Placental Changes Following Fetal Death in Rats*

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ABSTRACT

The effect of the death or removal of the fetuses at the middle and late stages of pregnancy to the placenta in rats is studied. Either death or removal of the fetuses during pregnancy brought rapid degeneration of all elements of the placenta, first in the yolk sac followed by labyrinth and junctional zone leading into ischemic necrosis secondary to the thrombosis of the maternal blood vessels supplying the placenta. Trophoblasts in the junctional zone survived for a short period of time, but no evidence of abnormal or continuous proliferation of them was noted. Shortly after the death or removal of fetuses cystic degeneration in the junctional zone was observed, but no definite evidence of mole like change was noted. Findings of periodic acid Schiff’s reaction, methyl-green pyronine staining and Feulgen reaction in the normal and degenerating placentas are described. The circulatory factors and structural differences between the rat and human placentas are discussed to account for differences observed in the placental changes following the death of fetuses in rats from those of human.

INTRODUCTION

Retained secundine following either abortion or full-term delivery is a rather common clinical condition, and the retained placental tissue may cause serious complications. However, the study on the fate of retained placental tissue is fragmentary, and no adequate study on the sequence of events which take place in the placenta follow-

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ing the death of the fetus is available.

Brews(1939), Kline(1951), and Novak(1953) reported that trophoblastic activities will recede rapidly as soon as fetal death takes place. Russell et al. (1957) found that urinary excretion of progesterone and estradiol dropped abruptly following intrauterine fetal death, and the placenta underwent infarction, although the dead fetus was retained for several weeks in the uterus. Hertig(1935) stated that cessation of fetal circulation with continuous maternal blood flow into the placenta will cause hydropic degeneration of chorionic villi and continuous survival of trophoblasts, which may eventually lead to the development of a hydatidiform mole and possibly choriocarcinoma. But, fortunately, the majority of cases will terminate in the stage of potential mole and only a few, one in two thousands pregnancies, will result in true mole formation.

Anatomy and histochemistry of the normal full-term placenta of the rat have been described by Duval(1891), Jenkinson(1902) and Bridgesman(1948). However, histologic and histochemical changes in the placenta following fetal death during the pregnancy have not been reported up to now. Therefore, the present study is undertaken to investigate histologic and histochemical changes of the rat placenta following the death of fetuses during pregnancy to facilitate the understanding of similar conditions in the human, with special attention to the changes of trophoblasts.
MATERIALS AND METHODS

A total of 112 female virgin albino rats, around 200 gms, were used for the experiments. Out of them, 10 rats were used for preliminary study to determine the estrus cycle, to achieve successful mating and to determine the duration of normal pregnancy.

The estrus cycle was checked by vaginal smear methods of Papanicolaou (1933), and mating was achieved by housing a female rat at pre-estrus cycle together with a male rat and the copulation was confirmed by rechecking of vaginal smears for the presence of spermatozoa. By this way it was found that the average estrus cycle is 96 hours and the duration of normal pregnancy is 21 days.

The remaining 102 rats were divided into 3 major groups and treated as follows: in the control group I (30 rats) the pregnancies were allowed to proceed normally; in group II (42 rats) the fetuses were killed in the middle stage of pregnancy (14th day) by electric coagulation with a hyfrecator (Birtcher Co., U.S. A.); and in group III (30 rats) the fetuses were surgically removed by Cesarean section in the late stage of pregnancy (18th day). Group II and III were divided again into two subgroups. In one subgroup all the fetuses in one uterine horn were killed or removed allowing continuation of pregnancy in the remaining uterine horn, and in the other subgroup all the fetuses of both uterine horns were killed or removed.

Specimens of the placentas were obtained by removing the entire uterus from 3 rats at different intervals of pregnancy in each group. In group I, specimens were obtained on the 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th, 20th and 21st days of pregnancy; in group II on the 15th, 17th, 19th, 21st, 23rd, 25th and 50th days of pregnancy; and in group III on the 19th, 21st, 23rd, 25th and 50th days of pregnancy. From each removed uterus, the numbers of conceptuses, the size of gravid segments, and the size of the placentas were checked. Then, two sections, cross and longitudinal, from each gravid segment were taken and embedded into paraffin after fixation in 10% neutral formalin. Microsections were prepared in 6μ thickness and they were stained with hematoxylin-eosin, periodic acid Schiff’s method, methyl-green pyronin, and Feulgen’s method.

RESULTS

A. Daily body weight changes of pregnant rats: An average daily increase of body weight during the three week period prior to pregnancy was from 0.65 to 0.81 gms. In the normal pregnancy group I it was 0.6±0.45 gm during the early stage (1~10 days), 2.6±0.57 gm during the middle stage (11~17 days) and 4.8±0.25 gm during the late stage (18~21 days) of pregnancy. In Group II the daily increase of body

Table 1. Average daily increase of body weight

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>1~10</th>
<th>11~17</th>
<th>18~21</th>
<th>22~25</th>
<th>26~50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>0.65±0.36</td>
<td>0.81±0.07</td>
<td>0.74±0.12</td>
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<tr>
<td>Normal pregnancy (group I)</td>
<td>0.60±0.45</td>
<td>2.60±0.57</td>
<td>2.80±0.25</td>
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<tr>
<td>Fetal death at A</td>
<td>0.95±0.11</td>
<td>1.60±0.04</td>
<td>1.80±0.60</td>
<td>0.76±0.02</td>
<td>1.10±0.14</td>
</tr>
<tr>
<td>14th day (group II) B</td>
<td>0.84±0.11</td>
<td>0.50±0.15</td>
<td>0.20±0.11</td>
<td>0.20±0.13</td>
<td>0.54±0.20</td>
</tr>
<tr>
<td>Fetal removal at A</td>
<td>0.81±0.13</td>
<td>2.67±0.43</td>
<td>0.86±0.14</td>
<td>0.76±0.70</td>
<td>0.65±0.04</td>
</tr>
<tr>
<td>18th day (group III) B</td>
<td>0.90±0.14</td>
<td>2.71±0.37</td>
<td>0.11±0.06</td>
<td>0.70±0.43</td>
<td>0.51±0.02</td>
</tr>
</tbody>
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A: Fetuses of unilateral uterine horn are killed or removed.
B: Fetuses of bilateral uterine horn are killed or removed.
Values: Mean±standard error. Unit: gm.
weight was equivalent to that of the normal control group during the early stage, but it was markedly lesser than the control group after the fetuses were killed and particularly so in the subgroup in which fetuses in both uterine horns were killed. In the group III, the daily increase was equivalent to those of the normal control group during the early and middle stages, but after the fetuses were removed the increment reduced markedly, particularly in the subgroup in which fetuses of both uterine horns were removed. These changes of body weight are tabulated in table 1.

B. Rate of successful mating and numbers of conceptuses:

Success rate of arbitrary mating was different by seasons. Success rate was much greater during the spring time than in the winter. The average number of conceptuses per rat was 9.8 ±2.2 with about equal numbers in each of the uterine horns, namely, 5.10±3.4 on the right and 5.19±2.9 on the left side.

C. Macroscopic findings of the uterus and gravid segmenta:

The blood vessels in the normal non-pregnant uterus consisted of fine capillaries, but they become engorged accompanying some edema on the 5th day of pregnancy and concentrated around the gravid segment. But it is not until the 7th day when one can recognize the demarcation of the individual gravid segment. On the 9th day, the site of placentation can be recognized as a dark brownish pad. Until the 15th day, the gravid segments enlarged equally between length and width, but from the 17th day the length became rapidly elongated and it reached 2.0±0.22 cm with the placentas of 0.7 cm in diameter.

The direction of fetuses can also be recognized from the outside on the 17th day, and the position of the fetal head in the majority of the cases directed counterclockwise. The amniotic fluid was aqueous until the 19th day, then it became viscid and the fetal movement was sluggish. The size of gravid segment was 2.7±0.13 cm and the diameter of the placenta 1.2 cm at the 21st day of the pregnancy.

When the fetuses were killed on the 14th day, the corresponding uterine horn became pale, inelastic, somewhat atrophic and the placenta discolored to a dark bluish appearance with marked disappearance of the blood vessels one day later. Then the gravid segments became progressively atrophic and the demarcation can no longer be recognized 3 days after the fetuses were killed. On the 23rd day of pregnancy, the uterine horn which maintained normal pregnancy returned to original appearance of the uterus after the fetuses were delivered spontaneously between the 21st and 23rd days of the pregnancy while the uterine horn in which fetuses were killed remained slightly enlarged. Only two animals showed marked cystic enlargement of the uterine segment on the 50th day of pregnancy due to abscess formation. The fetuses in the non-experimental uterine horns delivered at term without delay and the delivered fetuses grew normally, and furthermore that uterine horn could become pregnant again.

In the group III in which fetuses were removed surgically on the 18th day of pregnancy, the gravid segment became rapidly atrophic and pale, and the placenta became flattened with pale yellowish discoloration and can be easily detached from the uterine wall at 1 day after the fetuses were removed. The fetuses in the uterine horn which maintained normal pregnancy delivered with delay of 1 or 2 days than control group and the delivered fetuses were all dead. The placenta remained after the fetuses were removed also evacuated when the fetuses in the normal uterine horn delivered. In the group in which fetuses in both uterine horns were removed, the placenta remained a longer period, but as the days pass they became atrophic, necrotic and completely absorbed by the 50th day.
after conception.

D. Microscopic findings of the placenta:

Description and nomenclature of the rat placenta followed that of Bridgeman (1948).

In the normal control group, the placenta on the 5th day consisted mainly of decidual element, on the 7th day the yolk sac cavity is formed surrounded by decidual cells without any notable amount of trophoblasts. On the 9th day the yolk sac became enlarged and a small amount of sinusoidal network developed around a pole (fig. 1), and it was filled with non-nucleated maternal blood. On the 11th day, the yolk sac enlarged further and became eccentrically located pushing the decidual plate against the metrial gland (fig. 2). The sinusoidal network increased and took place in between the yolk sac and decidual plate and was recognizable as the labyrinth which was filled mostly with non-nucleated maternal blood and partly with nucleated fetal erythrocytes. At this time isolated trophoblasts were first noted scattered in the vascular wall of the labyrinth and between the labyrinth and decidual plate.

But it was not until the 13th day that the complete placental structure is formed. At this point, the yolk sac showed papillary foldings and glandular appearance; the labyrinth developed fully and was supplied half by fetal blood and half by maternal blood. The decidua is further compressed and diminished in amount and several layers of solid trophoblastic mass developed in between the decidua and labyrinth, the so-called junctional zone (fig. 3). Thereafter, the junctional zone and labyrinth increased in size and the decidua was further compressed and diminished in amount.

On the 18th day of pregnancy, the syncytial trophoblasts appeared at the periphery of the junctional zone and mild cytolysis developed at the center of junctional zone. The labyrinth increased in size and was mostly supplied by relatively mature but nucleated fetal erythrocytes. The decidua diminished further and became compressed to a narrow crescent-disc attached to the metrial gland. On the 21st day, immediately before the delivery, cleavage developed between the decidua and the junctional zone which filled with a small amount of blood and inflammatory exudates associated with congestion and thrombosis in the decidua and metrial gland (fig. 4).

When the fetuses were killed on the 14th day of pregnancy, the placenta showed various degenerative changes in all elements. One day later, the decidua showed marked congestion and beginning of thrombosis in engorged blood vessels; trophoblasts in the junctional zone started to lyse followed by cystic change (fig. 6), and then necrosis and inflammatory reaction developed in the yolk sac and peripheral portion of the labyrinth. As time passes, degenerative changes proceeded progressively as evidenced by coagulation necrosis of the yolk sac and labyrinth (fig. 7), associated with inflammatory reaction, cystic degeneration (fig. 8) of the junctional zone with cellular changes of the trophoblasts characterized by intense eosinophilia, hyaline degeneration of cytoplasm, and pyknosis or diffusion of the nuclear chromatin. On the 5th day after the fetuses were killed, hemorrhage and necrosis developed in between the decidua and junctional zone creating separation of the placenta from the uterine wall.

On the 7th day after the fetuses were killed (the 21st day of pregnancy), most of the placentas showed coagulation necrosis like a large ischemic infarct with only scattered stainable nuclei of the trophoblasts in the junctional zone and the labyrinth (fig. 8, 9), and in the majority of the cases the placenta became totally necrotic at 9 days after the fetus was killed, with early deposition of calcium. Thereafter, the placenta gradually liquified and was completely absorbed. There was no observed case of abnormal proliferation of trophoblasts or further survival of the trophoblasts beyond the 23rd day of pregnancy (9 days after the fetus was killed).
PLACENTAL CHANGES FOLLOWING FETAL DEATH IN RATS

There was no qualitative difference between the groups in which fetuses were killed in one uterine horn or both uterine horns, but the degenerative changes were more rapid and marked in the subgroup in which the fetuses in both uterine horns were killed.

If the fetuses were removed surgically on the 18th day of pregnancy, the changes in the placenta were similar to those seen following the fetuses were killed on the 14th day of pregnancy. But cystic changes in the junctional zone were more severe (Fig. 10) and the necrotic process was more rapid. There was also no notable qualitative difference in the changes of the placenta in animals regardless of whether the fetuses were removed from one or both uterine horns. One interesting finding was that the retained placenta, of the fetus-evacuated horn, was passed out at the same time when the normal fetuses in the remaining uterine horn were delivered. However the placentas, which had had the fetuses removed from both uterine horns, remained in place beyond the normal pregnancy of 21 days, and became progressively necrotic and finally absorbed.

PAS stained sections showed a small amount of PAS positive substance in the yolk sac epithelium and Reichert membrane as early as the 9th day of normal pregnancy, and on the 11th day, a small amount of PAS positive substance appeared in the trophoblasts of the junctional zone. In the mid-period of pregnancy, this substance increased in the junctional zone and the yolk sac, and was most intense in the Reichert membrane which stained like the basement membrane of other organs. The labyrinth and decidua showed a very mild degree of PAS positive reaction throughout the course of pregnancy, and in the labyrinth the positive reaction was mainly located in the wall of the blood vessels.

Following the death or removal of the fetuses, the PAS positive substance decreased markedly in the yolk sac epithelium and the trophoblasts of the junctional zone, but progressively increasing deposit was noted in the interstitial portion of the junctional zone and labyrinth. However the PAS reaction in the Reichert membrane was not notably altered from the normal control placenta.

Pyronophilic substance was found largely in the epithelial cells of the yolk sac and decidua cells during the early stage of pregnancy, and found most abundantly in the epithelial cells of the yolk sac and trophoblasts in the junctional zone during the middle stage of pregnancy, and then this substance gradually decreased during the late stage of pregnancy. Following the death or removal of the fetuses, cytoplasmic pyronophilic substance disappeared rather rapidly from the yolk sac epithelial cells, but that of trophoblasts in the junctional zone remained relatively longer. In the trophoblasts of the junctional zone, pyronophilic substance disappeared first from the cytoplasm, but the pyronophilic substance in the nucleoli became dispersed throughout the nuclei and remained visible until 11 days after the death of fetuses or removal of the fetuses even though the placenta appeared completely necrotic on hematoxylin and eosin stained sections. An interesting tinctorial reaction to methyl-green pyronin was staining of the erythrocytes. The normal mature non-nucleated red cells stained greenish blue while the immature nucleated red cells showed large amounts of pyronophilic substance in both cytoplasm and nuclei. As the immature red cells become mature the pyronophilic substance in the cytoplasm gradually disappeared.

Feulgen positive substance was found exclusively in the nuclei of various elements of the placental cells. Its amount increased somewhat during the middle stage of the pregnancy, and then gradually decreased in the late stage. After the fetus was killed or removed, the Feulgen positive substance gradually disappeared as the pyronophilic substance did. And in the later stage, degenerated nuclei gave a positive staining reac-
tion to both Feulgen and pyronin.

DISCUSSION

The results obtained by the present investigation showed that if the fetus is killed or removed without damaging the placenta during the pregnancy it is followed by congestion, inflammatory reactions, coagulation necrosis and finally an infarct like changes involving all elements of the placental tissue, but the first affected was the yolk sac followed by the labyrinth and junctional zone with cleavage between the decidual and the junctional zone with resulting autoamputation of the placenta from the uterine wall. Simultaneously or even prior to these histologic alterations, maternal circulation in the decidua and the metrial gland showed evidence of marked impairment characterized by congestion followed by thrombosis. Therefore, it may be reasonably stated that the severing of fetal circulation by either killing or removal of fetus seems to cause circulatory disturbances of the maternal blood flow into the placenta resulting in ischemic necrosis of the placental tissue.

Similar results were observed by Payne (1957) in a different set of experiments, namely, by injecting Brucella abortus organisms into the peritoneal cavity on the 12th or 13th day of pregnancy in rats, and he concluded that the degenerative changes in the placenta were due to the thrombosis in the maternal circulation in the decidua and in the metrial glands.

The mechanism by which maternal circulation become impaired and thrombosed when fetal circulation is severed is not clear. It is probably due to dissease phenomena or liberation of a certain biologically active substance influencing coagulation mechanisms. In view of the report that the placenta contains large amounts of anticoagulant substance (McKay et al. 1958), it is conceivable that the degenerating placenta loses its anticoagulant mechanism in addition to the liberation of tissue thromboplastic components.

In the study of circulatory factors governing the viability of the human placenta, Carter et al. (1963) reported that the constant changes following the severing of fetal circulation are degeneration of the yolk sac and umbilical cord, while the syncytial trophoblasts are entirely dependent upon the maternal circulation and the cytotrophoblasts and interstitial tissue of the chorionic villi are dually supplied by both fetal and maternal blood. Therefore, if the fetal circulation is cut off chorionic villi will undergo hydropic degeneration but trophoblasts may continue to survive (Hertig and Mankell 1956, Carter et al. 1963), in contrast to the placenta of the rat which undergo complete ischemic necrosis. This difference is probably due to the structural differences of the placenta of the human from that of the rat. There seems to exist two main differences in the structure of the human and rat placentas. The first is that there is no real chorionic villi to speak of in the rat placenta except a few short chorioallantoic processes projecting from the Reichert membrane into the labyrinth without continuous coverage of trophoblastic layers as seen in the human placenta (Sorokin and Padykura 1964). The second difference is that trophoblasts in the rat placenta are aggregated mainly as solid masses in the junctional zone, clearly demarcated both from the labyrinth and decidual plate without notable penetration into the decidua or metrial gland, which will cause complete detachment of trophoblasts from the uterine wall and the decidual layer.

Findings of PAS reaction were interesting. The strongest reaction was noted in the Reichert membrane and sinusoidal walls of the labyrinth, and was similar to the basement membrane in the other organs. This is very interesting in view of the recent speculation that glomerular changes in eclampsia are due to autoimmune reactions, i.e. the sensitization of the glomerular capillary basement membrane by the degeneration
of common antigenic elements of the placenta to that of glomerular capillary basement membrane (Boss 1963, Boss and Craig 1963). An increased interstitial deposit of PAS positive substance during the degeneration of the rat placenta was similar to the finding by McKay et al. (1958) who found increased amounts of glycoprotein deposits in the infarcted area of the human placenta.

The interesting findings of the methyl-green pyronin staining and the Feulgen reaction were the dispersion of pyronophilic substance, which normally is present only in the nucleoli, throughout nuclei, and the dispersion of Feulgen positive granular substance into a homogenous solid mass, and that of the pyronophilic substance and Feulgen positive substance were intermixed or superimposed when the trophoblasts undergo degeneration. These findings are likely due to the depolymerization of nucleolar RNA and nuclear DNA.

REFERENCES


Novak, E.: Obst. & Gynaec. 1: 8, 1953


LEGEND FOR FIGURES

Fig. 1. Rat placenta at the 9th day of normal pregnancy showing early yolk sac surrounded by a narrow zone of vascular network and thick layer of decidua. H. & E., × 100.

Fig. 2. Rat placenta at the 11th day of normal pregnancy showing enlarged yolk sac cavity, early labyrinth, narrow zone of junctional zone and decidua, from left to right. H. & E., × 100.

Fig. 3. Rat placenta at the 13th day of normal pregnancy showing full development of all placental structure, yolk sac, Reichtert membrane, labyrinth, junctional zone, and compressed decidua. H. & E., × 50.

Fig. 4. Labyrinth at the 17th day of normal pregnancy showing sinusoids filled by mostly relatively mature nucleated fetal red blood cells. H. & E., × 430.

Fig. 5. Rat placenta at the 21st day of normal pregnancy showing cleavage between decidua, right, and junctional zone, left, with hemorrhage, right upper corner. H. & E., × 100.

Fig. 6. Placenta at 1 day after the death of fetus showing cystic degeneration of junctional zones, and hemorrhage in between decidua and junctional zone. H. & E., × 100.
Fig. 7. Placenta at 3 days after the death of fetus showing coagulation necrosis of yolk sac, necrosis and inflammatory reaction in labyrinth. H. & E., × 100.

Fig. 8. Placenta at 7 days after the death of fetus showing a large infarct like necrosis with thrombosis in many blood vessels. H. & E., × 50.

Fig. 9. Placenta at 7 days after the fetus was killed, showing thrombosis surrounded by coagulation necrosis in the area in between decidua and junctional zone. H. & E., × 100.

Fig. 10. Placenta at 1 day after the fetus was removed at the 18th day of pregnancy, showing cytolysis and cystic changes in junctional zone. H. & E., × 100.

Fig. 11. Placenta at 9 days after the fetus was killed at the 14th day of pregnancy, showing diffusely stained pyronphilic substance in nuclei of trophoblasts in necrotic area of junctional zone. Methyl-green pyronin, × 430.

Fig. 12. Placenta at 9 days after the fetus was killed at the 14th day, showing diffuse and compact Feulgen positive reaction in the nuclei of trophoblasts in necrotic area of junctional zone. Feulgen, × 430.