A Pigment in the Rat’s Uterus

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With one plate (fig. 1)

SUMMARY

1. A yellowish-brown pigment was found at the old placental sites in rats killed at 10 and 20 days after littering.

2. The pigment contained ferric iron and therefore may be regarded as haemosiderin.

3. Other properties of the pigment suggest that there is also a lipid component present. Lipid is not usually associated with haemosiderin.

4. The lipid component behaves in many ways like the lipogenic pigments.

INTRODUCTION

SELYE and McKeown (1935) and Baker (1948) have noted the presence of pigment in the metrial glands of the uterus of the rat after littering. They found that it contained iron and concluded that it was haemosiderin. Baker also states that some of it stained with Sudan black in frozen sections. In a recent investigation of the post partum changes in the uterus of the rat (Warbrick, 1955), it was found that pigment appeared in the region of the placental sites on about the 2nd day post partum and that it was positive to the periodic acid / Schiff (PAS) test. As haemosiderin is not usually considered to be sudanophil or PAS-positive, this investigation was undertaken in order to study the characteristics of the uterine pigment in greater detail. Of the various techniques employed, many are such as could be expected to give a positive result with a substance containing lipid.

MATERIAL AND METHODS

Two rats were killed on the 10th day after littering and two on the 20th. On examining their uteri there was a small brown discoloration of the mesometrial part of the uterine horn at each placental site. Portions of the horns containing placental sites were removed and fixed in neutral 10% formalin for 24 hours. They were dehydrated in alcohol, cleared in cedarwood oil followed by benzene, and embedded in paraffin wax. Sections were cut, mounted, and treated with one or more of the following techniques:

1. For routine histological study, haematoxylin and eosin staining was used.

2. Staining for 10 minutes in 0.5% aqueous toluidine blue.

3. Perl’s reaction for ferric iron.

4. The PAS test, with diastase-treated sections as controls.

5. The performic acid / Schiff (PFA) reaction, as described by Pearse (1951).

6. The peracetic acid / Schiff reaction, by Lillie’s technique (1954).

7. Oxidation for one hour in 4% chromic acid followed by 45 minutes in Schiff reagent.
8. Exposure of section without previous oxidation to Schiff's reagent for periods up to 18 hours.
9. Staining in a saturated solution of Sudan black in 70% alcohol for 3 hours, rinsing in 70% alcohol, and mounting in glycerine jelly.
10. The long Ziehl-Neelsen method as employed by Pearse (1953).
12. Mallory's technique for haemofuscins.
14. (a) Bromination, and (b) treatment with 5% chromic acid for 1 hour, followed by the ferric ferricyanide reduction test.
15. The Masson-Fontana alkaline silver technique.
17. Bleaching in hydrogen peroxide for 48 hours.
18. Exposure to ultra-violet light for fluorescence.

Observations

The distribution, appearance, and properties of the pigment were the same in all the specimens that were examined, whether from the 10-day or 20-day rats. Pigment was found in large quantities at each of the old placental sites. All the pigment was intracellular and the cells containing pigment were grouped either in the remains of the metrial gland situated in the mesometrial triangle or in the mesometrial half of the endometrium, where they lay close to the myometrium (fig. 1, a). The pigment was yellow to light brown and formed granules that varied in size from fine particles to those that were 10 or more microns in diameter (fig. 1, b).

In assessing the results of the various techniques that were employed, allowance must be made for the original colour of pigment, because it is on to this that any second colour is superimposed. For instance, a method which gives a blue colour as a positive may with the pigment produce a green or greenish-blue shade. The observations described in detail below are summarized in table 1 (see p. 15).

The pigment was not stained by either haematoxylin or eosin, but with toluidine blue it became a deep greenish-blue, in parts appearing almost black. Perl's test for ferric iron gave a strong positive reaction and resulted in all the pigment granules becoming a deep blue, while a lighter bluish-green shade was imparted to the cytoplasm of the cells containing pigment. The pigment reacted strongly with the PAS technique, the granules becoming a deep brownish-red which could be readily distinguished from their normal colour. The same colouring of the pigment was also found in the diastase controls.

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**Fig. 1** (plate). A, transverse section of a uterine horn of a rat killed 20 days after littering. Note the distribution of the black pigment. B, a portion of A, at higher magnification.
and thus the possible presence of glycogen was excluded. The pigment remained uncoloured after exposure to Schiff's reagent for 18 hours when no previous oxidation was employed. This shows that the PAS method was giving a true positive reaction and was not responding to the presence of preformed aldehyde groups in the pigment. The PFA reaction, the peracetic acid / Schiff reaction, and oxidation with chromic acid followed by Schiff's reagent all failed to produce any colouring of the pigment. It was sudanophil, most of the particles giving a greysih colour with Sudan black, although some of the larger ones became a deep black. It was acid-fast, resisting the acid alcohol differentiation in the long Ziehl–Neelsen method, so that while the background was a pale pink the granules were a deep brownish-red. With the chrome alum / haematoxylin technique of Gomori the pigment took up some of the stain, and although the original yellow colour was not completely abolished, the effect of the haematoxylin could be easily recognized. After alcoholic differentiation in Mallory's technique for haemofuscins some of the basic fuchsin was retained by the pigment granules, which became a light reddish-brown. With the ferric ferricyanide reduction test the section as a whole was coloured a pale green but the pigment was more markedly affected. Nearly all the granules were coloured a deep green while some of the larger ones became a very deep greenish-blue. A positive reaction with this test is normally indicated by a blue colour. Nevertheless, the granules are regarded as being positive because they colour (although for the most part in green) much more deeply than the background. After bromination and the chromic acid treatment the granules remain uncoloured by the ferric ferricyanide method. Treatment with alkaline silver resulted in slight darkening of the granules which became browner. The Gmelin test for haematoidin was negative while the pigment was not bleached by hydrogen peroxide. There was no fluorescence under the microscope when ultra-violet light was employed.

**DISCUSSION**

This investigation confirms the findings of Selye and McKeown and of Baker that the pigment contains ferric iron. These writers were also undoubtedly right in regarding the pigment as haemosiderin as this substance is by definition a pigment which exhibits one or more of the reactions of ferric iron (Lillie, 1954). Also the uterine pigment resists bleaching by hydrogen peroxide and this is a characteristic of haemosiderin. According to Lillie (1954), three varieties of haemosiderin can be recognized, one of which stains with basic dyes, as does the uterine pigment with toluidine blue. Haemosiderin is one of the breakdown products of haemoglobin (Florey, 1954) and the pigment in the rat's uterus is certainly derived from the blood, as it is found almost entirely at the old placental sites into which haemorrhage occurs at the time of separation of the placenta.

Unlike the pigment in the rat's uterus, human haemosiderin is not PAS-positive (Lillie, 1950). According to Pearse (1953) haemosiderin is not acid-fast, does not stain with Sudan black nor colour with Gomori's chrome
alum / haematoxylin technique, is negative in the ferric ferricyanide test, and
does not darken alkaline silver. All these techniques as well as Mallory's
technique for haemofuscins give positive results when applied to the uterine
pigment. It is thus clear that while the uterine pigment may be regarded as a
haemosiderin, it possesses several properties that are not usually associated
with this substance.

The colouring with Sudan black suggests that there is a lipid element
present, and other positive reactions, as with the PAS reaction and the Ziehl–
Neelsen technique support this. A comparison may be made between the
uterine pigment and the lipogenic pigments, the properties of which have been
summarized by Gomori (1952), Pearse (1953), and Lillie (1954). These pig-
ments, which are fairly widely distributed, are known by a variety of names,
such as ceroid, luteolipin, wear and tear pigment, and lipofuscin. They all
arise as oxidation products of a lipid precursor (Pearse, 1953). Their reactions
with different histological and histochemical techniques vary and this may be
related to the degree of oxidation that they have undergone. Like the pigment
in the rat's uterus they withstand lipid solvents and can be identified in par-
affin sections. Again, many of the lipogenic pigments such as adrenal lipofuscin
are acid-fast and PAS-positive, react with ferric ferricyanide, and colour with
oil-soluble colouring agents in paraffin sections. They may darken with alka-
line silver, colour with Gomori's chrome alum / haematoxylin, and retain basic
fuchsin in Mallory's technique. In these respects the uterine pigment re-
sembles them. It differs from some such as ceroid which is positive to peracetic
acid/Schiff and fluoresces in ultra-violet light. Nor are the lipogenic pigments
usually associated with iron, although ceroid may occasionally contain a few
granules of pigments containing iron.

The negative Gmelin reaction indicates that haematoidin, the iron-free
breakdown product of haemoglobin, is absent. Although the uterine pigment
in some respects resembles melanin, it is not melanin, for granules of the latter
substance are darker in colour, do not stain with Sudan black nor give the
PAS-reaction, and are usually bleached within 48 hours by hydrogen peroxide.

It thus appears that the uterine pigment should be regarded as a variety of
haemosiderin in which the substance containing iron is mixed or perhaps com-
bined with a lipid component that has many properties in common with the
lipogenic pigments.

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Fund.
<table>
<thead>
<tr>
<th>Technique employed</th>
<th>Colour of pigment</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematoxylin and eosin</td>
<td>unchanged</td>
<td>basophil with a basic dye</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>deep greenish-blue</td>
<td>ferric iron present</td>
</tr>
<tr>
<td>Perl's method</td>
<td>deep blue</td>
<td>a strong positive</td>
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<tr>
<td>Periodic acid / Schiff</td>
<td>brownish-red</td>
<td>no preformed aldehyde group present</td>
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<tr>
<td>Schiff's reagent</td>
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<td></td>
</tr>
<tr>
<td>Performic acid / Schiff</td>
<td>unchanged</td>
<td>negative</td>
</tr>
<tr>
<td>Peracetic acid / Schiff</td>
<td>unchanged</td>
<td>negative</td>
</tr>
<tr>
<td>Chromic acid / Schiff</td>
<td>unchanged</td>
<td>negative</td>
</tr>
<tr>
<td>Sudan black</td>
<td>grey to deep black</td>
<td>suggests presence of a lipid</td>
</tr>
<tr>
<td>Long Ziehl–Neelsen</td>
<td>brownish-red</td>
<td>acid fast suggesting a lipid</td>
</tr>
<tr>
<td>Gomori's chrome alum / haematoxylin</td>
<td>greenish-blue</td>
<td>a positive reaction</td>
</tr>
<tr>
<td>Mallory's technique</td>
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</tr>
<tr>
<td>Ferric ferricyanide</td>
<td>green to greenish-blue</td>
<td>a positive reaction</td>
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<td>Bromination and ferric ferricyanide</td>
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<td>Alkaline silver</td>
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<td>Fluorescence (U.V.)</td>
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<td>no fluorescence observed</td>
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REFERENCES