EFFECT OF PREGNANCY ON CYTOPLASMIC AND MITOCHONDRIAL ENZYMES IN HUMAN AND ANIMAL MYOMETRIUM

By
Helmut Geyer and Michael Riebschläger

ABSTRACT

An investigation was made on the influence of pregnancy on the specific activities of cytoplasmic (lactate dehydrogenase\textsuperscript{2}, cytoplasmic malate dehydrogenase) and mitochondrial enzymes (glutamate dehydrogenase, mitochondrial malate dehydrogenase, cytochrome-c-oxidase) in the human and animal myometrium. The activities were related to DNA.

The specific activities of all the investigated enzymes increased. This rise in activity depended on the cellular localisation of the enzyme. The activity of all enzymes in one cellular compartment changed to the same extent. This change varied according to species. With regard to the human tissue, the increase of the cytoplasmic enzymes was larger than that of the mitochondrial enzymes. In the rat, however, a significantly larger increase of the mitochondrial enzymes was found.

The increase in the specific activities of the cytoplasmic enzymes in the human and rat was proportional to the protein-content and to the hypertrophy of the cells.

It was concluded that the number of mitochondria or their enzymatic activity increased in both species during pregnancy – in each species, however, to a different extent.

The pattern of the LDH-isoenzymes in the myometrium changed in the same manner in the human myometrium as in the rat. The percentage of M subunits of LDH compared to H subunits rose in both cases during pregnancy.

\textsuperscript{1} Essential parts of this investigation are quoted from the M. D. thesis of M. R.

\textsuperscript{2} List of abbreviations:
LDH: lactate dehydrogenase (EC 1.1.1.27).
MDH: malate dehydrogenase (EC 1.1.1.37).
GIDH: glutamate dehydrogenase (EC 1.4.1.3).
During pregnancy the uterus undergoes a development which can otherwise be seen only in tissues of early childhood, apart from pathological cases such as tumours.

Wetzstein (1965) and Jaeger (1966) reported an increase in the number of mitochondria in the human myometrium during pregnancy. Zinnari & Vallerino (1962) observed an increase in the number and size of the mitochondria caused by hormones. As we assumed that these morphological conditions were related to corresponding enzyme patterns, we investigated the influence of pregnancy on the activities of cytoplasmic and mitochondrial enzymes in the myometrium. Since, as opposed to the protein-content, the DNA of the cell remains almost constant, we related the enzyme activity to its DNA-content.

On the basis of previous investigations on human myometrium (Geyer 1968a, b) we examined the influence of pregnancy on the isoenzymes of the lactate dehydrogenase in the myometrium of the rat (Geyer & Riebschläger 1969, 1971).

**METHODS**

a) **Selection and preparation of the tissues**

The investigations were carried out on the myometrium of women, who had been admitted to the hospital for hysterectomies because of uterine prolap or myoma. The tissue was taken from the uterus immediately after the operation. The endometrium and serosa were removed and the remaining myometrium was washed in a physiological sodium chloride. No pathological alterations were visible by macroscopic and histological examination.

Tissues samples from Caesarian sections were prepared in the same manner.

For the study of animal tissue we used infantile female rats of the Sprague Dawley strain. The rats were kept in Macrolon-cages at room temperature. Their standard food consisted of Altromin from Altroge, Lage/Lippe, Germany, and water ad libitum. At the age of 30 days the animals were castrated. Since the animals also served as controls for other experiments, they received a daily injection of 0.5 ml of a mixture of physiological sodium chloride and 4% ethanol from the 35th to the 49th day. We also examined 2 groups each of 15 animals, which had not been previously treated, for their specific activities of lactate dehydrogenase and glutamate dehydrogenase. The mean values of lactate dehydrogenase (7800 mU/mg DNA) and of glutamate dehydrogenase (140 mU/mg DNA) were within the range of the corresponding values of pre-treated rats. The pre-treatment had no influence on the results.

At 40 days of age the rats were killed by decapitation. Pregnant animals were used at the end of gestation. The uteri were removed, washed and weighed after the endometrium had been scraped off. The experiments were always carried out at the same time of day. The further preparation was performed at a temperature of 0 to 4°C.

b) **Extraction**

The tissues were homogenized with an Ultraturrax-homogenizer. (Janke & Kunkel, Staufen i. Br., Germany) in 0.06 m phosphate buffer pH 7.4, to which Triton X-100

---

*Acta endocr. 77, 2*
had been added. We extracted human tissues with a threefold volume of phosphate buffer, which contained 0.04% of Triton X-100.

For the extraction of animal tissues a fivefold volume of phosphate buffer with 0.2% of Triton X-100 was used. These differences in the concentration of Triton X-100 resulted from the different effects of inhibition by Triton X-100 on cytochrome-c-oxidase in human and animal tissue. Neither the relative proportions of the tissue to phosphate buffer nor the different concentrations of Triton X-100 had any influence on the enzymatic activities. To confirm this we homogenized identical parts of human myometrium in phosphate buffer with increasing content of Triton X-100. The activity of glutamate dehydrogenase, used as a typical enzyme of mitochondria, was unaffected.

The homogenate was passed through a nylon sieve in order to remove connective tissues. Subsequently the DNA-content was measured. The homogenate was centrifuged at 600 × g for 10 min in order to remove undisintegrated material and cell debris. The supernatant was used to measure the activity of cytochrome-c-oxidase and the content of protein. Following a further centrifugation during a period of 20 min at 30,000 × g, we obtained a supernatant for the measurement of lactate dehydrogenase, malate dehydrogenase and glutamate dehydrogenase. This supernatant was also used for the electrophoretic separation of isoenzymes.

c) The electrophoretic separation of isoenzymes

The technique of the separation of isoenzymes has been described in previous publications as well as the calculation of the ratio H-LDH/M-LDH (Geyer 1968a,c).

d) Assays of the enzyme activities

Lactate dehydrogenase. – The measurement of lactate dehydrogenase has likewise already been described (Geyer 1968a).

Malate dehydrogenase. – The activity of malate dehydrogenase was determined according to the previous specification (Geyer 1968c).

Glutamate dehydrogenase. – The activity of glutamate dehydrogenase was assayed in an optical test according to the method of Schmidt (1962) with the use of a-keto-glutarate as substrate.

Cytochrome-c-oxidase. – The activity of cytochrome-c-oxidase was measured according to the method of Appelmans et al. (1955):

A 41 μM cytochrome-c-solution in a phosphate buffer pH 7.4 with 1 mM ethylenediamine-tetraacetate was reduced with sodium dithionite by 90%. The reaction was started by adding 0.1 ml of tissue extract which was diluted with 0.06 M phosphate buffer pH 7.4. The measurement was done in 1 cm cuvettes at 550 nm, 25°C, against a cytochrome-c-solution, which was completely oxidized with solid potassium ferri-cyanide.

One hundred ml of a solution contained one unit of the enzyme when the log of the optical density was decreased by 1.0 per min.

e) Measurement of the DNA content

The content of DNA was determined according to the method of Ceriotti (1952) as modified by Keck (1956).
f) Determination of the protein content

The content of soluble protein was determined by the biuret-method according to Beisenherz et al. (1953).

g) Fractionation of cell-organelles

To determine the localization of the MDH-isoenzymes in the cytoplasm or the mitochondria, the cells were fractionated according to the method of Berkeš-Tomašević & Holzer (1967).

RESULTS

1. Enzyme activities in the myometrium of non-pregnant and pregnant human uteri

The activities of lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase and cytochrome-c-oxidase were determined in the tissue homogenate which was prepared as has been described.

Malate dehydrogenase was split up into its 2 isoenzymes. Their activities were measured by an optical test. MDH-I was derived from the cytoplasm and MDH-II from the mitochondria. In this respect malate dehydrogenase from human myometrium did not differ from that of the rat.

Table 1 lists the mean $\bar{x}$ and the standard deviation $s$ of the specific activities of lactate dehydrogenase, cytoplasmic malate dehydrogenase (MDH-I), mitochondrial malate dehydrogenase (MDH-II), glutamate dehydrogenase and cytochrome-c-oxidase found in the myometrium of fertile, non-pregnant and of pregnant women. The activities were related to DNA. The means and standard deviations of the values $\frac{\text{mU GIDH}}{\text{mg DNA}}$ and $\frac{\text{mU LDH}}{\text{mg DNA}}$ were approximated from $\frac{\text{mU LDH}}{\text{mg protein}}$ respectively $\frac{\text{mU GIDH}}{\text{mg protein}}$ (Geyer 1968c) and $\frac{\text{mg protein}}{\text{mg DNA}}$ (Table 5) by Taylor's expansion.

The derived values are, accordingly,

$$\frac{\text{mU LDH}}{\text{mg DNA}} = \frac{\text{mU LDH}}{\text{mg protein}} \times \frac{\text{mg protein}}{\text{mg DNA}}$$

$$\frac{\text{mU GIDH}}{\text{mg DNA}} = \frac{\text{mU GIDH}}{\text{mg protein}} \times \frac{\text{mg protein}}{\text{mg DNA}}$$

Furthermore, Table 1 includes the percentage of MDH-I and MDH-II. During pregnancy this ratio shifted significantly in favour of MDH-I.

The specific activities of all the enzymes examined increased significantly during pregnancy. The cytoplasmic enzymes, however, increased to a significantly greater extent ($P < 0.05$) than the mitochondrial enzymes (Student's $t$-test).
Table 1.
Specific activities of lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase and cytochrome-c-oxidase and isoenzyme pattern of malate dehydrogenase in the human myometrium.

<table>
<thead>
<tr>
<th></th>
<th>mU LDH-1</th>
<th>mU MDH-I</th>
<th>mU MDH-II</th>
<th>mU GlDH2</th>
<th>mU cytochrome-c-oxidase3</th>
<th>% MDH-I</th>
<th>% MDH-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg DNA</td>
<td>mg DNA</td>
<td>mg DNA</td>
<td>mg DNA</td>
<td>mg DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>14 850</td>
<td>7090</td>
<td>2590</td>
<td>300</td>
<td>96</td>
<td>72.9</td>
<td>27.1</td>
</tr>
<tr>
<td>( s )</td>
<td>6890</td>
<td>2320</td>
<td>560</td>
<td>110</td>
<td>21</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>( n )</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>167 320</td>
<td>65 8502)</td>
<td>15 5603)</td>
<td>1540</td>
<td>1514)</td>
<td>80.75)</td>
<td>19.35)</td>
</tr>
<tr>
<td>( s )</td>
<td>61 420</td>
<td>23 540</td>
<td>6050</td>
<td>660</td>
<td>52</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>( n )</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \bar{x} \): mean  
\( s \): standard deviation  
\( n \): number of observations

1) derived from \( \frac{\text{mU LDH}}{\text{mg protein}} \times \frac{\text{mg protein}}{\text{mg DNA}} \)  
2) derived from \( \frac{\text{mU GlDH}}{\text{mg protein}} \times \frac{\text{mg protein}}{\text{mg DNA}} \)  
3) 100 ml of a solution contains one unit of the enzyme when the log of the optical density decreases by 1.0 per min.  
4) \( P < 0.05 \) significant on the 0.05 level (t-test).  
5) \( P < 0.001 \) significant on the 0.001 level (t-test).
2. Enzyme activities in the myometrium of infantile, castrated and pregnant rats

We compared the activities of the cytoplasmic and mitochondrial enzymes in the myometrium of the rat with those of the human myometrium.

In one test we collected, on the average, the uteri of 18 infantile animals, with an uterine weight of only 26.5 ± 3.0 mg. In the experiments with pregnant animals we used only one uterus, since the weight was 3.06 ± 0.66 g.

The specific activities of all the enzymes examined in the rat myometrium during pregnancy were likewise increased (Table 2). The first thing we noted was that the activities of the isoenzymes of malate dehydrogenase changed in inverse relation to those of the human myometrium. The ratio of \( \frac{\text{MDH-I}}{\text{MDH-II}} \) altered significantly from 78.6/21.4 in the non-pregnant uterus to 54.2/45.8 in the pregnant uterus, in favour of the mitochondrial MDH-II. The comparison of the increases in the specific activities indicates that the activities of mitochondrial enzymes in the rat are increased significantly over those of the cytoplasmic enzymes. This relation, however, was reversed in the human myometrium, where the activities of the cytoplasmic enzymes were greater.

3. Isoenzymes of lactate dehydrogenase in the myometrium of non-pregnant and pregnant human uteri

Very little is known about the physiological significance of the isoenzymes of lactate dehydrogenase. For this reason we shall report the investigations on the isoenzymes separately.

In the normal human myometrium we found a pattern of LDH isoenzymes which had a maximum activity in LDH-III (Geyer 1968a).

At the onset of pregnancy the maximum shifted to LDH-IV and the ratio \( \frac{\text{H-LDH}}{\text{M-LDH}} \) decreased significantly. The percentual H-content at different periods of pregnancy is illustrated in Fig. 1. This process occurred according to an exponential function. On the basis of the curve we may assume that the LDH- composition remains steady after the 27th week of pregnancy.

Fig. 1 shows the value measured in an 8 week old pregnancy. This value refers to a case of an extra-uterine pregnancy. The amount of H-subunits in the LDH of the myometrium was 41.1%. This was outside the 2 s-range of the values for the myometrium of non-pregnant uteri. Thus it is hardly likely that the change in the LDH pattern could be caused by a mechanical effect of the developing embryo on the uterus.

4. Isoenzymes of the lactate dehydrogenase in the myometrium of the rat during pregnancy

In addition to the enzymes mentioned above we investigated the isoenzymes
Table 2.
Specific activities of lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase and cytochrome-c-oxidase and isoenzyme pattern of malate dehydrogenase in the myometrium of the rat.

<table>
<thead>
<tr>
<th></th>
<th>mU LDH mg DNA</th>
<th>mU MDH-I mg DNA</th>
<th>mU MDH-II mg DNA</th>
<th>mU GIDH mg DNA</th>
<th>mU cytochrome-c-oxidase mg DNA</th>
<th>% MDH-I</th>
<th>% MDH-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>6050</td>
<td>5330</td>
<td>1490</td>
<td>130</td>
<td>71</td>
<td>78.6</td>
<td>21.4</td>
</tr>
<tr>
<td>( s )</td>
<td>2220</td>
<td>800</td>
<td>61</td>
<td>19</td>
<td>22</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>( n )</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>35 1101)</td>
<td>22 4001)</td>
<td>18 4501)</td>
<td>16901)</td>
<td>16001)</td>
<td>54.22</td>
<td>45.8</td>
</tr>
<tr>
<td>( s )</td>
<td>14 590</td>
<td>6230</td>
<td>6710</td>
<td>1090</td>
<td>700</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>( n )</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

\( \bar{x} \): mean  
\( s \): standard deviation  
\( n \): number of observations

1) \( P < 0.01 \) significant on the 0.01 level (t-test).
2) \( P < 0.001 \) significant on the 0.001 level (t-test).
3) 100 ml of a solution contains one unit of the enzyme when the log of the optical density decreases by 1.0 per min.
Fig. 1.
Percentage of H-type in the human uterus during pregnancy.
× Myometrium. ○ Myoma.

of the lactate dehydrogenase in the uterus of the rat. In Table 3 the composition of LDH in the myometrium of castrated rats is illustrated. The pattern deviates from that of LDH in the human myometrium.

The activity was distributed evenly between LDH-III and LDH-V. In the myometrium of pregnant rats it was concentrated on LDH-IV and LDH-V. The per cent of M-LDH increased significantly. The kind of change in the LDH-patterns was the same in human and animal tissues.

Table 3.
Isoenzymes of the lactate dehydrogenase in the myometrium of the rat.

<table>
<thead>
<tr>
<th></th>
<th>Distribution of activities (%)</th>
<th>H-type (%)</th>
<th>M-type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH-I</td>
<td>LDH-II</td>
<td>LDH-III</td>
</tr>
<tr>
<td>Castrated, infantile (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>7.7</td>
<td>17.6</td>
<td>24.9</td>
</tr>
<tr>
<td>s</td>
<td>2.9</td>
<td>0.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Pregnant (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>3.1</td>
<td>5.9</td>
<td>13.7</td>
</tr>
<tr>
<td>s</td>
<td>1.6</td>
<td>1.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1) \(P < 0.001\) significant on the 0.001 level \(t\)-test.
DISCUSSION

The investigation on the activities of cytoplasmic and mitochondrial enzymes in the uterine myometrium of the human and rat demonstrated that these activities are subject to fundamental changes under the influence of pregnancy.

During the course of pregnancy a hypertrophy and hyperplasia of the uterus occur. On the basis of the total weight and DNA-content of the uterus we were able to come to conclusions concerning the extent of this development in the rat (Table 4). As compared with castrated infantile rats, the weight of the uterus increased by a factor of 115 by the end of pregnancy. This increase was the result of cellular hypertrophy by a factor of 7 and a cellular hyperplasia by a factor of 16. In contrast to this, the alteration of the protein-content in the tissue was negligible. The cellular protein-content increased in accordance with the hypertrophy. As our results show, the activities of the cytoplasmic enzymes relating to the cell increased in proportion to the growth of the cells. However, the enzymes of the mitochondria (MDH-II, GIDH, cytochrome-c-oxidase) intensified their activity to a far greater extent (Table 2).


According to our results the concentration of soluble protein in the human myometrium remained constant. The amount of protein per mg DNA increased by a factor of 10 (Table 5). Thus the DNA-concentration of the tissue decreased to $\frac{1}{10}$. This indicates that the DNA-content of the human uterus during pregnancy remains constant until a growth of the uterus by the factor 10 is reached. Below this limit the uterine growth could be interpreted as being caused by hypertrophy and only a further growth is caused by hyperplasia. Through histoplanimetric investigations on the human uterus Strauss (1969) came to the same conclusions.

In the human myometrium we found an increase in the activity of the cytoplasmic enzymes relating to the cell in proportion to the growth of the cells. However, the enzymes of the mitochondria intensified their activity to a far greater extent.

Table 4.
DNA- and protein-content in the myometrium of non-pregnant and pregnant rats

<table>
<thead>
<tr>
<th></th>
<th>uterus-weight mg</th>
<th>DNA-content of the uterus mg</th>
<th>mg protein g wet weight</th>
<th>mg protein mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26.5 ± 3.0</td>
<td>0.21 ± 0.06</td>
<td>71.7 ± 20.3</td>
<td>8.7 ± 2.2</td>
</tr>
<tr>
<td>Pregnant</td>
<td>9</td>
<td>3020 ± 880</td>
<td>3.35 ± 0.83</td>
<td>64.7 ± 14.2</td>
</tr>
</tbody>
</table>

376
plasmic enzymes (LDH, MDH-I) by the factor 10 (Table 1). Thus we assume that in the human and rat myometrium the increase in the activities of the cytoplasmic enzymes corresponds to the degree of hypertrophy.

With regard to the human myometrium there is a minor increase in the mitochondrial enzyme activities (MDH-II, G6DH, cytochrome-c-oxidase). This could be correlated to the slight hyperplasia.

This leads to the conclusion that during pregnancy in the rat either the number of mitochondria per myometrium-cell increases to a greater extent than that in the cytoplasm or that the enzyme activities of the mitochondria are augmented, that is, there are qualitative changes in the mitochondria. This is not the case in the human myometrium. The increase in the number of the mitochondria or its enzyme activities is diminished as compared to the increase of the enzyme activities in the cytoplasm.

In humans we compared tissues of pregnant women with those of women in the fertile age.

In the case of the rat the tissues of pregnant rats were compared with those of castrated, infantile rats. A comparison with non-castrated rats was inadvisable because of the rapid alterations in the ovarian cycle in these animals.

Comparison of our results with those of other investigators is only possible in a few cases. In most of the publications the enzyme activities are related to either the fresh or the dry weight of the tissue or to the protein or nitrogen content. Schmidt et al. (1967) found a MDH-activity of 15 000 mU/mg DNA in the uteri of castrated Wistar rats weighing between 120–180 g. This is twice as high as our finding. They also specified the DNA-content of the fresh tissue (mg DNA/g uterus) at 11.8. With our test we found a value of 8.0. Telfer (1953), however, found a value of 14.8.

The previously discussed enzymes have been examined by us with regard to their localization in the cell. The same holds true for the distribution of the MDH-activity between the isoenzymes MDH-I and MDH-II. The isoenzymes of the lactate dehydrogenase are, on the other hand, localized in the same cell compartment. At present we can only speculate as to the physiological significance of the isoenzymes.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>mg protein</th>
<th>mg protein</th>
<th>mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g wet weight</td>
<td>mg DNA</td>
<td>g wet weight</td>
</tr>
<tr>
<td>Normal</td>
<td>14</td>
<td>48.0 ± 5.4</td>
<td>19.8 ± 6.3</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td>Pregnant</td>
<td>13</td>
<td>52.6 ± 6.4</td>
<td>188.0 ± 48.8</td>
<td>0.28 ± 0.08</td>
</tr>
</tbody>
</table>

Table 5.
Content of soluble protein and DNA in the human myometrium.

377
At an earlier date we reported on the effect of pregnancy on the isoenzymes of lactate dehydrogenase in the human myometrium (Geyer 1968a,b).

The results of this investigation are listed in Fig. 1, which demonstrates the dependence of the LDH-composition on the duration of pregnancy. In the course of pregnancy the per cent of H-subunits decreased according to an exponential function. The LDH pattern seems to be constant in the last trimester. This could be caused by a completion of uterine development at that time.

Reynolds (1967), on the basis of observations on rabbits, thought that hydration, hyperplasia and hypertrophy of the uterus were not induced by hormones. He believed this to be a response to the irritation produced by the expansion of the foetus. This expansion-irritation is, at least for the composition of lactate dehydrogenase, unimportant. We examined the LDH-isoenzyme pattern in the myometrium and the myoma of an extra-uterine pregnancy in the 8th week (Fig. 1). The values found for the percentage of H-subunits were outside twice the standard deviation for non-pregnant tissues. Thus we may assume that a mechanical cause – for instance, a change in the blood perfusion in the tissues resulting from growing pressure by the embryo – cannot be taken into consideration as a possible cause for a change in the LDH-composition. This has been confirmed by the data from Biron (1964), who observed an increase in LDH-V in pregnant rats in the non-implanted uterine horn as well as in the implanted one, as compared to normal rats.

The isoenzyme-pattern of the lactate dehydrogenase in the rat myometrium (Table 3) had reached its maximum of activity in LDH-IV. To this extent it deviated from the human myometrium, the maximum of activity of which was found in LDH-III.

During pregnancy, however, there was a significant increase in the percentage of M-type LDH in the rat as well as in the human, the maximum activity shifting to LDH-V.

In the myometrium of ovariectomized rats we found a per cent of H-subunits of 39.9, and in the myometrium of pregnant rats a value of 22.1. Battelino et al. (1971) found isoenzyme patterns in the same animal strain, which corresponded to percentages of H-subunits of 45.1 and 27.3 respectively. Their values were higher, however, the difference between the 2 sets of values was the same.

The changes in the isoenzyme-pattern of the lactate dehydrogenase as well as the modification of the specific activities of the examined enzymes, establish that the metabolism of the myometrium conforms to the demands of pregnancy. The investigations on the modification of the LDH-composition in the rat myometrium by oestradiol (Goodfriend & Kaplan 1964) and in cell cultures by varying the oxygen partial pressure (Goodfriend et al. 1966) indicate the relation between LDH-isoenzymes and the conditions of metabolism. It might
be possible that in the human and rat uterus the isoenzyme pattern of LDH (ratio $\frac{H}{M}$) varies during pregnancy according to the hypertrophy of the myometrium cell.

Our knowledge at the present stage of research, however, does not permit any further statements.

ACKNOWLEDGMENTS

The authors thank Mrs. E. Umseher for her excellent technical assistance and Dr. H. Bloedhorn, Institut für Medizinische Statistik und Dokumentation der Universität Freiburg i.Br., for the statistical evaluation.

REFERENCES

Biron P.: Rev. canad. Biol. 23 (1964) 497.

Received on December 21st, 1973.