was administered by intraperitoneal injection to immature (40–50g) female rats of the Wistar strain. The animals were killed by cervical dislocation at various times after hormone treatment and their uteri were removed.

Incubation system in vitro

Uteri were incubated in Fischer's medium (Grand Island Biological Co., Grand Island, N.Y., U.S.A.). Portions (3–5ml) of this chemically defined medium (Fischer & Sartorelli, 1964) were equilibrated in incubation vessels sealed with airtight rubber stoppers by gassing for approx. 20min at 37°C with O_2+CO_2 (95:5) before the addition of tissue. Radioactive substrates were injected through the rubber stoppers at approx. 5min after introduction of uteri. At the end of the incubation period the uteri were removed, rinsed briefly in ice-cold 0.9% NaCl, blotted dry and frozen in liquid N_2. Acid-soluble compounds were extracted and the ribonucleotide fraction was isolated as previously described (Oliver & Kellie, 1970). The acid-insoluble material was dried as described by Reel & Gorski (1968) and the DNA content of the lipid-free powder was determined by the method of Giles & Meyers (1965).

The decarboxylation of D-[1-^{14}C]glucose was measured during incubation of groups of five uteri in sealed, centre-welled vessels containing equilibrated Fischer's medium (5ml) plus uteri in the outer well. The reaction was stopped by the injection of 10% (v/v) $HClO_4$ (1ml) into the outer well, and $^{14}CO_2$ was collected in Hyamine 10X hydroxide (1ml) in the centre well during a further 20min of shaking at 37°C and was counted for radioactivity.

Assay of 5-phosphoribosyl 1-pyrophosphate amidotransferase

Uteri were homogenized for 30s at 4°C in a solution containing 10mM-tris–HCl, pH7.5, 10mM-sodium phosphate, pH7.5, 5mM-$MgSO_4$, 5mM-NaF, 0.1mM-glycine and 0.25M-sucrose (0.1ml/uterus), by using a Silverson tissue mixer–emulsifier. The suspensions were centrifuged at 4°C for 10min at 9000*g* in an MSE 18 centrifuge, the supernatant volumes were noted and 0.1ml portions of these solutions were immediately incubated with shaking at 30°C in 0.2ml of the same buffer containing 5-phosphoribosyl 1-pyrophosphate (0.4μmol) and L-[U-^{14}C]glutamine (22nmol; 0.1μCi). Control incubations contained no 5-phosphoribosyl 1-pyrophosphate. The reaction was stopped by placing the incubation tubes in a boiling-water bath for 1min, then they were cooled in ice and centrifuged at 4°C for 10min. Portions (50μl) of the supernatant solutions were applied across strips (2.5cm wide×20cm long) of MN300-cellulose-coated plastic chromatography sheets (Macherey, Nagel and Co., Düren, Germany) and the chromatograms were developed in propan-2-ol–99% (v/v) formic acid–water (20:1:5, by vol.). Reference strips were dipped into a solution of 1% (w/v) ninhydrin–0.1% (v/v) pyridine in acetone and heated to develop the characteristic blue colour of the amino acids. The areas corresponding to glutamine and glutamate on the remaining strips were cut out and transferred into counting vials, toluene scintillation fluid [0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene] was added and radioactivity was measured in a Packard model 3375 liquid-scintillation spectrometer.

Assay of adenine phosphoribosyltransferase

Uteri were homogenized in a solution containing 0.2M-tris–HCl buffer, pH7.4, 5mM-$MgSO_4$ and 1mM-GSH (0.1ml/uterus) and then were centrifuged at 9000*g* for 10min. Portions (0.1ml) of the supernatant solutions were incubated at 30°C for 10min with 0.2ml of the same buffer containing [8-^{14}C]-adenine (0.2μmol; 0.4μCi) and 5-phosphoribosyl 1-pyrophosphate (1.25μmol). Control incubations contained no 5-phosphoribosyl 1-pyrophosphate. The reaction was stopped by addition of 10% (v/v) $HClO_4$ (0.1ml) and the suspensions were centrifuged.

After neutralization of the supernatant solutions with 1M-KOH–0.1M-tris solution, residual adenine was removed by six replicate extractions with water-saturated butan-1-ol (1ml) as described by Henderson & Hori (1966). The organic phases were discarded and the aqueous phases, containing nucleotides, were counted for radioactivity in Bray's (1960) scintillation fluid.

Assay of ribose 5-phosphate pyrophosphokinase

Uteri were homogenized in a solution containing 50mM-sodium phosphate, pH7.6, 10mM-tris–HCl, pH7.6, 5mM-$MgSO_4$, 5mM-NaF, 2mM-dithiothreitol and 0.25M-sucrose (0.1ml/uterus). The homogenates were centrifuged at 9000*g* for 10min and 0.1ml portions of the supernatant solutions were incubated at 30°C in 0.2ml of the same buffer containing [6-^{14}C]orotic acid (13nmol; 0.1μCi), ATP (1μmol), ribose 5-phosphate (0.4μmol) and an ATP-regenerating system consisting of phosphoenolpyruvate (0.4μmol) and Sigma lactate dehydrogenase Type I, containing pyruvate kinase (4μl) (Sigma Chemical Co., St. Louis, Mo., U.S.A.). Ribose 5-phosphate was omitted in control tubes. The reaction was stopped by placing the incubation tubes in a boiling-water bath for 1min, then they were cooled in ice and centrifuged. Portions (50μl) of the supernatant solutions were chromatographed in 0.05M-LiCl on strips (2.5cm wide × 20cm long) of PEI (polyethyleneimine)-cellulose-coated plastic sheets for t.l.c. (J.T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.) with markers of orotic acid and UMP. Areas containing the pyrimidine compounds were located under u.v. light, cut from the plates and counted for radioactivity in toluene scintillation fluid.

Results

Purine nucleotide synthesis in vitro

Jervell *et al.* (1958) have shown that the incorporation of glycine, formate and serine into the purine nucleotide pool of uteri from oestrogen-treated (6h) rats is markedly higher than in control uteri. The results summarized in Table 1 confirm that purine nucleotide synthesis *de novo* from glycine is stimulated within 2h after oestrogen treatment and continues to rise over 6h of hormone action. A similar increase in nucleotide formation from the preformed base, adenine, occurs during the first 6h after hormone treatment.

Enzymic responses to oestradiol

The enzymes considered to regulate nucleotide formation from glycine and adenine are, respectively, 5-phosphoribosyl 1-pyrophosphate amidotransferase, the enzyme that catalyses the conversion of glutamine and 5-phosphoribosyl 1-pyrophosphate into glutamate and phosphoribosylamine, and adenine phosphoribosyltransferase, the enzyme that catalyses AMP synthesis from adenine and 5-phosphoribosyl 1-pyrophosphate (Murray, 1971).

The activity of 5-phosphoribosyl 1-pyrophosphate amidotransferase was measured in uterine extracts from the rate of 5-phosphoribosyl 1-pyrophosphate-dependent deamination of glutamine. A similar procedure has been used to assay the enzyme in tumour cell extracts (Tay *et al.*, 1969; Hill & Bennett, 1969). Fig. 1 shows a linear rate of glutamate formation during a 10min incubation of uterine extracts with glutamine and 5-phosphoribosyl 1-pyrophosphate. The reaction is cumulatively inhibited by addition of AMP and GMP in the incubation mixture (Table 2); this is a characteristic regulatory property of 5-phosphoribosyl 1-pyrophosphate amidotransferase in mammalian cells (Murray, 1971). The results summarized in Table 3 show that pretreatment of animals between 0 and 6h with oestradiol does not change the activity of 5-phosphoribosyl 1-pyrophosphate amidotransferase in uterine extracts.

The activity of adenine phosphoribosyltransferase in uterine extracts, determined from the rate of nucleotide formation from adenine and 5-phosphoribosyl 1-pyrophosphate, is 5nmol/min per mg of

Table 1. *Synthesis of purine nucleotides in vitro*

Duplicate groups of six uteri from control and oestrogen-treated rats were incubated in Fischer's medium (4ml) containing [2-^{14}C]glycine (0.5μmol; 4μCi) or [8-^{14}C]adenine (1μmol; 1μCi) for 60min and 30min respectively. The acid-soluble compounds were extracted with 4% (v/v) $HClO_4$ and the nucleotide fractions were isolated from the neutralized perchlorate extracts and counted for radioactivity.

Time after oestradiol administration (h)	Ribonucleotide synthesis	
	[^{14}C]Glycine as substrate (nmol/60min per mg of DNA)	[^{14}C]Adenine as substrate (nmol/30min per mg of DNA)
0	2.56	47.5
2	4.22	63.5
4	4.79	76.8
6	5.04	83.6

DNA. This is much higher than the rate of AMP synthesis measured in the intact tissue (Table 1) and it does not increase in response to oestradiol.

Synthesis and supply of 5-phosphoribosyl 1-pyrophosphate

The rate of nucleotide synthesis from adenine and endogenous 5-phosphoribosyl 1-pyrophosphate in the intact uterus is low (47.5 nmol/30 min per mg of DNA in control tissue) and increases in response to oestradiol, whereas the rate measured in uterine extracts supplemented with 5-phosphoribosyl 1-pyrophosphate is higher (5 nmol/min per mg of DNA) and is unaltered after hormone treatment. Purine and pyrimidine bases enter uterine cells freely by the hormone-insensitive process of passive diffusion (Oliver, 1972) and so changes in adenine uptake are not responsible for the differences between rates of AMP synthesis and effects of oestradiol in intact uteri and tissue extracts. An alternative explanation is that the availability of 5-phosphoribosyl 1-pyrophosphate limits nucleotide synthesis in the uterus and that oestradiol promotes an increased supply of this substrate.

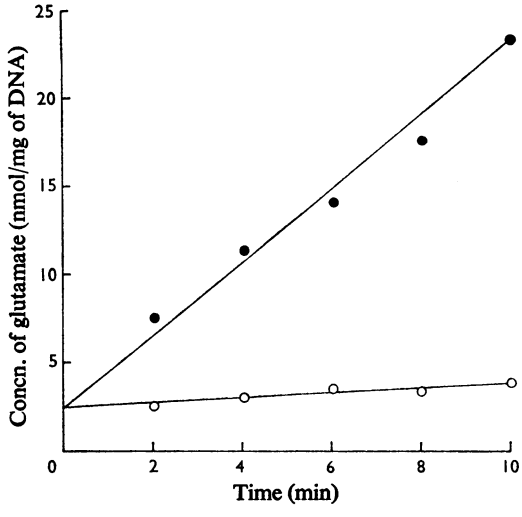

Fig. 1. *Activity of 5-phosphoribosyl 1-pyrophosphate amidotransferase in uterine extracts*

The 5-phosphoribosyl 1-pyrophosphate-dependent conversion of L-[^{14}C]glutamine into L-[^{14}C]glutamate was measured in duplicate in uterine extracts from groups of ten rats. These results correspond to a reaction rate of 1.9 nmol of glutamate/min per mg of DNA. ●, Complete incubation mixture; ○, 5-phosphoribosyl 1-pyrophosphate omitted from the incubation mixture.

The results summarized in Table 4 are supporting evidence that AMP synthesis is controlled by the supply of 5-phosphoribosyl 1-pyrophosphate. The presence of an equimolar concentration of guanine in the medium decreases nucleotide formation from adenine in the intact tissue by 20%. The uptake of guanine and its phosphoribosylation by hypoxanthine/guanine phosphoribosyltransferase is independent of the uptake and phosphoribosylation of adenine by adenine phosphoribosyltransferase. Therefore the inhibition of AMP synthesis must be due to competition between the two phosphoribosyltransferases for the limiting amounts of the common substrate, 5-phosphoribosyl 1-pyrophosphate. AMP synthesis is also decreased in the presence of formycin {7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine}. This *C*-glycoside analogue of adenosine does not affect the activity of adenine phosphoribosyltransferase in uterine extracts. However, Henderson and co-workers (Henderson *et al.*, 1967; Caldwell *et al.*, 1969) have shown that it is phosphorylated by adenosine kinase (EC 2.7.1.20) and inhibits the formation of 5-phosphoribosyl 1-pyrophosphate by ribose 5-phosphate pyrophosphokinase (EC 2.7.6.1). Therefore a continued synthesis of 5-phosphoribosyl 1-pyrophosphate is required to maintain AMP formation.

An increased rate of 5-phosphoribosyl 1-pyrophosphate synthesis in response to oestradiol could be

Table 2. *Activity of 5-phosphoribosyl 1-pyrophosphate amidotransferase in uterine extracts*

The enzyme was measured in duplicate in uterine extracts as described in Fig. 1. Purine nucleotides were present at 5 mM in the assay mixtures. The incubation period was 10 min.

Additions to the reaction mixture	5-Phosphoribosyl 1-pyrophosphate amidotransferase activity (nmol of glutamate/min per mg of DNA)
None	1.94
AMP	0.81
GMP	1.05
AMP+GMP	0.36

Table 3. *Effect of oestradiol on 5-phosphoribosyl 1-pyrophosphate amidotransferase*

The enzyme was measured in duplicate in uterine extracts from control and oestrogen-treated rats as described in Table 2.

Time after oestradiol administration (h)	5-Phosphoribosyl 1-pyrophosphate amidotransferase activity (nmol of glutamate/min per mg of DNA)
0	2.0
2	2.1
4	2.3
6	2.1

Table 4. *5-Phosphoribosyl 1-pyrophosphate-dependent nucleotide synthesis in the immature uterus*

Duplicate groups of three uteri were incubated for 30min in Fischer's medium (5ml) containing [^{14}C]adenine (0.55μmol; 0.2μCi). Residual adenine was removed from neutralized $HClO_4$ extracts by butan-1-ol extraction (Henderson & Hori, 1966) and the nucleotides were counted for radioactivity. The compounds indicated were present in the incubation mixtures for 30min before the introduction of [^{14}C]adenine.

Additions to the medium	Ribonucleotide synthesis (nmol/30min per mg of DNA)
None	50.0
Guanine (0.1mM)	39.2
Formycin (0.2mM)	41.1
Methylene Blue (0.1mM)	49.2
Phenazine methosulphate (0.22mM)	48.5
Phenazine methosulphate (0.22mM)+guanine (0.1mM)	38.7

Table 5. *Oxidative decarboxylation of* D-[1-^{14}C]*glucose*

Groups of five uteri were incubated in sealed centre-welled vessels in Fischer's medium (5ml) containing D-[1-^{14}C]glucose (27.5μmol; 0.1μCi). After 1h, 70% $HClO_4$ was added to the outer well and $^{14}CO_2$ was collected in the centre well in Hyamine 10X hydroxide.

Additions to the medium	$^{14}CO_2$ released (c.p.m./100mg wet wt. of tissue)
None	915
Methylene Blue (0.1mM)	3916
Phenazine methosulphate (0.22mM)	13742

due to activation of ribose 5-phosphate pyrophosphokinase and/or to an increased supply of its substrate, ribose 5-phosphate, resulting from hormonal activation of the pentose phosphate pathway of glucose metabolism (McKerns, 1967).

The results in Tables 4 and 5 indicate that an increased synthesis of ribose 5-phosphate is unlikely to have a direct effect on the availability of 5-phosphoribosyl 1-pyrophosphate in the immature rat uterus. Methylene Blue and phenazine methosulphate are electron acceptors that stimulate the oxidative branch of the pentose phosphate pathway by replenishing $NADP^+$ (Henderson & Khoo, 1965*a*,*b*; Hershko *et al.*, 1969). Although these compounds increase the conversion of [1-^{14}C]glucose into $^{14}CO_2$ by 4-fold and 16-fold respectively (Table 5), the resulting increase in the supply of ribose 5-phosphate does not alter the availability of 5-phosphoribosyl 1-pyrophosphate for nucleotide synthesis from adenine and it does not relieve the inhibition of AMP formation caused by the presence of guanine in the medium.

Ribose 5-phosphate pyrophosphokinase catalyses 5-phosphoribosyl 1-pyrophosphate synthesis from

ribose 5-phosphate and ATP. This enzyme was measured in uterine extracts by including orotic acid as well as ribose 5-phosphate and ATP in the incubation mixture. Orotate phosphoribosyltransferase (EC 2.4.2.10) converts orotate and 5-phosphoribosyl 1-pyrophosphate into OMP*, and the highly active, irreversible enzyme OMP decarboxylase converts OMP into UMP. The rate of 5-phosphoribosyl 1-pyrophosphate synthesis is measured from the rate of UMP formation. A linear rate of UMP synthesis occurs in uterine extracts (Fig. 2), which increases more than 4-fold to 2nmol of UMP/min per mg of DNA when 5-phosphoribosyl 1-pyrophosphate replaces ribose 5-phosphate in the incubation mixture. Therefore UMP synthesis is limited only by the synthesis of 5-phosphoribosyl 1-pyrophosphate and not by the activities of the coupled enzymes of pyrimidine metabolism.

*Abbreviation: OMP, orotidine monophosphate.

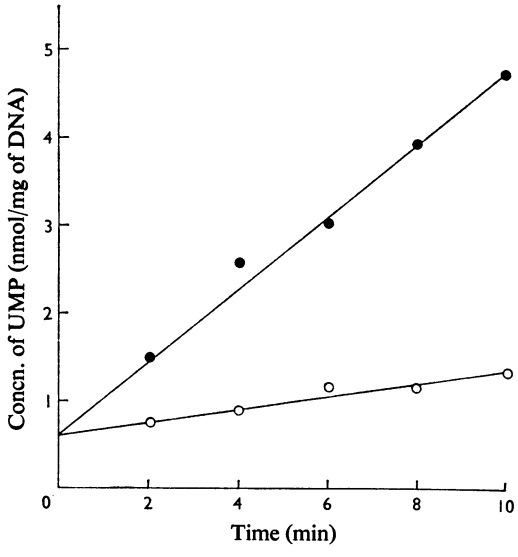

Fig. 2. *Activity of ribose 5-phosphate pyrophosphokinase in uterine extracts*

The enzyme was measured in duplicate in uterine extracts from groups of ten rats from the ribose 5-phosphate-dependent conversion of [6-^{14}C]orotic acid into UMP. These results correspond to a reaction rate of 0.31 nmol/min per mg of DNA. ●, Complete incubation mixture; ○, ribose 5-phosphate omitted from the reaction medium.

The results in Table 6 show that the rate of ribose 5-phosphate-dependent UMP synthesis is doubled within 2h after oestrogen treatment and continues to increase over 6h of hormone action. This indicates a rapid increase in ribose 5-phosphate pyrophosphokinase activity in response to oestradiol.

Discussion

Purine nucleotides are required as substrates, coenzymes and allosteric effectors for a wide range of oestrogen-sensitive processes in the immature rat uterus (see Segal & Scher, 1967). Therefore it is not surprising that the pathways of synthesis of these compounds both *de novo* and from preformed substrates is stimulated during the acute response (0–6h) of the immature rat uterus to oestradiol. The present work was undertaken to find the mechanism of this hormonal response.

When all of the required substrates are present, it is most likely that direct control of purine synthesis *de novo* is exerted at the first obligate step in the pathway, 5-phosphoribosyl 1-pyrophosphate amidotransferase (Murray, 1971). However, the increased synthesis of purines from glycine in the immature rat uterus is not accompanied by changes in the activity of this enzyme in uterine extracts. The activity of adenine phosphoribosyltransferase in uterine extracts is high and is also unaffected by oestradiol. Therefore the hormonal stimulation of purine nucleotide formation from glycine and adenine does not reflect changes in the potential activities of these enzymes. It is possible that changes in feedback inhibition, pH etc. may change

Table 6. *Effect of oestradiol on ribose 5-phosphate pyrophosphokinase*

The enzyme was measured in duplicate in uterine extracts from control and oestrogen-treated rats as described in Fig. 2. The incubation period was 10min.

Time after oestradiol administration (h)	Ribose 5-phosphate pyrophosphokinase activity (nmol of UMP/min per mg of DNA)
0	0.30
2	0.55
4	0.64
6	0.67

the proportion of these activities that are expressed *in vivo*; these factors cannot be evaluated from the present results.

An alternative explanation for the hormonal stimulation of purine nucleotide synthesis is that oestradiol may increase the supply of a rate-limiting substrate. 5-Phosphoribosyl 1-pyrophosphate was studied because this is the only substrate for nucleotide formation not freely available from Fischer's medium and because, in the absence of hormonal effects on adenine uptake and adenine phosphoribosyltransferase activity, an increased supply of 5-phosphoribosyl 1-pyrophosphate is the most likely explanation for the increased phosphoribosylation of adenine in the intact uterus after oestrogen treatment.

The proposal that the availability of 5-phosphoribosyl 1-pyrophosphate controls nucleotide synthesis is supported by the decrease in AMP synthesis from adenine in whole tissue when guanine and formycin are present in the incubation medium. Neither compound affects the uptake of adenine or the activity of adenine phosphoribosyltransferase, but both decrease the supply of 5-phosphoribosyl 1-pyrophosphate: guanine promotes an alternative route of 5-phosphoribosyl 1-pyrophosphate utilization (the phosphoribosylation of guanine by hypoxanthine/guanine phosphoribosyltransferase) and formycin is an inhibitor of ribose 5-phosphate pyrophosphokinase.

An increased supply of 5-phosphoribosyl 1-pyrophosphate in the uteri of oestrogen-treated rats could result from hormonal activation of ribose 5-phosphate pyrophosphokinase, the enzyme that catalyses 5-phosphoribosyl 1-pyrophosphate synthesis from ribose 5-phosphate and ATP, and/or from an increased supply of ribose 5-phosphate resulting from hormonal stimulation of the pentose phosphate pathway of glucose metabolism (McKerns, 1967).

The latter proposal is unlikely, since Methylene Blue and phenazine methosulphate, electron acceptors that stimulate ribose 5-phosphate formation via the oxidative route of pentose–phosphate synthesis, do not increase the availability of 5-phosphoribosyl 1-pyrophosphate for nucleotide synthesis from adenine in control uteri.

However, the activity of ribose 5-phosphate pyrophosphokinase is low in uterine extracts and increases in parallel with the rate of nucleotide synthesis measured in the intact tissue after hormone treatment.

It is concluded that the stimulation of purine nucleotide formation from preformed bases in the uteri of oestrogen-treated (0–6h) rats is due to an increased availability of 5-phosphoribosyl 1-pyrophosphate mediated by activation of ribose 5-phosphate pyrophosphokinase. The increase in purine synthesis *de novo* may also be due to the increased availability of 5-phosphoribosyl 1-pyrophosphate for 5-phosphoribosyl 1-pyrophosphate amidotransferase; however, the possibility that changes may occur in the activities of other enzymes of this pathway during the first 6h of hormone action has not yet been excluded. Further work is required to find if oestradiol affects the amount and/or the catalytic activity of ribose 5-phosphate pyrophosphokinase in the immature rat uterus.

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