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HISTOCHEMICAL STUDIES OF DECIDUOMATA PRODUCED IN RAT UTERUS

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Introduction

Since Loeb(5) first observed the deciduoma reaction in the guinea pig uterus, many investigators have contributed to the biological studies of it. Ershoff and Deuel(2) and Peckham and Greene(7) found that the presence of deciduomata extended the period of pseudopregnancy in the rat, contrary to Astwood and Greep(1) and Kamell and Atkinson(3) who reported that it possessed no such influence in the rat and mouse. Recently, Olsen et al (6) and Velardo et al (9) stated that the presence of massive deciduomata prolonged the period of pseudopregnancy in the rat until about the normal time of parturition. They also found that this prolongation has relation with the amount of deciduoma cells which were produced in the uterine mucosa; the presence of a large amount of deciduoma cells prolonged the period of pseudopregnancy, whereas the presence of a small amount of them did not.

From the histological and histochemical point of view, there are few who have studied the deciduomata. Velardo et al (8) reported that the deciduoma reaction in the rat, occurred first in the antimesometrial region of the uterine mucosa, then in the mesometrial one. Krehbiel (4), who obtained similar results, also reported that mesometrial and antimesometrial deciduoma cells always contained a large amount of lipid and glycogen.

In this investigation, I have dealt with the appearance of glycogen, lipid, RNA and alkaline phosphatase in the deciduoma cells, and compared them with the cytochemical natures of true decidua cells.

Materials and Methods

Twelve female rats which exhibited a regular oestrous cycle were used. The deciduomata were produced in the empty uterus of the rat in unilateral pregnancy. Prior to the time of copulation, the oviduct of the left side was tied. At the 4th day after copulation, the empty uterus was stabbed with a

steel needle. The rats were killed by decapitation at the desired time after copulation. The uteri which had deciduomata were fixed in 95 per cent alcohol and in 10 per cent neutral formalin. As controls, the pregnant uteri were used. The materials fixed in 10 per cent neutral formalin were made into frozen sections of 30μ thick. The materials fixed in 95 per cent alcohol were embedded in celloidin and sectioned 20μ thick.

For the demonstration of glycogen, the sections were stained by the periodic acid-Schiff method modified by Lillie (PAS). The identification of glycogen was made by means of the salivary test at 37°C in an incubator. For RNA, thionin was used. RNA was confirmed by ribonuclease treatment. For lipid, Sudan III and Sudan black B were used. For the demonstration of alkaline phosphatase, Gomori's revised method was employed by using glycerophosphate as a substrate. McJunkin's method for peroxydase and Schultz's method for cholesterol were used. Morphology of the deciduomata was made by hematoxylin-eosin stain, Mallory's stain, Van Gieson's stain and Gomori's aldehyde-fuchsin stain.

Results

1. Production of deciduomata.

As shown in Table 1, the massive deciduomata were formed in the empty uterus of all the rats in unilateral pregnancy, whereas they were not formed in the uterus of non-pregnant rats. The number of deciduomata nearly coincided with that of stimulation; for example, four deciduomata were found in the four stimulated parts of the empty uterus. The deciduomata were not defined by size. This may be due to the degree of intensity of stimulation.

Table 1. Deciduomata produced by mechanical stimulation.

Rat No.	Gestation age at time stimulated (day)	Gestation age at time killed (day)	No. of embryos in normal uterus	No. of deciduomata produced	No. of stimulated part	Instrument used for stimulation
1	4	7	10	3	4	steel needle
2	4	9	5	3	4	"
3	4	10	5	4	4	"
4	4	12	5	4	5	"
5	4	15	5	5	5	"
6	4	8	8	4	4	"
7	4	6	3	5	5	"
8	4	6	4	2	4	"
9	4	10	8	3	5	"
10	4	8	5	5	5	"
11	—	(2)	—	0	5	"
12	—	(3)	—	0	5	"

Note: ()..... Age from stimulation to decapitation.

2. Histochemical natures of deciduomata.

General histological and cytological aspects. The development of deciduomata

Table 2. Histochemical aspects of deciduomata.

Staining methods	Fixing fluids	Deciduoma cells	Decidua cells (Pregnant)	Connective tissue cells of endometrium (non-pregnant)
PAS	95% alcohol	++ to +++	+++	—
Best's carmine	"	++ to +++	+++	—
PAS after salivary test	"	—	—	—
Sudan III	10% neutral formalin	+ to ++	++	—
Sudan black B	"	++ to +++	++	—
Schultz	"		—	—
Thionin	95% alcohol	+++	+++	—
Thionin after RNA-ase	"	—	—	—
Alkaline phosphatase peroxidase	"	+++	+++	—
	"	—	—	—
Hematoxylin-eosin	"	light red	light red	light red
Mallory	"	light blue	light blue	light blue
Van Gieson	"	yellow	yellow	yellow
Aldehyde-fuchsin	"	light purple	light purple	—

closely resembled that of decidua: The deciduoma reaction occurred first in the antimesometrial region, then in the mesometrial one. A wedge-shaped projection of uterine lumen was formed towards the antimesometrium (Figs. 1 and 2). The uterine glands were pushed out towards the basal zone. The sinus of capillary developed among the massive deciduoma cells. They were more abundant in the mesometrium. The differences between the development of deciduomata and that of decidua are as follows: Firstly, the uterine epithelium was lost in the early stage of decidua formation, but not lost through the course of deciduoma formation. Secondly, the implantation zone was not formed through the course of deciduoma formation, but it was distinctly formed in the early stage of decidua formation.

The deciduoma cells were polygonal in shape, having frequently one or more projections, and possessed a round nucleus which was coarsely stained by the Feulgen's method. As shown in Table 2, the cytoplasm of deciduoma was stained in light red color by hematoxylin-eosin stain, light blue by Mallory's stain, yellow by Van Gieson's stain and purple by aldehyde-fuchsin stain.

Glycogen. When the deciduoma cells were stained by the PAS method, red-purple granules appeared in the cytoplasm. Stained by the Best's carmine method, red granules appeared. Since the PAS and Best's carmine positive substance was digested by saliva, this was apparently glycogen. As shown in Table 2, the amount of glycogen in the deciduoma cells was always abundant as in the case of the normal decidua cells (Fig. 3).

Lipoid. When the deciduoma cells were stained with Sudan III, the brown colored droplets, and stained with Sudan black B, the black colored droplets, showing the presence of lipoid, appeared in the cytoplasm (Fig. 4). The amount of lipoid was especially abundant in the cells near the uterine epithelium at the antimesometrial region. This aspect nearly coincided with that of the normal decidua cells.

RNA. Stained with thionin, purple colored granules appeared in the cytoplasm, showing the presence of RNA. The amount of RNA was always abundant, nearly coinciding with that of normal decidua cells (Fig. 5).

Alkaline phosphatase. As shown in Table 2, an intense alkaline phosphatase reaction appeared in the deciduoma cells as in the normal decidua cells (Fig. 6). This reaction also appeared intensely in the endothelial cells of the capillary sinus.

Other substances. The Schultz reaction was negative in the deciduoma cells, showing the absence of cholesterol. McJunkin's reaction was also negative, showing the absence of peroxydase.

The uterine epithelium contained a small amount of lipoid, RNA and alkaline phosphatase.

Krehbiel(4) studied the production of deciduomata in the empty uterus of pregnant rats associating with its histological and histochemical natures and reported that the deciduoma cells contained a large amount of glycogen and lipoid. In this investigation, the deciduoma cells possessed a large amount of glycogen and lipoid; the latter was especially abundant in the cells near the uterine epithelium at the antimesometrium, agreeing with the results of Krehbiel.

In the present investigation, the deciduoma cells also contained a large amount of RNA and alkaline phosphatase. This shows that the deciduoma cells have a high metabolic activity.

From the biological point of view, Olsen et al (6) and Velardo et al (9) stated that the presence of massive deciduoma prolonged the period of pseudo-pregnancy in the rat until about normal time of parturition. Considering from their reports and from the cytochemical natures of the deciduoma cells found in the present investigation, it is considered that the function of the deciduoma cells resembles extremely that of the decidua cells.

Summary

The results obtained in this investigation are summarized as follows :

1. The deciduoma reaction occurred first in the antimesometrial region, then in the mesometrial region. This closely resembled the development of normal decidua.
2. The experimentally produced deciduoma cells contained a large amount

of glycogen, lipid, RNA and alkaline phosphatase, showing the high metabolic activity of the cells.

3. From the fact that the cytochemical natures of deciduoma cells were the same as those of the decidua cells, it seems that the function of the deciduoma cells resembles extremely that of the decidua cells.

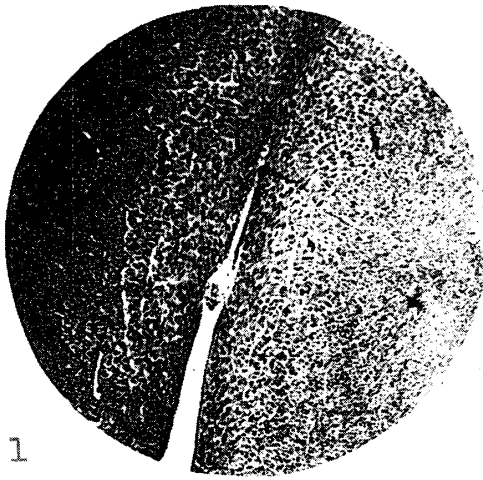
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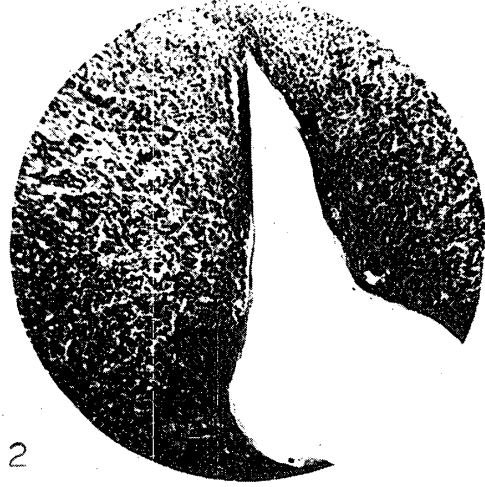
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Explanation of Figures

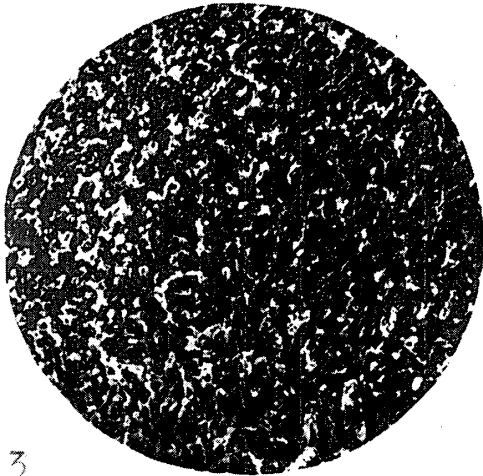
- Fig. 1. Uterus of rat at 7th day of pregnancy. $\times 50$. Hematoxylin-eosin stain.
The blastula implanted and the decidua cells developed.
- Fig. 2. Stimulated empty uterus of pregnant rat. $\times 50$. Hematoxylin-eosin stain.
The uterine lumen projected towards the antimesometrial region and the uterine glands were pushed out to the basal zone. The deciduoma cells developed.
- Fig. 3. Experimentally produced deciduoma. $\times 100$. PAS stain.
The deciduoma cells contained a large amount of glycogen.
- Fig. 4. Experimentally produced deciduoma. $\times 100$. Sudan black B stain.
The deciduoma cells possessed a large amount of lipoid.
- Fig. 5. Experimentally produced deciduoma. $\times 100$. Thionin stain.
The deciduoma cells had a large amount of RNA.
- Fig. 6. Experimentally produced deciduoma. $\times 100$. Gomori's stain.
The deciduoma cells showed an intense alkaline phosphatase reaction.



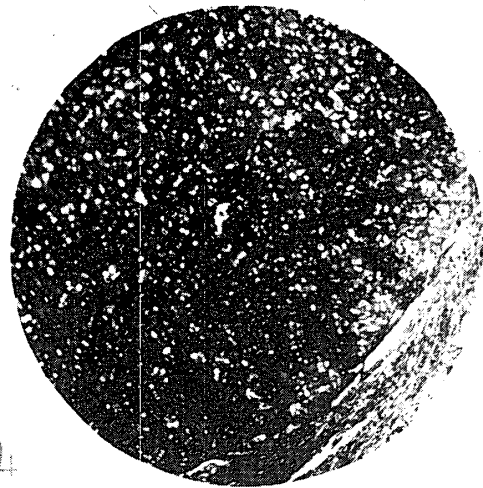
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