OVARIAN ACTIVITIES OF PYRIDINE NUCLEOTIDE DEPENDENT DEHYDROGENASES IN THE RAT DURING PREGNANCY AND LACTATION

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ABSTRACT

The activities of soluble enzymes catalyzing electron transfer in the systems malate:NADP, glucose-6-phosphate:NADP, 6-phosphogluconate:NADP, isocitrate:NADP and malate:NAD were determined in the ovaries of rats at several stages of reproduction. The enzymic activities (as μmol of product formed per min per g tissue) during late pregnancy (33–7–5–28–48, respectively) differed appreciably from the activities during the oestrous cycle (7–13–3–30–23) and also at parturition (13–10–3–26–26). During pregnancy and parturition as well as during lactation and weaning there was an inverse relationship between the malate enzyme (malate:NADP) and the glucose-6-phosphate dehydrogenase. The ratio between the activities of these enzymes varied within a wide range (0.5–5) and appeared to reflect luteal function. The enzymic activities on day 6 of lactation in the involuted corpora lutea of pregnancy (7–15–3–41–32) and in the corpora lutea deriving from the post partum ovulation (15–7–4–39–36) were in agreement with this view. The possible role of the malate enzyme in the ovarian synthesis of steroids is discussed with reference to its presumed role in lipogenesis in the rat.

NADPH is required as a reductant in several of the reactions involved in the biosynthesis of lipids including steroids. The generation of cytoplasmic NADPH (cf. Rogstad & Katz 1966) may take place by oxidation of glucose via the

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hexose monophosphate pathway, by oxidation of isocitrate and of malate, as well as by NADH - NADPH transhydrogenation. The relative importance of these and other possible reactions for the supply of NADPH in ovarian tissue has not been established. The observations that bovine and human corpora lutea are very rich in glucose-6-phosphate dehydrogenase (Savard et al. 1963) have indicated that NADPH required for ovarian steroidogenesis may be mainly provided by oxidation of glucose via the hexose monophosphate pathway. In support of the view that this pathway may also be highly important in rat ovaries McKerns (1965) found considerable fluctuations of the glucose-6-phosphate dehydrogenase during the oestrous cycle and, moreover, that the activity of this enzyme increased in response to gonadotrophins. The present investigation was prompted by an observation that the activity of the glucose-6-phosphate dehydrogenase in pregnant rat ovaries appeared to be much lower than those of two other NADP dependent enzymes, namely the isocitrate dehydrogenase and the malate enzyme. Studies conducted to assess enzyme activities at various stages of reproduction indicated that the malate enzyme in particular may be of physiological significance in rat ovaries.

**MATERIALS AND METHODS**

The animals used were virgin or first pregnancy Sprague-Dawley rats obtained from Berkeley Pacific Laboratories. The reproductive stages of the animals when sacrificed and the number of animals in each group were as follows:

- Oestrous cycle, random phases: 15
- Pregnancy, day 6-11: 6
- Pregnancy, day 15-18: 6
- Parturition: 4
- Lactation, day 2: 6
- Lactation, day 6: 6
- Lactation, day 15-20: 8
- Weaned, day 1: 5
- Weaned, day 5: 6

In these groups the intact ovaries were processed in pairs as obtained from each of the animals.

Another group comprised 4 animals all of which had lactated for 6 days. In these the old corpora lutea of pregnancy were separated from the young corpora lutea of lactation by needle dissection, the residual tissue being discarded.

The adrenals as well as the pituitary gland were sampled in a total of 13 animals during the oestrous cycle (4), pregnancy (5) and lactation (4).

Bovine ovaries were obtained at the slaughterhouse from non pregnant animals.

The organs were always cooled on ice immediately after removal. Preparation of homogenates and assays of the 30 000 × g supernatant for enzymic activities by spectrophotometry at 340 μν were carried out essentially as previously described by Baldwin & Milligan (1966).

The activity of the malate enzyme was determined under conditions similar to those
used for the isocitrate dehydrogenase, i. e. at pH 7.6 in the presence of manganese ions.

RESULTS

The activities of the following enzymes were determined: glucose-6-P dehydrogenase\(^1\), 6-P-gluconate dehydrogenase\(^2\), isocitrate dehydrogenase\(^3\), malate (»malic«) enzyme\(^4\) and malate dehydrogenase\(^5\).

The highest activities of the glucose-6-P dehydrogenase were found in the ovaries of cycling animals, in animals at parturition and in animals after weaning (Fig. 1). The activity of 6-P-gluconate dehydrogenase was highest during pregnancy when the lowest values of glucose-6-P dehydrogenase were found.

During pregnancy the activity of the malate enzyme increased to values almost 5 times as high as in cycling animals. There was a clear tendency for this enzyme to vary inversely in activity with that of glucose-6-P dehydro-

\[\text{Fig. 1.}\]

Means of enzymic activities as \(\mu\)mol of product formed per min per g tissue in the ovaries of rats during the oestrous cycle (C), pregnancy (P), lactation (L) and weaning (W). The standard errors of the means are indicated by vertical lines.

a: Malate enzyme (malate: NADP),
b: Glucose-6-phosphate dehydrogenase (glucose-6-P: NADP),
c: 6-Phospho-gluconate dehydrogenase (6-P-gluconate: NADP).

1. D-Glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49.
2. 6-Phospho-D-gluconate: NADP oxidoreductase (decarboxylating) EC 1.1.1.44.
3. \(L_s\)-Isocitrate: NADP oxidoreductase (decarboxylating), EC 1.1.1.42.
4. L-Malate: NADP oxidoreductase (decarboxylating), EC 1.1.1.40.
5. L-Malate: NAD oxidoreductase, EC 1.1.1.37.
genase through all the stages investigated. Thus low values of the malate enzyme were found at parturition and during early lactation as well as after weaning.

The ratio between the activities of the malate enzyme and of glucose-6-P dehydrogenase (Fig. 2) changed within a range of 0.5 to 5, a 10-fold increase. The activity ratio between the 6-P-gluconate dehydrogenase and glucose-6-P dehydrogenase varied according to a similar pattern but to a smaller degree, viz. from 0.15 to 0.75, a 5-fold increase.

The activity of the NAD dependent malate dehydrogenase increased about two-fold during pregnancy and decreased toward parturition (Fig. 3). The isocitrate dehydrogenase activity did not change appreciably during pregnancy. The highest values of this enzyme were found during early lactation and the lowest values after weaning.

The corpora lutea of pregnancy and the corpora lutea of lactation obtained 6 days after parturition differed in that the young set had a higher activity of the malate enzyme and a lower activity of glucose-6-P dehydrogenase.

![Fig. 2](image1)

**Fig. 2.**
Means of the ratios between the activities of the malate enzyme (malate: NADP) and the glucose-6-P dehydrogenase (glucose-6-P: NADP) in the ovaries of rats during the oestrous cycle (C), pregnancy (P), lactation (L) and weaning (W). The standard errors of the means are indicated by vertical lines.

![Fig. 3](image2)

**Fig. 3.**
Enzymic activities in rat ovaries, cf. text Fig. 1.

a: Malate dehydrogenase (malate: NAD),
b: Isocitrate dehydrogenase (isocitrate: NADP).
Both sets contained high levels of isocitrate dehydrogenase in agreement with the results obtained in whole ovaries on day 6 of lactation.

The activities of the malate enzyme in bovine corpora lutea were much lower than in rat ovaries whereas the glucose-6-P dehydrogenase activities were considerably higher (Table 1).

The enzyme activities in the adrenals and in the pituitary glands were not found to change consistently during pregnancy and lactation and the data for each animal were pooled for the purpose of comparison with the results obtained in the ovaries (Table 1).

The adrenal activities of the malate enzyme and of glucose-6-P dehydrogenase were similar to those in the ovaries of cycling animals. Otherwise the adrenals differed from non luteinized ovaries particularly in having high activities of 6-P-gluconate dehydrogenase relative to glucose-6-P dehydrogenase and to the activities of isocitrate dehydrogenase, which were low.

The lowest levels of the malate enzyme were found in the pituitary glands. Conversely the activities of the NAD dependent malate dehydrogenase in the pituitary glands exceeded those recorded in the other tissues examined. The pituitary gland activities of glucose-6-P dehydrogenase were low but approached those found in luteinized ovaries.

**DISCUSSION**

*Kerns* (1965) found considerable fluctuations in ovarian glucose-6-P dehydrogenase during the oestrous cycle, the activities being about twice as high during oestrus as during dioestrous. In the present investigation the measurements in cycling animals were carried out regardless of the phase of the cycle. Thus, as could be expected, the variation in the values obtained for the glucose-6-P dehydrogenase of this group was relatively large (Fig. 1).

According to *Eto et al.* (1962) the ovarian vein blood content of progesterone during pregnancy increases until about day 16, after which time the content rapidly decreases to a minimum toward parturition. The requirements for NADPH during the period of maximal luteal activity with regard to steroid production is presumably increased. It was unexpected therefore that during this period the lowest activities were found of glucose-6-P dehydrogenase which is considered to be the rate limiting enzyme for NADPH generation by the hexose monophosphate pathway. On the other hand, the activity pattern of the malate enzyme during pregnancy and parturition clearly resembled that reported for progesterone secretion. The two sets of corpora lutea present during lactation were remarkably uniform with regard to the activities of 6-P-gluconate dehydrogenase, isocitrate dehydrogenase and NAD dependent malate dehydrogenase. The finding of an inverse relationship between glucose-
Table 1.

Enzymes in two sets of corpora lutea on day 6 of lactation, in adrenal and pituitary glands in the rat and in bovine corpora lutea. Means ± S.E. of activities calculated as µmol of product formed per min per g tissue. R: ratio between the activities of the malate enzyme and the glucose-6-P dehydrogenase.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Malate: NADP</th>
<th>Glucose-6-P: NADP</th>
<th>6-P-Gluconate: NADP</th>
<th>Isocitrate: NADP</th>
<th>Malate: NAD</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpora lutea of pregnancy</td>
<td>4</td>
<td>7.15 ± 3.25</td>
<td>14.6 ± 1.1</td>
<td>3.49 ± 0.47</td>
<td>41.1 ± 5.5</td>
<td>32.0 ± 4.7</td>
<td>0.49 ± 0.20</td>
</tr>
<tr>
<td>Corpora lutea of lactation</td>
<td>4</td>
<td>15.3 ± 1.5*)</td>
<td>7.30 ± 1.22*)</td>
<td>3.99 ± 0.13</td>
<td>38.5 ± 4.9</td>
<td>36.8 ± 5.6</td>
<td>2.15 ± 0.42</td>
</tr>
<tr>
<td>Adrenals</td>
<td>13</td>
<td>5.95 ± 1.81</td>
<td>15.1 ± 3.4</td>
<td>13.3 ± 5.1</td>
<td>7.12 ± 2.66</td>
<td>50.6 ± 13.9</td>
<td>0.41 ± 0.14</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>13</td>
<td>0.78 ± 0.29</td>
<td>6.38 ± 1.63</td>
<td>1.01 ± 0.36</td>
<td>4.92 ± 1.98</td>
<td>67.7 ± 24.2</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Bovine corpora lutea</td>
<td>4</td>
<td>2.40 ± 0.31</td>
<td>29.0 ± 9.5</td>
<td>6.52 ± 1.46</td>
<td>16.1 ± 1.9</td>
<td>30.1 ± 4.9</td>
<td>0.09 ± 0.02</td>
</tr>
</tbody>
</table>

*) Student's t-test for paired differences between corpora lutea of pregnancy and corpora lutea of lactation:
  malate: NADP, P < 0.01,
glucose-6-P: NADP, P < 0.001.
6-P dehydrogenase and the malate enzyme in the two sets is in good agreement with the changes taking place during regression of luteal activity before parturition. The trends of the enzymic activities during lactation and during weaning resembled those observed during pregnancy and parturition and would seem to be partially accounted for by corresponding ovarian changes, i.e. by growth of the young corpora lutea followed by involution with subsequent development of follicles.

In general the results indicate that highly functional luteal tissue in the rat is characterized by high malate enzyme activities and low activities of glucose-6-P dehydrogenase. The relatively high ovarian activities of glucose-6-P dehydrogenase during the oestrous cycle, at parturition and after weaning suggest that this enzyme may be more important in follicular tissue. Changes in the proportions of cell types present in the ovaries during the different stages of reproduction might obviously contribute to the changes observed in absolute, as well as relative enzymic activities. Histochemical studies in rat ovaries indicate that thecal and interstitial cells are rich in glucose-6-P dehydrogenase and that the activity of this enzyme may be correlated with oestrogen synthesis (cf. Kidwell et al. 1966). The finding in the present investigation of a relatively high activity of glucose-6-P dehydrogenase in involuted corpora lutea of pregnancy is compatible with the histochemical observation that the activity of the enzyme in corpora lutea of cycling animals increases continuously throughout the course of their involution (Pupkin et al. 1966). Histochemical studies on the distribution between various cell types of the malate enzyme in ovarian tissue are apparently not available.

The inverse relationship between glucose-6-P dehydrogenase and the malate enzyme during pregnancy as well as in old and young corpora lutea deserves some further comments since it seems related to the functional state of luteinized cells rather than to a difference in cell types, and since it does not appear to apply to rat liver tissue, in which the aggregate activity of the two NADP dependent dehydrogenases of the hexose monophosphate pathway largely parallels that of the malate enzyme when this is changed by dietary or hormonal manipulations (Tepperman & Tepperman 1964, 1965). The relatively low activity of glucose-6-P dehydrogenase in highly functional luteal tissue of pregnancy and in the young corpora lutea of lactation is of considerable interest since the enzyme occupies a key position at a branching point of glucose metabolism by competing for glucose-6-P which is also the substrate for the isomerase giving rise to fructose-6-P and subsequent glycolysis via the Embden-Meyerhof pathway. Low activity of glucose-6-P dehydrogenase due to inhibition or simply to low absolute levels of the enzyme would thus tend to favour glycolysis and, hence, diminish the NADPH generation potential inherent in glucose degradation to pyruvate. The possible role of pyruvate in the generation of NADPH will be discussed below in connection with the malate
enzyme. Highly functional luteal tissue might conceivably contain steroid metabolites in high concentrations. From _in vitro_ studies on mammalian enzymes it is known that glucose-6-P dehydrogenase, but not 6-P-gluconate dehydrogenase, is susceptible to inhibition by a number of naturally occurring steroids such as pregnenolone, 17-OH-pregnenolone and dehydroepiandrosterone (Marks & Banks 1960). The possibility that glucose-6-P dehydrogenase may be inhibited selectively by endogenous steroids seems to be supported by the following comparison of its activity in various tissues, with that of 6-P-gluconate dehydrogenase which catalyzes the subsequent reaction within the hexose monophosphate pathway. There was a marked trend for 6-P-gluconate dehydrogenase to increase in activity during the apparent suppression of glucose-6-P dehydrogenase in pregnant animals and then to decrease toward parturition. Thus during pregnancy the activity of the former enzyme changed about as the activities of the malate enzyme and of the NAD dependent malate dehydrogenase, seemingly in relation to changes in general cellular activity associated with luteal function, as judged from steroid secretion. The increase in glucose-6-P dehydrogenase at parturition might be due to some extent to development of follicles but also to the large predominance in the ovaries of involuting corpora lutea, the steroid production of which is presumably very low (cf. Eto et al. 1962). The activity of 6-P-gluconate dehydrogenase in the corpora lutea of lactation was about the same as in the old corpora whereas glucose-6-P dehydrogenase activities differed by a factor of 2, being highest in the old set. High activities of glucose-6-P dehydrogenase relative to the activities of 6-P-gluconate dehydrogenase were also found in cycling animals in which the ovarian steroid production is smaller than during pregnancy (Eto et al. 1962). As in highly functional luteinized ovaries, the activity of glucose-6-P dehydrogenase in adrenal glands barely exceeded that of 6-P-gluconate dehydrogenase (Table 1). In contrast, the ratio between the activities of these enzymes in pituitary glands amounted to about 6:1. These observations would seem to warrant investigations on the biochemistry of ovarian glucose-6-P dehydrogenase with a view to its possible regulation by steroids. Recent studies on the rat mammary gland glucose-6-P dehydrogenase suggest that its inhibition by steroids may involve interference with the steric configuration of the enzyme (cf. Levy 1963; Levy et al. 1966).

In the bovine corpus luteum the level of the malate enzyme was much lower than that of glucose-6-P dehydrogenase, the ratio between their activities being about 1:10. Similar results were reported by Savard et al. (1963). In human ovaries too (Nielson & Warren 1965) the malate enzyme seems to be of minor importance since its activity has been found to be very low in several types of ovarian tissue including functional corpora lutea in which it is exceeded in activity by glucose-6-P dehydrogenase by a factor of about 10. During the menstrual cycle the corpus luteum activity of glucose-6-P dehydrogenase
parallels luteal function in so far as the activity remains high for about 10–12 days after ovulation and then decreases progressively during luteal involution. The relatively high ovarian activities of glucose-6-P dehydrogenase in the bovine and in the human are consistent with in vitro studies on glucose metabolism which indicate the existence of an active hexose monophosphate pathway in ovarian tissue of both species (Field et al. 1960). In luteinized rat ovaries, on the contrary, it seems that relatively small proportions of glucose are metabolized via this pathway (Armstrong & Greep 1962). In luteal tissue of this species, most of the glucose is apparently metabolized via the Embden-Meyerhof pathway under in vitro conditions (Armstrong 1963). These results, which were obtained by incubation of tissue slices, indicate that the activity of the hexose monophosphate pathway may be comparatively small in luteinized rat ovaries and that the relative activity of glucose-6-P dehydrogenase in intact luteal cells in this species may perhaps be even lower than those observed here by methods involving cell disruption and preparation of highly diluted extracts.

The possible significance of the malate enzyme in the rat ovary may be discussed with reference to recent enzymic and metabolic studies concerning lipogenesis in the rat (e.g. Wise & Ball 1964; Tepperman & Tepperman 1964; Rogstad & Katz 1966; Ballard & Hanson 1967). It appears that the malate enzyme is involved in a sequence of reactions favouring NADPH generation as well as a supply of activated acetate fragments to the cytoplasm in the following manner. Pyruvate, primarily originating from glycolysis, may be carboxylated either in the mitochondria or in the cytoplasm (Ballard & Hanson 1967) to form oxaloacetate. Cytoplasmic oxaloacetate is converted to malate by hydrogen transfer from NADH. This reaction is supported by the soluble malate dehydrogenase, the activity of which increases during pregnancy as does the activity of the NADP dependent malate enzyme. The latter enzyme catalyzes an oxidative decarboxylation of malate whereby pyruvate is formed again. The reaction sequence, referred to as the malate transhydrogenation cycle (Ballard & Hanson 1967), results in generation of NADPH at the expense of NADH:

\[
\text{pyruvate} + \text{CO}_2 \rightarrow \text{oxaloacetate} \xrightarrow{\text{NADH}} \text{malate} \xrightarrow{\text{NAD}} \text{pyruvate} + \text{CO}_2 \]

Mitochondrial oxaloacetate may combine with acetyl-CoA to give citrate which is capable of diffusing into the cytoplasm where it is cleaved by the citrate cleavage enzyme. Activated acetate fragments are thereby made available for cytoplasmic synthesis of lipids. If the citrate cleavage pathway is followed to provide acetyl-CoA required for lipogenesis large amounts of oxaloacetate are formed, the retrieval of which is effected by hydrogenation to form malate as
described. These reactions represent a compensatory alternative to the hexose monophosphate pathway for the generation of NADPH, the functional role of which in the rat is indicated by the finding of high activities of the malate enzyme associated with low activities of the glucose-6-P dehydrogenase. The possibility that ovarian steroidogenesis in the rat may be dependent on the reactions outlined for the supply of acetyl-CoA to the cytoplasm, remains to be substantiated.

The role of the soluble, NADP dependent isocitrate dehydrogenase, the activity of which remained rather high at all stages of reproduction, is less well understood than that of the NAD dependent mitochondrial enzyme which catalyzes one of the reactions of the Krebs cycle. The possibility exists that the soluble enzyme may support the reductive carboxylation of $\alpha$-ketoglutarate, resulting in the formation of isocitrate which on conversion could yield a cytoplasmic substrate for the citrate cleavage enzyme (D'Adamo & Haft 1965). The soluble isocitrate dehydrogenase might thus participate in a mechanism for acetyl-CoA generation requiring NADPH for its operation:

$$\begin{align*}
\text{NADPH} & \quad \alpha\text{-ketoglutarate} + \text{CO}_2 \\
\text{isocitrate} & \quad \text{aconitate} \\
\text{NAD} & \quad \text{citrate} \\
\text{oxaloacetate} + \text{acetyl-CoA} & \quad 
\end{align*}$$

The activity of the isocitrate dehydrogenase did not change appreciably through pregnancy and parturition but increased as lactation ensued. In a group of rats deprived of their litters immediately after parturition, the ovarian activity of the isocitrate dehydrogenase 6 days later was about $1/2$ that found in normally lactating animals at this stage. Similarly, low activities were found in rats weaned for 5 days after the normal period of lactation (Fig. 3). The significance of these observations is obscure. In human corpora lutea the isocitrate dehydrogenase activity has been found to increase progressively with the age of the structure (Nielsen & Warren 1965).

Administration of gonadotrophins has usually been found to result in increased activities of the ovarian glucose-6-P dehydrogenase in the rat (cf. Kidwell et al. 1966; McKerns 1965). However, a decrease in the activity of this enzyme was noted after FSH and LH in combination (Wiest et al. 1963). The possibility that gonadotrophins may affect the ovarian malate enzyme level does not seem to have been explored. Large doses of 17$\beta$-oestradiol administered to rats during mid-lactation resulted in a considerable increase in the ovarian activity of the malate enzyme and enhanced the physiological decrease in the activity of the glucose-6-P dehydrogenase (Lunaas & Baldwin 1967), possibly due both to interference with secretion and the luteotrophic action of prolactin.
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