

HISTOCHEMICAL STUDIES OF ALKALINE AND ACID PHOSPHATASE ACTIVITY IN THE UTERUS AND PLACENTA OF PREGNANT WHITE RATS

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At the present time in the study of enzymes, much time is devoted to the phosphatases which are so widely distributed throughout the animal and plant worlds. Histochemical methods of analysis [2, 4] have been developed so that their localization within the tissues has become possible. The investigation of the distribution of alkaline and acid phosphatases within the placenta has considerable importance as it might indicate the functional peculiarities of the pharmacology of this organ.

Wislocki, Deane and Dempsey [6] studied alkaline and acid phosphatases within the placenta of mice, guinea pigs, rats, hamsters and rabbits. Their work presents general data concerning the enzymatic activities of the tissues of the placenta without taking into adequate account the duration in time of the pregnancy. The alkaline phosphatase, which appears in the placenta, gradually increases until term, while the acid phosphatase is found only at the end of the pregnancy. However, Hard [5], who made quantitative studies of the distribution of alkaline phosphatase within the guinea pig placenta from the 11th to the 68th day of pregnancy, came to the conclusion that the amount of the enzyme increased until the 55th day after which its quantity diminished. Wislocki and Dempsey [7] investigated the activity of the acid phosphatase in six human uteri 8 to 16 weeks pregnant.

Thus the findings in the literature concerning the alkaline and acid phosphatase content of placenta, especially of rodents, are contradictory and incomplete. Furthermore, in the literature available to us, we were unable to find data bearing on the enzymatic activity of the maternal tissues, in particular that of the uterus, during the very earliest stages of pregnancy when the embryo was being implanted so that we were unable to get a fuller concept of this activity for the entire duration of the pregnancy. Our work is an attempt to clarify the problems just raised.

In the present investigation we used the uterus and placenta of the white rat. The material taken from the rats in various stages of pregnancy was fixed in acetone. The alkaline and acid phosphatases were determined by the Gomori method. The slices were 6 to 8 μ in thickness and incubated in the corresponding substrate 2 and 21 hours. The place of alkaline phosphatase activity acquired a black tint on the sections while the acid phosphatase turned the tissue brown.

We believed it possible to distinguish four degrees of phosphatase concentration using the following arbitrary designations. The presence of the enzyme within the nucleolus only we termed trace; within the nucleus only — little; within the nucleus and cytoplasm of cell — much; while the darkening by the one or the other phosphatase to the extent that the cell structure became obscured we characterized as very high concentration of the phosphatase.

Alkaline Phosphatase. In the rat uterus on the 6th day of pregnancy the embryo, consisting of one type embryonic cells, is still either not implanted [1] or in the process of implantation. At this stage, in the portion of the tunica propria of the mucous membrane adjacent to the uterine lumen, the connective tissue cells are crowded together and within them are traces of alkaline phosphatase. Individual cells lying directly next to the blood capil-

laries exhibit much alkaline phosphatase. The number of blood capillaries is relatively increased here and within the endothelium of these capillaries there is also much alkaline phosphatase, at times the concentration becoming very high (Fig. 1, a). In cells of the precapillary type (Fig. 1, b) some of the endothelial cells contain little or only traces of the alkaline phosphatase. The walls of the larger vessels (Fig. 1 c) contain no enzyme.

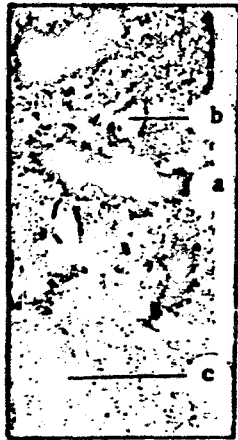


Fig. 1. Alkaline phosphatase in the tunica propria of the uterine mucous membrane of a white rat 6 days pregnant.
a) Capillary; b) blood vessel of the precapillary type; c) large vessel.
Ocular 7X, objective 40.



Fig. 2. Alkaline phosphatase in the uterus of a white rat 10 days pregnant. Sagittal section.
a) Mesometral side of the uterus; b) antimesometral side of the uterus; v) muscular layer of the uterus; d) termination of uterine lumen; e) lacunae with maternal blood; f) embryo- g) line of future uterine lumen; h) ectoplacental conus; i) connective tissue. Oct. 2X, ob. 3.7.

In the other portion of the tunica propria, lying nearer to the muscle layer of the uterus where the connective tissue is looser, no alkaline phosphatase could be found. However, on the antimesometral side in the region of the future embryonal implantation within the connective tissue cells of the tunica propria, beginning from the uterine lumen and extending almost to the muscular layer, alkaline phosphatase is found. In some cells there is much, while in occasional instances there is a very high concentration of the alkaline phosphatase.

On the 10th day of pregnancy the embryo is in the stage of a late gastrula (Fig. 2, f). By this time, the embryo is separating from the extraembryonic tissues. There is an amnion. There is still no contact between the chorionic plate and the allantois. The tunica propria tissues of the uterine mucous membrane have grown and become enriched with blood containing lacunae (Fig. 2, e), which penetrate the ectoplacental conus (Fig. 2, h) which represents the mesometral portion of the prophoblast. Thus, at this stage of pregnancy the elements of the future placenta have been laid down and the further development of the embryo has been assured. At this time within the rat uterus the quantity of alkaline phosphatase increases. On the antimesometral side (Fig. 2, b) within the enlarged cells of the growing tunica propria there is much alkaline phosphatase while, within the decidual cells adjacent to the walls of the blood bearing capillaries the concentration is very high. The same condition is to be observed within the endothelium of the blood-bearing capillaries and the connective tissue at the site of the future uterine lumen (Fig. 2, g).

On the mesometral side (Fig. 2, a) the forming lacunae (Fig. 2, e) have various dimensions. The larger ones are disposed around the ectoplacental conus (Fig. 2, h) and contain traces of the enzyme. The decidual tissue between the large lacunae, especially in direct contact with the ectoplacental conus, has no enzyme. In the mid-portion of the mesometral side (Fig. 2, c) there is much of the enzyme, while in the direction of the muscular

layer (Fig. 2, *h*) and the terminal uterine lumen (Fig. 2, *d*) it diminishes and disappears altogether. However, a thin layer of connective tissue, lying along the muscular layer (Fig. 2, *i*), darkens intensively. This darkening is to be observed for some distances from the ends of the uterine lumen and, at times, spreads to the whole length of the muscular layer.



Fig. 3. Alkaline phosphatase within the placenta of a white rat on the 17th day of pregnancy.

a) Allantoic mesenchyme; b) placental labyrinth; c) border zone; d) zone of giant cells; e) maternal placenta; f) line of future placental separation; g) basal decidua layer; h) wall of embryonic egg yolk sac; i) central placental vein; j) leukocytes (black) within the venous sinus of the maternal blood. Ocular 7X. Objective 3.7.

stage contain much enzyme. The trophoblastic cells within the placental border zone (Fig. 3, *c*) contain much alkaline phosphatase; among the glycogen-containing cells of this zone are encountered groups of encapsulated cells, there being an especially large number of them around the central vein (Fig. 3, *i*) of the placenta, while within each of these encapsulated groups can be counted from 2 to 5 cells of varying size all containing much enzyme. In the other glycogen-containing cells, among which are to be seen doubly-nucleated cells resulting from amitotic division, there is little alkaline phosphatase. The zone of the giant cells (Fig. 3, *d*) is thinned as compared with earlier developmental stages becoming interrupted in some areas; the giant cells are more rounded and smaller. Within the cytoplasm phagocytosed remnants of degenerated cells and also of blood cells are to be seen. Within the giant cells there is either only a little or traces of the alkaline phosphatase. While within the maternal placenta (Fig. 3, *e*) and the basal decidua layer (Fig. 3, *g*) there is none.

Acid Phosphatase. The uterus of a white rat pregnant 6 days does not contain acid phosphatase.

By the 10th day it begins to appear in almost the same uterine areas as the alkaline but in much smaller concentrations. In the middle portion of the antimesometrial side there is much of the enzyme which decreases in the direction of the muscular layer of the uterus and the direction of the embryo gradually disappearing. At the site of the future uterine lumen the connective tissue cells display traces of the acid phosphatase. On the mesometrial side in the areas of decidua tissue, there is much acid phosphatase around the larger lacunae lying directly

By the 15th day of the pregnancy the placenta is fully formed. The cells of the fetal mesenchyme contain no alkaline phosphatase. It is to be observed in the zone of the placental labyrinth. Here the trophoblast is represented by an intensely darkened syncytial net having nuclei of different sizes. In the loops of the net are lacunae with maternal blood while, within the trabeculae of the trophoblastic syncytium, separating the maternal lacunae, lie fetal vessels accompanied by allantoic mesenchyme. The cells of the maternal tissue lying in this zone form islets of varying dimensions containing little enzyme. The elements of the trophoblasts of the border zone are represented by relatively scattered narrow lacunae and large fields of clear, glycogen-containing cells and show rarely a very high concentration of the enzyme, more often there being but little of it. The trophoblastic elements more distant from the lacunae and the cells of the maternal tissues contain traces of the enzyme. The giant cells of the trophoblast contain little phosphatase while the maternal placenta and the basal decidua layer have none. Along the line of the future placental separation within the connective tissue there is a very high concentration of the alkaline phosphatase, this being usually localized not along the whole line but only along its peripheral portions.

On the 17th and 18th days of the pregnancy, when the placenta attains its maximum dimensions, the alkaline phosphatase is still absent from the fetal mesenchyme (Fig. 3, *a*) while within the zone of the placental labyrinth (Fig. 3, *b*) the very high concentration is maintained. Within the placental labyrinth the trophoblastic syncytium is thinned and intensively darkened; the elements of the maternal tissues which are occasionally still seen at this

around the ectoplacental conus. In the direction of the muscular layer and the terminal uterine lumens, the enzyme rapidly diminishes and disappears.

By the 15th day of pregnancy acid phosphatase is present in all the placental zones. There is much in the zone of the allantoic mesenchyme while within the elements of the trophoblasts of the placental labyrinth and the border zone the concentration is very high. Very high concentrations are also present in some giant cells although in others there is but little. The acid phosphatase is much in evidence within the islets of maternal tissue present in the placental labyrinth and the glycogen-containing cells. In the maternal placenta and the basal decidual layer the enzyme is distributed unequally: in some areas the concentration is very high while in others there is much.

By the 17th and 18th days of the pregnancy the amount of acid phosphatase present in the rat placenta increases and is found in all the zones. In some areas of the allantoic mesenchyme the amount of enzyme is small while in other areas there is much. The enzyme is found present in very high concentrations within the thinned trophoblastic syncytium and the islets of the placental labyrinth of the maternal tissue, the trophoblast and the glycogen cells of the border zone. Within the giant cells and the cells of the maternal placenta and the basal decidual layer by the 17th day the enzymatic concentration is very high, there still being much of it present on the 18th day.

Much alkaline and acid phosphatase is found within the leukocytes of the maternal blood (Fig. 3, j) and the cells of the embryonic blood. The enzyme localizes first in the anterior portion of the nucleoli, the nucleus and then in the cell cytoplasm.

The alkaline phosphatase appears within the uterus at the very beginning of pregnancy, increasing gradually until the very end. There is no acid phosphatase within the uterus in the first days of pregnancy. It appears on the 10th day, the amount increasing sharply at the end of the term.

The large placental content of acid and alkaline phosphatases indicates the activity of the biochemical processes going on. Of special interest is the fact that the largest quantity of phosphatase is found within the layer of the trophoblastic syncytium which envelops the fetal vessels and borders the lacunae filled with maternal blood. The data now available indicate that it is precisely in these areas that the trophoblast absorbs the amino acids from the maternal blood and, very possibly, resynthesizes the protein bodies entering the blood of the fetus [3].

SUMMARY

In investigating the activity of the alkaline and acid phosphatases in the uterine and placental tissues on the 6th, 10th, 15th, 17th and 18th day of pregnancy certain regular cytochemical features are revealed.

Phosphatases are localized mainly in the nucleoli, nucleus and then in the cellular cytoplasm. Large amounts of alkaline and acid phosphatases are found in the leukocytes of the maternal blood and in the embryoblasts. Alkaline phosphatase appears in the uterus at the onset of pregnancy and gradually increases until its termination. Acid phosphatase appears only on the 10th day and greatly increases until the end of the pregnancy.

The largest amounts of phosphatase are revealed in the trophoblastic syncytium which covers the fetal blood vessels and lies next to the lacunae filled with maternal blood.

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*In Russian.