# DEVELOPMENT OF THE RESPONSE OF RABBIT FETAL TISSUES TO THYROXINE

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The development of the response of rabbit fetal tissues to thyroxine was investigated by determining changes in α-glycerophosphate dehydrogenase activity of the liver mitochondria of pregnant rabbits and their fetuses during administration of thyroxine at different times of pregnancy. Until the 23rd day the activity of this enzyme increased only in the mitochondria of the maternal liver, whereas in the fetuses it remained at the control level. After the 24th day of pregnancy an increase in activity of this enzyme was observed in the fetuses also, where it can be explained by the appearance of the response of the rabbit fetal tissues to maternal thyroxine at this stage of development, and increasing until the end of pregnancy.

KEY WORDS: thyroxine; pregnancy; α-glycerophosphate dehydrogenase.

A deficiency of thyroid hormones during pregnancy is known to predispose to prematurity [5] and it can be prevented by timely treatment with exogenous thyroxine [12]. However, administration of certain hormones during pregnancy, even in therapeutic doses, can lead to the development of various developmental anomalies, congenital diseases, and perinatal mortality [3], especially if their administration coincides with the so-called critical periods of ontogeny [1]. Furthermore, many investigations have shown that developmental anomalies in children are associated with diseases of the maternal thyroid gland. Barkhatova [2] found various disturbances, most frequently affecting the CNS and cardiovascular system, less frequently the reproductive, endocrine, and musculoskeletal systems, in one-third of children whose mothers suffered from diffuse toxic goiter.

The discovery of the role of hormonal factors in mammalian ontogeny, especially in the embryonic period, when processes of development and morphogenesis are particularly intensive, must be considered from the standpoint of the embryo's requirement of maternal hormones; this implies essential changes in the sensitivity of the fetal tissues to maternal hormones at different stages of intrauterine development. Thyroxine has been shown to increase the oxygen uptake in embryos of intact chickens at all stages of development [10].

However, modern views on the mechanism of action of thyroid hormones assume that the change in basal metabolism is secondary relative to the induction of nucleic acid and protein synthesis as a result of interaction between thyroid hormones and the specific receptor in the nucleus [15]. One enzyme which plays an important role in the regulation of energy metabolism in the liver cells and the synthesis of which is specifically induced by thyroid hormones is mitochondrial α-glycerophosphate dehydrogenase, by contrast with the cytoplasmic form of this enzyme [13]. An increase in α-glycerophosphate dehydrogenase (αGPD) activity after administration of thyroxine correlates distinctly with the increase in the ability of the mitochondria to oxidize α-glycerophosphate, i.e., with the αGPD activity of the liver mitochondria [13].

The object of this investigation was to study the sensitivity of metabolism of rabbit fetuses to changes in the thyroxine concentration in maternal blood as reflected in changes in the αGPD activity of the liver mitochondria of the fetus and mother at different times of pregnancy. Since thyroxine may have a toxic action on the embryo [17], its doses and the times after administration of the hormone when it caused considerable activation of mitochondrial αGPD in the maternal liver, but gave no appreciable embryotoxic effect by the time of the experiments, were investigated.

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## EXPERIMENTAL METHOD

Experiments were carried out on 72 pregnant chinchilla rabbits. Thyroxine was injected intravenously into the female in a dose of 5 mg/kg body weight 24 h before sacrifice. Mitochondria were isolated from the maternal and fetal liver in 0.25 M sucrose containing 0.01 M Tris-HCl and 0.005 M EDTA (pH 7.4) by the usual method [16]. Mitochondrial $\alpha$ GPD activity was measured polarographically. The incubation medium of the mitochondria contained 10 mM $\alpha$-glycerophosphate, 2 mM ATP, 20 mM Tris-HCl, pH 7.4, 3 mM $MgSO_4$, 1 mM $Na_2HPO_4$, and 100 mM KCl. To rule out any effect of the degree of coupling of the mitochondria, 2,4-dinitrophenol was added to the polarographic cell in a concentration of $5 \cdot 10^{-5}$ M. Protein was determined by Lowry's method [16].

## EXPERIMENTAL RESULTS

The results of measurement of mitochondrial $\alpha$ GPD activity in the maternal and fetal liver from the 18th to the 30th days of pregnancy inclusive are shown in Table 1. Before the 23rd day thyroxine caused an increase in maternal $\alpha$ GPD activity without any corresponding increase in fetal $\alpha$ GPD activity. This result may be interpreted as the onset of sensitivity to maternal thyroxine in the cells of the fetal liver. The appearance of this response evidently reflects the readiness of the tissue to respond adequately to the hormone and it is an indication of the need for the hormone to take its part, starting from that period, in the complex mechanisms of cell processes. This problem is of fundamental importance to the understanding of the particular features of formation of individual tissues and organs in the course of embryonic development and the participation of individual hormones in this process.

The results of this investigation demonstrate the high demand of the fetus for thyroxine during the period studied and confirm the results of a previous investigation showing an increase in the intensity of hormone formation in the maternal and fetal rabbit thyroid gland at these times [8]. The fact will be noted that on the 27th-28th days of pregnancy the degree of activation of the test enzyme was higher in the fetuses than in adult rabbits.

Considering the results of administration of high doses of KI during pregnancy (leading to a reduction in the blood thyroid hormone level [7]), according to which the highest fetal mortality arises as a result of the action of this compound on the 27th day of pregnancy [6], and also data showing the high response to thyroxine at this period, it can be tentatively suggested that the last 3 or 4 days before parturition constitute the critical period of development in rabbits. The hypothesis that the developing organism is highly sensitive or has low resistance to the action of stimuli is usually linked with the concept of critical periods in mammalian antenatal development [4]. The critical period of manifestation of the characteristic effects of hypothyroidism varies for animals of different species: In rats it is the postnatal period, whereas in sheep it covers the whole of period III of pregnancy [11].

There is as yet no information in the literature on the sensitivity of mammalian embryonic tissues to thyroid hormones. Khamidov et al. studied the action of thyroxine on respiration and oxidative phosphorylation of the liver and heart mitochondria of chick embryos and showed high sensitivity to thyroxine in the period of active cell differentiation in these organs [9]. However, these observations must be considered independently, for chick embryos have no placenta and their development is independent of the state of the mother.

TABLE 1. $\alpha$-Glycerophosphate Dehydrogenase Activity (in $\mu$atoms $O_2$/min · mg protein) of Liver Mitochondria of Pregnant Rabbits and Their Fetuses Following Administration of Thyroxine to the Mother at Different Times of Pregnancy

| Day of pregnancy | Number of animals | Mother | | | Fetus | | |
|---|---|---|---|---|---|---|---|
| | | control | experiment | in % of control | control | experiment | in % of control |
| 18th | 4 | 7,61±0,73 | 11,62±1,50 | 152,69 | 7,40±0,52 | 6,29±0,29 | 85,00 |
| 21st | 4 | 7,76±2,11 | 12,73±0,53 | 164,05 | 12,89±3,35 | 10,54±2,55 | 81,77 |
| 22nd | 8 | 5,51±0,55 | 8,28±0,74 | 150,27* | 9,85±0,72 | 8,91±0,42 | 90,46 |
| 23rd | 12 | 5,24±0,67 | 8,62±1,06 | 164,50* | 8,02±0,67 | 8,14±0,70 | 101,50 |
| 24th | 20 | 6,75±0,88 | 11,54±1,43 | 170,96* | 7,50±0,65 | 11,20±1,01 | 149,33* |
| 25th | 10 | 5,12±0,84 | 8,12±0,71 | 158,59* | 9,01±1,08 | 13,52±1,77 | 150,05 |
| 26th | 6 | 11,48±1,87 | 20,88±0,99 | 174,77* | 10,00±1,13 | 16,40±1,62 | 163,78* |
| 27th | 6 | 10,22±1,32 | 17,38±0,71 | 170,06* | 6,79±0,17 | 15,32±0,96 | 225,63* |
| 29th | 4 | 9,75±1,14 | 17,50±0,85 | 179,48* | 7,48±1,32 | 18,13±1,05 | 242,38* |

Legend. Differences for which $P < 0.05$ marked by asterisk.

For an unequivocal solution to the problem of the role of the placental barrier in the present investigation it would be necessary to study the metabolic response to thyroxine when given both to the mother and to the fetus, and this will be the subject of future investigations.

The results showing the development of sensitivity of embryonic tissues to thyroxine administered to the mother must therefore be interpreted in relation to the concept of unity of the maternal and fetal organisms. In order to safeguard the normal development of the fetus during administration of hormonal preparations to the mother, the times of pregnancy must be taken into account.

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