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Histochemical Study of the Umbilical Cord in the Developing Human Fetus

(umbilical cord/development/histochemistry)

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We observed histochemically the monthly changes in 50 umbilical cords from fetuses aborted at various stages of gestation. The investigation was focused on the Amnion epithelium, Wharton's jelly, A. umbicalis and V. umbicalis in the umbilical cord, collagenous, reticular and elastic fibers.

The umbilical cord acts as a channel for the supply of oxygen and nutrients which are necessary for the life of the fetus as well as for the excretion of waste products. Functions of the umbilical cord have been studied from the aspects of morphology (1), embryology (2), biochemistry (3), neurology (4–5) and histology (3, 4, 6–10). Histochemical studies have also been done (11–17). As there are few histochemical and systematic studies on the development of the human umbilical cord and its transition in the course of development, related investigations were done out in our laboratory. Our findings are reported herein.

MATERIALS AND METHODS

Cross-sections of fifty normal umbilical cords of human fetuses at gestational ages between 2 and 10 months were examined. All the materials were fixed in 10% formalin, dehydrated, embedded in paraffin and then sliced. The following stains were used for the histology : Hematoxylin-Eosin (HE), Van-Gieson (VG), Azan-Mallory (AM, Masson's Trichrome method (MT), Weigert's Resorcin-Fuchsin (EL) stain, and Watanabe's Silver Impregnation (GF) method. For the histochemical examinations, the Periodic Acid Schiff (PAS) reaction, Lille's method, Metachromasia of Ohno's method, Alcian blue method, Rinehart's Colloid iron reaction and R. Mayer's Mucicarmin (MC) method were used.

RESULTS

During tissue development of the human umbilical cords, amnion epithelium (squamous epithelium), Wharton's jelly, and umbilical arteries and veins were studied.

Histological Findings

a) Second fetal month (Photos 1—2 and 11)

Amnion epithelium (AE) : consisted of a layer of plain epithelial cells immedietely beneath the epithelium fibers which stained deeply with VG and AM and presented somewhat thick lines.

Wharton's jelly (WJ) : cell components in the stroma were small in number, presented a stellate or fusiform shape, and possessed intercellular cytoplasmic processes, presenting a reticular structure. The fibers along the cell surfaces were strongly stained with VG, and rather strongly with AM. In the stroma, only collagen fibers (CF) were evident, and elastic fibers (EF) or reticular fibers (RF) were not detected, even with the GF method.

A. and V. umbilicalis (Vasa umbil) : The Vasa umbil at this stage had comparatively thin walls, and presented a round or elliptical structure. The endothelial cells lining the luminal surface were clearly visible with HE stain. Distinction between the internal, intermediate, and external muscle layers was not clear. When stained with VG, there were muscle fibers which stained yellow, and CF which stained red. The latter slightly outnumbered the former. The same results were obtained after staining with AM. With the GF method, reticular fibers RF were present in the area assumed to be the internal layer of the Vasa umbil. These fibers presented a very fine and irregular reticular structure. Elastic fiber was not detected in any layer.

b) Third fetal month (Photos 3 and 12)

AE : The findings were the same as those obtained at the 2nd fetal month.

Wasa umbil : The vascular lumen was small, but presented a round or elliptical structure, and the wall was thick. The muscle layers could be distinguished clearly. At this stage, circularly distributed and extremely fine EF was detected for the first time in the inner layer of the Vasa umbil. There were relatively large amounts of EF in the arteries and very small amounts in the veins.

RF : The distribution of RF was the same as that observed in the 2nd fetal month.

c) Fourth fetal month (Photos 4 and 13)

AE : Findings were much the same as observed at the 2nd and 3rd fetal months.

WJ : Fine fibers were circularly distributed, rather densely and perivascularly in the stroma, and thick fibers were coarsely distributed near the epithelium, in a reticular fashion. Von Gieson staining of WJ in the area around the blood vessels was moderately positive, while near the epithelium it was moderately or strongly positive. These findings were the same when the Masson method was used. Neither EF nor RF was observable.

Vasa umbil : The arterial walls were hypertrophic and the diameter enlarged while the venous walls were thin and the vascular lumen enlarged. The Arteria umbilicalis (A. umbil) was round, and the Vena umbilicalis (V. umbil) was elliptical in shape. Also, a slight increase in EF was observed in the

vascular walls. Otherwise, the findings were the same as those obtained in the 3 month old fetuses.

d) Fifth fetal month (Photos 5 and 14)

AE : was strongly stained with Van Gieson. No other marked changes were observed.

WJ : At this stage, there were numerous fibers in the stroma and around the blood vessels, the reaction was moderate to strongly positive, with the VG method. Near the epithelium, the scattered fibers became coarse and thick in a reticular structure and the reaction was strongly positive. The same results were observed with AM.

Vasa umbil : The diameters of the blood vessels increased considerably. The wall became thicker in A. umbil. The V. umbil dilated to a size more than twice that of the A. umbil, but the wall of the V. umbil was thin. The muscle fibers in the walls of the blood vessels were now discernible, and the inner, mid, and outer layers were clearly distinguishable. Muscle fibers and CF could be easily distinguished with VG and AM. The muscle fibers were somewhat more abundant in the A. umbil than in the V. umbil. RF was distributed in all the layers of the vascular walls, in a reticular form. An increase in EF was observed, and the structure of the blood vessels was more distinct.

e) Sixth fetal month (Photos 6 and 15)

AE : the same as in the 5th fetal month.

Vasa umbil : An increase in the EF was observed in the vascular walls, otherwise there were no differences from the 5th fetal month.

f) Seventh fetal month (Photo 7)

AE, WJ : No remarkable change was observed.

Vasa umbil : Both arteries and veins were clearly visible. The arterial walls had thickered, while venous walls were dilated, and muscle fibers could be clearly observed either with VG or AM. There was very little CF in the vascular walls. The EF and RF findings were the same as those obtained in the 6th fetal month.

g) Eighth fetal month (Photos 8—9)

AE, WJ : No remarkable change was observed.

Vasa umbil : The findings were much the same as those obtained in the 7th fetal month, however, EF in the vascular walls had markedly increased. In A. umbil, 3 to 5 lines of thick fibers were distributed on the inner 1/3 of the vascular wall. In V. umbil, 1 to 2 lines of thick fibers were distributed only immediately beneath the endothelium.

h) Ninth fetal month

AE, WJ : There was no remarkable change.

Vasa umbil : An increase of EF and RF was apparent in the arteries and veins.

i) Tenth fetal month (Photo 10)

AE : No remarkable change was observed. Immediately beneath the epithelium, there were filiform fibers stained strongly with VG and AM.

WJ : Fibers which stained strongly with VG and AM were fine around the blood vessels and coarse and thick near the epithelium. No EF or RF were observed.

Vasa umbil : The muscle layers in the arteries and veins were the same as those observed when the fetuses were 8 to 9 months old. EF was distributed in all layers in the Vasa umbil and particularly in the A. umbil, both thick and fine fibers were numerous in the inner layer, and many fine fibers were noted in the muscle layer. Fine fibers were sometimes observed in the outer layer. In the V. umbil, 1 to 3 lines of thick fibers were found in the inner layer, in addition to a few in the mid layer. No fibers were noted in the outer layer. A reticular formationy RF was observed in all the layers. All these findings are summarized in Table I.

Histochemical Findings (With Regard to Polysaccharides)

a) Second fetal month (Photos 1—2 and 11)

AE : At this stage, many large or small glycogen granules with consistently positive PAS reactions were seen in the amnionic epithelial cells. They reacted negatively at any pH when the metachromasia (M) or the alcian blue (AB) method was used. But by means of the colloid iron (CI) method, slightly positive reactions were observed in the epithelial cells. The filiform fibers immediately beneath the epithelium also reacted positively with the CI method. By means of MC, AE reacted positively. A site immediately under the epithelial cells which reacted positively and occasionally rather strongly positive was also observed.

WJ : A few glycogen granules were found in the stroma. In the perivascular cells and tissues more glycogen granules were observed. These reacted negatively to M and AB methods, but positively to CI. With the mucicarmin method, they reacted positively in the perivascular tissues and moderately positive near the epithelium.

Vasa umbil : There were numerous glycogen granules which reacted strongly positive to the PAS reaction on the inner layer of the vascular walls of the Vasa umbil, but at pH 4.1 and 7.0 there was no positive reaction with the M method. However, compared to the A. umbil, the reaction of the V. umbil was rather weak. The Vasa umbil stained negatively with Alcian blue. With the CI and M methods, the reaction was moderate at the inner and mid layers of the vascular walls, and minimal at the outer layer.

Allantios : Glycogen granules were observed by means of the PAS reaction, but negative results were obtained with the and AB methods. Moderately positive results were obtained using the CI and M methods.

b) Third fetal month (Photos 3 and 12)

AE : By means of the PAS reaction, more glycogen granules were observed in AE compared with findings of the 2nd fetal month. Using the M method, the reaction was negative at pH 2.5 and weakly positive at pH 4.1 and pH 7.0. Using the AB method, the reaction was negative, and with the CI and M methods, the findings were much the same as in the 2nd fetal month.

WJ : By means of the PAS reaction, more glycogen granules were found

TABLE I. *Histological Changes of the Umbilical Cord of the Human Fetus*

Fetal age(month)	Staining / Object / Localization	HE / Tissue aspects	VG	AM	MT	EL	GF
			For differentiation of collagenuous fiber and muscle			Elastic fiber	Reticular fiber
2	(A)	(TS) round	(C)± - +	(C)± - +	(C)± - +	-	+
		(VW) thin	(M)± - +	(M)± - +	(M)± - +		
	(V)	(TS) round	(C)+	(C)±	(C)±	-	+
		(VW) thin	(M)± - +	(M)± - +	(M)± - +		
	(WJ)	(PV) fiber dense	(C)+ - ++	(C)+ - ++	(C)+ - ++	-	-
		(NE) fiber loose					
	(B)		(C)+	(C)+	(C)+	-	-
	(AM)	one layer	-	-	-	-	-
3	(A)	(TS) round	(C)± - +	(C)± - +	(C)± - +	inner layer +	+
		(VW) thick	(M)+ - ++	(M)+ - ++	(M)+ - ++		
	(V)	"	(C)± - +	(C)± - +	(C)± - +	inner layer +	+
	(WJ)	fiber loose	(C)+ - ++	(C)++	(C)++	-	+
	(B)		(C)+	(C)+	(C)+	-	-
	(AM)	one layer	-	-	-	-	-
4	(A)	(TS) round	(C)±	(C)±	(C)±	+	+
		(VW) thick	(M)+	(M)+	(M)+		
	(V)	(TS) oval	(C)±	(C)±	(C)±	+	+
		(VW) thick	(M)+	(M)+	(M)+		
		(L) dilation					
	(WJ)	(PS) fiber dense	(C)+ - ++	(C)+ - ++	(C)+ - ++	-	-
		(NE) fiber loose	(C)++ - +++	(C)++ - +++	(C)++ - +++	-	-
	(B)		(C)+	(C)+	(C)+	-	-
	(AM)	one layer	-	-	-	-	-
5	(A)	(VW) swelling	(C)+	(C)+	(C)+	+	++
			(M)++	(M)++ - +++	(M)++ - +++		
	(V)	(VW) dilation thin	(C)+	(C)+	(C)+	+	++
			(M)++	(M)++	(M)++ - +++		
	(WJ)	(PS) fiber dense	(C)++ - +++	(C)++ - +++	(C)++ - +++	-	-
		(NE) fiber loose	(C)+++ - ++++	(C)++++	(C)+++ - ++++		
	(B)		(C)+ - ++	(C)+ - ++	(C)+ - ++	-	-
	(AM)	one layer	-	-	-	-	-
6	(A)	(VW) swelling	(C)+	(C)+	(C)+	++	++
			(M)++	(M)++	(M)++		
	(V)	(VW) dilation	(C)+	(C)+	(C)+	+	++
			(M)++	(M)++	(M)++		
	(WJ)	(PS) fiber dense	(C)++	(C)++	(C)++	-	-
		(NE) fiber loose	(C)+++	(C)+++	(C)+++		
	(B)		(C)+ - ++	(C)+ - ++	(C)+ - ++	-	-
	(AM)	one layer	-	-	-	-	-
7	(A)	(VW) swelling	(C)+	(C)+	(C)+	++	++
			(M)+++	(M)+++	(M)+++		
	(V)	(VW) dilation	(C)+	(C)+	(C)+	+ - ++	++
			(M)+++	(M)+++	(M)+++		
	(WJ)	(PS) fiber dense	(C)++	(C)++	(C)++	-	-
		(NE) fiber loose	(C)+++	(C)+++	(C)+++		
	(B)		(C)++ - +++ ++		++	-	-
	(AM)	one layer	-	-	-	-	-

8	(A)	(VW) swelling	(C)+	(C)+	(C)+	+++ - ++++	++
			(M)+++	(M)+++	(M)+++		
	(V)	(VW) dilation	(C)+	(C)+	(C)+	++ - +++	++
			(M)+++	(M)+++	(M)+++		
	(WJ)		(C)+++	(C)++ - +++	(C)++ - +++	-	-
	(B)		(C)+ - ++	+	+	-	-
	(AM)		-	-	-	-	-
9	(A)		(C)+ - ++	(C)+ - ++	(C)+ - ++	+++ - ++++	+++
			(M)+++	(M)+++	(M)+++		
	(V)		(C)+ - ++	(C)+ - ++	(C)+ - ++	++ - +++	+++
			(M)+++	(M)+++	(M)+++		
	(WJ)		(C)+ - ++	(C)+++	(C)+++	-	-
	(B)		(C)+ - ++	(C)+++	(C)+++	-	-
	(AM)		-	-	-	-	-
10	(A)		(C)+ - ++	(C)+ - ==	(C)+ - ++	+++++	+++
			(M)+++	(M)+++	(M)+++		
	(V)		(C)+ - ++	(C)+ - ++	(C)+ - ++	+++ - ++++	+++
			(M)+++	(M)+++	(M)+++		
	(WJ)		(C)+++ - ++++	(C)+++	(C)+++	-	-
	(B)		+ - ++	+	+	-	-
	(AM)		-	-	-	-	-

Abbreviations : (A) A. umbilocalis, (V) V. umbilicalis, (WJ) Wharton's Jelly, (B) Immediately beneath amnion epithelium, (AM) Amnion Epithelium, (TS) Tissue Shape, (VW) Vascular Wall, (PS) Perivascular, (NE) Near Epithelium, (L) Lumen, (C) Collagenous fiber, (M) Muscle, HE : Hematoxylin-Eosin, VG : Van-Gieson, AM : Azan-Mallory, MM : Masson's Trichrome, EL : Resorcin-Fuchsin, GF : Silver Impregnation.

in amnionic epithelial cells, particularly in the perivascular tissues, compared with observations at the 2nd fetal month. With the M method, the reaction was negative at pH 2.5 and weakly positive at pH at 4.1 and 7.0. For the first time, at this stage, areas where fibers were fine and rather close to the epithelium reacted positively with the AB method. Near the epithelium in the area where the fibers were coarse and thick, the stellate and spindle shaped cells reacted positively with the AB method. Results of the CI and M methods were much the same as observed in the 2 month old fetuses.

Vasa umbil : With respect to the PAS reaction, the findings were much the same as those observed in the 2 month old fetuses. With the M method, the reaction was negative at pH 2.5 and distinctly positive at pH 4.1 and 7.0. These reactions were strong on the inner layer of the vascular wall, comparatively weak in the mid layer and only slight in the outer layer. With the AB method, as in WJ, weakly positive to positive reactions were observed in the vascular walls. This reaction was strong in the inner layer and very weak in the outer layer. The reactions to the CI and M methods were the same as those observed in the 2 month old fetuses.

c) Fourth fetal month (Photos 4 and 13)

WJ : Using the Pas reaction, the number of glycogen granules in the stroma was less than when the fetuses were 3 months old. With the M method, the reaction was negative at pH 2.5 and distinctly positive at pH 4.1 and 7.0. With the AB and DI methods, a strongly positive reaction was seen

more in stellate and spindle shaped cells and in large thick fibers closer to the epithelium than in the perivascular cells and fine fibers. With the M method, the reaction was the same as that observed when the fetases were 3 months old.

Vasa umbil : The reactions were much the same as those observed at 3 months of gestation, with each method.

d) Fifth fetal month (Photos 5 and 14)

AE : By means of the PAS reaction, many fine and, strongly positive glycogen granules were found in the epithelium. Otherwise, the reactions were much the same as observed in the 4 month old fetuses.

WJ : With the PAS reaction, many glycogen granules were seen in the perivascular stroma and the number of granules was diminished near the epithelium With the M method, the findings were much the same as observed when the fetases were 4 months old. With the AB method, the reaction was weaker. With the CI method, the reaction was moderately positive in the perivascular stroma, and moderate to strong in the stroma near the epithelium. The reactions were somewhat stronger than those observed when the fetuses were 4 months old.

Vasa umbil : By means of the PAS reaction test, a particularly strong positive appearance of glycogen granules was found in all the Vasa umbil layers. These reactions were particularly strong in the inner layers. With the M and AB methods, the reactions were the same as those when the fetuses were 4 months old. With the CI method, both A. and V. umbil reacted strongly positive at the inner and mid layers, but rather weakly at the outer layer. Compared with the A. umbil, the V. umbil reacted rather weakly. With the M method, the reaction was the same as that observed in the 4 month old fetuses.

e) Sixth fetal month (Photos 6 and 15)

AE : With the PAS reaction, the glycogen granules stained strongly positive. With the M and CI methods, the reactions were much the same as those observed in the 5th fetal month. The mucous substance attached to the epithelial surface layer, however, reacted strongly positive, with each method. At the epithelium and immediately beneath the epithelium, the reaction was positive and sometimes strongly positive, with the M method.

WJ : By means of the PAS reaction, more glycogen granules were found in the stroma than at the 5th fetal month. At this time, with M method and at pH 2.5, weakly positive and sometimes positive reactions were observed. Furthermore, at pH 4.1 and 7.0, strongly positive reactions were observed. With the AB method, the reaction was moderately positive in the perivascular stroma yet moderately to sometimes strongly positive at the coarse stroma near the epithelium. With the CI method, the reaction was moderately to strongly positive at the perivascular stroma, and strongly positive at the coarse stroma near the epithelium. With the M method, the reaction was positive at the perivascular stroma and moderately positive at the stroma close to the epithelium.

Vasa umbil : With the PAS reaction test, the most positive appearance of glycogen granules was observed as in the case of the 5th fetal month. With the M method, the A. umbil reacted weakly positively, and V. umbil very weakly and sometimes weakly positively at pH 2.5. But at pH 4.1 and 7.0, the reactions of both A. and V. umbil were strongly positive. With the AB method, the reaction was rather strong compared with findings at the 5th fetal month. With the CI method, Vasa umbil reacted strongly positive. With the M method, the reaction was the same as in the 5th fetal month.

f) Seventh fetal month (Photo 7)

AE : Using the PAS reaction test, the number of glycogen granules in AE was found to be smaller. The reactions were negative at each pH with the M and AB methods. With the CI and M methods, the reaction was somewhat weaker than that observed in the 6th fetal month.

WJ : With the PAS reaction, some decrease in the glycogen granules in the stroma was observed, however, some glycogen granules in the perivascular stroma did react moderately positive. With M, CI and AB methods, the reactions ranged from positive to moderately positive, showing a narrower range than in the 6th fetal month.

Vasa umbil : With the PAS reaction, Vasa umbil reacted moderately positive to very strongly positive. V. umpil, however, showed a decrease in glycogen granules when compared to A. umbil. With the M method, A. umbil showed a weakly positive result and sometimes positive reactions at pH 2.5. TheV. umbil showed only a weakly positive reaction. With the AB method, the reaction was much the same. With the CI method, the reaction of A. umbil was strongly positive, and that of V. umbil was moderately positive. With the M method, the reaction of A. umbil was stronger than when the fetuses were 6 months old, but the reaction of V. umbil was weak.

g) Eighth fetal month (Photos 8—9)

AE : With the PAS reaction test, the appearance of glycogen granules in the epithelium was most evident in the 5th and 6th fetal month. With the M method, the reaction was negative at pH 2.5, and very weak or sometimes weakly positive at pH 4.1 and 7.0. With the AB method, the reaction was negative, with the CI method, the reaction was weakly positive and with the M method, the reaction was weaker than in the 7th fetal month.

WJ : With the PAS reaction, M and AB methods, the reactions were the same as in the 7th fetal month. But with the CI method, the reaction was positive to moderately positive at the perivascular stroma, and moderately to strongly positive in the coarse stroma near the epithelium. With the M method, the reaction was somewhat weaker.

Vasa umbil : The reactions were much the same as in the 7th fetal month. However, with the M method, the reaction at the A. umbil was strongly positive at pH 4.1 in the 7th fetal month, but was weaker yet still positive in the 8th fetal month. With the M method, the reaction was rather weak.

h) Ninth fetal month

AE : With the PAS reaction test, a marked decrease of glycogen granules

was observed in the epithelium, compared with the findings in the 8th fetal month. With the M method, the reaction was negative at any pH, and with AB and CI methods, the reactions were the same as in the 8th embryonic month. With the M method, reactions were positive, and sometimes strongly positive.

WJ : With the PAS reaction and M method, the reaction was the same as in the 8th fetal month. But with the CI method the reaction was from moderately to strongly positive. Also with the M method, the reaction was rather strong.

Vasa umbil : With the PAS reaction test, the reaction was the same as in the 8th fetal month. But with the M method, the reaction at the Vasa umbil was stronger than in the 8th fetal month. The reaction in the A. umbil was stronger than in the V. umbil. With the CI method, the reaction was the strongest at A. umbil and moderately positive at the V. umbil. Also with the M method, the reaction was rather strong at A. umbil, while the reaction at V. umbil was the same as in the 8th fetal month.

i) Tenth fetal month (Photo 10)

AE : With the PAS reaction, M, AB, and CI method, the reactions were the same as in the 9th fetal month. With the M method, the reaction was weaker than that observed when the fetuses were in the 9th month of gestation.

WJ : With the PAS reaction test, a slight decrease in glycogen granules was observed. With the M method, the reaction was negative at pH 2.5, and positive at pH 4.1 and 7.0. With the AB method and CI methods, the reactions were the same as in the 9th fetal month.

Vasa umbil : With the PAS reaction test, a slight decrease in glycogen granules at the Vasa umbil was observed. With the M method, the reaction was negative at Vasa umbil at pH 2.5. At pH 4.1 and 7.0, the reaction was moderately positive at A. umbil and positive at V. umbil. With the AB method, the reaction was rather weak. However, with the CI method, the reaction at A. umbil was rather weak, while at V. umbil the reaction was about the same as in the 9th fetal month. All these findings are summarized in Table II.

DISCUSSION

Collagen Fiber (CF)

The CF was found immediately beneath the epithelium at the 2nd fetal month. According to Kondo (4) and Tomita (7), endothelial tissue in the blood vessels is surrounded by CF and most of CF is present in the perivascular area. Tomita (7) found that the CF showed a tendency to surround the blood vessels. With development of the fetuses, the CFs were decreased and muscle fibers were increased. The distinction of the muscle fibers from the CF was very clear when VG, AM and MT stains were used.

TABLE II. *Histochemical Changes of the Umbilical Cord of the Human Fetus*

Fetal age(month)	Staining	MC	PAS	Diastase digestion test	Metachromasia pH2.5 4.1 7.0	Alcian blue	Colloid iron
	Object	Glycoprotein	Glycogen				
	Localization	Mucous substance	Neutral mucopolysaccharide	Glycogen	Acid mucopolysaccharide		
2	(A)	+ - ++	+++	±	- ± + ± + ± +	-	++
	(V)	+ - ++	+++	±	- ± + ± + ± +	-	++
	(WJ)	+ - ++	+	±	- - -	-	+
	(B)	+ - ++	+ - ++	±	- - -	-	+ - ++
	(AM)	++	++ - +++	±	- - -	-	± - +
3	(A)	+ - ++	+++	±	- + +	± +	++
	(V)	+ - ++	+++	±	- + +	± +	++
	(WJ)	+ - ++	++ (PS)	±	- ± ±	± +	+
	(B)	± - +	+	±	- - -	-	+ - ++
	(AM)	++	+++	±	- - ± - ±	-	± - +
4	(A)	± - +	+++	±	- + +	+	+ - ++
	(V)	± - +	+++	±	- + +	+	+ - ++
	(WJ)	+ - ++ (PS)	+	±	- + +	++ +++	+ - +++
	(B)	±	±	±	- ± ±	+	+ - ++
	(AM)	± - ±	+	±	- ± + ± +	+ ++	± - +
5	(A)	± - +	++++	±	- + +	± +	+++
	(V)	± - +	++++	±	- + +	± ++	++ - +++
	(WJ)	± - +	+	±	- ± + ± +	± ±	+ - ++
	(B)	±	+	±	- ± ± ± ±	± +	++ - +++
	(AM)	+ - ++	++++	±	- ± ± ± ±	± ±	± - +
6	(A)	± - +	++++	±	± +++ +++	+	+++
	(V)	± - +	++++	±	± ± +++ +++	+	+++
	(WJ)	++	++	±	± + +++ +++	++ +++	+++
	(B)	++	+	±	- ± + ± +	± +	± +
	(AM)	+ - +++	++++	±	- ± + ± +	± +	± - +
7	(A)	± - ++	++++	±	± + +++ +++	+ ++	+++
	(V)	± - +	+++	±	- ± ±	± ±	++
	(WJ)	± - ++	± - ++	±	- ± ±	+ ++	+
	(B)	± - +	+	±	- - -	+	±
	(AM)	+ - ++	+++	±	- - -	-	±
8	(A)	± - +	++++	±	- + +	± +	+++
	(V)	± - +	+++	±	- + +	± ±	++
	(WJ)	± - +	+	±	- + +	+	+ - ++
	(B)	±	+	±	- - -	-	+++
	(AM)	± - +	++++	±	- ± + ± +	-	±
9	(A)	+ - ++	+++	±	± + +	++ +++	++++
	(V)	± - +	++ - +++	±	± + +	+ ++	++
	(WJ)	+ - ++	+ - ++	±	- ± + ± +	+ ++	++ - +++
	(B)	+	+	±	- - -	-	+
	(AM)	± - ++	+ - ++	±	- - -	-	+
10	(A)	± - +	++	±	- ++ ++	+ ++	+++
	(V)	± - +	++	±	- + +	± +	++
	(WJ)	+	+	±	- + +	± +	+++
	(B)	± - +	+	±	- - -	-	+
	(AM)	± - +	+ - ++	±	- - -	-	+

Abbreviations : (A) A. umbilicalis, (V) V. umbilocalis, (WJ) Wharton's Jelly
(B) Immediately beneath amnion epithelium, (AM) Amnion epithelium,
(PS) Perivascular, M. C. Mucicarmin, P. A. S. Periodic Acid Schiff.

Elastic Fiber (EF)

At the 2nd fetal month, no EF was observed. EF appeared when immediately beneath the endothelium of the vascular muscle fibers, especially the A. umbil (4). Though opinions differ as to the development of EF, it is generally agreed that EF gradually increases between the 4th and the 7th fetal months, and marked increase takes place only after the 8th fetal month.

Reticular Fiber (RF)

The RF was observed in the muscle layers of the vascular walls of the umbilical cord at the 2nd or 3rd fetal months and formed either spiny or irregular networks. The distribution was most evident in the internal layers of the Vasa umbil. However, RF was not detected in the WJ. These observations are in agreement with the data of Tomita (7).

Changes in Glycoprotein Fluid

At the 2nd fetal month, the reaction was positive with the M method in Vasa umbil, WJ and AE and this phenomenon continued to the 9th fetal month. At the 10th fetal month, the reaction decreased somewhat.

Glycogen and Neutral Mucopolysaccharide

At the 2nd fetal month, glycogen granules were observed using the PAS stain. Takami (6) examined glycogen by means of the Best Carmin method and concluded that glycogen was present in the gland area. Hama (11) also stated "Glycogen was seen from the umbilical cord formation period to its maturity and child delivery period. The highest glycogen level was seen at the 3rd fetal month, and in the latter half of pregnancy the glycogen level decreased and mainly existed in the vascular walls of the Vasa umbil. A significant amount was seen in the protoplasma and also in some other areas. The appearance was mostly granular, but partly in aberrant or a semilunal shape."

Changes of Acid Mucopolysaccharide

Nagano (16) examined the acid mucopolysaccharides by means of the thionine stain confirmation method in order to check M. He used thionine solution pH 2.5 as a buffer at the time of separation of hyaluronic acid and Chondroitin sulphate, and also used, for histochemical confirmation, (1) PAS method, (2) CI method, and (3) M method. According to his study, the mucopolysaccharides in the umbilical cord exist mainly in the form of hyaluronic acid, which is seen in WJ and in the areas between muscle fiber bundles at the tunica media of the blood vessels and also at the tunica externa. With growth of the fetuses, this amount increases. At the end of the fetal development, chondroitin sulphate can be observed, though the amount is scanty.

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LEGENDS

Photo 1. Second fetal month. The wall of the umbilical artery is thin and cell components in Wharton's jelly are distributed loosely and reticularly.
Hematoxylin-Eosin stain × 400

Photo 2. Second fetal month. Collagenous fibers in the umbilical artery and Wharton's jelly (A), and in the umbilical vein and Wharton's jelly (B).
Van-Gieson stain × 200

Photo 3. Third fetal month. The wall of the umbilical artery has thickened and muscle and collagenous fibers have increased.
Hematoxylin-Eosin (A), Van-Gieson stain (B) ×200

Photo 4. Fourth fetal month. Reticular fibers in the umbilical artery (A), and the umbilical vein (B).
Silver impregnation stain × 100

Photo 5. Fifth fetal month. Elastic fibers were noted in the inner layer of the umbilical artery (A), and umbilical vein (B).
Resorcin-fuchsin stain × 400

Photo 6. Sixth fetal month. Elastic fibers are numerous in the umbilical artery (A), and umbilical vein (B).
Resorcin-fuchsin stain × 400

Photo 7. Seventh fetal month. The difference of the thickening in the umbilical artery (A), and umbilical vein (B).
Hematoxylin-Eosin stain ×200

Photo 8. Eighth fetal month. Elastic fibers increase in the umbilical artery (A), and umbilical vein (B).

Resorcin-fuchsin stain × 200

Photo 9. Eighth fetal month. Collagenous fibers in Wharton's jelly. Azan-Mallory stain (A), Van-Gieson (B) × 200

Photo 10. Tenth fetal month. Elastic fibers clearly increase in the umbilical artery (A), and umbilical vein (B).
Resorcin-fuchsin stain × 100

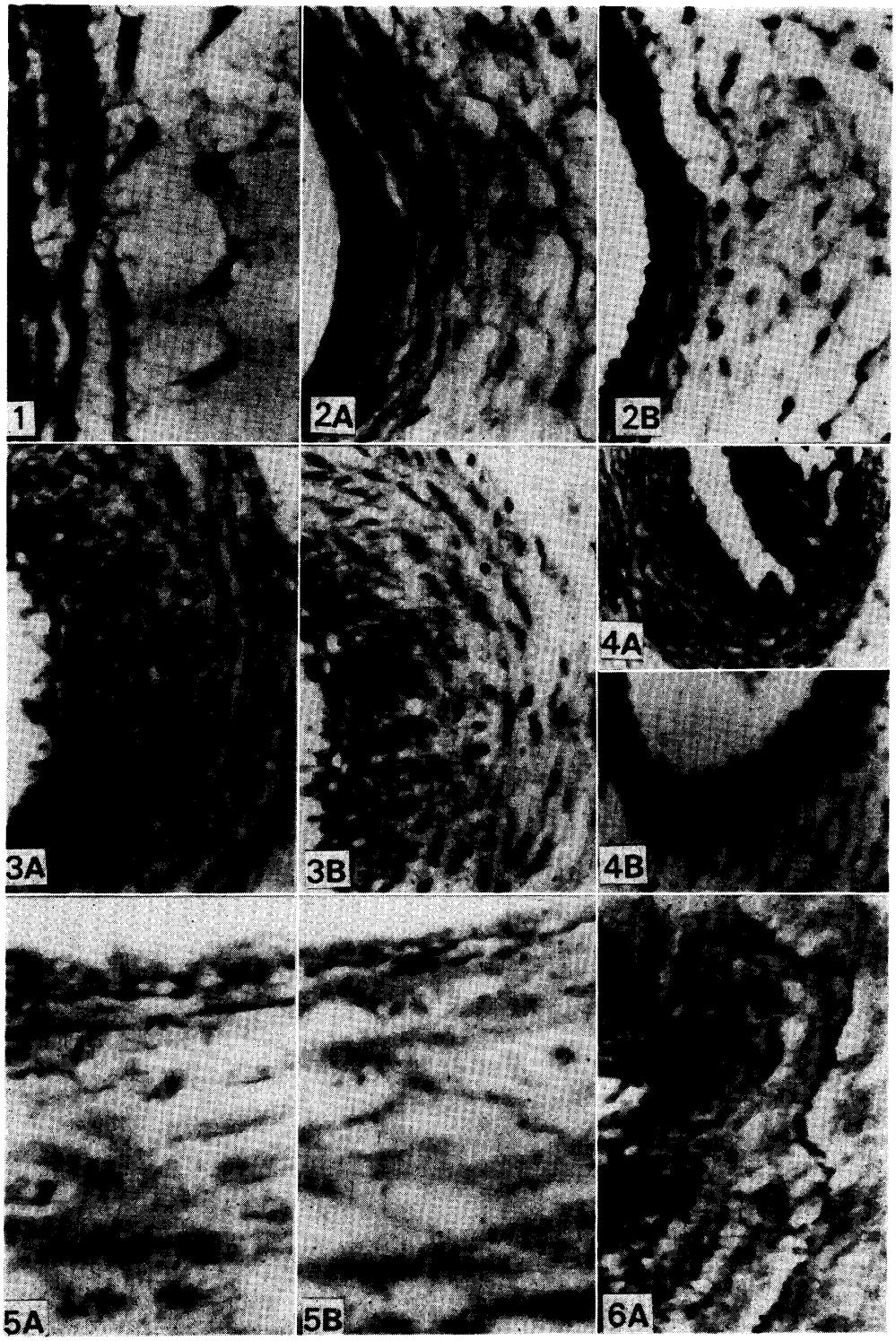
Photo 11. Second fetal month. Glycogen granules are noted in the wall of the umbilical artery (A). The same section is digested by silva to identify glycogen (B).
PAS reaction × 400

Photo 12. Third fetal month. Remarkably positive response in the umbilical artery, Wharton's jelly and amnion epithilium (A), and in the umbilical vein, Wharton's jelly and amnion epithelium (B).
Colloid iron reaction × 400

Photo 13. Fourth fetal month. The tissue of Wharton's jelly shows a positive response. Mucicarmin stain (A), Alcian blue stain (B). × 400

Photo 14. Fifth fetal month. The strong positive reaction in the umbilical artery (A), and umbilical vein (B).
Periodic Acid Schiff reaction × 400

Photo 15. Sixth fetal month. Hyaluronic acid in Wharton's jelly shows a negative response at pH 2.5 (A) and positive one at pH 4.1 (B).
Metachromasia reaction × 400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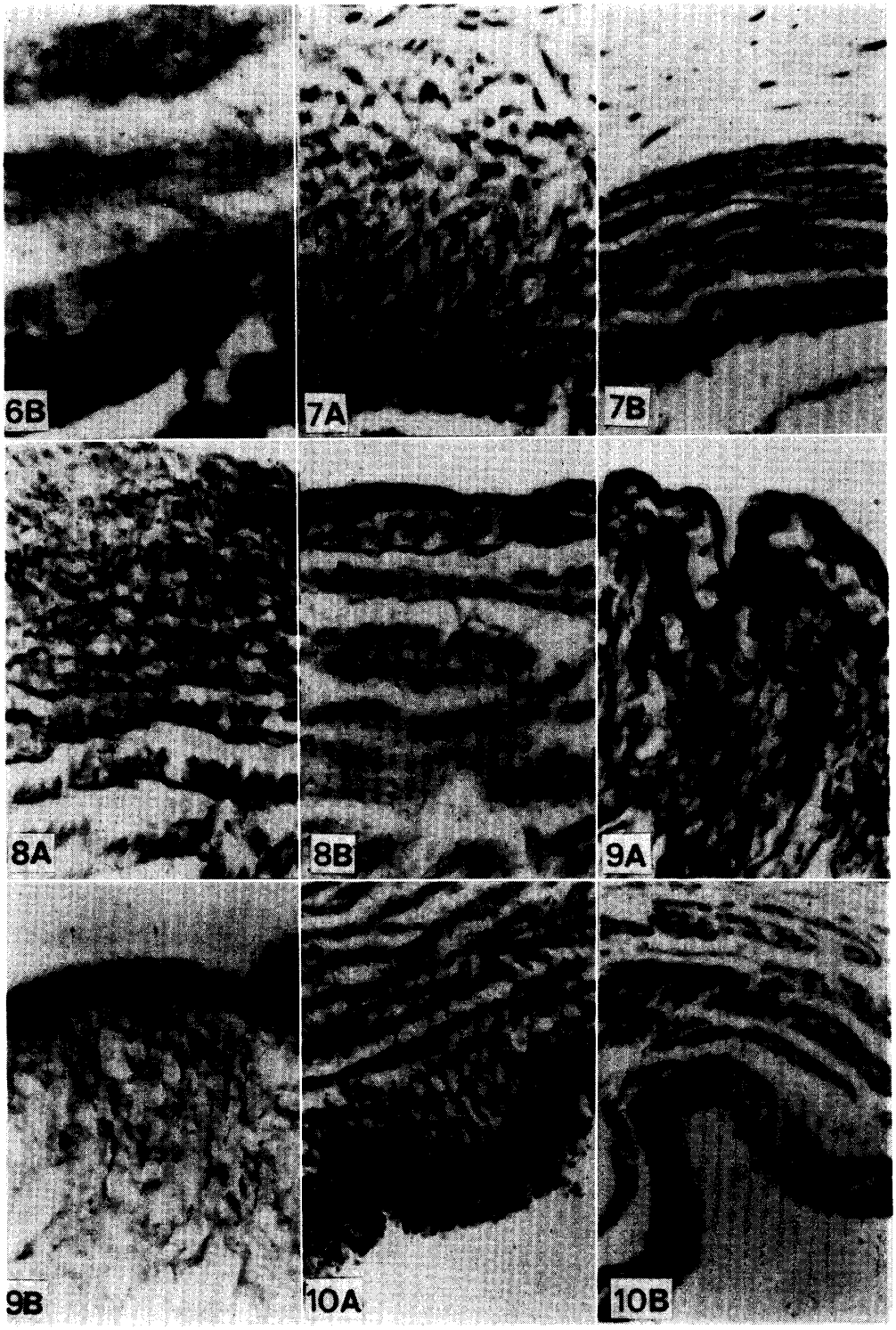
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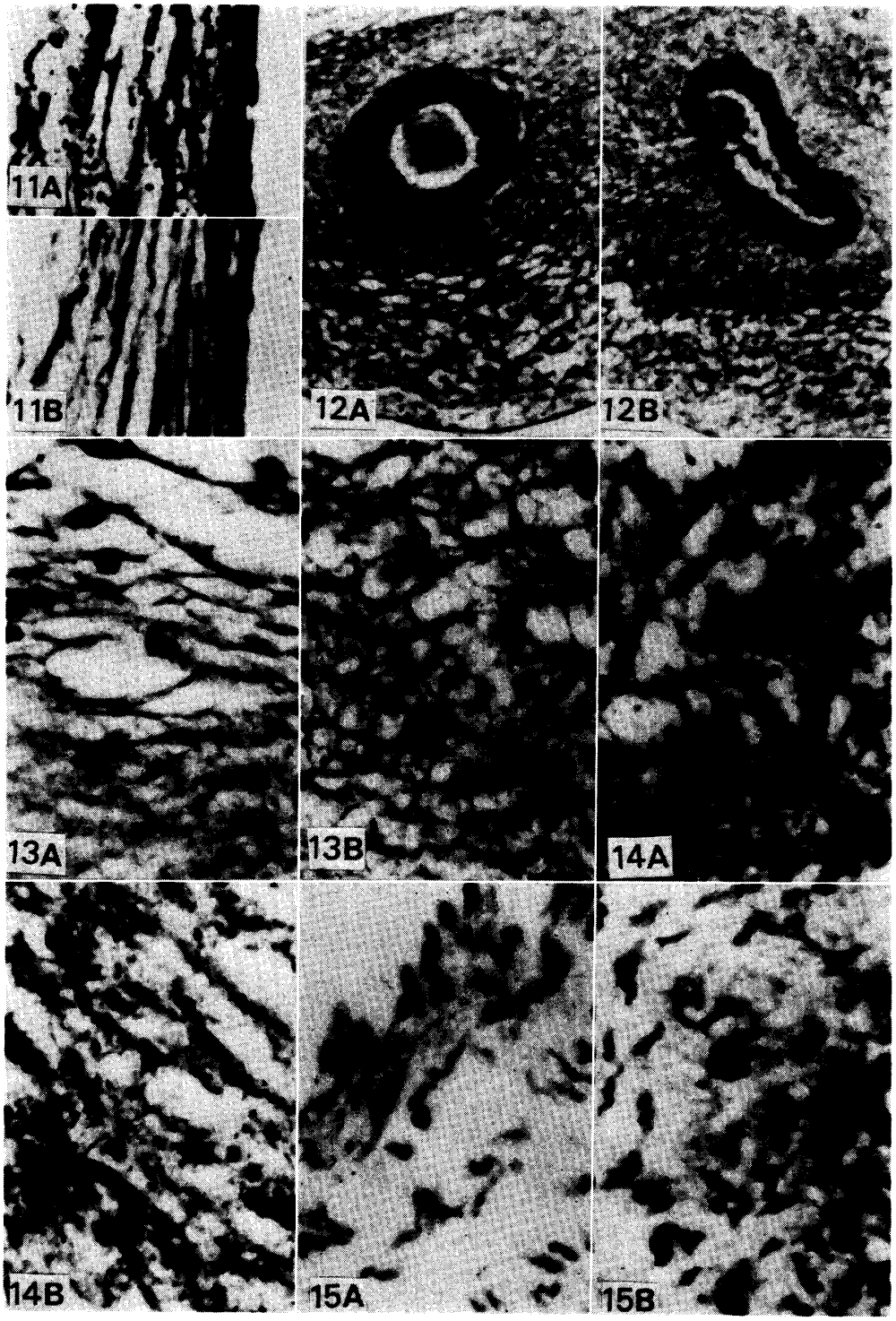
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