ALKALINE PHOSPHATASE AND ACID PHOSPHATASE ACTIVITY OF HUMAN OVARY DURING PREGNANCY AND PUERPERIUM

by

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The biological function of alkaline phosphatase is not well understood. It has been related to transport mechanism, growth and differentiation, formation of fibrous proteins or site of steroid production (Cohen *et al* 1964).

Acid phosphatase is now well known for its proteolytic and hydrolytic activity and also for its function of intracellular catabolism, detoxification and autolysis. Moreover, the function of the enzyme is under the influence of steroids. With increased secretion of steroids the acid phosphatase activity diminished. Lobel *et al* (1962) and Nov'koff *et al* (1961) stated that acid phosphatase occurs mainly in the lysosomes. Cohen *et al* (1964) has assigned another function that is the enzyme is suggestive of the site of steroid production. The present study is undertaken to determine the content of phosphatases in the ovary, so as to understand its intrinsic cell function when placental hormone production is predominant.

Material and Methods

Ovarian tissues were procured from 45 patients admitted to the indoor department of Kamala Nehru Memorial Hospital. The specimens were collected from patients subjected to hysterotomy and sterilization, lower segment caesarian section, puerperal sterilizations and laparotomy for ectopic pregnancy (Table I).

TABLE I
Material

Groups	Number of cases
1. Normal pregnancy group:	
(a) Early pregnancy	3
(b) Term pregnancy	27
2. Puerperal group:	13
3. Miscellaneous group:	
(a) Ectopic pregnancy	2
Total	45

Alkaline Phosphatase: The azo dye coupling method using substituted napthols and fast Red RR salt (Burstone 1958) has been used at PH 8.6. Dark red granular material indicated the presence of this enzyme.

Acid Phosphatase: Burstone's (1958) method gave consistent results. Acid phosphatese was seen in the form of red intracellular granules at PH 5.2.

Results

Surface Epithelium

The alkaline phosphatase activity in

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the cells of the surface epithelium was minimal. No acid phosphatase was seen.

Primordial, graafian and atretic follicles

Alkaline phosphatase activity in the cytoplasm of the oocyte and the granulosa cells though less was more than the surrounding stroma, whereby it was easy to recognise them under the microscope.

In the oocyte of the preantrum and the antrum follicle the acid phosphatase activity was minimal. Comparatively the granulosa cells showed more activity.

The activity of the alkaline phosphatase and acid phosphatase in the granulosa cells of the graafian follicle varied greatly from one cell to the other. Alkaline phosphatase activity was either moderate or good. Acid phosphatase activity was minimal to moderate in the theca and granulosa cells. The theca interna cells had comparatively more of both the enzymes.

The layers of the follicular cyst had the same activity as seen in the graafian follicle, the theca interna cells were heavily stained and formed a red zone. The cells towards the cavity had more of activity than those towards the periphery. The vascular endothelium was rich in alkaline phosphatase. Activity of acid phosphatase did not show much change with atresia (Fig. 1).

Interstitial gland tissue

Alkaline (Fig. 2) and acid phosphatase activity was present in most of the cells of the interstitial gland tissue. The cells nearer the centre had more activity than the peripheral ones. Some scattered cells showed intense activity of the enzyme.

Corpus luteum

The staining with alkaline phosphatase emzyme was highly selective in this tissue. Granulosa lutien cells showed strong reaction. Activity was granular and was more towards the plasma membrane. Most of the cells were loaded with it.

The intensity of reaction in the functioning corpus luteus was the same whether seen during early pregnancy, term on puerperium. There was no enzyme activity in the corpus albicans. Strong reaction was exhibited by the fibroblasts traversing the substance and macrophages surrounding the albicans.

The granulosa lutein cells had moderate to good activity of acid phosphatase. The granules of acid phosphatase were mainly perinuclear. Paralutien cells did not show the presence of enzyme. The blood vessels in the fibrous septa were deviod of it. In the corpus albicans no enzyme was seen. Macrophages showed good enzyme activity (Fig. 3).

Stroma

The alkaline phosphatase activity of the stromal cells was more than the acid phosphatase. Intense activity of the alkaline was in the blood vessels.

Discussion

Histochemically observations can thus be summarized.

1. There was an overall increase of alkaline phosphatase content at term. During puerperium the activity of graafian follicle, artretic follicle and corpus luteum was the same but that of interstitial gland tissue decreased.

2. Acid phosphatase activity of corpus luteum increased with the advancing gestation and decreased during puerperium. No appreciable change was observed in the other constituents of the ovary.

Alkaline Phosphatase

In the present study, primordial

follicles showed the presence of the alkaline phosphatase in the cytoplasm of the oocyte and the granulosa cells. Mc Kay *et al* (1961) found peripheral rim of enzymes around the ovum.

Alkaline phosphatase increased as the follicle matured. The granulosa cells showed moderate activity of the enzyme and theca interna cells were rich in it. With luteinization there was a further increase. It was abundent in the vascular endothelium.

Mc Kay *et al* (1961) reported almost similar results as of the present series. The activity seen by them in the granulosa cells was patchy. Some cells had moderate activity and some had negligible. The presence of alkaline phosphatase in the theca cells had been consistently reported by Mc Kay *et al* (1961) Deane *et al* (1962) and Govan (1968). No activity in the granulosa cells was observed by Govan (1968).

With the onset of atretic changes in the follicle enzyme activity diminished but continued to be seen as a thick red band of theca around the follicle. The blood vessels had more activity than the cells. With the development of interstitial gland tissue the activity was enhanced. Govan (1968) found a strong reaction in the theca cells prior to atresia. With atresia the activity progressively decreased which is in accordance with the present results. However, he reported the persistance of the enzyme in some solid thecal masses. McKay *et al* (1961) found patchy distribution of slight to moderate enzyme activity in the theca cells of atretic follicle.

Granulosa lutein cells of the corpus luteum were rich in the alkaline phosphatase. The activity was granular and was more towards the plasma membrance. There was very little of alkaline phosphatase in the theca lutein cells of the corpus luteum studied. The growing blood vessels were rich in it. In the active corpus luteum Mc Kay *et al* (1961) noticed abundant alkaline phosphatase in the theca cells and the endothelial cells of the ingrowing capillaries supplying the theca and granulosa layers. With degeneration activity of the enzyme is less than in the active corpus luteum and is found in a ribbon like pattern involving the granulosa lutein cells near the central coagulum.

No activity was observed by them in the corpus ablicantia except for the macrophages which were rich in it as also observed in this study.

Acid Phosphatase

In both primary and preantrum follicles the activity of acid phosphatase was minimal in the cytoplasm of the oocytes and was comparatively more in the granulosa cells. These results compare well with those of McKay *et al* (1961) but Potter (1963) did not find any activity in the primordial folicles.

Deane, *et al* (1962) stated that acid phosphatase activity was not detectable in the healthy looking follicles but had developed in atreic ones, apparently first isolated, small cells, in the theca interna and later in granulosa. In the follicles of human ovary a rise in the acid phosphatase reaction appears to characterize incipent atresia.

On the contrary McKay *et al* (1961) noticed that the acid phosphatase activity was regularly present in the theca of mature follicles and commonly in the granulosa also. It was less marked than that of alkaline phosphatase in the theca and was not seen in the blood vessels. Ons can not distinguish between theca and granulosa layer by the use of acid phosphatase stain (McKay *et al* 1961).

In this study granulosa and theca cells showed minimal to moderate amount of activity. The activity with atresia remained the same.

McKay *et al* (1961) and Deane *et al* (1962) observed that acid phosphatase activity was high in all of the corpora lutea of pregnancy. In the 6 week specimens, activity occurred predominantly, in the lutein cells, generally, in small perinuclear granules, but it also existed in some phagocytes. By 12 weeks, parenchymal reaction was greater and occurred in large cytoplasmic clumps, reactive phagocytes appear numerous. Thereafter the reaction became intense throughout, although, postpartum the over all activity of the corpora lutea of pregnancy appreared some what reduced, as more and more groups of lutein cells were replaced by connective tissue. In our series the acid phosphatase of the corpus luteum was more at term than that of early pregnancy or puerperium. In the corpora albicanita macrophages were rich in it.

Summary

Fourtyfive ovarian biopsies were taken from patients subjected to hysterotomy, caesarian section, laparotomy for ectopic pregnancy and during puerperal sterilisations. Good activity of alkaline and acid phosphatase was present in the theca cells of the Graafian follicle, theca cell of the atretic follicle, interstitial gland tissue, corpusluteum and macrophages. Blood vessels and granulosa cells of the graafian follicle were rich in alkaline phosphatase. Some activity of the enzymes were also seen in the stromal cells, primordial follicle. Granulosa cells of the graafian follicle showed minimal acid phosphatase activity.

The above findings showed that the ovary is rich in acid and alkaline phosphatase through out pregnancy. These enzymes are significant in conserving its intrinsic cell function.

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See Figs. on Art Paper I