The Use of Sex Chromatin in Identifying Embryonic and Maternal Tissues in the Placenta: New Observations on the Haemochorial Nature of the Cat Placenta

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WITH FOUR PLATES

INTRODUCTION

Barr’s method of sex detection on the basis of quantitative determination of the sex chromatin content in the cell nuclei (1949) is widely used in clinical medicine. It seems to be useful for solving a number of disputed theoretical problems as well. For instance, in the study of placentae there is an urgent need to have recourse not only to histological and histochemical methods, but also to those affording reliable evidence for the origin of different placental elements as embryonic or maternal. This is especially important when identical cells, tissues, and structures of a species are considered to be embryonic in origin by some authors and maternal by others, thus causing controversy concerning its placental type.

The aim of the present investigation was to determine the origin of the so-called ‘endothelial’ and ‘decidual’ cells in the foetal placenta of the cat. If their origin (either embryonic or maternal) could be established we should know whether the cat placenta is endotheliochorial (Duval, 1894; Heinricius, 1891; Grosser, 1909), vasochorial (Wislocki & Dempsey, 1946; Amoroso, 1952, 1955; Vaček, 1955; Dempsey & Wislocki, 1956), or haemochorial (Zhemkova, 1958).

To resolve this question, Barr’s method (1949, 1951, 1954) of quantitative determination of the sex chromatin body content in the cell nuclei was used. The reliability of this method has been confirmed in numerous investigations by different authors. The sex difference has been found not only in animal and human nerve-cell nuclei, but also in interkinetic cell nuclei of somatic tissues (Bertram, Bertram, & Lindsey, 1950; Barr, 1951; Moore & Barr, 1954, 1955;

The sex chromatin body formed in consequence of the fusion of heteropyknotic parts of the sex chromosomes is clearly visible in female interphase nuclei. In somatic tissues it is often found lying against the nuclear membrane, on the average in 69-78 per cent. of cells in females and in 2-13 per cent. in males. Barr's method of nuclear sexing used in the present investigation affords the most reliable sex determination and can therefore be used to define the origin of the elements tested when the foetus is male. Before giving the evidence obtained with this method, we consider it necessary to make a brief mention of the histological observations on the development of the cat placenta which suggested that it was of the haemochorial type.

MATERIALS AND METHODS

In the present study 103 cats, at various stages of gestation from the 11th day or at term, were used. The pregnant uteri with the foetuses and placentae were fixed in Carnoy's and Davidson's fluids, in Zenker's formol, or in 12 per cent. formalin, and embedded in paraffin. Sections cut at 7-8 μ were stained with haematoxylin-eosin, methylgreen-pyronin, Weigert's, and Heidenhain's haematoxylin, using Feulgen's, Mallory's, Best's, Bauer's, and Foot's methods.

The sex chromatin was examined in 33 cat placentae at various stages of gestation. For the investigation of the sex chromatin sections of placenta were stained by the Feulgen (F), Heidenhain's iron haematoxylin (IH), and haematoxylin and eosin (HE) methods. Only large chromatin particles, not less than 0.7-1.5 μ and adjacent to the nuclear membrane, were counted as sex chromatin.

RESULTS

Observations on the histological structure of the cat placenta

In the cat implantation is of the central type. During its development the placenta becomes belt-like. The trophoblast, not involved in this development, remains free and forms the paraplacental parts. On the 11th–12th day of gestation the blastocyst is demonstrable in the lumen of the uterus. Its implantation into the uterine wall occurs on the 13th day (Plate 1, fig. A). At this time the
trophoblast, underlined by a thin mesenchymal layer, consists of large cells which are in some places high and prismatic and are arranged in 1, 2, or 3 layers. It lies closely against the mucous membrane surface, its folds being embedded in the uterine crypts. The uterine epithelium at the sites where the trophoblast had been in contact with it has disappeared and the latter comes into contact with the very loose edematous connective tissue and with the dilated maternal blood-capillaries. The connective-tissue cells and the endothelial cells of the superficial capillaries adjacent to the trophoblast lose their staining properties and the distinctness of their cell boundaries. Here and there the endothelium is found to be detached from underlying tissues. Already at this stage sites where the endothelial capillary lining is partially replaced by the trophoblastic cells may be seen. The disappearance of the uterine epithelium, superficial connective-tissue cells and endothelial cells seems to be associated with the lytic property of the trophoblast. A similar pattern may be also seen outside the developing placenta at those sites at which paraplacental trophoblast joins the apices of the mucous membrane folds, in the region where eventually the brown border is formed (Plate 1, fig. B). Within the zone of contact between the embryonic and maternal tissues, the uterine epithelium begins to disappear and the trophoblast approaches the walls of the maternal capillaries (Plate 1, fig. B). The destruction of the uterine capillaries by the trophoblast is followed by the formation of extraplacental haematomes, which is a characteristic of Carnivore placentae.

By the 15th day of gestation the trophoblast involved in the development of the placenta consists of a number of folds and massive strands fixed in the superficial layers of the mucosa. In locations where the embryonic and maternal tissues are in direct contact, a continuous boundary layer is formed by the trophoblastic cells and only maternal blood penetrates this layer. In the foetal placenta connective-tissue cells of maternal origin are absent as at the implantation period they have been dissolved and resorbed by the trophoblast along with the lining epithelium and endothelium of the superficial capillaries of the uterine mucosa. By this time the uterine capillaries are entirely deprived of their endothelium and form parts of the foetal placenta, having been converted into wide irregular blood lacunae completely lined by trophoblastic cells. At this stage of gestation the latter are of the same type almost in all parts of the placenta, oval, round, or polygonal. Only those with their bases lying on the mesenchyme and their apical endings facing the lumens of the uterine glands, have a tall prismatic form. In their size, shape, and staining properties the cells lining the blood spaces of the foetal placenta are similar to the adjoining trophoblastic elements. Only some of them become more flattened because of the changes in conditions of their activity (Plate 1, fig. C). Thus, by the 15th day of gestation the cat foetal placenta consists of the trophoblast, mesenchyme occupying only the embryonic surface of placenta, and maternal blood flowing in the lacunal spaces lined by trophoblastic cells. From this time on the size of the foetal
placenta increases rapidly due to the growing trophoblast, mesenchyme, and foetal vessels.

By the 20th day of gestation the labyrinthine and the intermediate portions are readily distinguishable. In sections of the labyrinthine portion, structures formed by the cellular trophoblast have the appearance of tubes or laminae with maternal blood channels passing through their central part. At the embryonic surface of the placenta these channels widen and form lacunae. The tube-like structures are separated from each other by wide layers of mesenchyme with a poorly developed embryonic capillary net. The trophoblastic tubes of the labyrinth are composed of cells similar in size and shape (Plate 2, figs. D, E). Only the cells bordering the maternal blood-stream undergo a number of changes. Some of them preserve their former oval and round shape even at this stage. Others become flattened and assume elongated, spindle-like, and semilunar forms in section. Their cytoplasm stains more deeply than that of other cells. Furthermore, this layer becomes separated from the remaining cellular mass by a thin streak of a structureless substance. Its formation undoubtedly bears a relation to the activity of the trophoblast as among these structures there are no connective-tissue elements, neither of embryonic nor of maternal origin.

The intermediate layer of the foetal placenta lies on the apices of the folds in the mucosa. It consists of trophoblastic cells forming a massive almost continuous layer which separates the foetal placenta from the uterine mucosa as the basal lamina does in the human placenta.

In the cat maternal placenta, unlike that of rodents and of man, the mucous membrane assumes the appearance of branched folds. The connective tissue does not undergo decidual modifications, while the uterine gland epithelium accumulates glycogen, becomes hyperplastic, and gives rise to meshworks that are either plasmodial or stratified. These structures gradually disintegrate, transform into detritus, and become part of the embryotropho.

By mid-gestation the trophoblast of the labyrinthine portion differentiates in three distinct directions. (1) The cells surrounding the blood-channel lumina become arranged in a single-row layer, its elements being spindle-shaped and semilunar. The nuclei of these cells are central. The cytoplasm is basophilic due to the presence of ribonucleo-proteins. The cells belonging to the same layer, but lining the lumina of wide lacunae at the embryonic placenta surface, retain their oval shape, are large, and occasionally become prismatic (Plate 2, fig. E). (2) The cells resting on the septae of the mesenchyme assume a low prismatic shape and are often arranged in two layers. Their basal portions are usually occupied by the nucleus and in their apical portions single, rather large vacuoles are present. In this layer intercellular boundaries are not clearly visible. (3) The cells of the middle portion of the tubes differentiate into large, round, or oval, separate elements (Plate 3, figs. G, H). In their cytoplasm the nuclei are surrounded by accumulating basophilic granules. Mitotic figures are seen in numerous cells.
The cells reach their maximum size in the second half of gestation when the usage of the name 'giant cells' is quite justified. At this period bi-nucleate and tri-nucleate cells are increasingly apparent while mitotic figures become progressively rarer. In late gestation the cytoplasm of these cells becomes oxyphil and not infrequently vacuolated.

In the intermediate layer of the placenta the trophoblastic cells modify in the same manner as those of the middle portion of labyrinth. They enlarge and become 'giant' (Plate 3, fig. J). In their appearance and arrangement they are very much like decidual cells of the human maternal placenta, but lack glycogen deposits in their cytoplasm.

The structureless substance, the formation of which was shown at early stages of the foetal placenta development, continues to be deposited at the later stages, chiefly in the labyrinth just beneath the trophoblastic layer adjoining the maternal bloodstream. The most massive deposits are seen around the lacunae situated at the embryonic surface of the placenta. The giant cells of the labyrinth and of the intermediate zone are surrounded by the structureless substance. When the preparations, especially those of late placentae, are treated by Foot's method, the structureless substance becomes impregnated with silver. However, the argyrophil materials are not similar to the fibrillar or reticular formations of the general connective tissues or of the foetal mesenchyme. They assume the appearance of structureless homogeneous layers of varying thickness.

Near term the labyrinthine portion of the foetal placenta is composed of thin trophoblastic tubes which adhere closely to one another, but are separated by narrow mesenchymal layers and surrounded by numerous foetal capillaries. The trophoblastic cells lining the blood channels of the labyrinth become progressively thinner and acquire a resemblance to endothelium (Plate 3, fig. K).

Thus the investigation of the histological structure and development of the cat placenta has shown a number of peculiarities. The early period of its formation reveals a striking similarity to that of rodents. The trophoblast, while implanting into the uterine wall, dissolves the uterine epithelium, the underlying layer of the loose edematous connective tissue, and the endothelium of the superficial capillaries, and forms a continuous cellular boundary layer adjoining the more compact maternal tissues. Hence, even before the foetal circulation is established it can be stated that maternal tissues, except blood, are absolutely lacking in the placenta. The blood spaces of the foetal placenta are lined by trophoblastic cells, the form of which is changed in accordance with their new function. They become progressively thinner and in the second half of gestation assume the appearance of endothelial cells. It is noteworthy that in the cat placenta (unlike that of rodents) the trophoblast coming into contact with maternal blood retains its cellular structure and does not convert into a syncitium. The giant elements of the labyrinth and intermediate zone, which reveal striking similarity to the decidual cells, are derived from the trophoblast. The structureless substance present in the foetal placenta of the cat is produced by
trophoblastic cells, but not by the connective-tissue elements of the uterus as the foetal placenta is deprived of these elements.

The above-mentioned observations have suggested that the structure of the cat placenta belongs rather to the haemochorial than to the endotheliochorial or vasochorial type. The results obtained are thus contrary to the data from the modern literature. To avoid possible mistakes in so important a question as placenta typing, it has become necessary to settle the question of the origin of the endothelium-like and giant (decidual according to contemporary authors) cells of the foetal placenta of the cat. To solve this problem the sex chromatin bodies in the nuclei of the two above-mentioned forms of cells in placentae taken from the male embryos have been counted (Zhemkova, 1961).

The importance of sex chromatin determination for detecting embryonic and maternal tissues in the placenta of the cat

Although decisive results are to be expected from the sex chromatin body studies of the placentae of male foetuses, in the present investigation placentae from female foetuses have been examined as well. It was necessary to establish the quantitative difference of the sex chromatin body-content in cells of the same type in placentae belonging to foetuses of each sex. In the foetal placenta the sex chromatin countings were performed (1) in the nuclei of embryonic connective tissue and foetal vessel endothelium, (2) in the nuclei of the paraplacental trophoblast, (3) in the nuclei of giant, so-called 'decidual' cells, and (4) in the nuclei of endothelium-like cells of the labyrinth.

Mesenchymal cells and the embryonic vessel endothelium as well as the paraplacental elements of the trophoblast were used as controls since they are undoubtedly of embryonic origin and hence of foetal sex. For the sake of comparison the following tissues known with certainty to be maternal were investigated: (1) uterine gland epithelium, and (2) uterine vessel endothelium lying beneath the foetal placenta in the mucous and muscular membranes. In each of the 6 cell groups investigated 300 nuclei were examined. The sex chromatin was determined in interkinetic nuclei with a regular well-defined contour.

In the cells examined the general chromatin was in the form of small granules. Among the latter large deeply stained sex chromatin bodies at the nuclear membrane were readily distinguishable. These bodies were of various shape: wedge-shaped, planoconvex, and oval (Plate 4). Occasionally the sex chromatin could be identified in the form of a local thickening of the nuclear membrane. The size of the sex chromatin bodies ranged from 0.7 to 1.5 μ. There was usually a single sex chromatin body, but quite frequently double bodies were encountered (Plate 4, figs. A, H). In binucleate giant cells sex chromatin bodies could be found in both nuclei. Occasionally large chromatin particles lying against the nucleolus were seen, but they were not considered to be sex chromatin.

In 15 placentae the sex of the foetuses had been identified before fixation. The sex of the foetuses in remaining placentae was unknown. However, on the basis
of the sex chromatin content in the nuclei it was quite easy to determine the sex of the cellular elements of these placentae.

**Table 1**

Sex chromatin adjacent to the nuclear membrane (% of nuclei counted) in the cat placenta from male foetuses

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Length of the embryo (cm.)</th>
<th>Staining</th>
<th>Embryonic connective tissue and foetal vessel endothelium</th>
<th>Paraplacental trophoblast</th>
<th>So-called 'decidual' cells</th>
<th>Endothelium-like cells of the labyrinth</th>
<th>Uterus gland epithelium</th>
<th>Uterus vessel endothelium</th>
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* In these foetuses the sex was known from anatomical evidence.

**Table 2**

Sex chromatin adjacent to the nuclear membrane (% of nuclei counted) in the cat placenta from female foetuses

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<th>Animal No.</th>
<th>Length of the embryo (cm.)</th>
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* In these foetuses the sex was known from anatomical evidence.
The cells of the same type appeared to contain a different quantity of sex chromatin in male and female foetal placentae (Tables 1 and 2). In the placentae of male foetuses the sex chromatin bodies were present on the average: in the nuclei of embryonic connective tissue and endothelial cells (A) in 4.53 per cent.; in the cells of the paraplacental trophoblast (B) in 4.78 per cent.; in the nuclei of the so-called ‘decidual’ cells (C) in 5.53 per cent.; and in the nuclei of endothelium-like cells (D) in 5.13 per cent. (Table 1). In the placentae of female foetuses the sex chromatin was identified on the average: in group A' in 15.66 per cent., in group B' in 17.04 per cent., in group C' in 16.06 per cent., and in group D' in 15.93 per cent. (Table 2). Thus the sex chromatin was more abundant in the placentae of female foetuses.

The difference in the average values of sex chromatin in the placentae of male and female foetuses was computed by the Student–Fisher method (Fisher, 1954) and proved to be statistically significant. For the groups A–B and A'+B' (i.e. for the groups of tissues undoubtedly embryonic) $t = 17.3; \, n = 58; \, P<0.001$. For the groups C–D and C'+D' (i.e. for the groups of tissues the origin of which was to be established) $t = 17.4; \, n = 58; \, P<0.001$. This means that the cells examined (the so-called ‘decidual’ and endothelium-like cells) show the same statistically significant sex difference as the cells of undoubtedly embryonic origin.

Table 3

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It appeared that even on the 13th day of gestation, at the period of foetal implantation, the trophoblastic cells and the mesenchyme show two highly different types of quantitative sex chromatin content, and thereby the sex of the foetuses could be determined (Table 3). On this basis in two ‘loculi’ the foetuses were identified as female and in one as male.

The results of the investigation of the placentae of male foetuses are convincing (Table 1). The comparison of the average sex chromatin content in the cells of so-called ‘maternal’ origin (C and D) with the control tissues of undoubtedly embryonic origin (A and B) reveals their striking similarity (5.53 per cent., 5.13 per cent., 4.53 per cent., and 4.78 per cent. respectively). At the same time

1 ‘Loculus’ is a local thickening of the uterine horn caused by the presence of the developing embryo with its membranes and with the correspondingly changed maternal tissues.
all these tissues are quite different from the true maternal tissues, uterus gland epithelium and uterus vessel endothelium (E and F), as their sex chromatin content amounts on the average to 19-64 per cent. and 17-73 per cent. respectively. Thus, in the labyrinth of the cat foetal placenta taken from male foetuses cells of ‘maternal’ origin were male as to their sex chromatin content. The average difference in the sex chromatin content in the control group $A+B$ from that in the investigated group $C+D$, was proved statistically and appeared to be insignificant ($t = 1.93; n = 58; 0.1 > P > 0.05$). This means that both groups of the cellular elements mentioned belong to the same sex (male) and therefore the cells studied (endothelium-like and ‘decidual’) are of embryonic, trophoblastic, and not of maternal, origin.

**DISCUSSION**

According to Grosser’s (1909) classification the type of the placenta is defined by the maternal tissue with which the chorion establishes direct contact. The placentae of Carnivora are considered to be of the endotheliochorial type (Duval, 1894; Heinricius, 1891; Schoenfeld, 1903; Grosser, 1909; Strahl & Ballmann, 1915; Rau, 1925; Wislocki & Dempsey, 1946; Amoroso, 1952, 1955; Vaček, 1955; Enders, 1955; Dempsey & Wislocki, 1956).

However, different conclusions were reached by these investigators as to the histological structure of the placenta. The cat placenta may serve as an example of this. Duval (1894) and Grosser (1909) indicated that in the foetal placenta of the cat the only maternal tissues were blood and uterine vessel endothelium, the trophoblast coming into direct contact with the latter. Later a number of authors (Wislocki & Dempsey, 1946; Amoroso, 1952, 1955; Vaček, 1955; Dempsey & Wislocki, 1956) established that not only endothelial cells, but also large cells formerly considered to be trophoblastic, are maternal connective-tissue decidual cells. Thus in accordance with modern conceptions, present in the labyrinthine portion of the cat placenta, along with embryonic tissues (trophoblast, allantoic mesenchyme, and foetal vessels) are maternal tissues (uterus vessel endothelium, connective-tissue decidual cells, and ground substance). This already offers adequate reasons for considering the cat placenta syndesmochorial. Since the chorion is in contact with unchanged maternal vessel walls, Wislocki has suggested to regard this placenta as vasochorial. However, the results of our investigation (Zhemkova, 1958) do not agree with the above opinion and let us consider the cat placenta belonging to the haemochorial type. Similar controversy is found in the works of Sansom (1937), Wimsatt & Wislocki (1947), and Owers (1960). Sansom considered the placenta of *Crocidura caerulea* as haemochorial; Wimsatt & Wislocki described it as endotheliochorial; and according to Owers it is endothelio-endothelial.

The evidence available from the sex chromatin body counts in the nuclei of cells hitherto considered maternal, convincing indicates their embryonic origin. The histological investigations have also demonstrated that the trophoblast
cells can assume the appearance of endothelial and decidual elements because of the specific conditions of their activity in the labyrinth and in the intermediate layer of the placenta. In successive stages of the cat placenta throughout gestation these changes may be followed step by step. For an understanding of the relationship between maternal and embryonic tissues it is the early period of the placenta formation that is most important, starting from the time of implantation.

From the observations described the conclusion may be reached that the cat placenta is haemochorial, rather than vasochorial or endotheliochorial as Duval, Grosser, Wislocki & Dempsey, Amoroso, and Vaček have stated.

Countings of the quantative sex chromatin body content in the nuclei can be recommended for solving a number of questions connected with the establishment of the origin of tissues.

**SUMMARY**

1. Histological investigation of the cat placenta at various stages of development has led to the conclusion that it belongs to the haemochorial type.
2. Study of the sex chromatin bodies in the nuclei of the so-called ‘endothelial’ and ‘decidual’ cells in the cat placentae of male foetuses has shown that these cells are derived from the embryo and are trophoblastic.
3. The results of the study of the sex chromatin bodies have confirmed the data of the histological investigation. Consequently the cat placenta should be considered as haemochorial, and not syndesmo-, vaso-, or endotheliochorial.

**RÉSUMÉ**

*Utilisation de la chromatine sexuelle pour identifier les tissus embryonnaires et maternels dans le placenta: observations nouvelles sur la nature hémochoriale du placenta de Chatte*

1. L’examen histologique du placenta de Chatte à divers stades de son développement a conduit à la conclusion qu’il appartient au type hémochorial.
2. L’étude des granulations de chromatine sexuelle dans les noyaux des cellules dites ‘endothéliales’ et ‘déciduales’ du placenta des fœtus mâles a montré que ces cellules proviennent de l’embryon et sont trophoblastiques.

**REFERENCES**


**EXPLANATION OF PLATES**

**PLATE 1**

Fig. A. Cat. 13th-day gestation. Implantation of the trophoblast into the uterus mucous membrane. Methylgreen-pyronin. 10 oc, 20 ob. 1, trophoblast; 2, mesenchyme; 3, connective tissue of uterus mucous membrane; 4, uterus blood capillaries; 5, uterus glands.

Fig. B. Cat. 13th-day gestation. Extraplacental trophoblast. Haematoxylin-eosin. 10 oc, 40 ob. 1, points of junction of the extraplacental trophoblast and the apices of the uterus mucous membrane folds; 2, uterus capillaries; 3, mucous membrane folds.

Fig. C. Placenta of a cat on the 15th day of gestation. Iron haematoxylin. 10 oc, 40 ob. 1, trophoblast; 2, lacunal spaces filled with maternal blood lined by trophoblast cells.

**PLATE 2**


Fig. F. Placenta of a cat. Mid-gestation. Haematoxylin-eosin. 10 oc, 40 ob. Wide blood lacunae situated at the embryonic surface of the placenta. 1, trophoblast cells lining the blood lacunal spaces; 2, structureless substance.

**PLATE 3**

Figs. G. and H. Placenta of a cat near term. Foetal length, 13 cm. Labyrinth. Azure II—eosin. 10 oc, 40 ob. 1, giant mononuclear trophoblast cells; 2, giant binucleate trophoblast cells; 3, endothelium-like trophoblast cells; 4, trophoblast cells adjoining mesenchymal septae; 5, mesenchyme of the septae; 6, foetal vessels.
1, trophoblastic cells in the border layer; 2, mucous membrane folds.

FIG. K. Placenta of a cat near term. Foetal length 13 cm. Labyrinth. Weigert’s haematoxylin.
15 oc., 40 ob. 1, endothelium-like trophoblastic cells; 2, giant trophoblastic cells.

PLATE 4

FIGS. L–Q. Labyrinth of a cat foetal placenta. Sex chromatin in the nuclei.
FIGS. L. and M. Of the so-called ‘decidual’ cells.
FIG. N. Of the endothelium-like cells.
FIGS. O, P, and Q. Of the trophoblastic cells of the labyrinth.
FIG. R. Of the uterus epithelium.
FIG. S. Of the uterus vessel endothelium. Feulgen. 15 oc., 90 ob. oil-immersion.

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