MORPHOLOGICAL AND CYTOCHEMICAL STUDIES OF PIGMENT CELLS IN THE RAT UTERUS

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By

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(*Received December, 19, 1955*)

Introduction

In his investigation on the development of the rat placenta, Bridgman (1) reported that in the uterus just after copulation certain wandering cells containing lumps of brownish pigment which reacted with osmic acid and Sudan III were found in the stroma, and in the rats which had been previously pregnant these cells congregated densely in groups under the attachment of the mesometrium. He also stated that these wandering cells stored trypan blue as well as the uterine histiocytes.

In this investigation, I have dealt with the appearance and the morphological and cytochemical features of the pigment cells, corresponding to "Wandering cells of Bridgman", in the uterus of the non-pregnant and pregnant rats.

Materials and Methods

About 4 months old rats which were bred with the same diet were used; these included 8 non-pregnant rats, of which 3 had been previously pregnant, and 23 pregnant rats at various stages of pregnancy. The uteri were fixed in 10 per cent neutral formalin and in 95 per cent alcohol. The materials fixed in 10 per cent neutral formalin were made into frozen sections of 25 μ thick. The materials fixed in 95 per cent alcohol were embedded in celloidin or paraffin and were cut at 3 to 15 μ.

The staining methods used in this investigation were as follows; Azure II eosin and hematoxylin stain (Maximow) for morphology, thionin stain for RNA, Sudan black B stain and Sudan III stain for lipids, Ziehl-Neelsen stain for acid-fast lipofuscin, alkaline silver reaction for lipopigments, Schmorl method and chrome alum hematoxylin method for lipofuscin, bleaching test with 10 per cent H_2O_2 for pigments, PAS method for polysaccharides, PFAS method for SS group, plasmal reaction after blocking with hydroxylamine for acetal phosphatide, Schultz method for choresterol, Gomori method for alkaline phosphatase, Turnbull blue method for ferrous iron and Perls method for ferric

iron. For the vital staining of the endometrium, intraperitoneal injections with 0.5 ml of 0.5 per cent trypan blue performed into 2 non-pregnant and 3 pregnant rats every day for a week.

Results

1. *Morphological features of pigment cells*

The pigment cells were found in the endometrium through the uterus. These cells possessed no definite shape, and had hardly no projections of cytoplasm (Fig. 1). The nucleus was oval in shape like that of macrophages. The cytoplasm usually contained a large amount of pigment granules of various sizes. These pigment cells degenerated as follows: The nucleus showed piknosis, while the cytoplasm ruptured to disperse the pigment granules, and finally the cells completely disappeared. The size of the pigment cells was measured about 9–43$\mu \times$5–14μ. The detailed descriptions concerning the size of the pigment cells will be given in the section "Appearance of pigment cells".

To investigate whether the pigment cells have a phagocytic faculty the vital staining with trypan blue was carried out on the non-pregnant and pregnant rats by the method already stated. The results are as follows: A small number of macrophages appeared in the endometrium. They stored trypan blue closely packed in the cytoplasm. The monocytes in the blood also stored trypan blue abundantly. The eosinophils which appeared abundantly in the endometrium stored trypan blue in the cytoplasm. The pigment cells did not store trypan blue at all. The cells of the intermediate type of macrophages and pigment cells were frequently found in the endometrium. They contained few yellowish pigment granules in the cytoplasm and stored slightly trypan blue (Fig. 2). From the result just stated, it was inferred that the pigment cells originate from the macrophages.

Bridgman (1) reported that in the rat uterus certain wandering cells containing lumps of brownish pigment appeared in the stroma. He further stated that the pigment cells stored trypan blue in the cytoplasm.

In this investigation, the pigment cells stored trypan blue agreeing with Bridgman's report, while it was confirmed that the pigment cells originated from the macrophages.

2. *Cytochemical features of pigment cells*

The cytochemical natures of the pigment cells in the various stages of pigmentation are given in Table 1.

As shown in Table 1, when the sections were stained with Azure II eosin and hematoxylin, the pigment granules showed a blue colour. With the increment of pigmentation the granules stained more intensely. Stained with

Table 1. Cytochemical natures of pigment cells

Stains	Fixatives	Sections	Pigment cells	
			early stage of pigmentation	later stage of pigmentation
Colour of pigment unstained	Formalin Alcohol	Frozen Paraffin Celloidin	Yellow	Brown
Azure II eosin and hematoxylin	Formalin	Paraffin	Blue	Deep blue
Vital staining with trypan blue	"	"	###	–
Thionin	Alcohol	"	+	###
Thionin after RNA-ase	"	"	+	###
Sudan black B	Formalin	Frozen	###	##
Sudan III	"	"	###	+
Ziehl-Neelsen	"	Paraffin	+	±
Alkaline silver	"	Frozen	±	+
Schmorl	"	"	+	##
Chrome alum hematoxylin	"	Paraffin	##	###
Bleaching with 10% H_2O_2	"	Frozen	48 hours	48 hours
PAS	Alcohol	Paraffin Celloidin	±	±
PFAS	Formalin	Frozen	+	+
Plasmal	"	"	–	–
Schultz	"	"	–	–
Alkaline phosphatase	Alcohol	Paraffin	–	–
Turnbull blue	"	"	–	–
Perls	"	"	–	–

thionin, the pigment granules exhibited an intense blue colour. Since the blue coloured substance did not digest with RNA-ase, it was not RNA.

Stained with Sudan black B, the pigment cells showed a deep black colour (Fig. 3) and with Sudan III, they took a brown colour, showing the presence of lipids in their cytoplasm.

When the sections were stained by the Ziehl-Neelsen method for acid-fast lipofuscin, the pigment cells showed a bright red colour. By the alkaline silver method, the Schmorl method and the chrome alum hematoxylin method for lipofuscin, the pigment cells were stained as follows; by the first method, the pigment granules showed a dark brown colour showing the activity of the alkaline silver reduction, by the second method, they displayed a dark blue colour showing that the ferricyanide reduced to ferrocyanide (Fig. 5), and by the third method, they showed a dark blue colour showing the presence of lipofuscin (Fig. 4).

Treated with 10 per cent H_2O_2 for 48 hours, the pigment granules were bleached completely.

As shown in Table 1, stained by the PAS method for polysaccharides, the pigment cells displayed a pale red colour. Since the red coloured substance resisted saliva, it was not glycogen. Stained by the PFAS method for SS group, the pigment cells exhibited a light red colour showing the presence of SS group. By the plasmal method after the blocking with hydroxylamine, the pigment cells were not stained showing the absence of acetal phosphatide.

As shown in Table 1, when the sections were treated by the Schultz method for choresterol, Gomori method for alkaline phosphatase, Turnbull blue method for ferrous iron and Perls method for ferric iron, the pigment cells of various stages of pigmentation showed no reaction.

From the results described above, it is clear that the pigment granules are composed of lipofuscin. To support the view just stated, I noticed the following relations: First, the pigment granules discoloured with 10 per cent H_2O_2 treatment for 48 hours; second, they stained with Sudan dyes such as Sudan black B and Sudan III; third, they showed positive reactions by the Schmorl method, alkaline silver method and chrome alum hematoxylin method; fourth, they contained no irons of both ferrous and ferric type.

The macrophages in the endometrium contained RNA in a small amount, lipids in a large amount, but no PAS reactive substance.

Pearse (11) reported that the lipofuscin became more basophilic with the increment of pigmentation. In this investigation, the brownish granules in the later stage of pigmentation were more basophilic than the yellowish granules in the early stage of pigmentation, agreeing with Pearse's report.

Pearse (11) further stated that the lipofuscin was stained by the PFAS method. McManus (8) reported that the lipofuscin was reacted by the PAS method and by the plasmal method. He further mentioned that the lipofuscin of ceroid type, corresponding to the lipofuscin in the early stage of pigmentation, gave a positive reaction by the PAS method and by the plasmal method.

In the present investigation, the pigment granules gave a positive reaction by both the PFAS method and the PAS method, agreeing with the reports of Pearse and of McManus. On the contrary, however, the pigment granules gave a negative result with the plasmal reaction, disagreeing with McManus' report.

3. *Appearance of pigment cells*

The appearance of pigment cells in the uteri of the non-pregnant and pregnant rat is given in Table 2, and the size of them in Table 3.

Non-pregnant animals

As shown in Table 2, in the uteri of the rats which had not been previously pregnant, a large number of cells in the early stage of pigmentation appeared dispersedly in the endometrium. No pigment cells of aggregated type were found.

In the uteri of the rats which had been previously pregnant, the appearance of the pigment cells was the same as those in the uteri of the rats which had not been previously pregnant, except that a small number of pigment cells aggregated in groups under the attachment of the mesometrium. It seems that this aggregated cell masses originated from the pigment cells which appeared during the previous pregnancy.

No variations in the distribution and number of pigment cells were found during the estrous cycle.

Table 2. Appearance of the pigment cells in the uteri of the non-pregnant and pregnant rats

Age of pregnancy (day) \ Type of cells		Dispersed type		Aggregated type	
		early stage of pigmentation	later stage of pigmentation	early stage of pigmentation	later stage of pigmentation
Non-pregnant	not previously pregnant	++ to +++	−	−	−
	previously pregnant	++ to +++	−	−	+
Pregnant	1	++ to +++	−	−	−
	2	++ to +++	−	−	−
	3	++ to +++	−	−	−
	4	+++	−	−	−
	5	+ to ++	−	−	−
	6	++ to +++	−	−	−
	7	+	+	−	−
	8	+	+	−	− to +
	9	+	+	−	− to ++
	10	+	+	−	− to +
	12	−	+	−	+
	16	−	+	−	+++
	18	−	−	−	++
	21	−	−	−	+++

Table 3. Size of pigment cells

Age of pregnancy (day) \ Type of cells		Dispersed type		Aggregated type	
		early stage of pigmentation	later stage of pigmentation	early stage of pigmentation	later stage of pigmentation
Non-pregnant		13–20 × 5–9 (μ)	not seen	not seen	not seen
Pregnant	1 to 6	14–23 × 5–13	,,	,,	,,
	7 to 10	13–24 × 5–12	10–24 × 5–13	,,	10–32 × 5–14
	11 to 21	not seen	16–25 × 5–12	,,	9–43 × 5–7

Pregnant animals

Only the rats which had been previously pregnant were used for the study

of the pigment cells in the pregnant uteri.

1st to 6th day of pregnancy: As shown in Table 2, a moderate to a large number of pigment cells appeared dispersedly in the endometrium, but they did not appear in the decidua. All cells were of the early stage of pigmentation.

7th to 10th day of pregnancy: As shown in Table 2, a small number of pigment cells in the early to later stage of pigmentation appeared in the connective tissue of muscle layers of uteri, whereas they did not appear in the stroma, decidua and placenta. The masses of the cells in the later stage of pigmentation appeared frequently near the blood vessels in the connective tissue of the muscle layers (Fig. 6).

11th to 21th day of pregnancy: As shown in Table 2, a small number of pigment cells in the later stage of pigmentation was found in the connective tissue of the muscle layers, whereas those in the early stage of pigmentation they disappeared. The aggregated pigment cells increased in number. Frequently the degenerating pigment cells were found in all specimens.

As shown in Table 3, no variation in size of pigment cells of dispersed type was found in the non-pregnant and pregnant uteri, measuring about 9–25μ×5–13μ, while the pigment cells of the aggregated type found in the later stage of pregnancy were somewhat larger than those of the dispersed type, measuring about 9–43μ×5–14μ.

Lillie et al. (4, 5) and Edwards and White (3) reported that the lipofuscin of ceroid type appeared in the cirrholic liver cells of animals maintained on inadequate diets. Edwards and Dalton (2) described a similar pigment occurring in the liver cells of mice treated with carbon tetrachroride. Martin and Moore (6, 7) reported that the lipofuscin of ceroid type was found in various tissues, especially liver, muscle, ganglion cells in vitamine E deficiency of rats and of other laboratory animals. Nishikawa and Horie (9) and Onuma and Nishikawa (10) reported that the pigment cells containing lipofuscin appeared in the testis of the horse at various stages of development, whereas it did not appear in the testis of the goat and mouse.

In the present investigation, the pigment cells containing lipofuscin in the early stage of pigmentation were found in the endometrium of non-pregnant and pregnant rats. With the progress of pregnancy the pigment cells containing lipofuscin in the later stage of pigmentation appeared dispersedly in the endometrium. At the later stage of pregnancy they were frequently observed in groups in the connective tissue of muscle layers of uteri.

Summary

The results obtained in this investigation may be summarized as follows:

1. The pigment cells had no definite shape, measuring about 9–43μ×5–14μ. The nucleus was oval and the cytoplasm usually contained a large amount of

pigment granules of various sizes.

2. The macrophages in the endometrium stored trypan blue closely packed in the cytoplasm, while the pigment cells did not. The cells of the intermediate type of macrophages and pigment cells were frequently found in the endometrium, suggesting that the pigment cells originated from the macrophages.

3. The pigment granules were composed of lipofuscin; they discoloured with the 10 per cent H_2O_2 bleaching for 48 hours, stained with Sudan dyes, showed positive reactions by the Schmorl method, alkaline silver method and chrome alum hematoxylin method, and contained no irons.

4. In the non-pregnant rats, a large number of pigment cells appeared dispersedly in the endometrium.

5. During the early stage of pregnancy, yellowish cells in the early stage of pigmentation appeared dispersedly in the stroma of uteri. During the middle stage of pregnancy, the brownish cells in the later stage of pigmentation appeared dispersedly in the connective tissue of muscle layers of uteri. During the later stage of pregnancy, the yellowish cells in the early stage of pigmentation disappeared, while the brownish cells in the later stage of pigmentation were frequently found in groups in the connective tissue of the muscle layers.

Acknowledgment: I wish to express my hearty thanks to Prof. Dr. Y. Toryu for his valuable guidance and criticism throughout the entire course of this work.

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Explanation of Figures

Fig. 1. The pigment cells in the rat uterus on the 4th day of pregnancy.
Azure II eosin and hematoxylin stain. ×800.
The blue coloured granules are closely packed in the cytoplasm.

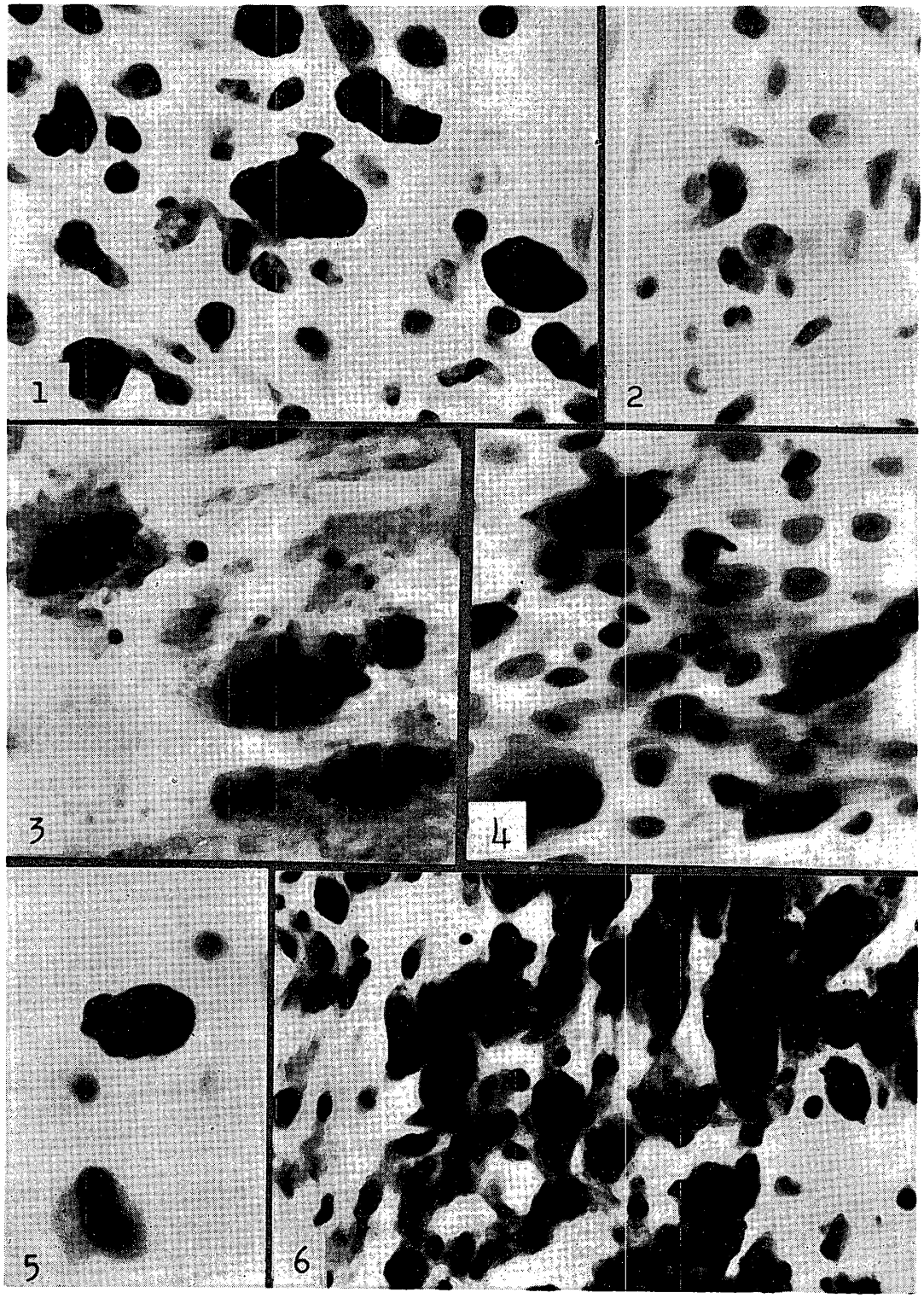
Fig. 2. The pigment cells in the uterus of non-pregnant rat.
Vital staining with trypan blue. ×800.
The yellowish cells in the early stage of pigmentation store slightly trypan blue.

Fig. 3. The pigment cells in the uterus of non-pregnant rat.
Sudan black B stain. ×800.
The pigment cells exhibit a deep black colour, showing the presence of lipids.

Fig. 4. The pigment cells in the rat uterus on the 6th day of pregnancy.
Chrome alum hematoxylin stain. ×800.
The pigment cells display a deep blue colour, showing the presence of lipofuscin.

Fig. 5. The pigment cells in the rat uterus on the 7th day of pregnancy.
Schmorl method. ×800.
The pigment cells display a deep blue colour, showing the presence of lipofuscin.

Fig. 6. The pigment cells in the rat uterus on the 16th day of pregnancy.
PAS stain. ×800.
The brownish cells in the later stage of pigmentation aggregate in the endometrium.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