

## Histochemical Study of the Placenta in Prolonged Pregnancy, Toxemia of Pregnancy and Small-For- Dates Infants

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The functions of placenta in prolonged pregnancy, toxemia of pregnancy, small-for-dates infant (SFD) and in normal delivery at term were investigated. Histochemical studies on the ramus chorii which is considered to be most functional parts of the placenta, such as fetal nutritional supply, oxygenation, hormonal secretion and enzyme.

The following results were obtained :

### 1. Chorional epithelium of the ramus chorii

#### (1) Feulgen reaction

(a) Most of the Karyoplasm in syncytial cells of the chorional epithelium surrounding the ramus chorii had a granular appearance.

(b) In the S. K., the G/A ratio increased in normal delivery and prolonged delivery cases, but it decreased in the toxemia of pregnancy cases.

#### (2) Methyl-green-pyronin stain

The karyoplasm of the S. K. cells was extremely weak in staining comparing with other cases. This tendency was particularly observed in the S. K.

### 2. The ground substance of the ramus chorii

Acid mucopolysaccharides which were contained in the placenta are particularly considered to be hyaluronic acid. In the cases with toxemia of pregnancy, parturient eclampsia and prenatal death of fetus showed a tendency of strong staining in the colloidal iron reaction.

In conclusion, the amounts of DNA (Feulgen reaction), RNA (methyl-green-pyronin stain) and acid mucopolysaccharides (colloidal iron reaction) in SFD were quite different from those in other cases. This suggests that SFD shows some abnormalities in placental functions.

(Key Words : Histochemical Study, Placenta, Small-for-dates Infant, Toxemia of Pregnancy)

## INTRODUCTION

There have been many pathological and histological investigations of the placenta concerning infarct, thick profusion or matting of the villi, the appearance of fibrinoid and changes in the syncytial knot (S. K.) (25, 3, 8, 23, 17). In many cases, however, the placenta has large capacity, and partial abnormalities do not affect the overall function of the placenta. Therefore, the morphology of the placenta often does not correlate with fetal

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development. We conducted histochemical studies on the ramus chorii which is considered to be most functional part of the placenta in prolonged pregnancies, toxemia of pregnancies and small-for-dates infants (SFD)

## MATERIALS & METHODS

### (1) MATERIALS

i) Placenta in normal delivery at term	29
ii) Placenta in prolonged pregnancy	27
iii) Placenta in toxemia of pregnancy	14
iv) Placenta in SFD	12

These cases were selected as follows.

*Normal delivery at term* : Normal delivery was defined as vaginal delivery of a healthy child after from 39 to 42 weeks of gestation without particular complications. Fetuses weighing more than 4,000 g were excluded.

*Prolonged pregnancy* : These cases included fetuses delivered after 43 weeks of gestation. Cases born of mothers who had irregular menstruation were excluded.

*Toxemia of pregnancy* : Delivery was between 38 to 41 weeks of pregnancy after receiving therapy for three complications, i. e. edema, hypertension and albuminuria. Parturient eclampsia patients were also included as special cases.

*SFD* : Cases were selected according to the criteria by Funakawa's Standard (9) in deliveries after from 38 to 42 weeks of pregnancy.

### (2) METHODS

According to the above criteria, immediately after afterbirth of the placenta, the parts which appeared normal were sampled and fixed with 10% formalin and Carnoy's fixative. These specimens were all processed under the same conditions.

The staining methods were the hematoxylin-eosin stain, Feulgen reaction, methyl-green-pyronin stain, colloidal iron reaction, metachromasia (pH 4.1 and 2.5), and Alcian-blue stain. Ribonuclease and hyaluronidase digestion tests were carried out whenever required.

*Feulgen reaction* : 200 pieces of S. K. in ramus chorii were counted types and classified into granular and agglutinated types (Fig. 1, 2). The ratio of granular to agglutinated types (G/A) was calculated with the latter taken as 1.

*Methyl-green-pyronin stain* : Staining by pyronin in each case was compared with the corresponding clinical data. (Fig. 3, 4)

*Colloidal iron reaction* : Colloidal iron staining by Mowry's modification of Hale's reaction (Sano 1972) in each case was also compared with the clinical data. (Fig. 5, 6, 7 and 8).

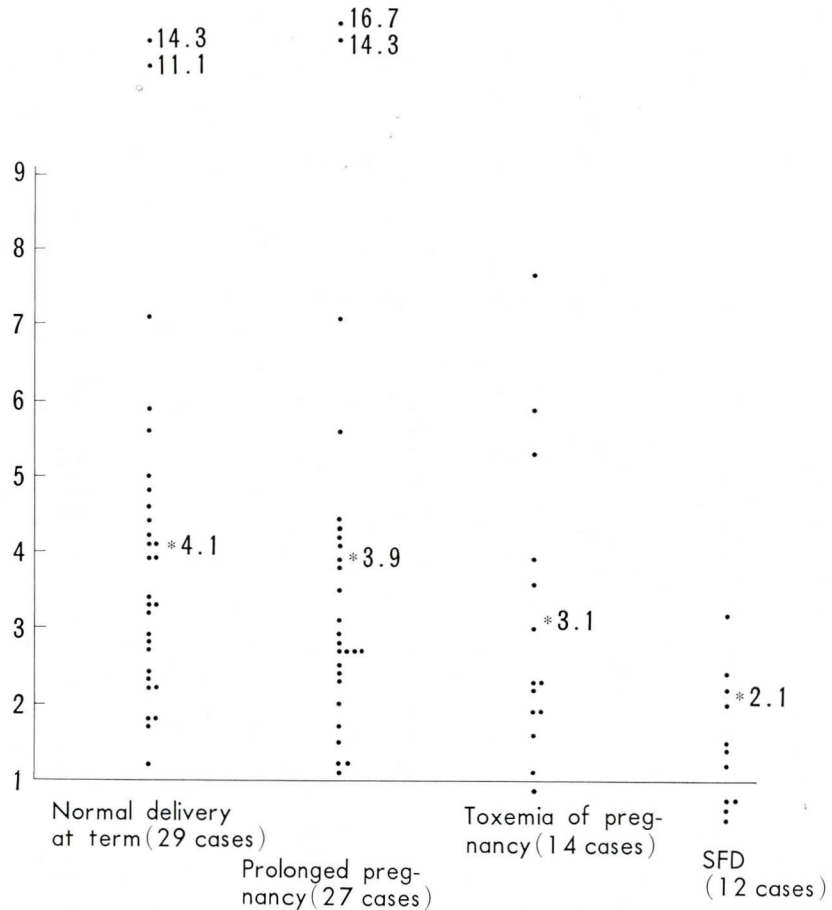
## RESULTS

### (1) Appearance of the chorionic epithelium

#### i) *Feulgen reaction*

Most of the karyoplasm in syncytial cells of the chorionic epithelium surrounding the ramus chorii had a granular appearance. In S. K., the G/A ratio increased in normal delivery and prolonged pregnancy cases, but it decreased in the toxemia of pregnancy cases (Table 1).

#### ii) *Methyl-green-pyronin stain*

**Table 1.** Feulgen reaction in syncytial knot (granular/agglutinate) \*average

In all cases, both chorional epithelium and S. K. showed staining of 1+ to 2+, but SFD revealed only traces of positive staining ( $\pm$ ) (Table 2).

This tendency was observed particularly in S. K..

(2) Appearance of the ground substance of ramus chorii

i) *Metachromasia and Alcian-blue*

Metachromasia was positive at pH 4.1 and negative at pH 2.5. the Alcian-blue reaction was positive.

A comparative study among the cases was difficult and is thus omitted in this report.

ii) *Colloidal iron reaction*

Most of cases showed staining in the range of 1+ to 2+, but in SFD, staining was slight and was in the range of 1+ to  $\pm$ , and for the toxemia of pregnancy cases, the strong staining of 1+ to 2+ was noted.

However, two out of 14 cases of toxemia of pregnancy revealed very weak staining as light as  $\pm$ , making it difficult to determine a consistent tendency for the toxemia cases (Table 3).

The observations above are summarized in Table 4 according to the various clinical states.

*Prolonged pregnancy* : Staining was similar to that of the normal deliv-



**Table 2.** Ramus chorii (methyl-green-pyronin stain)

Group	Normal delivery at term		Prolonged preg- nancy		Toxemia of pregnancy		SFD	
	E. L.(%) *	S. K.(%) **	E. L.(%)	S. K.(%)	E. L.(%)	S. K.(%)	E. L.(%)	S. K.(%)
A : (3+)+2+(1+)	9(31)	6(21)	4(15)		2(14)	1( 7)		
B : (3+)+1+(2+)	19(66)	16(55)	20(75)	13(48)	10(72)	7(50)	9(75)	2(17)
C : (±)+1+				1( 4)	1( 7)			
D : (2+)+±(1+)	1( 3)	7(24)	3(10)	11(41)	1( 7)	5(36)	3(25)	10(83)
E : ±								
F : (1+)+∓(+)				2( 7)		1( 7)		
G : ±								
TOTAL	29		27		14		12	

\* E. L.=Epithelial layer

\*\* S. K.=Syncytial knot

**Table 3.** Ground substance of Ramus Chorii (colloidal iron reaction)

Group	Normal delivery at term(%)	Prolonged pregnancy(%)	Toxemia of pregnancy(%)	SFD(%)
A : 2+		2( 7)	2(14)	
B : 2+(1+)	1( 3)		5(36)	1( 8)
C : 1+(2+)	2( 7)	4(15)		
D : 1+	19(66)	18(67)	5(36)	7(59)
E : ±	5(17)	2( 7)		2(17)
F : ±(∓)	2( 7)	1( 4)	2(14)	1( 8)
G : ∓				1( 8)
TOTAL	29	27	14	12

**Table 4.** Histochemical findings of ramus chorii (summary)

Case	S. K. (Feulgen reaction) Granular/Agglutinate	Epithelial layer (M. G. P. stain)	Ground substance (Colloidal iron reaction)
Normal delivery at term	4.1	1+~2+	1+
Prolonged pregnancy	3.9	1+	1+
Toxemia of pregnancy	3.1	1+	2+~1+
SFD*	2.1	1+~2+	1+~±

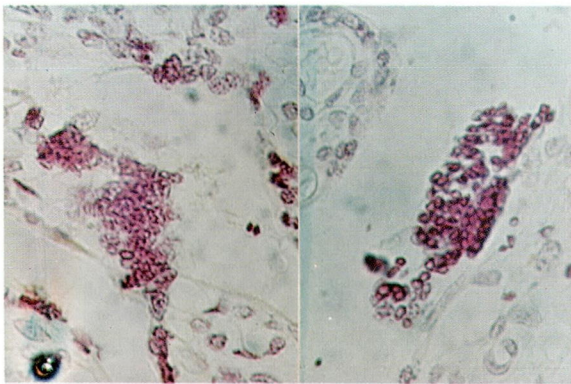
\* without toxemia complications

S. K.=Syncytial knot M. G. P. stain=Methyl-Green-Pyronin stain

ery in the Feulgen reaction and colloidal iron reaction but somewhat weaker in the methyl-green-pyronin stain. (Table 4)

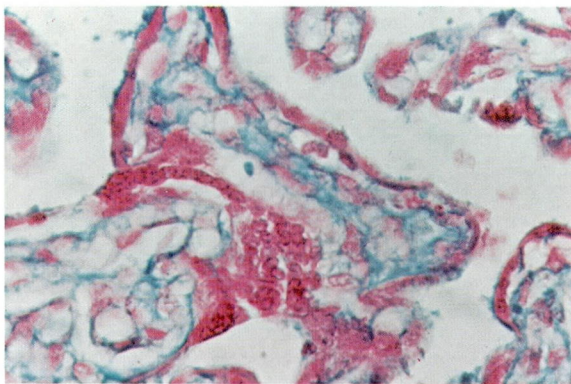
*Toxemia of pregnancy*: there were few cases, there were impressive features: cases with parturient eclampsia and prenatal death of the fetus showed a G/A ratio in the feulgen reaction approaching one and a strong staining in the colloidal iron reaction (Fig. 7). (Table 4, 5)

*SFD*: In the Feulgen reaction, the G/A ratio was smaller than that in the other cases, but in the methyl-green-pyronin stain, the staining was slight on the whole. In the colloidal iron reaction, there were specimens which were stained the least of any (Fig. 6).

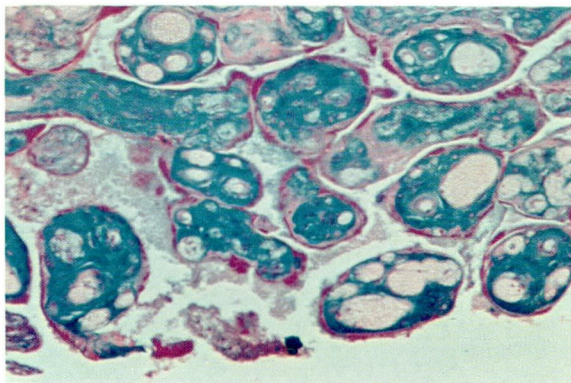


**Fig. 1** Feulgen reaction of ramus chorii and S.K. Karyoplasm is granular ( $40 \times 40$ ): Left

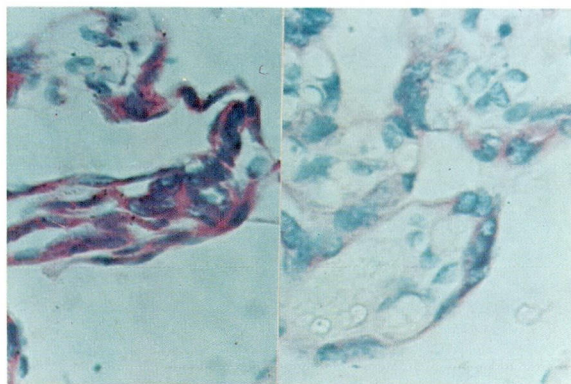
**Fig. 2** Feulgen reaction of ramus chorii and S.K. Agglutinated karyoplasm ( $40 \times 40$ ): Right



**Fig. 5** Colloidal iron reaction. Ground substance of ramus chorii is stained blue (1+). Normal full term placenta. ( $40 \times 40$ )

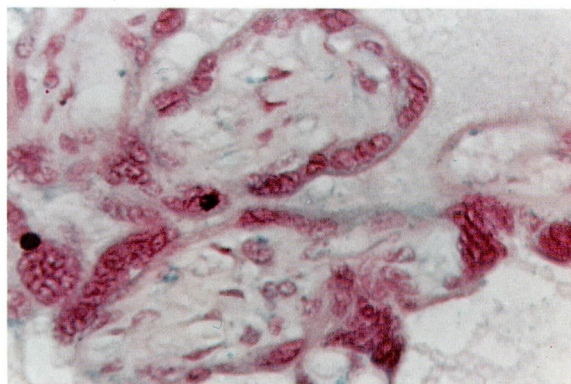


**Fig. 7** Colloidal iron reaction. Ground substance of ramus chorii stained dark blue (2+). A case of parturient eclampsia. ( $40 \times 10$ )

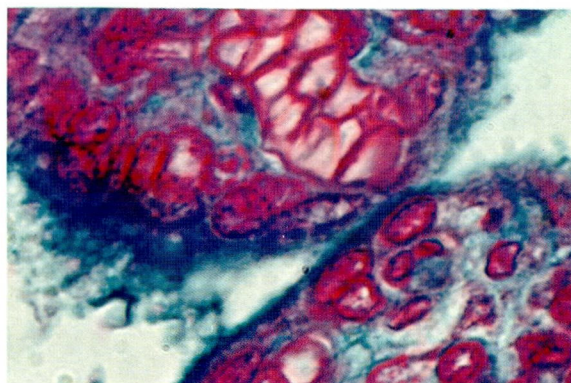


**Fig. 3** Methyl-green-pyronin stain. Cytoplasm stained strongly with pyronin (2+) ( $100 \times 10$ ): Left

**Fig. 4** Methyl-green-pyronin stain. Cytoplasm stained to a medium degree (1+). ( $100 \times 10$ ): Right



**Fig. 6** Colloidal iron reaction. Ground substance of ramus chorii is stained weakly. (±) A case of SFD. ( $40 \times 10$ )



**Fig. 8** Colloidal iron reaction: Ground substance of ramus chorii stained dark blue and at the same time exuded over the surface through chorionic cells. A case of prenatal death of the fetus. ( $100 \times 10$ )





**Table 5.** Histochemical findings of placenta of toxemia

Case	S. K. (Feulgen reaction) Granular/Agglutinate	Epithelial layer (M. G. P. stain)	Ground substance (Colloidal iron reaction)
SFD*	3.5	1+~±	2+~1+
Eclampsia at pregnancy	1.0	1+	2+
Intrauterine fetal death	1.6	2+	2+
Toxemia of pregnancy (without complication)	3.7	1+~±	1+~±

S. K. = Syncytial knot

M. G. P. stain = Methyl-Green-Pyronin stain

\* With toxemia complications

## DISCUSSION AND CONCLUSION

### (1) Chorional epithelium of the ramus chorii

#### i) *Feulgen reaction*

The feulgen reaction is a histochemical staining method of DNA which was established by Feulgen and others (1924). Application of this method to syncytial cells proved that in the nucleus of syncytial cells of the chorional epithelium surrounding the chorional tissue, most of the Feulgen reaction-positive karyoplasm was granular while the karyoplasm of the S. K. cells was either granular or agglutinated. A comparison of G/A in S. K. with the clinical data revealed that there was no significant difference between normal delivery at term and prolonged pregnancy, while a marked decline in granular nuclei was noticed in the toxemia of pregnancy and particularly in SFD.

Many investigators have reported the mechanism of formation and functions of the S. K.

It has been speculated that the S. K. is due to degeneration of the chorional epithelium (21) that it is an amoebic motion of the villi (2) that it is a supporting apparatus of the plate of the villi (12) that it is formed by functional inactivity of the syncytium (10), that it is due to active syncytial proliferation (1, 16), and that it is the consequence of hypoxia or reduction in blood circulation in the villi (22, 18). Tatsumiya (1973) reported that the structure of S. K. is a nuclear mass of about 24 to 72  $\mu$  from serial sections of the villi.

Although there are many reports such as the above, there have not been any convincing theories on the physiological significance of S. K.. In our study, the findings concerning the relationship between the distribution of nucleic acid as demonstrated by the Feulgen reaction and the clinical data was considered to be very interesting will be investigated further in the future.

#### ii) Methyl-green-pyronin stain

The methyl-green-pyronin stain is a method established by Brachet (1940, 1942). The pyronin-positive part is considered to be RNA.

Investigations of the placenta using MGP have been very few.

According to Murae (1960), cytoplasmic RNA of S. K. cells identified by pyroin is very small in quantity.

In our study, both the chorional epithelium and S. K. in SFD showed weak pyronin positivity.

Slight differences were noticed between the normal delivery at term, prolonged pregnancy and toxemia of pregnancy. The staining was the strongest in the normal delivery at term, followed by prolonged pregnancy, toxemia of pregnancy and SFD in that order.

In particular, SFD staining was extremely weak compared with that of other cases.

(2) Ground substance of ramus chorii

Histochemical studies of the ground substance of ramus chorii have not been extensive.

Since the ground substance was positive with Alucian-blue, positive with colloidal iron reaction, positive with methachromasia at pH 4.1 and negative at pH 2.5, the material contained in the placenta is considered to be acid mucopolysaccharides, particularly hyaluronic acid, when we stress the role of the placenta in permeation.

Although the biological and chemical significances of the mucopolysaccharides have not yet been clarified, they are believed to play an important role in normal maintenance of the connective tissue, especially in protection from the invasion of toxins.

It seems likely that hyaluronic acid is produced mainly in the fibroblast essential to the connective tissue and its turnover is considered to be faster than that of other acid mucopolysaccharides (Meyer, 1956).

The authors divided the staining degrees in the colloidal iron reaction into 7 stages, and compared them with the clinical data. While no significant difference was noticed between normal delivery at term (Fig. 5) and prolonged pregnancy, the SFD showed a tendency for weak staining (Fig. 6). Toxemia of pregnancy and cases which were thought to have marked asphyxia such as prenatal parturient eclampsia and death of the fetus showed strong staining (Fig. 7.8). This suggests that strong asphyxia is followed by accumulation of hyaluronic acid due to some unknown factors.

In SFD, staining was weaker than in the other cases. This may be due to low hyaluronic acid production by the stroma cells.

In conclusion, the amounts of DNA (Feulgen reaction), RNA (methyl-green-pyronin stain) and acid mucopolysaccharides (colloidal iron reaction) in SFD were quite different from those in other cases. This suggests that SFD shows some abnormalities in placental functions.

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