HISTOCHEMICAL STUDIES OF RAT EMBRYO AND UTERUS WITH SPECIAL REFERENCE TO IMPLANTATION AND FORMATION OF FETAL MEMBRANES

HISTOCHEMICAL STUDIES OF RAT EMBRYO AND UTERUS WITH SPECIAL REFERENCE TO IMPLANTATION AND FORMATION OF FETAL MEMBRANES

By

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Introduction

Morphological investigations on the implantation and the formation of fetal membranes and of placenta were made by many workers. Alden(1) studied the implantation of the rat ova and the alteration of the lipid contents in the uterine epithelium during the implantation stage. He (2) futher investigated on the origin and development of the trophoblastic giant cells of the rat embryo. Bridgman (4, 5) studied morphologically the development of the rat placenta. Krehbiel(11) investigated on the decidual reaction of the rat during the early pregnancy and the production of deciduomata. He also observed the appearance of lipid and glycogen in these tissues.

From the histochemical point of view, Loveland, Maurer and Snyder (12) studied the distribution of glycogen in the rabbit placenta and reported that it diminished during last third period of pregnancy. Hard (9) studied the localization of phosphatase in the placenta and fetal membranes of the guinea pig. Baker(3) investigated on the appearance of RNA, glycogen and lipoid in the metrial glands of the rat during pregnancy and lactation. Orsini (13) studied cytochemically the trophoblastic giant cells and endovascular cells of the hamster during pregnancy. Wislocki, Dempsey and his co-workers(6 to 8, 17 to 24) performed serial investigations concerning the histology and histochemistry of the placenta of women and of various kinds of animals as rodents, cats and pigs. Wimsatt (14) studied cytochemically the fetal membranes and placenta of the bat. He (15, 16) further studied the binucleated giant cells of the ruminants. However, there are few who have histochemically studied the embryos and uteri in the early stage of pregnancy : Krehbiel(11) and Bridgman (4) investigated on the fine changes of glycogen and lipid in the decidua and placenta of the rat from the 5th to 12th day of pregnancy, employing Best's

carmine for glycogen and Sudan III for lipid.

In the previous paper (10), I have cytochemically studied the cleaved ova in the oviduct and uterus of the rat and reported that a large amount of glycogen was found in the just ovulated ova, decreasing gradually with the process of segmentation.

In the present investigation, I have dealt with the appearance of glycogen, RNA and alkaline phosphatase in the rat embryo and uterus with special remarks to the implantation and the formation of fetal membranes.

Materials and Methods

Thirty two pregnant rats were killed by decapitation at the desired intervals. The embryos and the uteri were fixed in 95 per cent alcohol, embedded in celloidin and sectioned serially 20μ thick.

For the demonstration of glycogen, the sections were stained by the periodic acid-Schiff method (PAS) modified by Lillie. The identification of glycogen was made by means of the salivary test at 37°C in an incubator. For RNA, thionin and methylene blue were used. RNA was confirmed by ribonuclease treatment. For the demonstration of alkaline phosphatase, Gomori's revised method was employed by using sodium glycerophosphate as a substrate. Morphology of the embryos and of uteri was made by hematoxylin-eosin stain and Mallory's connective tissue stain.

Results

1. *Results obtained for glycogen.*

The appearance and distribution of glycogen in the embryos and uteri from the 5th to 10th day of pregnancy are given in Table 1.

Pre-implantation stage.

At the 5th day of pregnancy, the blastula of about 130μ was always found in the antimesometrial part of the uterine lumen. It retained an ovoidal shape and consisted of the inner cell mass of 30 to 40 cells and the surrounding trophoblastic layer. As shown in Table 1, both the inner cell mass and the trophoblastic cells contained a small amount of glycogen (Fig. 1). The uterine epithelium, the cells of which are columnar, contained no glycogen. Uterine glands were numerous in the antimesometrial mucosa, but scarce in the mesometrial one. The glands possessed no glycogen, but the mucin in their lumen was intensely stained by the PAS method.

At the 6th day, the blastula increased in size, estimating to be about 250μ in long diameter. The inner cell mass contained no glycogen, though the trophoblastic cells contained a small amount of it (Fig. 2). The cells of the uterine epithelium near the blastula were depressed and became flattened in shape. This is known as "Implantation cup of Graves"(11). Many subepithelial

Table 1. Distribution of glycogen in the embryo and uterus.

Tissues		Stage of development		
		Pre-implantation (5th to 6th day)	Impantation (7th day)	Placentation (8th to 10th day)
Embryo	Inner cell mass (blastula)	+ to −	not seen	not seen
	Trophoblastic layer (")	+ to ±	+	"
	Endoderm (")	not seen	+	+
	Embryoblast (egg-cylinder)	"	not seen	−
	Chorionic ectoderm (")	"	"	−
	Germinal layer of embryonic shield	"	"	−
	Träger	"	"	++
	Giant cell	"	"	+
	Reichert's membrane	"	"	(++)
	Allantoic stalk	"	"	−
	Amnion	"	"	−
Uterus	Epithelium	−	not seen	not seen
	Uterine gland	−	"	"
	Primary decidua	++	−	"
	Secondary decidua	not seen	++	++
	Implantation zone	"	(++)	(++)
	Basilar decidua	"	not seen	++ to ++
	Capsular decidua	"	"	++ to −

Note : ( ) ..... Not digested by saliva.

cells hypertrophied and transformed into decidua cells which laded a large amount of glycogen (Fig. 2). The area composed of these decidua cells is known as "Primary decidua zone of Krehbiel"(11). Subsequently the antimesometrial stroma cells proliferated from the primary decidua zone towards the myometrium, pushing the uterine glands towards the basal zone of the stroma. The wide area compsed of hypertrophied decidua cells is known as "Secondary decidua zone of Krehbiel" (11). As shown in Table 1, the decidua cells contained a large amaunt of glycogen.

Implantation stage.

At the 7th day, the embryo became a so-called cylinder stage, estimating to be about 400μ in long diameter : The endodermal cells formed a distinct single layer along the inner surface of the trophoblastic layer. As shown in Table 1, both the endoderm and trophoblast contained a small amount of glycogen (Fig. 3). The egg-cylinder existed in the antimesometrial groove of the uterine lumen. Afterwards, it was enclosed by the endometrial cells, thereby the uterine epithelium disappeared after its lumen was constricted off. At this stage the secondary decidua cells possessed a large amount of glycogen, whereas the primary decidua cells lost it (Fig. 3). The cells consisting the implantation

zone, the inner part of the primary decidua zone, contained a large amount of PAS positive glycoprotein.

Placentation stage.

In the early course of the 8th day, the egg-cylinder became well implanted and the differentiation of it highly progressed: The embryoblast and the chorionic ectoderm were clearly developed. The primitive amniotic cavity appeared in the chorionic ectoderm, subsequently the amnio-chorionic cavity became completed. As shown in Table 1, although the endodermal cells contained a small amount of glycogen, the embryoblastic cells and the chorionic ectodermal ones possessed none of it. In this stage, some of trophoblastic cells formed a Träger in the mesometrial area. These cells contained a moderate amount of glycogen (Fig. 4). Trophoblastic giant cells appeared near the Träger. These cells possessed a small amount of glycogen (Fig. 4). When the giant cells migrated into the decidua, a so-called Reichert's membrane appeared. The evenly scattered endoderm cells attached to the inner surface of this membrane. The Reichert's membrane contained a large amount of PAS positive glycoprotein and the attached endodermal cells neither glycogen nor glycoprotein.

In the middle course of the 8th day, the ring-shaped amniotic folds appeared at the boundary of the embryoblast and chorionic ectoderm. After a while, these folds closed with each other, separating the amniotic cavity from the chorionic cyst. The decidua cells between the mesometrial and the antimesometrial area possessed a large amount of glycogen. The sinusoidal capillaries appeared in this zone. Some of the mesometrial decidua cells contained a moderate amount of glycogen.

In the last course of the 8th day, the embryonic shield consisting of the ectodermal, mesodermal and endodermal layers appeared. As shown in Table 1, this contained no glycogen (Fig. 5). Afterwards, a bud of allantoic stalk projecting from the caudal end of the embryo into the extra-embryonic celom appeared. This stalk contained no glycogen. The Träger contained a moderate amount of glycogen and the basilar decidua a large amount of it (Fig. 6).

At the 9th day, the placental cone was formed. This possessed persistently no glycogen (Fig. 7).

At the 10th day, the allantoic stalk attached to the placenta. The union between the placental cone and the basilar decidua was still loose; the wide blood lacunae presented clearly in this region. The basilar decidua contained a large amount of glycogen (Fig. 8), while the highly developed capsular decidua a moderate amount of it. Afterwards the capsular decidua degenerated rapidly, thereby the uterine lumen was reformed.

Many embryological investigations were carried out on the rat embryo and endometrium during pregnancy. Most of them were performed at about the

period from the establishment of the antimesometrial implantation to the formation of placenta. Recently, Bridgman(4, 5) studied the development of the placenta of the rat during the whole period of pregnancy. Thus, although the implantation, decidual reaction and development of the fetal membrane of the rat have been well investigated, there are few who have studied the glycogen which appeared in these processes. Krehbiel (11) studied the appearance of glycogen in the decidua of the rat during the early pregnancy and obtained the following results; first, the glycogen appeared in the antimesometrial decidua cells on the 6th day after insemination. Subsequently, it appeared in the mesometrial decidua cells on the 8th day. The feature of glycogen in the decidua, obtained in the present investigation, nearly coincided with the results obtained by Krehbiel.

From the results obtained in the series of this investigation the amount and the localization of glycogen differed in different stages. As already stated in the previous paper (10), a large amount of glycogen in the ovulated ova diminished gradually with the segmentation processes and a small amount of it was found in both the trophoblastic layer and inner cell mass of the blastula in the early stage. In this investigation, a small amount of glycogen was found in the trophoblastic layer, but not in the inner cell mass of blastula in the later stage. In the egg-cylinder stage, a small amount of glycogen appeared in the endodermal cells and the trophoblastic cells, but not in the embryoblast, chorionic ectoderm and embryonic shield.

2. *Results obtained for RNA.*

The appearance and distribution of RNA in the embryos and uteri are given in Table 2.

Pre-implantation stage.

As shown in Table 2, at the 5th and 6th day of pregnancy, the blastula contained a small amount of RNA. The uterine epithelium and gland possessed a moderate amount of it. Many decidua cells had always a large amount of RNA.

Implantation stage.

At the 7th day, the blastula transformed into the egg-cylinder. The ectodermal cells contained a large amount of RNA, while the endodermal ones which formed a single layer along the inner surface of the trophoblastic layer a small amount of it (Fig. 11). Many decidua cells possessed a large amount of RNA (Fig. 11).

Placentation stage.

As shown in Table 2, at 8th day of pregnancy, both the embryoblast and chorionic ectoderm contained a large amount of RNA. The cells of the Träger and the trophoblastic giant cells also possessed a large amount of it, while the Reichert's membrane and the cells of the implantation zone none of it. The

Table 2. Distribution of RNA in the embryo and uterus.

Tissues		Stage of development		
		Pre-implantation (5th to 6th day)	Implantation (7th day)	Placentation (8th to 10th day)
Embryo	Inner cell mass (blastula)	+	not seen	not seen
	Trophoblastic layer (")	+	+	"
	Endoderm (")	not seen	+	++
	Embryoblast (egg-cylinder)	"	not seen	+++
	Chorionic ectoderm (")	"	"	+++
	Germinal layer of embryonic shield	"	"	+++
	Träger	"	"	+++
	Giant cell	"	"	+++
	Reichert's membrane	"	"	−
	Allantoic stalk	"	"	+
	Amnion	"	"	±
Uterus	Epithelium	++	not seen	not seen
	Uterine gland	++	"	"
	Decidua cell (early stage)	+++	+++	"
	Implantation zone	not seen	−	−
	Basilar decidua	"	not seen	+++
	Capsular decidua	"	"	++

embryonic shield contained a large amount of RNA.

At the 9th day of pregnancy, the allantoic stalk possessed a small amount of RNA. The basilar decidua contained persistently a large amount of RNA, while the capsular one a moderate amount of it (Fig. 12).

Baker(3) studied the histochemical variations in the uterine stroma of the rat during pregnancy and lactation and reported that at the 6th of pregnancy, many of the mesenchyme-like connective tissue cells contained RNA. This increased during the next several days, accompanied by the cellular enlargement. Wislocki and Dempsey(20 to 22) studied the histochemical reactions in the decidua and placenta of the cats, pigs and women and reported that during the early pregnancy the outermost portion of the tunica propria, including the epithelium and decidua cells, contained a large amount of RNA. In the present investigation of the rat the decidua cells contained a large amount of RNA and the uterine epithelium and gland a moderate amount of it, whereas the cells of implantation zone none of it. The accumulation of RNA probably plays a role in protein synthesis during a period of active endometrial growth and secretion.

I (10) have already reported that many cleaved ova and blastula in the early stage contained always a small amount of RNA. In the present investigation of the blastula in the later stage, the trophoblastic cells and the cells of each

germinal layer possessed the RNA abundantly.

3. *Results obtained for alkaline phosphatase.*

The appearance and distribution of alkaline phosphatase in the embryos and uteri are given in Table 3.

Table 3. Distribution of alkaline phosphatase in the embryo and uterus.

Tissues		Stage of development		
		Pre-implantation (5th to 6th day)	Implantation (7th day)	Placentation (8th to 10th day)
Embryo	Inner cell mass (blastula)	±	not seen	not seen
	Trophoblastic layer (")	+	+	"
	Endoderm (")	not seen	±	+
	Embryoblast (egg-cylinder)	"	not seen	+
	Chorionic ectoderm (")	"	"	+
	Germinal layer of embryonic shield	"	"	++
	Träger	"	"	+++
	Giant cells	"	"	++
	Reichert's membrane	"	"	+++
	Allantoic stalk	"	"	+
	Amnion	"	"	−
Uterus	Epithelium	++	not seen	not seen
	Uterine gland	++	"	"
	Decidua cells (early stage)	+++	+++	"
	Implantation zone	not seen	−	−
	Basilar decidua	"	not seen	+++
	Capsular decidua	"	"	+++

Pre-implantation stage.

As shown in Table 3, at the 6th day of pregnancy, the blastula showed a weak alkaline phosphatase reaction. The uterine epithelium and gland exhibited a moderate reaction, and many decidua cells an intense reaction.

Implantation stage.

When the embryo formed the egg-cylinder, at the 7th day of pregnancy, both the endodermal and ectodermal cells exhibited a weak alkaline phosphatase reaction (Fig. 15). The decidua cells constituting the uterine mucosa always showed an intense reaction of alkaline phosphatase.

Placentation stage.

As shown in Table 3, at the 8th day of pregnancy, both the embryoblast and chorionic ectoderm showed a weak reaction of alkaline phosphatase. The cells of Träger and the trophoblastic giant cells exhibited a moderate reaction of it. The Reichert's membrane showed an intense reaction, while the cells of the

implantation zone exhibited no reaction. All decidua cells persistently showed an intense reaction of alkaline phosphatase. The embryonic shield exhibited a moderate reaction of it.

At the 9th day of pregnancy, the allantoic stalk showed a weak reaction of it, while the amnion no reaction. The basilar decidua and the capsular decidua exhibited an intense reaction of it (Fig. 16).

Hard (9) studied the alkaline phosphatase in the placenta and fetal membrane of the guinea pig and reported that the embryonic endoderm, yolk-sac endoderm and decidual regions showed alkaline phosphatase reaction, whereas the fetal mesenchyme negative reaction. Wislocki and Dempsey (18) studied the histochemical reactions of the endometrium in pregnancy and reported that the endometrium of rats, guinea pigs, sows, cats and women showed the alkaline phosphatase reaction; however, its amount and distribution varied widely, being present in a small amount in the uterus of cats, guinea pigs and rats and a large amount in the uterus of sows and women. In the present investigation of the rat, the decidua cells showed an intense alkaline phosphatase reaction, the uterine epithelium and gland a moderate reaction, whereas the cells of implantation zone no reaction.

In the previous paper (10), I have reported that the cleaved ova and blastula showed a weak alkaline phosphatase reaction. In the present investigation, the embryonic endoderm and ectoderm showed a weak reaction of it, the cells of Träger a moderate reaction and the Reichert's membrane an intense reaction, showing that these distributions are intimately associated with the glycogen contents.

4. *Results obtained from the placenta.*

As materials, the placenta from the 12th to 21th day of pregnancy were used. After separated from the embryo, these were fixed in 95 per cent alcohol, embedded in celloidin and sectioned 20μ thick. The glycogen, acid polysaccharide, RNA and alkaline phosphatase were demonstrated by routine techniques. The results are given in Table 4.

Table 4. Distribution of glycogen, acid polysaccharide, RNA and alkaline phosphatase in the rat placenta.

Tissues		Glycogen	Acid polysaccharide	RNA	Alkaline phosphatase
Fetal placenta	Labyrinth	−	−	++	− to ++
	Syncytial trophoblast	++ to −	−	++	++ to +
	Trophoblastic giant cell	++ to −	−	++	++ to +
	Junctional zone	−	−	−	++
Maternal placenta	Basilar decidua	++ to +	− Unknown cell ++	++	++

a) *Glycogen and acid polysaccharide.*

As shown in Table 4, at the 12th day of pregnancy, a large amout of glycogen appeared in the syncytial trophoblast, trophoblastic giant cells and basilar decidua cells, but not in the labyrinth and junctional zone (Fig. 9). Afterwards, the glycogen in the syncytial trophoblast and trophoblastic giant cells decreased gradually and finally disappeared at the 18th day of pregnancy. The glycogen in the basilar decidua also decreased gradually and the amount became small at the 21th day of pregnancy (Fig. 10).

Acid polysaccharide appeared in the unknown woundering cells which were found in the basilar decidual zone during the 12th to 18th day of pregnancy. These cells were especially abundant around the blood vessels. They were round or oval in shape and had a round nucleus, negative for eosin, aldehyde-fuchsin and neutral red, metachromatic for basic dyes such as methylene blue and thionin.

b) *RNA.*

The RNA appeared abundantly in the syncytial trophoblast, trophoblastic giant cells and moderately in the labyrinth cells and the basilar decidua cells (Figs. 13 and 14). On the contray, it did not appear in the junctional zone. No marked variation in its amount was found during the pregnancy.

c) *Alkaline phosphatase.*

As shown in Table 4, at the 12th day of pregnancy, an intense reaction was found in the junctional zone, syncytial trophoblast and trophoblastic giant cells, and a moderate reaction in the basilar decidua, whereas there was no reaction in the labyrinth (Fig. 17). The alkaline phosphatase reaction in the syncytial trophoblast and trophoblastic giant cells decreased gradually and became weak on the 21th day of pregnancy. This feature coincided with the decrease of glycogen in these cells. No alkaline phosphatase reaction was found in the labyrinth at the 12th day of pregnancy. Its reaction increased with the development of the blood vessels and reached the maximum at the 21th day of pregnancy (Fig. 18), showing the same level throughout the course of the pregnancy.

Loveland, Maurer and Snyder(12) studied the distribution of glycogen in the rabbit placenta and reported that it diminished there during the last third period of pregnancy. Dempsey and Wislocki (6 to 8), Wislocki, Deane and Dempsey(17), Wislocki and Dempsey(18 to 22) and Wislocki and Wimsatt(24) studied histochemically the placenta of women and various kinds of animals and reported as follows : The glycogen was not demonstrated in the placental labyrinth but occurred abundantly in the basilar decidua. The RNA was abundant in the labyrinth and it decreased gradually during pregnancy. The alkaline phosphatase occurred plentifully in the labyrinth and increased steadily up to the end of pregnancy. Hard(9) studied the localization of the alkaline

phosphatase in the placenta of the guinea pig and reported that the following structures showed an intense reaction of it; labyrinth, junctional zone and basilar decidua. Whereas the following structures showed persistently negative reaction; ectoplacental trophoblast and trophoblast of the spongy zone.

In the present investigation the glycogen was demonstrated in the basilar decidua agreeing with the reports of Wislocki and his co-workers, and it was also proved in the syncytial trophoblast and trophoblastic giant cells. The glycogen in the basilar decidua decreased gradually and the amount became small at the 18th day of pregnancy, nearly coinciding with Loveland's report on the rabbit placenta.

The RNA appeared in the labyrinth, trophoblast and basilar decidua, agreeing with the reports of Wislocki and his co-workers. In the present investigation, it did not decrease during pregnancy, differing from the reports of Wislocki and his co-workers in which he described the decrease of RNA in the labyrinth during pregnancy.

The alkaline phosphatase was found in the junctional zone, trophoblast and basilar decidua, agreeing with the reports of Wislocki and his co-workers, but disagreeing with Hard's report in which he mentioned that no reaction was found in the trophoblast. No alkaline phosphatase was found in the labyrinth at the 12th day of pregnancy as already stated, disagreeing with the reports of Wislocki and his co-workers in which he described the alkaline phosphatase reaction in the labyrinth.

From the results obtained it was noticed that the glycogen appeared in the decidua, which occupied the greater part of the uterine mucosa, during the early stage of pregnancy, and it became restricted to the basilar decidua during the middle stage and afterwards it decreased gradually. It was also found that the alkaline phosphatase reaction was not demonstrated in the labyrinth during the early stage of pregnancy and it increased with the development of the blood vessels in the labyrinth.

These facts show that during the early stage of pregnancy the embryo which has no more glycogen in itself takes the glycogen as the nutriments from the surrounding decidua, that is, capsular decidua and basilar decidua, and after the establishment of the placenta in which the blood vessels well develop during the later stage of pregnancy it takes the glycogen from the basilar decidua.

Summary

The results obtained in this investigation are summarized as follows:

1. In the blastula and egg-cylinder of the rat, from the 5th to 10th day of pregnancy, a small amount of glycogen appeared in the inner cell mass, trophoblastic layer and endoderm of blastula and trophoblastic giant cells, and

a moderate amount of it in the Träger. In the uterus a large amount of glycogen appeared in the decidua.

2. In the blastula and egg-cylinder a small amount of RNA was found in the inner cell mass, trophoblastic layer and endoderm of blastula, allantoic stalk and amnion, and a large amount of it in the embryoblast and chorionic ectoderm of egg-cylinder, germinal layer of embryonic shield, trophoblastic giant cells and Träger. In the uterus a large amount of RNA occurred in the decidua.

3. In the blastula and egg-cylinder the alkaline phosphatase reaction was weakly found in the inner cell mass, trophoblastic layer and endoderm of blastula, embryoblast and chorionic ectoderm of egg-cylinder, germinal layer of embryonic shield and allantoic stalk, and intensely in the Träger and Reichert's membrane. In the uterus it occurred moderately in the uterine epithelium and gland, and intensely in decidua.

4. In the placenta, from the 12th to 21th day of pregnancy, a large amount of glycogen appeared in the syncytial trophoblast, trophoblastic giant cells and basilar decidua. The glycogen in these tissues decreased gradually.

5. In the placenta, a large amount of acid polysaccharide appeared in the unknown woundering cells which were found in the basilar decidua during the 12th to 18th day of pregnancy.

6. In the placenta a moderate amount of RNA was found in the labyrinth and basilar decidua, and a large amount in the syncytial trophoblast and trophoblastic giant cells. No marked variation in its amount was found during the pregnancy.

7. In the placenta a moderate alkaline phosphatase reaction was found in the basilar decidua, and an intense reaction in the junctional zone, syncytial trophoblast and trophoblastic giant cells. The alkaline phosphatase reaction was not demonstrated in the labyrinth in the early stage of development and it increased with the development of the blood vessels in the labyrinth.

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Plate 1

Explanation of Figures

Figures 1 to 10 were stained by PAS method for glycogen, Figures 11 to 14 by thionin stain for RNA and Figures 15 to 18 by Gomori's revised method for alkaline phosphatase.

Fig. 1. Blastula on 5th day of pregnancy. ×100.
A small amount of glycogen was found in the inner cell mass and trophoblastic layer.

Fig. 2. Blastula on 6th day of pregnancy. ×100.
A small amount of glycogen was found in the trophoblastic layer and a large amount of it in the primary decidua.

Fig. 3. Egg-cylinder in early course of 7th day of pregnancy. ×50.
A small amount of glycogen was found in the endoderm and a large amount of it in the secondary decidua.

Fig. 4. Egg-cylinder in early course of 8th day of pregnancy. ×50.
A small amount of glycogen was found in the endoderm and trophoblastic giant cells, a moderate amount of it in the Träger and a large amount of it in the decidua.

Fig. 5. Egg-cylinder in last course of 8th day of pregnancy. ×50.
A moderate amount of glycogen was found in the capsular decidua.

Fig. 6. Träger in last course of 8th day of pregnancy. ×50.
A moderate amount of glycogen was found in the Träger and a large amount of it in the basilar decidua.

Plate 1

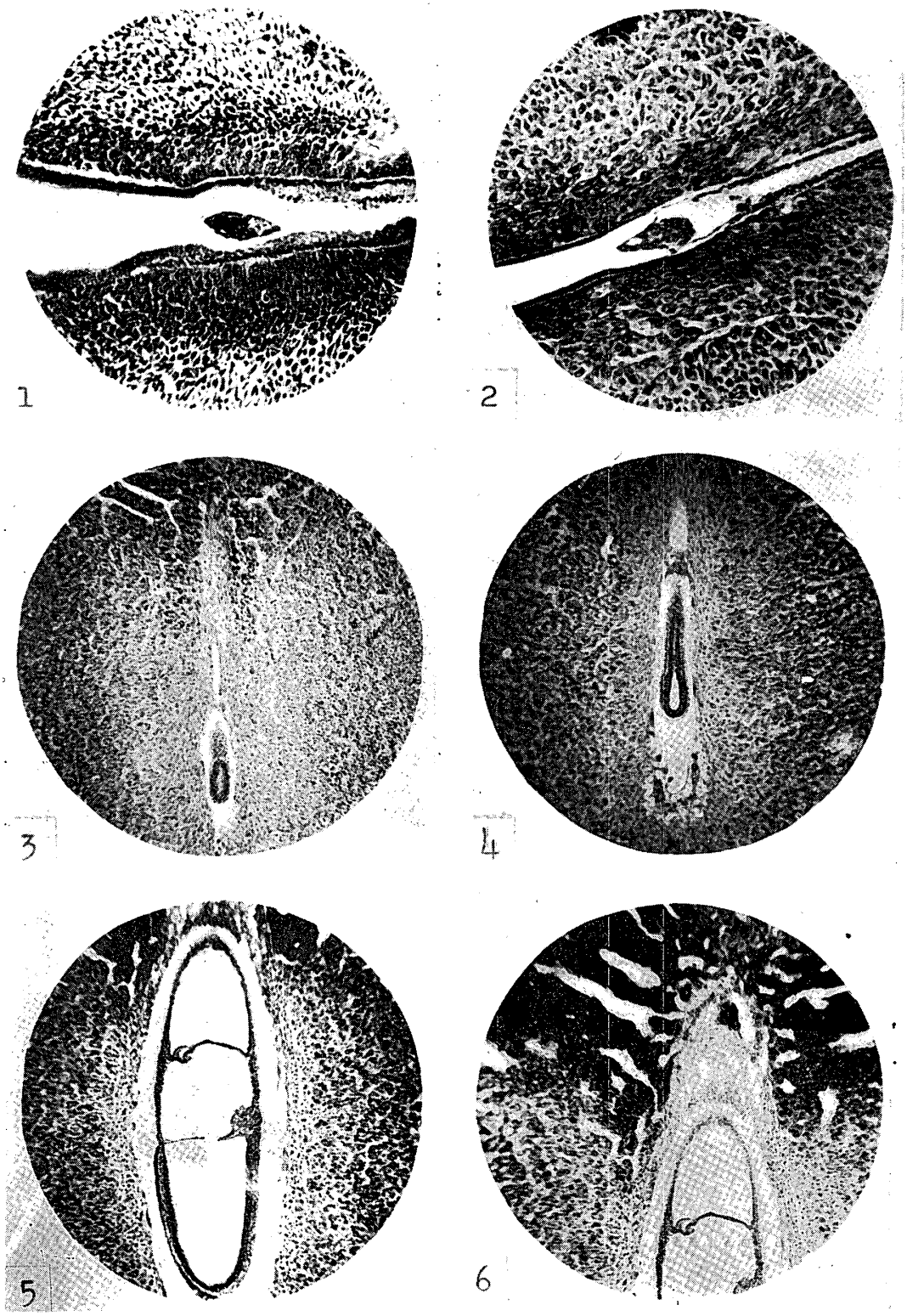


Plate 2

Explanation of Figures

Fig. 7. Placenta on 9th day of pregnancy. ×50.
A moderate amount of glycogen was found in the syncytial trophoblast, a large amount of it in the basilar decidua and none of it in the placental cone.

Fig. 8. Placenta on 10 th day of pregnancy. ×50.
A large amount of glycogen was found in the syncytial trophoblast and basilar decidua and none of it in the placental cone.

Fig. 9. Placenta on 12th day of pregnancy. ×50.
A large amount of glycogen was found in the basilar decidua and syncytial trophoblast, and none of it in the labyrinth.

Fig. 10. Placenta on 21th day of pregnancy. ×50.
A small amount of glycogen was found in the basilar decidua and none of it in the labyrinth and syncytial trophoblast.

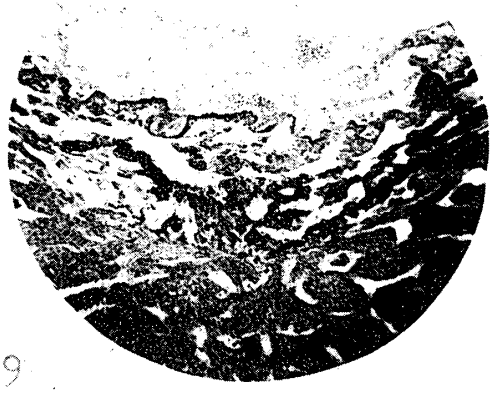
Fig. 11. Egg-cylinder on 7th day of pregnancy. ×100.
A large amount of RNA was found in the ectoderm of egg-cylinder and decidua.

Fig. 12. Embryo on 9th day of pregnancy. ×50.
A moderate amount of RNA was found in the embryo and capsular decidua.

Plate 2

7

8



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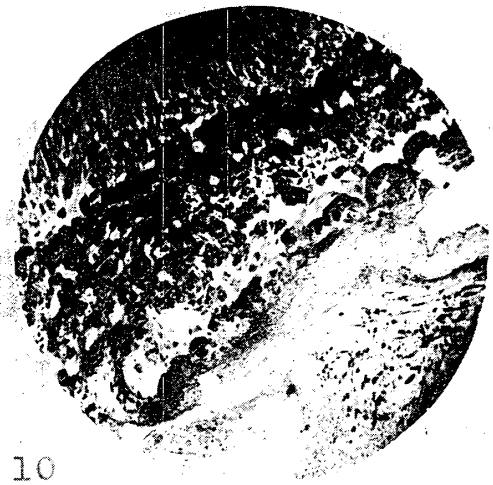

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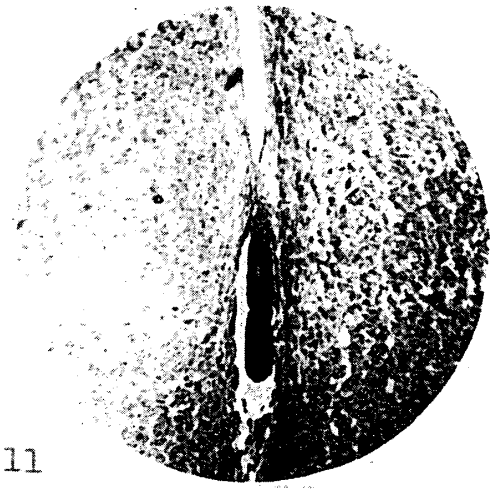

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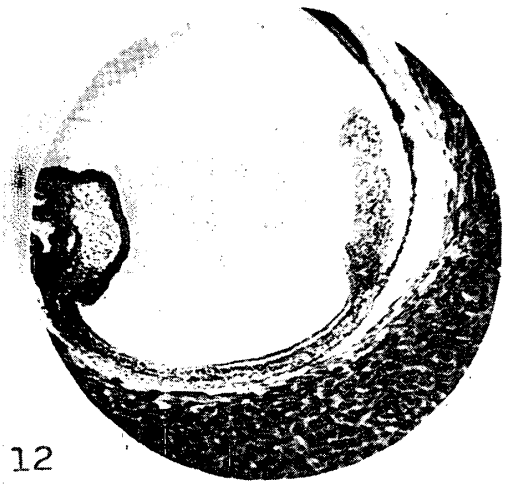

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Plate 3

Explanation of Figures

Fig. 13. Placenta on 12th day of pregnancy. ×50.
A moderate amount of RNA was found in the labyrinth and basilar decidua and a large amount of it in the syncytial trophoblast.

Fig. 14. Placenta on 18th day of pregnancy. ×50.
A moderate amount of RNA was found in the labyrinth, and a large amount of it in the syncytial trophoblast.

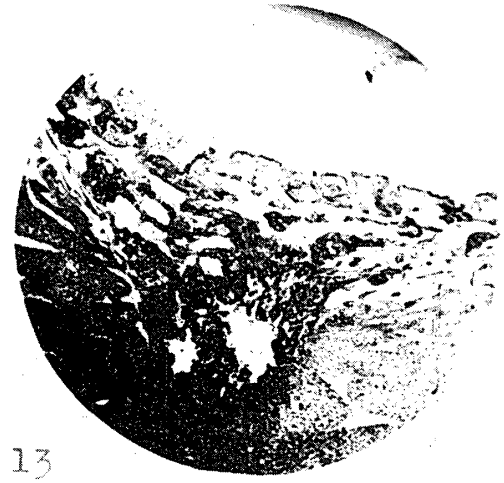
Fig. 15. Egg-cylinder on 7th day of pregnancy. ×50.
A weak reaction of alkaline phosphatase was found in the egg-cylinder, and an intense reaction of it in the decidua.

Fig. 16. Embryo on 9th day of pregnancy. ×50.
A moderate reaction of alkaline phosphatase was found in the embryo and an intense reaction of it in the decidua.

Fig. 17. Placenta on 12th day of pregnancy. ×50.
A moderate reaction of alkaline phosphatase was found in the basilar decidua, an intense reaction of it in the syncytial trophoblast and none of it in the labyrinth.

Fig. 18. Placenta on 21th day of pregnancy. ×50.
An intense reaction of alkaline phosphatase was found in the labyrinth.

Plate 3



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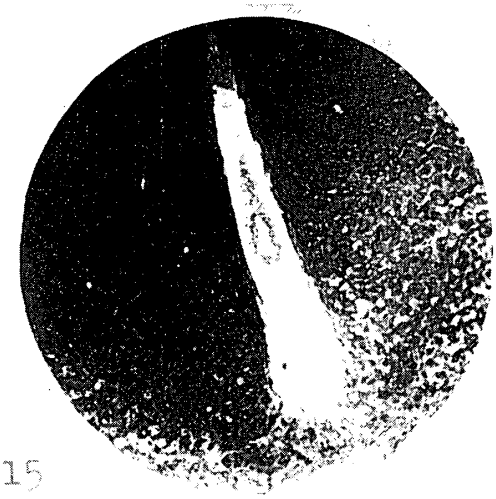

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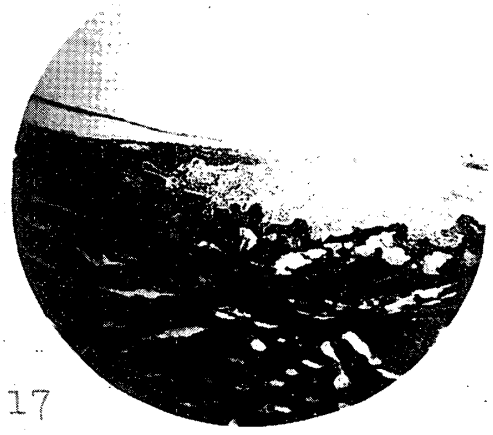

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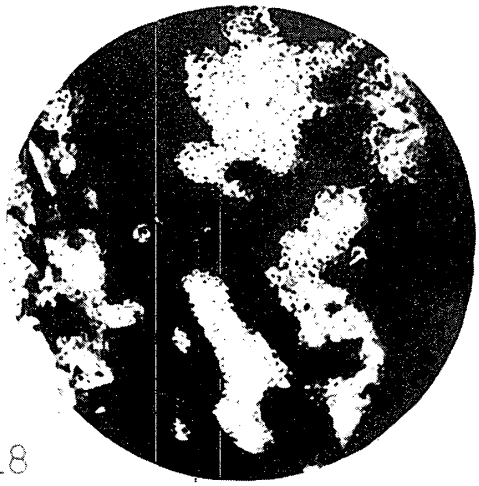

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