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HORMONAL INFLUENCE ON THE PHOSPHORYLASE ACTIVITY OF THE HUMAN MYOMETRIUM*, **

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INTRODUCTION

Profound changes in the composition and structure of the human myometrium have been shown to take place under the influence of ovarian hormones[1,2]. The glycogen-content, especially, exhibits an impressive increase in connection with hormonally induced growth of this tissue. Thus, it could be demonstrated that the amount of glycogen per tissue unit*** is increased about 50 times in a pregnant uterus at term as compared with the value found in a post-menopausal uterus[2].

It may be postulated that the hormone-induced changes in the myometrial glycogen-content are mediated by a series of enzyme systems, and that the action of substances which regulate cellular metabolism may be elucidated by studying their effect on these systems. This paper reports some investigations on the phosphorylase

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** The abbreviations or contractions used in the paper include AMP, adenosine-5'-phosphate; DNAP, deoxypentose nucleic acid-phosphorus; EDTA, ethylenediaminetretaacetate.

*** The expression "tissue unit" is used in the sense of a statistical unit and implies the average amount of extracellular and intracellular tissue per nucleus. For further explanation, see BRODY[1].

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activity in the human myometrium. No data on this enzyme in the myometrium seem to have been reported earlier, either in man or animals. As the investigation was planned with special emphasis on the effect of the ovarian hormones, uteri under the influence of various hormone levels have been investigated. Specimens have been analyzed from uteri of women in the post-menopausal period, of non-pregnant women with existing ovarian activity, of pregnant women at different stages of pregnancy and of women a few days after parturition. The material to be presented may be regarded as a series of target-organ specimens from the stage of absent or minimal trigger-substance influence (post-menopausal group) to the stage of maximal influence (term of pregnancy) followed by the period of regressive changes (puerperal group).

MATERIALS AND METHODS

Material was obtained as described earlier[1]. The specimens were immediately put in a beaker immersed in dry ice.

The phase of the cycle was determined by microscopic investigation of an endometrial biopsy.

The tissues were homogenized in a cysteine-glycerophosphate buffer at pH 6.0 with a Bühler homogenizer. Precautions were taken to keep the temperature close to 0°C. The buffer always contained NaF and, in some experiments, EDTA. The homogenate was centrifuged at 2000 r.p.m. for 10 minutes and when not otherwise stated, the activity was determined on the supernatant. The activity, however, was referred to the dry weight or DNAP of the total homogenate.

When not otherwise stated, the activity measurements have been made in the presence of AMP. The reaction was run at 30°C, preincubation with glycogen was carried out for 20 minutes, and incubation with glucose-1-phosphate and AMP run for 10, 15 and 20 minutes. The final concentration of NaF was 0.05 *M*, that of the EDTA 0.001 *M*. The final concentration of the other reaction constituents as well as the further treatment of the incubation mixture were those described by ILLINGWORTH AND CORI[3]. The phosphorus was determined according to TEORELL'S[4] modification of FISKE AND SUBBAROW'S method. Satisfactory first order kinetics were observed and all the activities were expressed in phosphorylase units as defined by CORI, CORI AND GREEN[6] and calculated per mg dry weight or per tissue unit (see below).

DNAP was determined as described earlier[7].

After nucleic acid extraction, the tissue residue was washed several times with distilled water. The residue was dried to constant weight and the dry weight determined.

The colorimetric determinations were carried out in a Beckman DU quartz spectrophotometer with a 1 cm cell. Centrifugations were performed in a M.S.E. refrigerated centrifuge.

RESULTS AND DISCUSSION

As a first step in this investigation, the recovery of the enzyme in water extracts was investigated. Homogenates were prepared as described above, and the phosphorylase activities were determined before and after centrifugation at 2000 r.p.m. for 10 minutes. The results were identical.

Human myometrial phosphorylase required the addition of glycogen for activity. In the absence of glycogen, a negligible amount of inorganic phosphorus was released, probably due to the action of phosphoglucomutase and glucose-6-phosphatase. The presence of NaF in a final concentration of 0.05 *M* suppressed this effect.

The error of the determination method has been evaluated by statistical analysis[8]. The standard deviation expressed as a percentage of the mean value is 6.1.

The existence in liver and cross-striated muscle of phosphorylase in two forms, *a* and *b*, has been demonstrated by CORI, CORI AND GREEN[9–13]. In some experiments the relative amounts of phosphorylase *a* and *b* in the human myometrium were determined. Specimens were chosen from non-pregnant uteri as well as from pregnant ones. Two of the last-mentioned uteri were from the 20th week of pregnancy and two from the 40th week during labor. The tissue was homogenized in the presence of EDTA. This

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substance has been shown by KREBS AND FISCHER[14] and FISCHER AND KREBS[15] effectively to block the conversion of phosphorylase *b* to *a in vitro*. The myometrial phosphorylase activity in the absence of AMP in all the different samples was between 65 and 70% of that found in the presence of AMP. It has been found by GREEN AND CORI[9] that phosphorylase *a* is approximately 65% active without AMP. The values presented here would thus indicate that practically all the measurable myometrial phosphorylase is present in the *a* form. A similar high phosphorylase *a* content has been demonstrated in heart muscle[16]. It is interesting to observe that in all the different states of the myometrium, and irrespective of whether the muscle is contracting or not, the relative phosphorylase *a* activity is the same throughout.

In this connection it should be pointed out that freezing of the specimen with the method applied in this investigation increases the relative amount of phosphorylase *a* in cross-striated muscle-tissue[16]. It is hardly probable, however, that this procedure *per se* would eliminate possible differences in the myometrial specimens representing different states of growth and muscular activity. Freezing of cross-striated muscle has been used by LEONARD[17]. Differences in phosphorylase *a* activity were clearly discernible as between tissues under different pre-treatment. This point is now being made the subject of further investigation.

Table I is a summary of the results. The phosphorylase activities determined in the presence of AMP have been calculated with reference to dry weight and per tissue unit. The first standard of reference is the conventional one, but the disadvantages,

TABLE I

CHANGES IN PHOSPHORYLASE ACTIVITY OF HUMAN MYOMETRIUM

State of uterus	*Enzyme units per mg dry weight*	*Enzyme units per tissue unit · 10^6*
Post-menopausal		
	13	1.8
	11	1.8
Non-pregnant		
menstruation	23	8.5
6th day	23	8.5
8th day	22	10.4
21st day	19	9.1
24th day	30	11.0
26th day	22	7.8
Pregnant		
20th week	57	47.1
20th week	40	46.0
40th week	57	153.4
40th week	70	152.1
36th week	69	160.3
39th week	72	147.7
Puerperal		
4th day after parturition	58	86.9
4th day after parturition	63	94.0

The enzyme activity per tissue unit calculated on the assumption of an average DNAP-content per nucleus of 0.65 pg (see text). For data on nucleic acid analyses the reader is referred to BRODY[1].

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especially in connection with studies on the growth and development of an organ, have been pointed out earlier[1,18]. It has been clearly demonstrated that the protein-content of myometrial tissue shows a close correlation to the state of growth. Changes in the cellular constituent under study will therefore be obscured by concomitant changes in the standard of reference. A biochemically more precise approach is the reference to the tissue DNAP. This calculation is based on the repeatedly confirmed finding of a constant amount of DNAP in the somatic cells of a given species. For a detailed discussion the reader is referred to BRODY[1].

On the assumption that the average amount of DNAP per nucleus is 0.65 pg (1 pg = 10^{-12} g), the phosphorylase activities have been determined per tissue unit. In this case, where it is matter of a tissue-constituent manufactured and localized within the cell, the activity per tissue unit is identical with the average activity per cell. As is seen in Table I, the enzymic activities in the post-menopausal group are comparatively slight. An increase is observed under the influence of ovarian hormones, moderate in the non-pregnant group and considerable during pregnancy. The highest values are observed at the term of pregnancy. After parturition there is a considerable and rather rapid drop.

Increased phosphorylase *a* activity has been associated with accelerated glycogenolysis[19,20]. This opinion is based, *inter alia*, on evidence that epinephrine and the hyperglycemic factor of the pancreas increase this enzymic activity, which effect is accompanied by a concomitant decrease in glycogen concentration. The experimental results have been interpreted as indicating that the actual level of phosphorylase *a* is determined by an interaction between enzyme systems inactivating and resynthesizing phosphorylase *a*. Epinephrine and the hyperglycemic factor seem to promote resynthesis of phosphorylase *a* and at the same time influence the equilibrium glucose-1-phosphate $\underset{+P}{\overset{-P}{\rightleftharpoons}}$ glycogen in the direction of glycogen breakdown. It was possible to demonstrate that the relative activities in the liver-test systems corresponded to the hyperglycemic response in the intact animal[21].

The glycogen concentration in human myometrium has been determined in an earlier investigation[2]. It was there demonstrated that hormonal stimulation caused a considerable accumulation of glycogen in the myometrial cell. A uterus at the term of pregnancy exhibits a glycogen concentration that is about 50 times that of a post-menopausal uterus. The present investigation has revealed a close correlation between the average amount of glycogen and phosphorylase activity per tissue unit. Fig. 1 gives a graphic survey.

It thus seems permissible to assume a hormonal stimulation of the phosphorylase activity. In the above discussion, the hormonal influence on the human uterus has been dealt with without any reference to the relative effect of the estrogens and the progesterone. In all the actual states under ovarian hormone influence, with the possible exception of the proliferative phase of the menstrual cycle, both types of hormones are operating. No conclusions as to the respective roles played by the groups of hormones can be drawn from this investigation. However, experimental evidence gained from investigations on castrated and hormone-treated rats indicates estrogens as the only hormones effective in stimulating glycogen deposition in the myometrium[22]. Studies on the cyclic rat point in the same direction[23–25]. The close correlation demonstrated here between glycogen concentration and phosphorylase activity makes it

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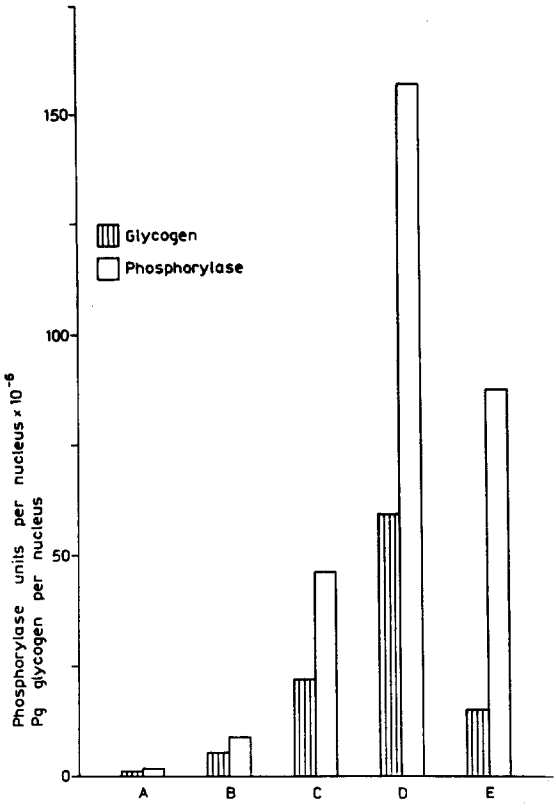

Fig. 1. Survey of changes in phosphorylase activity and glycogen content of human myometrium. State of uterus: A, post-menopausal; B, non-pregnant with ovarian function; C, pregnant 20th week; D, pregnant, term of pregnancy; E, puerperal, 4th day after parturition. The expression per nucleus is identical with per tissue unit. For further explanation, see text.

seem probable that the changes demonstrated in the phosphorylase activity are, directly or indirectly, induced by changes in the level of estrogens.

As regards the intracellular function of the enzyme, it should be mentioned that during glycogen accumulation (from the post-menopausal level to the term of pregnancy) the ratio between the average phosphorylase activity per tissue unit and the average glycogen-content per tissue unit is increasing, being 1.50 in the post-menopausal group, 1.67 in the non-pregnant uteri with existing ovarian activity, 2.10 in the 20th week and 2.64 in the 40th week of pregnancy. This increasing ratio of the enzyme and one of its substrates in combination with increasing amounts of glycogen may point to a change of the equilibrium in the direction of glycogen synthesis, the rate of which gradually increases. This will result in an increased net-synthesis. At parturition and during the involution of the uterus, there is a rapid decrease in the amount of glycogen per tissue unit. In connection with this reduction there is a considerable increase in the enzyme/substrate ratio, which amounts to 5.8 on the 4th day after parturition. One possible interpretation of this finding is that there is a reversal of the phosphorylase activity in the direction of glycogen breakdown induced by the considerable hormonal changes taking place during and after parturition, a reversal which

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results in an increased net-breakdown. However, the elucidation of this problem requires further investigation with respect to other enzyme systems and intermediates involved in glycogen synthesis and breakdown. These are now in progress.

SUMMARY

The phosphorylase activity in human myometrium from women in the post-menopausal period, from non-pregnant women with ovarian function, from pregnant women at different stages of pregnancy and from women in the puerperal period has been determined.

The accuracy of the determination method has been evaluated by statistical analysis, the standard deviation being 6.1% of the average.

In all these different states, the phosphorylase *a* activity (the activity determined in the absence of added adenosine-5'-phosphate) amounts to approximately 65% of the total phosphorylase activity (the activity determined when adding AMP to the incubation mixture). This implies that practically all the measurable myometrial phosphorylase activity is present in the *a* form.

The phosphorylase activity exhibits a considerable increase during the hormone-induced growth of the human uterus. The activity in a post-menopausal uterus is $1.8 \cdot 10^{-6}$ enzyme units per tissue unit and the activity at the term of pregnancy $156 \cdot 10^{-6}$ units per tissue unit. After parturition there is a considerable and rapid decrease in the phosphorylase activity. Four days after parturition the activity is $87 \cdot 10^{-6}$ units per tissue unit.

It has been demonstrated that the changes in the phosphorylase activity are accompanied by changes in the average amount of glycogen per tissue unit. The possible implications of these findings in relation to the endocellular function of the enzyme are discussed.

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