HISTOCHEMICAL CHARACTERISTICS OF CORPUS LUTEUM CELLS

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The RNA and ascorbic acid content is low in the lutein cells from the first until the 17th day of pregnancy and also from the 1st until the 20th day of lactation. Succinate dehydrogenase (SDH) and nonspecific esterase activity during these periods is high. From the 17th day of pregnancy until birth and during involution of the corpus luteum, the content of RNA and ascorbic acid in the lutein cells rises, but SDH activity and nonspecific esterase activity fall sharply. Alkaline phosphatase activity, which is detected only in the interstitial cells, rises considerably before parturition.

Studies of the histochemistry of the corpus luteum [1, 2, 8-19] have mainly been concerned with the individual stages of its development in different mammals. The fragmentary data obtained by a variety of methods are frequently contradictory and they do not paint a complete picture of the function of the corpus luteum during pregnancy and lactation.

The object of the present investigation was to make a histochemical study of the corpus luteum cells in different stages of its development during pregnancy and lactation. The dynamics of changes in RNA, ascorbic acid, succinate dehydrogenase (SDH), nonspecific esterase, and alkaline phosphatase, all of which participate in hormone synthesis and reflect the functional activity of the cell, was studied.

EXPERIMENTAL METHOD

Adult albino rats weighing 180-200 g, consisting of 50 pregnant and 70 lactating females, were used in the investigation. The material was taken on the 1st, 7th, 12th, 17th, and 21st days of pregnancy and on the 1st, 5th, 10th, 15th, 20th, and 25th days of lactation. RNA was detected by the method of Brachet and Einarson. Before staining, control sections were treated with deoxyribonuclease and ribonuclease. The method of Schneider and Meibaum was used for the quantitative biochemical analysis. SDH activity was detected by Nachlas's method with nitro-BT, and it was determined quantitatively by means of an MF-4 photometer. Nonspecific esterase activity was detected by the use of α-naphthyl acetate after preliminary fixation of fresh sections in cold acetone for 2 min. Alkaline phosphatase was detected by the azo-coupling reaction and ascorbic acid by the method of Giroud and Leblond.

EXPERIMENTAL RESULTS

Previous work [6] showed the principal periods in the development of the corpus luteum during pregnancy and lactation: the pre-implantation period (1st-7th days of pregnancy), the period of active function of the corpus luteum of pregnancy (7th-17th day), the prenatal period (from the 17th day of pregnancy until parturition), and the period of lactation and involution of the corpus luteum.

Changes in the RNA content could be used to assess the intensity of progesterone synthesis by the lutein cells during these periods of functions of the corpus luteum. In the period of preimplantation and active function of the corpus luteum of pregnancy, only single RNA granules could be seen in the cytoplasm of the lutein cells. In the prenatal period the RNA content rose sharply to reach a maximum by the 21st

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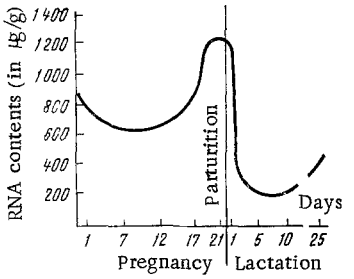

Fig. 1. Changes in RNA content in the corpus luteum at various stages of pregnancy and lactation.

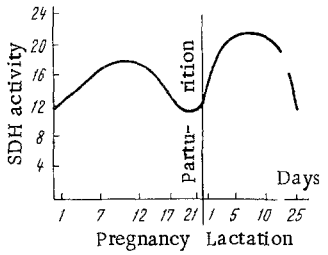

Fig. 2. Changes in SDH activity (in relative units based on photometric readings) in lutein cells during pregnancy and lactation.

day of pregnancy. The RNA level rose to 2.5 times its value in the previous periods of pregnancy. At the same time the RNA granules in the cytoplasm of the lutein cells formed clusters resembling the masses of tigroid substance in the neuron. At all stages of pregnancy the nucleoli in the lutein cells were deeply stained. After perturition and for 15-20 days of lactation, practically no RNA could be detected in the lutein cells. The biochemical control showed that during this period the RNA content fell almost to one-sixth of its level before parturition (Fig. 1). During the first days after parturition the nucleoli of the lutein cells were weakly stained, but toward the end of lactation the intensity of their staining increased. Single basophilic granules reappeared in the cytoplasm of the lutein cells on the 20th day after parturition, i.e., from the time when lactation ceased and with the beginning of involution of the corpus luteum. The RNA content during the period of involution of the corpus luteum was twice that observed in the preceding days of lactation.

The lutein cells contained ascorbic acid during the first 17 days of pregnancy in the form of a few granules located around the periphery of the nuclei and in the region of their cell membranes. In the prenatal period, numerous ascorbic acid granules accumulated in the cytoplasm of the lutein cells. After parturition, during the first days of lactation the ascorbic acid content again fell sharply. During involution of the corpus lutein, solitary large and irregularly distributed granules of ascorbic acid were seen in the cytoplasm of the lutein cells. The periods of waxing and waning of the ascorbic acid content in the lutein cells coincided with those of RNA.

Differences in the functional state of the lutein cells in the course of pregnancy and lactation were also demonstrated by changes in SDH activity. SDH is connected with the respiratory activity of the mitochondria. The reaction for SDH in the lutein cells during the first day of pregnancy was manifested by deposition of diformazan granules in their perinuclear zone, and in some of them it reached a high level. The greatest SDH activity in the corpora lutea of pregnancy occurred in the period from the 7th to the 12th day, when it was 1.6 times higher than on the first day of pregnancy (Fig. 2). Activity of the enzyme decreased until birth and reached the level of the first days of pregnancy. In the period of lactation, a sharp increase in SDH activity took place in the lutein cells. During the first days after parturition, diformazan granules were mainly located around the lipid droplets, while on subsequent days they were more uniformly distributed throughout the cytoplasm of the lutein cells. SDH activity reached its maximum on the 10th-15th day of lactation and fell gradually on the following days. In the period of involution of the corpus luteum the activity of the enzyme fell still more, but individual groups of lutein cells still retained a high content of the enzyme.

Nonspecific esterase is concerned with the hydrolysis of esters of fatty acids [12], the components of which are used in progesterone synthesis. Its activity in the cytoplasm of the lutein cells differs at different stages of pregnancy and lactation. It was lowest on the 1st day of pregnancy. On the following days the activity of this enzyme increased and it reached a maximum on the 12th-15th day of pregnancy. Before parturition and during the first two days of lactation the nonspecific esterase activity was lowered, but later it again began to rise until the 10th-15th day. During involution of the corpus luteum, hardly any nonspecific esterase could be seen. The dynamics of the changes in nonspecific esterase activity during pregnancy and lactation closely resembled those of SDH, but the former was higher during pregnancy and lower during lactation.

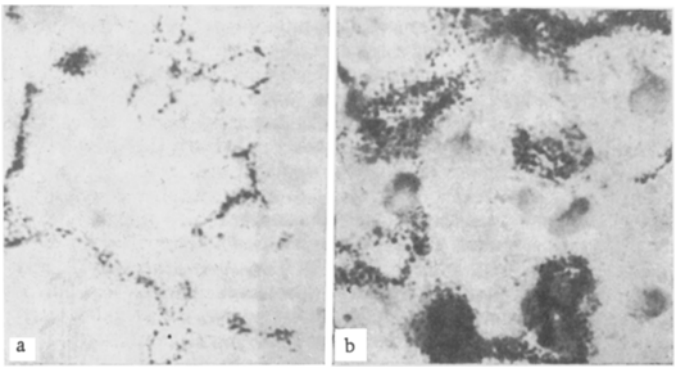

Fig. 3. Alkaline phosphatase activity in interstitial cells of the corpus luteum: a) 17th day, b) 21st day after pregnancy. Stained by azo-coupling method, 850 ×.

Interesting results were obtained during investigation of alkaline phosphatase activity. Alkaline phosphatase is concerned in the synthesis of fibrillary proteins, in the transport of metabolites through the cell membrane, and in the synthesis of estradiol [1, 7, 9, 11, 15, 17]. In the ripe ovarian follicle alkaline phosphatase was detected only in the cells of the tunica interna, which synthesize estradiol [13], whereas in the follicular epithelium the results of the reaction for this enzyme were always negative. After ovulation, in the period of formation of the corpus luteum the cells of the tunica interna proliferate, invade the granulosa-cell layer, and are converted into interstitial cells. Because of their positive reaction for alkaline phosphatase activity in the interstitial cells of the corpus luteum differs at different periods of pregnancy and lactation. In the early periods of development of the corpus luteum, alkaline phosphatase activity in the interstitial tissue is slight. Toward the period of active function of the corpus luteum (the 7th-17th days of pregnancy), when the interstitial tissue is well developed, the alkaline phosphatase content was increased (Fig. 3a). A sharp increase in the intensity of the reaction for this enzyme in the interstitial cells took place in the prenatal period (Fig. 3b), at a time when function of the lutein cells was sharply depressed. In the stages of lactation, alkaline phosphatase activity in the interstitial cells fell again, although it continued to be detected during the period of involution of the corpus luteum.

Most investigators nowadays consider that the corpora lutea function only during the first third or half of pregnancy [2, 3, 18]. According to clinical evidence [4, 5, 8, 15], the highest progesterone content in the blood is detected in the first half of pregnancy, whereas in the second half it gradually falls to reach a minimum at birth. It is important to note that the highest progesterone concentration in the blood corresponds to the lowest accumulation of lipids in the lutein cells, and the lowest concentration occurs at a time of considerable accumulation of lipids in the lutein cells [6]. The blood estradiol level rises gradually throughout pregnancy and increases sharply just before birth [3-5, 14].

The present investigations show that the dynamics of the histochemical changes in the corpus luteum are directly related to the incretory activity and functional state of its cells. In the preimplantation period, and also during the period of active function of the lutein cells of the corpus luteum of pregnancy, there is a decrease in the content of RNA and of granules of ascorbic acid, while the activity of the respiratory enzyme and of nonspecific esterase is at its maximum. The corpus luteum at these times is exerting its function of progesterone synthesis at maximum intensity, as the results of clinical and biochemical investigations have shown [4, 5, 16].

In the prenatal period, hormonal changes connected with the impending parturition takes place in the body. As the result, the blood progesterone concentration falls sharply, while the estradiol concentration rises [4, 5, 8]. Substrates participating in progesterone synthesis (RNA, ascorbic acid) accumulate in the cytoplasm of the lutein cells at this time, while the activity of the enzymes catalyzing this process (SDH, nonspecific esterase) is lowered. The same changes are observed during involution of the corpus luteum, although to a less marked degree.

During lactation, progesterone stimulates development of the mammary glands and milk secretion [3]. In this same period, the function of the lutein cells is stimulated, as is shown by the rapid utilization of RNA and ascorbic acid and the increase in activity of the respiratory enzyme and nonspecific esterase.

The gradual increase in alkaline phosphatase activity in the interstitial cells in the preimplantation period and the period of active function, with a sharp increase in the period immediately before parturition are in agreement with results [1, 4, 5, 9, 13-15] showing an increase in the blood estradiol concentration.

The histochemical changes observed are thus connected with differences in the incretory function of the corpus luteum (synthesis of progesterone and estradiol) in the period of pregnancy, parturition, and lactation.

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