THE HISTOLOGICAL DISTRIBUTION OF THE BLOOD GROUP SUBSTANCES IN MAN AS DISCLOSED BY IMMUNOFLUORESCENCE

III. The A, B, and H Antigens in Embryos and Fetuses from 18 mm in Length*, ‡

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Plates 59 to 62

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The group antigens A, B, and H in the tissues and secretions of adults and larger fetuses have been described in two previous communications (1, 2).

Relatively little is known concerning the blood group antigens in early stages of intrauterine life. The information available in the literature concerns the A,B antigens of the erythrocytes, demonstrable in fetuses from the end of the 2nd month (3, 4), from 7 to 50 cm crown to heel (5), and during the period of 3 to 6 months (6), while the water-extractable antigens of the tissues and meconium were reported for fetuses from 5 to 9 months (7), 3.1 to 44 cm crown to heel (8), and in tissues of fetuses from the first half of the 3rd month of pregnancy onward (9). More recently, cells from various organs of fetuses above 3 months of age grown in tissue culture were shown to possess the A, B antigens by the mixed agglutination technique (10).

The present communication is concerned with the mapping out of the ABH antigens in the tissues and with the elucidation of their behavior during the earlier stages of intrauterine life, characterized by the early development of several organs from their primordia and by the maturation and assumption of function by many others. By describing the natural developmental history of this set of well defined genetical traits, which are now becoming translatable into chemical terms (11–13), it hopes to contribute to the understanding of gene action on cell and tissue level and to the elucidation of its role in embryonic development. In a more particular sense, implicit in the nature of the antigens under study and their homologous antibodies, it underlines the problem of their especial biological significance for fetal-maternal relationships.

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Materials and Methods

Embryos and Fetuses.—Embryos and fetuses from 18 to 125 mm. CR (crown to rump length) encompassing the span from 5 to 14½ weeks OA (ovulation age)[1] were available at the Boston Lying-in Hospital. The lower limits of the series represent the smallest (18 mm.) and the youngest (5 weeks) specimens available. The upper limit was chosen arbitrarily, as representing the stage at which the adult status with respect to the ABH antigens is virtually reached.

About two-thirds of the specimens were products of therapeutic hysterotomies or of laparotomies for tubal pregnancies; they were obtained, bled out, and frozen within an hour of surgery. Acceptable specimens from spontaneous abortions were processed with minimum delay.

Freezing, Cutting, and Staining of Sections.—Rapid freezing was effected in a routine manner, as described before (1). Whole embryos or blocks prepared from larger specimens were cut *seriatim* at 4 micra in a cryostat. Topographical orientation was achieved by means of sections from selected levels, quickly stained with Giemsa stain, and kept for permanent collection.

Sections were fixed in acetone as a routine, but an alternative quick dip in formalin (10 per cent), followed by a wash in buffered saline, was often found necessary when dealing with tissues holding mucus. Staining was effected by using a single or a double layer method.

Antisera and Conjugates.—The principal reagents, anti-A, anti-B, and anti-H conjugates were as described in detail previously (1, 2).

Several additional sera were used in conjuction with the principal reagents. *A rabbit anti-A serum,* prepared by the method of Glynn *et al.* (14) had a titer of 2000; employed with a goat anti-rabbit conjugate as a staining layer, it gave a most powerful specific fluorescence. A few *human hyperimmune anti-A and anti-B sera* with a minimal titer of 1000 became available during this study through the courtesy of the Blood Grouping Laboratory of Boston and were used on random sections from all group A, B specimens, with a horse antihuman globulin conjugate as a staining layer. These additional experiments were included in order to confirm further the results obtained with the principal reagents; no deviation from findings obtained with the latter was ever encountered.

The *goat anti-rabbit globulin conjugate* was prepared using a serum (obtained through the kindness of Dr. S. Leskowitz of the Massachusetts General Hospital) by coupling it with fluorescein isothiocyanate. The *horse antihuman globulin conjugate* coupled with fluorescein isothiocyanate was obtained commercially (Sylvana Chemical Company, Orange, New Jersey). It elicited no significant background staining in embryonic and fetal material, no doubt on account of the low concentration of globulins prevailing therein.

All sera and conjugates were absorbed with human or animal liver powder, repeatedly if necessary.

Control Sera and Conjugates.—All the anti-ABH reagents were used in conjunction with their control counterparts prepared by absorption with homologous erythrocytes or secretor saliva (1).

Grouping of Embryos and Fetuses.—Human hyperimmune anti-A,B and Bombay sera were employed for agglutination of embryonal or fetal erythrocytes. Satisfactory results were consistently obtained, provided that the cells were washed thoroughly and that typing was performed on the same day.

RESULTS

The ABH antigens disclose a remarkably rich pattern, demonstrable in the earliest group of specimens, 18 to 30 mm, 5 to 7½ weeks OA (Table I). The

[1] Ovulation age is used henceforth as more truly representing the actual age of the conceptus. It is calculated as taking place 2 weeks after the 1st day of the last menstrual period.

group is characterized by the widespread distribution of the *antigens outlining the cell walls* of the endothelium and of sundry epithelia, while the *water-soluble* antigens appear at the stage of 35 to 40 mm, 8 weeks OA.

The *endothelial cell wall antigens* throughout the cardiovascular system are permanent and persist into postnatal life. The *epithelial cell wall antigens* of the earliest group of specimens are found in the epithelia of the integument, the pharynx, the alimentary, respiratory and lower urinary tracts, and of the mesonephric and Müllerian ducts. They present a pattern of maximal antigen distribution since continuation of these antigens into adult life is seen only in the epithelia forming stratified squamous, transitional, or simple confining membranes; *i.e.*, those of the skin, the pharynx, the mouth and the esophagus, and of the lower urinary tract from the collecting tubules to the urethra. On the contrary, the cell wall antigens of the epithelia of the gastrointestinal tract, including the pancreas, of the thyroid and pituitary (anterior lobe), of the respiratory and upper female genital tracts undergo a gradual recession upon further development of the fetus. Chronologically, the waning of the antigens can be said to coincide generally with the morphologic differentiation and functional maturation of the organ concerned, as set out in a systematic presentation below.

The *water-soluble antigens* appear relatively late, at the 35 to 40 mm CR stage, borne in the earliest secretions of the developing salivary glands and of the gastrointestinal tract. The antigens of the pancreatic secretions are the last to appear, becoming demonstrable at 100 to 125 mm CR length (13 to 15 weeks OA). Two cases of *aberrant secretion* (15) in salivary glands (submaxillary and sublingual) were encountered (Table I), without the corresponding phenomenon being observed in the secretions of the gastrointestinal tract.

Both the H and the A,B antigens conform to an identical spectrum of patterns determined by the developmental stage of the individual. The relations of the H to the A,B antigens have been investigated as described in detail in the previous study (2), by comparing the intensity of fluorescence obtained in non-O group tissues with the Bombay and the anti-A,B conjugates, respectively. The consistent parallel of the H and A,B antigens demonstrated therein is confirmed for the present series; the epithelia of the anterior lobe of the pituitary gland and of the lung present special quantitative features.

It is convenient to present the detailed results separately for (*A*) the cell wall antigens, as found in the youngest group of embryos, 18 to 30 mm, and thence tracing their subsequent behavior, and for (*B*) the water-soluble antigens, starting at 35 to 40 mm CR, the stage of their first appearance, and similarly following them throughout the series.

A. Cell Wall Antigens

The Endothelial Cell Wall Antigens.—These antigens fully outline the cardiovascular system by ready staining of the endothelium in the heart and in

TABLE I

Embryos and Fetuses Analyzed for the H and A, B Antigens

Length, *mm* CR	18	19	20	23	24	27	30	35	40	42	45	45	45	45	47	50	50	50	52	55	58	60	60	65	70	70	75	80	80	85	85	85	88	90	100	100	110	115	125
Wks., OA	6½	7	5	7	6	6½	7½	7½	8			8½	8½		9	9		11	10	9½		10	9½			9½	11			11½	11		11½	15	15	13	14	
Age‡				6	6	6½	7	7½	8	8	8½	8½	8½	8½	8½	9	9	9	9½	9½	9½	10	10	10	11	11	11	11½	11½	12	12	12	12	12	13	13	13½	14	14½
Blood group	A	O	A	A	A	A	O	B	B	B	O	O	A	O	B	O	O	O	O	A	O	O	A	O	O	A	A	O	B	O	A	O	A	O	O	O	A	B
Origin of specimen	H	Tu	Tu	H	H	H	Sp	H	Tu	H	H	H	H	Sp	H	Sp	H	H	H	H	H	H	H	Sp	Sp	H	Sp	Sp	Sp	H	H	H	Sp	H	H	H	H	H
Adrenal	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−		−		−	−			−			−								−	−	
Bladder	+	+		+	+	+	+	+	+	+									+		+		+				+			+	+	+				+		+	
Brain	−	−	−	−	−	−	−	−	−	−						−			−		−															−		−	
Bronchi	+	+	+	+	+	+	+	+	+							+											±		±		−		−				−		
Colon	+	+	+	+	+	+	+	+	+	+		− *							± *	± *	± *		− *			− *							− *	− *	− *	− *		− *	− *
Esophagus	+	+	+	+	+	+	+	+	+	+ **	+	+	+			+ **			+		+		+				+ **	+ **		+ **				+	+		+	+ **
Gonad	−	−	−	−	−	−	−	−	−	−	−	−	−			−			−		−											−	−						
Liver	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−			−			−				−					−	−		−
Mesonephros	+	+	+	+	+	+	+	+	+	±		+				+			+		+																	
Metanephros	+	+	+	+	+	+	+	+	+ **	+	+	+	+	+	+	+	+	+	+	+	+	+	+ **	+	+					+ **	+ **							+

Mouth, tongue	+	+	±	+		+	+			+		+	+	+	+	+	+	+		+		+				+		+								+
Müllerian system							+	+	+	±		+						+																		+
Muscle	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−				−	−	−						−	−
Pancreas	±	−	−	−	−	±	−	±	±	±	−	−		−				−	±		±		±		−		±		±	±			±		±	−	−
										*																				*			*		*	*	*
Parotid	+						+		−									−	−	±		−	−	−				−	−	−		−		−	−	
									*									*	*	*		*	*	*				*	*	*		*		*	*	
Pituitary	+	+	+	+		+		+			+	+						+		±		+					−		−				−	−	−
Rectum	+	+	+	+	+	+	+	+							+				±		−															
																			*		*																
Skin	+	+		+	+	+	+	+	+		+		+	+			+	+	+	+	+	+	+		+	+		+	+	+			+		+	+
Small gut	+	+	+	+	+	+	+	±	±	−	±	±	±	−		−	−	−		±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
								*	*	*	*	*	*	*		*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Stomach	+	+		+	+	+	+	±	±	±		−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−		−	−		−	−	−	−
								*	*	*		*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*		*	*		*	*	*	*
Salivary glands	+	+		+			+		±	Ab		NS	NS	−			−	−	−			−		−		Ab	−	−	−	−			−				
									*					*			*	*	*			*		*			*	*	*	*			*				
Thymus	+	+	+	+		+	+		+																+									+		
Thyroid	+					+	+		+	+			+					+						±			±		−				−	−	−
Trachea	+	+	+	+	+	+	+		+	+	+		+			+		+		+		+				+	+						+		+
																⁂										⁂	*						*			*

CR, crown to rump length. OA, ovulation age. H, hysterotomy or hysterectomy; Tu, tubal pregnancy; Sp, spontaneous abortion. Muscle: skeletal, cardiac, and smooth. Salivary glands: submaxillary and/or sublingual.

+ epithelial cell wall antigen present; ±, epithelial cell wall antigen waning; −, epithelial cell wall antigen absent.

* secretion-borne antigen; ⁂ coating of lumen by soluble antigen.

Ab, aberrant secretor; *i.e.*, expected A, B antigen absent. NS, secretion-borne antigen absent.

‡ Streeter, G. L., Weight, sitting height, head-size and menstrual age of the human embryo, *Carnegie Institution of Washington, Pub. No. 274, Contrib. Embryol.*, 1920, **11**, 145.

vessels of all calibers (Figs. 1 to 3). The rich capillary network in the adrenal (Fig. 24) attests to that gland's already established endocrine function in contrast to the contemporary, morphologically primitive, and relatively avascular anterior lobe of the pituitary (Figs. 7 and 8) or to the thyroid (Fig. 11). The liver sinusoids, (Fig. 3) like those of the adult organ, tend to stain weakly, presumably due to rapid postmortem degradation of the antigens.

The Epithelial Cell Wall Antigens.—These antigens are at their maximal distribution in the earliest available specimens, 18 to 30 mm, and are best described as seen in that morphologically virtually homogeneous group. They outline the vast majority of epithelia, the chief exceptions being the already morphologically mature liver (Figs. 3 and 4) and adrenal (Fig. 24). The cells of the central (Fig. 10) and peripheral nervous system are also free of antigens. The *hemopoietic elements,* best seen in the liver and thought to be overwhelmingly erythrocytic (16), seem to possess less antigen than the epithelial cells and are best demonstrated by staining by a double layer method (Fig. 4). *The epidermis of the integument* (Fig. 5) shows clear specific staining which continues into adult life. The cells of the multiplying layers remain for some time morphologically undifferentiated, and the absence of antigens from the basal layer, as seen in all stratified squamous epithelia, does not become apparent until the 40 mm stage (Fig. 6). *The buccal epithelium* resembles that of the integument. The epithelium of the *nasal cavities* is also largely derived from that of the integument and retains the antigens until the time of appearance of mucoid goblet cells, between 50 to 60 mm CR. The epithelium of the *internal ear,* derived from the ectoderm by a process of surface invagination, demonstrates the cell wall antigens often in an irregular fashion; they are not to be found after the 50 mm CR stage.

The *pharynx* and the remains of the pouches are lined by a thin, generally two cell deep epithelium rich in the cell-outlining antigens which are retained in sectors destined to become permanently stratified or "transitional." The *Eustachian tube* and the *tympanic cavity* derived from the first pouch are lined by a thin epithelium identical with that of the parent viscus. The earliest primordium of pharyngeal origin, that of the *thyroid,* is at the earliest stage of this series a solid epithelial crescent without acinar formation and without the characteristic rich capillary network of an endocrine organ. The parenchyma readily demonstrates the epithelial cell wall antigens (Fig. 11) which wane at the stage of 70 to 80 mm CR (12th week OA); after this time, the gland shows definite acini and begins to resemble the final adult histological picture (Fig. 12). *The thymus* (Fig. 11), an exclusively epithelial organ at the present early stage, is rich in the antigens which persist permanently in the Hassall's corpuscles, in conformity with the rest of the stratified squamous epithelia elsewhere. *The parathyroid glands,* often difficult to identify with certainty, seem to possess the antigens; these, however, are not destined to become permanent, as in glands from several adults no antigens could be demonstrated.

The pituitary gland: The anterior lobe clearly betrays its origin from the roof of the stomatodeum (Rathke's pocket); the antigens outline the cells of the solid cords of the developing organ (Fig. 7) until they commence to recede about the 60 mm CR stage, 10th week OA. From 12 weeks onwards the parenchymal cells are completely free of the antigens (Fig. 10). Throughout the positive interval, the A,B antigens are consistently markedly weaker than the H antigen (Fig. 8). The posterior lobe, derived from the brain, is free of the antigens (Fig. 9).

The esophagus (Fig. 11) is lined by an epithelium which in some of the earliest specimens (Fig. 13) shows deep stratification at the time of appearance of the permanent lumen. The antigens are to persist in the stratified squamous epithelium of the subsequent stages. The epithelia of the *gastrointestinal tract*, primitive and resembling that of the esophagus, are rich in antigens (Figs. 16 and 22); the latter, however, are to wane and disappear upon further development of the mucosae of the various segments, each proceeding at its own pace. Thus, the mucosae of the body of the stomach (Figs. 16 to 19), of the pylorus, and of the small intestine rapidly lose their cell wall antigen at 35 to 40 mm CR, while the colon and the rectum follow at 45 to 60 mm CR. In each case the antigen loss coincides with the assumption of the specialized function of mucus secretion.

The epithelia of the parenchymatous organs derived from the small bowel demonstrate little or no antigen. Thus, *the pancreas* manifests varying, rather small amounts, to be lost upon further development of the gland (22, 23) while *the liver* is free of the antigens, even in the earliest available specimens (Fig. 3).

The trachea and main bronchi are lined by a primitive epithelium resembling the digestive tract and are equally well equipped with the antigens (Fig. 13). Those of the trachea persist throughout fetal life, albeit with great attenuation towards the end of that period, and are characterized by scarcity and inconstancy in postnatal life. A unique peculiarity of the developing bronchial tree is the consistent gross preponderance of the A,B antigens over the H antigen. At the same time, a reverse relationship holds for the antigens of the pulmonary capillary endothelium: the latter stains poorly with the anti-A,B reagents and brilliantly with the Bombay conjugate (Figs. 14 and 15). The bronchial antigens are not found after the 85 mm CR stage (12 weeks OA).

The bladder and the urogenital sinus are lined by an epithelium which at the early stages is identical with that of the hindgut from which they were earlier partitioned off by the cloacal septum. The cell wall antigens are accordingly very well represented here. Their permanence is in agreement with the general endowment with the antigens, of the stratified epithelia, in this case the transitional epithelium of the bladder (Fig. 26) and the stratified squamous epithelium of the vestibule and the lower vagina.

The mesonephros shows brilliant outlining of the collecting tubules and of the main ducts. The antigens could be found as long as the organ remained identifiable; *i.e.*, up to 58 mm CR (10th week OA). Vestiges of the ductal system in the adult female (*e.g.* tubules of Kobelt) showed persistence of the antigens in occasional cells. Similarly, the epididymis of newborns showed occasional stainable epithelial cells. The epithelium of *the metanephric duct* and its ramifications stain uniformly brilliantly in the earliest embryos (Fig. 24). The antigens remain permanent throughout life. Noteworthy are the persistence and consistently large amounts of cell wall antigens in the epithelium of the collecting tubules (Fig. 25), the only simple epithelium in late fetal and extrauterine life thus endowed.

In *the Müllerian ducts* the epithelium consistently evinces the presence of the antigens. No correlation was obtained between the amounts of the Müllerian antigens and the sex of the individual. The antigens are found outlining the epithelium of the largest specimen in this series and are seen in neonatal, but not in adult, endometria. The *gonads*, including the germ cells, are devoid of antigens.

The *adrenal cortical* epithelium is consistently negative (Fig. 24), but the possibility that the antigens were present during earlier stages cannot be excluded. Cells of the chromaffin system show no antigens.

B. Water-Soluble Antigens

These antigens, borne in the secretions of *the salivary glands* (Fig. 20) and of the mucosa of *the gastrointestinal tract* (Figs. 17 to 19), first appear at 35 to 40 mm CR (8 weeks OA). They are demonstrable in *the submaxillary, sublingual, and parotid glands* as soon as the lumens in the primordia become discernible.[2] At the same stage, *the pylorus* begins to secrete large amounts of antigens, with smaller amounts found in *the body of the stomach* and in the goblet cells of *the small bowel*; *the duodenum* commences the secretion of the antigens rather late, at 65 mm CR. *The large bowel* seems to assume antigenic secretion at 45 mm CR, with *the rectosigmoid* entering last, at 58 mm CR.

The goblet cells and small mucous glands of *the respiratory tract* have not been traced in detail. The presence of goblet cells and of submucosal glands secreting the antigens has been easily observed in specimens above 80 mm CR.

The pancreas (Fig. 22) remains free of the secreted antigens until the 100

[2] The nature of the salivary and gastrointestinal secretions has not been investigated beyond elucidating positive staining with PAS. It is noteworthy that both the submaxillary and the sublingual glands, destined to secrete large proportions of mucus, as well as the parotid, destined to serous secretion, are indistinguishable both morphologically and with respect to the antigen content of their secretions. But, whereas the antigens persist in the mucous elements of the mixed glands throughout life, they disappear from the secretion of the parotid shortly after birth.

to 125 mm CR stage. Commencing secretion is illustrated in one of the largest specimens of this series (Fig. 23).

Antigen presumably excreted in *the urine* and accreted on the renal calyceal-pelvic epithelium has been observed in several cases, the youngest 40 mm CR. This recalls the appearance seen in postnatal material in secretor individuals (1, 2).

The water-soluble antigens continue to be secreted during subsequent fetal development and during extrauterine life by all the organs described. Two fetuses, 45 mm CR each (Table I) showed lack of the secretion-bound antigens in the salivary glands (Fig. 21) with no significant diminution of those of the gastrointestinal tract. These individuals are presumed to be *non-secretors*, especially since each had one non-secretor parent. The problem of non-secretion in intrauterine life remains to be studied further.

DISCUSSION

Epithelial Cell Wall Antigens.—The most noteworthy finding in this investigation is the widespread distribution of the epithelial cell wall antigens early in intrauterine life and their subsequent orderly recession. The survival of the antigens beyond the 12th week OA, 70 to 80 mm CR, is restricted to stratified or simple confining epithelia (integument, esophagus, lower urinary tract, vagina), while epithelia proceeding to further morphological and functional complexity (gastrointestinal tract, lung, thyroid, pituitary) undergo antigenic loss in a manner and at a stage peculiar to each tissue. Thus, *the recession of the cell wall antigens* takes place in close coincidence with recognizable steps in histo-differentiation and commencement of mucous secretion in the respective segments of the gastrointestinal tract; it occupies a brief span, the 12th week OA, during which the thyroid begins to demonstrate definite acini and is reported to commence specific function by accumulation of iodine (17). The behavior of the antigens in the cells of the anterior lobe of the pituitary presents a complex problem not least due to the gradualness of the changes and the multiplicity of the cell types involved (18) and has not been studied in sufficient detail. Little is known about pituitary function during early fetal life; the demonstration of small amounts of extractable growth hormone in a gland from a 12 weeks OA (85 mm CR) fetus (19), however, serves to indicate the presence of functional activity in a gland in which antigen loss is complete.

The striking freedom from antigens on the part of the liver, the adrenal gland (cortex), and the nervous system is compatible with their morphologically advanced state when first seen at the 18 to 35 mm (5 to 8 weeks OA) stage and is explicable by assuming that these cell systems have already undergone antigenic loss. In the adrenal, the morphological advancement and extraordinary vascularity are parallelled by highly specific enzymatic competence, demonstrated by biochemical techniques (20) in fetuses from 40 mm

CR on; in the liver, cholesterol synthesis has been demonstrated at 5 weeks OA (21).

The virtually universal prevalence of the epithelial cell wall antigens early in development leads to the question as to their function in embryonal life. Since they are situated at the cells' surface, the possibility must be entertained that they are the serological representatives[3] of a *sui generis* blood group substance contributing to the molecular profile of the cell wall and play a part in such processes as specific cell-to-cell contact (22, 23) of especial significance in embryogenesis (24). The waning of the antigens signifies either a change in the blood group macromolecule or its disappearance and coincides with the switching by the cells to more specific metabolic activities, responsible for recognizable steps in morphological advancement and/or commencement of functional activity.

While the mucopolysaccharide nature of the blood group substances has been demonstrated for the water-soluble entities derived from saliva, gastric juice, and mucinous ovarian cyst (11, 12), nothing is known concerning the water-insoluble substances of the epithelial and endothelial cell walls, save for the serological individuality of the reactive groupings. That there are differences between the underlying macromolecules of the various kinds of substances is suggested by the finding that the blood group substance isolated from erythrocytes is chemically significantly different from that of the secreted mucoids (25).

The Significance of the Embryonal Antigens in Fetal-Maternal Relationships. —A large body of statistical evidence exists pointing to the selective intrauterine loss of progeny caused by fetal-maternal incompatibility within the ABO system (reviewed in references 26, 27), that is, to loss of group A,B conceptuses, whose antigens (inherited from their fathers) are homologous with existing maternal anti-A,B isoagglutinins. The early appearance, large amounts, and the strategic disposition of the cell wall antigens herein described provide an insight into the process leading to the loss of incompatible embryos by revealing these antigens as potential targets for maternal antibodies early in pregnancy.[4] The importance of the water-soluble antigens is presumed to lie in their ability to penetrate into the fetal plasma (28) and thus to serve as a buffer against maternal anti-A,B isoagglutinins. It is significant in this connection that the majority of abortions, perforce including those due to ABO incompatibility, take place about the 8th week OA, following the death of

[3] That the oligosaccharidic side chains imparting the ABH specificities themselves are unlikely to be essential in any universal reaction or process is implicit in the existence of individuals devoid of one (group A or B), two (group O), and all three (Bombay) of the markers.

[4] The notion that the ABH antigens so well established at 18 mm appear even earlier has been borne out by finding the A antigen in a 5 mm specimen (27 days OA) studied at the time of writing of the present communication and to be published later.

the embryo (29), at a stage well before the protective buffering action of the water-soluble substances could come into play.

SUMMARY

The ABH antigens have been mapped out in the tissues of embryos and fetuses, 18 to 125 mm crown to rump length, 6 to 14½ weeks ovulation age.

Both the H and A,B antigens have the same distribution, and their spatial and temporal parallel obtains in intrauterine as well as extrauterine phases of life.

The cell wall antigens are present in their maximal distribution in the youngest specimens available. They outline the endothelium of the cardiovascular system, and the cells of most of the epithelia throughout the body. The exceptions are the liver, the adrenal, and the nervous system, presumed to have lost the epithelial antigens at stages antedating the youngest specimens here described.

The antigens of the stratified epithelia (and of the simple epithelia of the renal collecting tubules), together with the endothelial antigens, are permanent and persist into and throughout adult life. All other cell wall antigens disappear at a time characteristic for each organ. The antigenic recession coincides with recognizable steps of morphological advancement and often with assumption of function by the organ concerned; it is completed at about the end of the first trimester of pregnancy.

The secretion-borne antigens first appear at the 35 to 40 mm stage (8 weeks ovulation age) in the salivary glands and in the stomach, to be followed in a constant sequence by the rest of the gastrointestinal tract, respiratory system, and pancreas. The secretion of these antigens persists throughout life.

The early presence and wide distribution of the cell wall A,B antigens render them likely potential targets for maternal anti-A,B antibodies in heterologous pregnancies; the advent of the water-soluble substances at 8 weeks ovulation age may be providing a buffer shielding the fetal cell wall antigens by mopping up the maternal isoagglutinins.

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EXPLANATION OF PLATES

PLATE 59

Endothelium, Erythrocytes

FIG. 1. Heart interatrial septum, 24 mm embryo, group A; anti-A conjugate. Well staining endothelium, desquamated in places. × 170.

FIG. 2. Ventricular outflow tract and arterioventricular valve, 20 mm embryo, group A; Bombay conjugate. Identical picture obtained with anti-A reagents. Continuous layer of lining endothelium outlined by antigen. × 170.

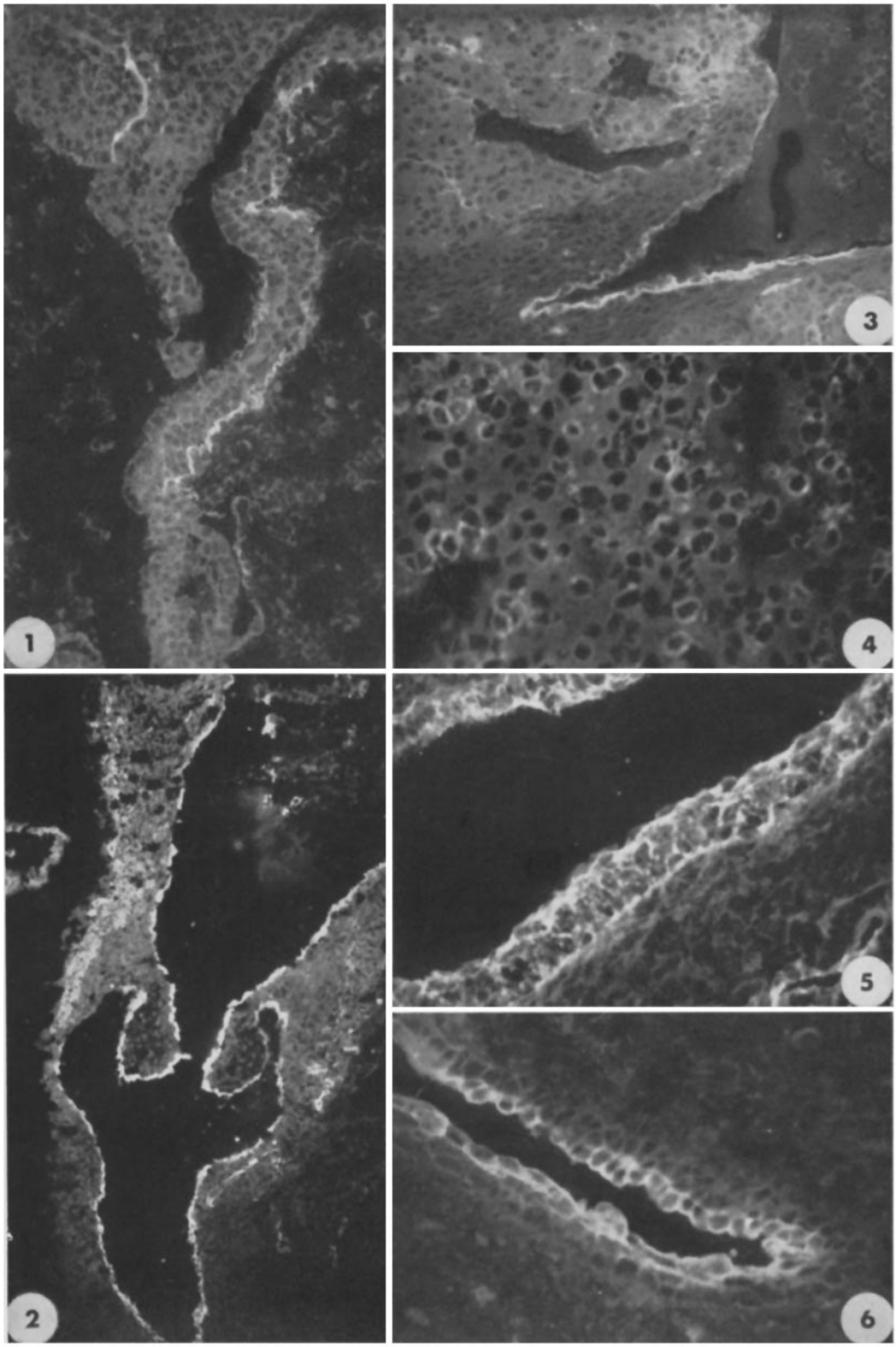
FIG. 3. Sinus venosus and hepatic sinusoids, 18 mm embryo, group A; Bombay conjugate. Identical picture obtained with anti-A reagents (except for more distinct staining of hemopoietic cells). Feeble but discernible staining of the endothelium of the hepatic sinusoids. Note the complete outlining of individual endothelial cells in the sinus venosus. × 170.

FIG. 4. Liver and hemopoietic cells, embryo 18 mm, group A; rabbit anti-A serum and anti-rabbit conjugate. A high power view of clusters of immature erythrocytes, well outlined by double layer staining, against the background of negative liver parenchyma. × 300.

Integument

FIG. 5. Skin, junction of lower limb with trunk, 18 mm embryo, group A; Bombay conjugate. Identical picture obtained with anti-A reagents. Well outlined stratified epithelium; basal layer well stained. × 300.

FIG. 6. Skin, same situation as in Fig. 5, 40 mm embryo, group B; Bombay conjugate. Identical picture obtained with anti-B reagents. Thin, well staining stratified epithelium; negative basal layer. × 300.



(Szulman: A, B, and H antigens in human embryos)

PLATE 60

Pituitary Gland

FIG. 7. Pituitary gland, general view, 35 mm embryo, group B, Bombay conjugate. The epithelium proliferates in solid masses into a vascular mesenchymal stroma. The lumen of Rathke's pouch is compressed to a potential space except where it widens, at the right extremity of the pars posterior. The epithelium is specifically outlined, as are the small vessels of the mesenchyme in the center; the contrast with the non-staining pars posterior is well demonstrated. The cartilage in the left upper corner shows up due to autofluorescence. × 70.

FIG. 8. Pituitary, one "wing" of anterior lobe, 27 mm embryo, group A stained with rabbit anti-A serum followed by anti-rabbit conjugate to demonstrate the striking deficiency of A antigen. The endothelium shows the usual abundant A antigen. × 170.

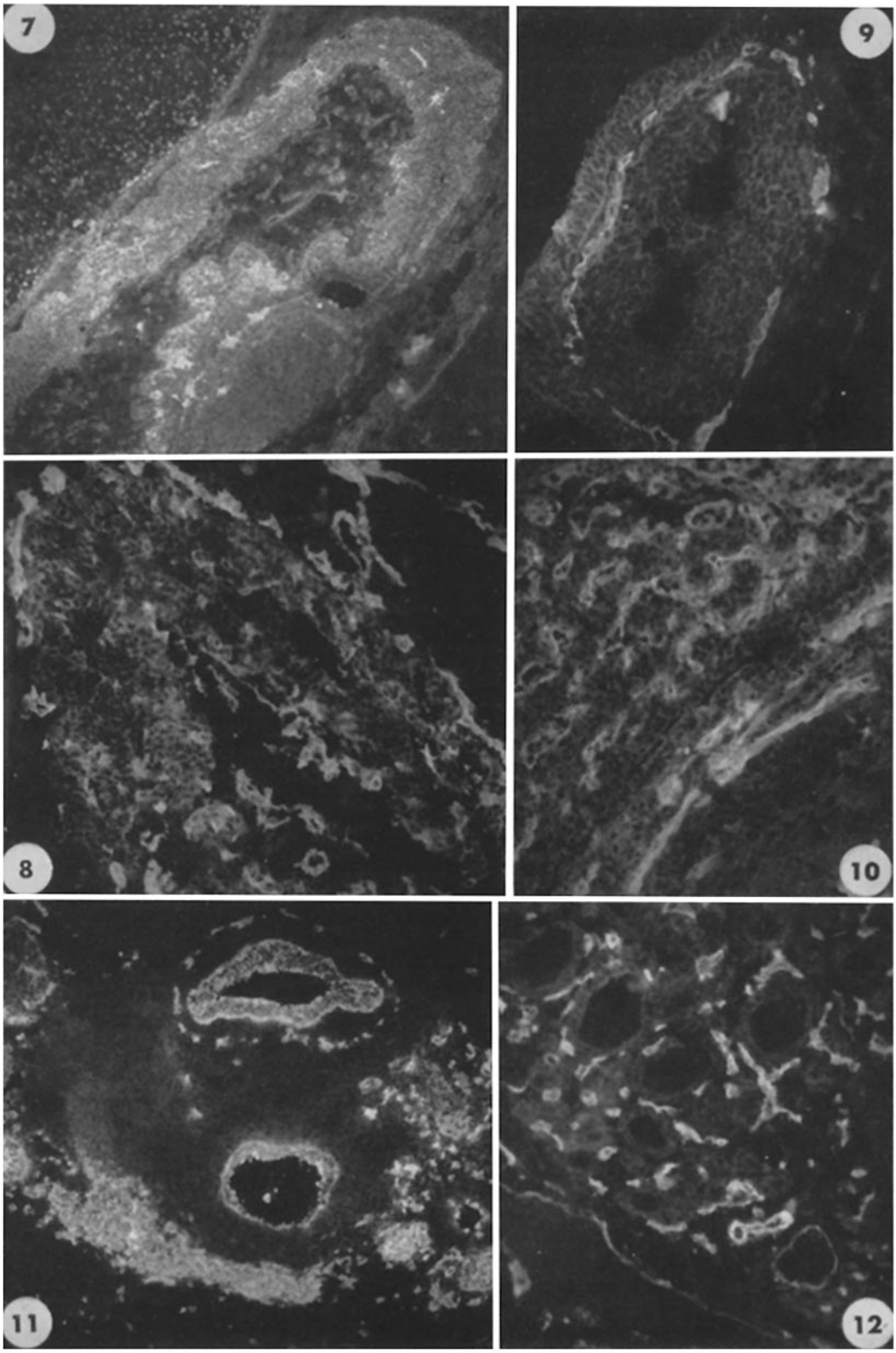
FIG. 9. Pituitary posterior lobe and pars intermedia, 35 mm embryo, group B, (from the same case as Fig. 7); human anti-B conjugate. The pars posterior is devoid of antigens; the pars intermedia stains feebly with the anti-B reagent (compare with the brilliant endothelium of vessels running along the periphery of pars posterior). The original lumen of Rathke's pouch is represented by the space in left upper corner. X 170.

FIG. 10. Pituitary at about 25 weeks' gestation, group 0, Bombay conjugate. The anterior lobe, upper left, has long lost the antigen completely; it shows the rich vascularity of an endocrine, as does the pars intermedia (below and to the right of the residual lumen of Rathke's pouch identifiable as a narrow diagonal slit). The pars posterior, lower right, shows comparatively poor vascularity. × 170.

Thyroid, Thymus

FIG. 11. Thyroid, thymus, trachea, and esophagus, 18 mm embryo, group A; human anti-A conjugate. The thyroid is seen as a solid, specifically staining crescent of epithelium in the lower part of the photograph, lying in front of the trachea and the esophagus. The thymus is represented by the two anlagen seen as two small round masses of specifically stained epithelium one at each extremity of the arc of the thyroid. The primitive trachea and esophagus demonstrate well the specific staining of their epithelia. × 70.

FIG. 12. Thyroid, fetus 150 mm CR (mid-pregnancy), group 0; Bombay conjugate. A high power view to illustrate the absence of antigen from the cell walls of the acini; periacinar capillaries well visualized by virtue of specific endothelial staining. × 200.



(Szulman: A, B, and H antigens in human embryos)

PLATE 61

Respiratory System

FIG. 13. Mediastinum, 19 mm embryo, group 0; Bombay conjugate. The bifurcation of the trachea (two central tubes) and the right main bronchus (lower right of photograph) are well visualized by virtue of specific outlining of the epithelium. The esophagus with its well staining epithelium is seen below the tracheal bifurcation (compare Fig. 11). The aorta, in the left lower corner, and the atria at the upper edge of the photograph demonstrate the usual endothelial specific staining. × 70.

FIGS. 14 and 15. Primitive lung from the same 18 mm embryo, group A. In Fig. 14, note the abundance of A antigen in the bronchial epithelium and its scarcity in the capillary endothelium (human anti-A conjugate). Fig. 15 shows the scarcity of H antigen in the bronchi and large amounts of it in the capillaries (Bombay conjugate). × 70.

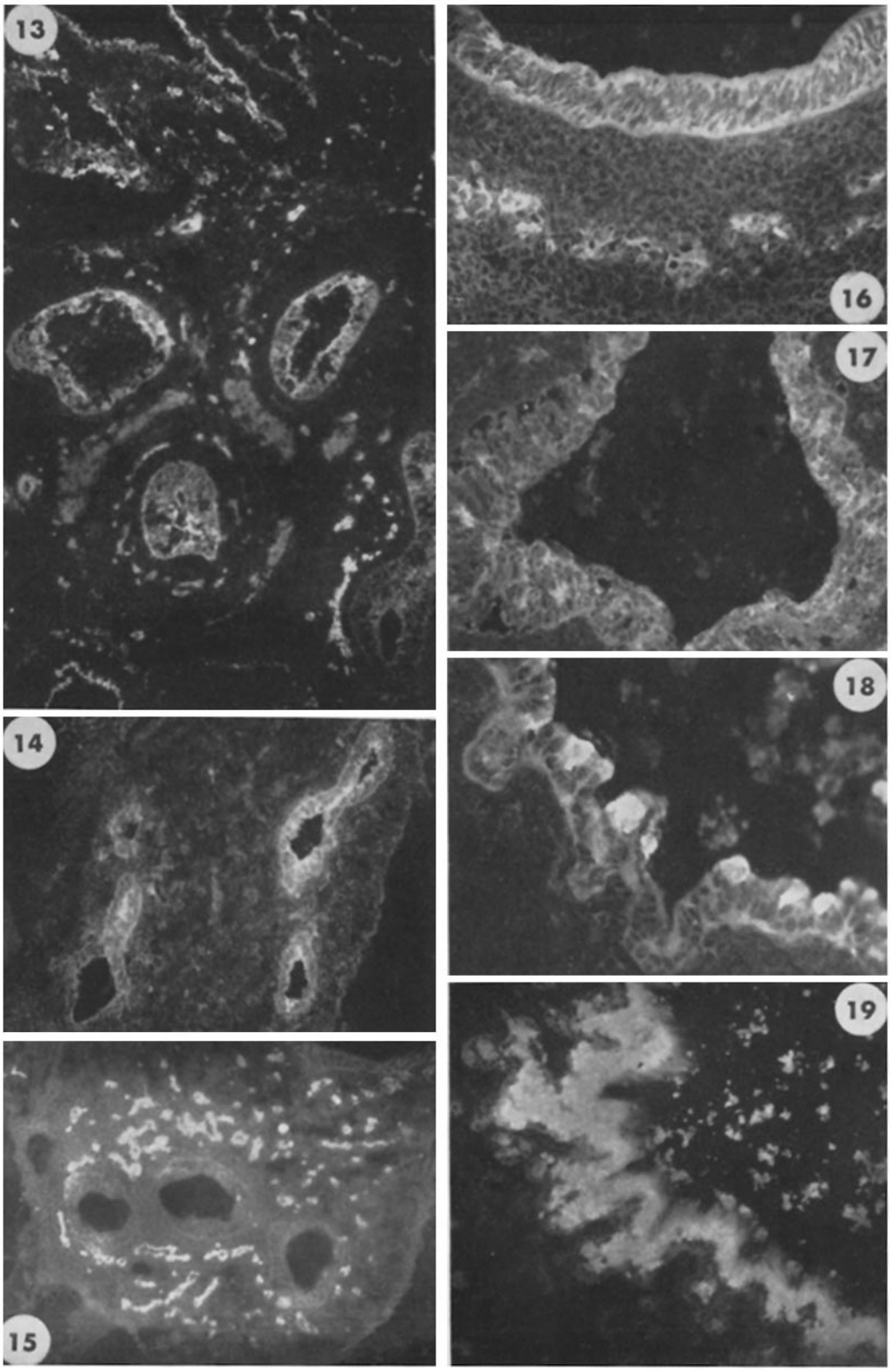
Stomach (Corpus)

FIG. 16. Lining epithelium, embryo 18 mm, group A; Bombay conjugate (identical picture obtained with anti-A reagents). Epithelium well outlined by antigen; note uniform, undifferentiated character of the membrane. × 170.

FIG. 17. Mucosa in an older specimen, 40 mm CR, group B; human anti-B conjugate. Note structural advancement of the mucosa; the cell wall antigen is waning rapidly and several blebs of secretion bearing the antigen have appeared. × 170.

FIG. 18. Mucosa from fetus 70 mm CR, group 0; Bombay conjugate. Secretion of the mucus-borne antigen is well illustrated; the cell wall antigen is absent; some mucus coating of epithelial cells has taken place. × 300.

FIG. 19. General view of mucosa of secretor type, fetus 115 mm CR, group A; rabbit anti-A serum and anti-rabbit conjugate. Abundant mucus-borne antigen; no staining of cell wall antigens. × 70.



(Szulman: A, B, and H antigens in human embryos)

PLATE 62

Salivary Glands

FIG. 20. Submaxillary gland, fetus 40 mm crown to rump, group B; human anti-B conjugate. (Similar picture obtained with the Bombay conjugate.) Commencing secretion rich in antigen. × 170.

FIG. 21. Submaxillary gland, fetus 45 mm CR, group 0; Bombay conjugate. No secreted antigens demonstrated in serial sections of this gland and of the parotid; this case is presumed to be a non-secretor. × 170.

Pancreas

FIG. 22. The formation of the gland by an outgrowth from the duodenum, 18 mm embryo, group A; human anti-A conjugate. Note the well marked cell-bound antigen in the duodenum and in the pancreatic duct, and its absence from the tubules. × 70.

FIG. 23. Commencing secretion of antigens in some pancreatic acini in a fetus 110 mm CR, group 0; Bombay conjugate. × 300.

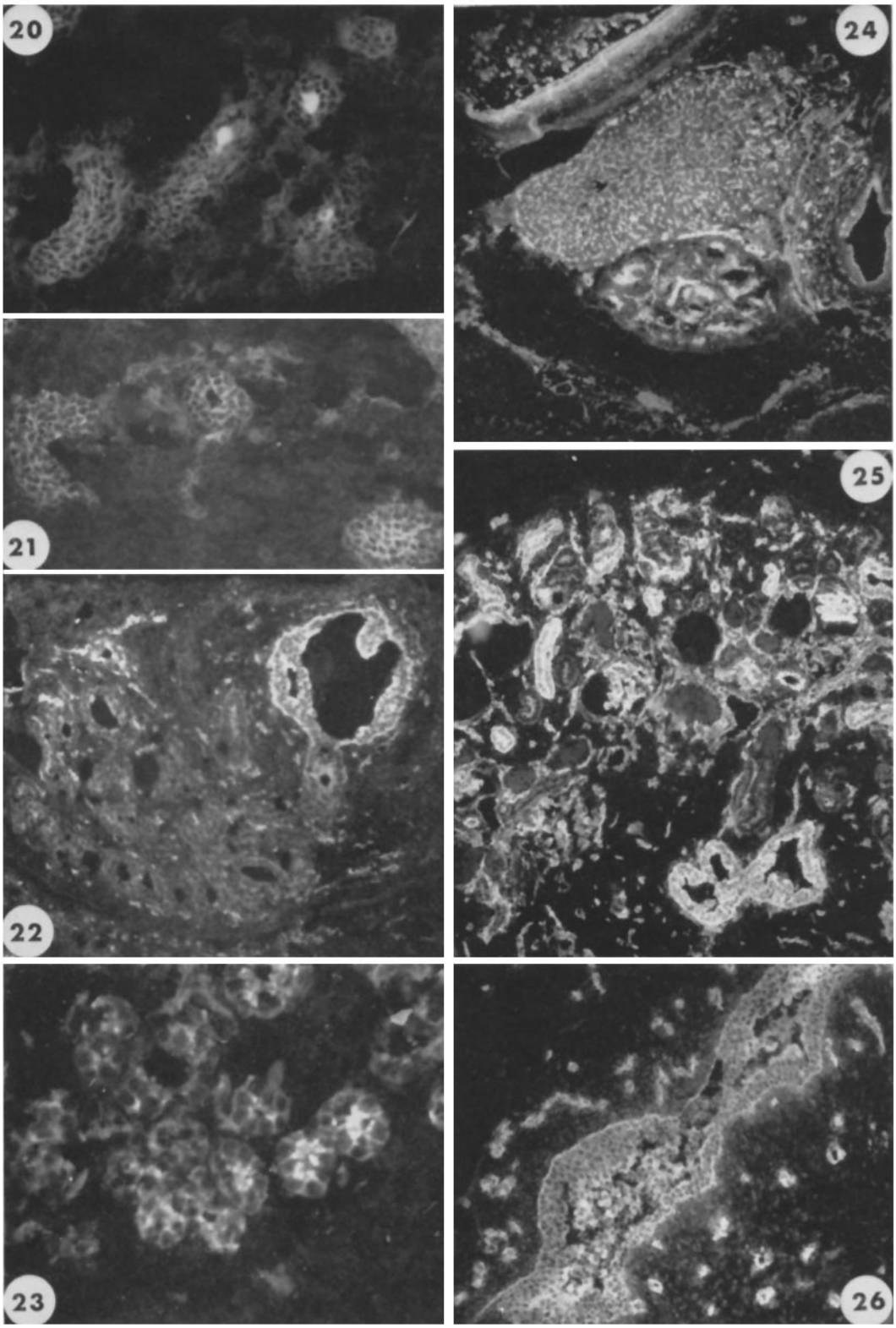
Kidney, Adrenal, Bladder

FIG. 24. Anlage of the permanent kidney and adrenal, embryo 27 mm, group A; human anti-A conjugate. The early kidney shows the well established branching of the ureteral bud. Note the abundance of the cell wall antigen of the epithelium, a feature to persist into adult life from the collecting tubules down to the ureter.

The adrenal is seen between the stomach (above) and the metanephros (below). There is no cell wall antigen in the parenchymal elements; there is remarkable vascularity visualized due to endothelial specific staining. The vessel at the right edge of the photograph is the aorta. × 30.

FIG. 25. Kidney, fetus 65 mm crown to rump, group A; rabbit anti-A serum and anti-rabbit conjugate. Continued abundance of antigen in the branching ureteral bud, as it forms the pelvis, calyces, and collecting tubules; other tubules devoid of antigen. The glomeruli stain by virtue of endothelial antigen. × 70.

FIG. 26. Urinary bladder, fetus 115 mm CR, group A; human anti-A conjugate. Sharp outlining of cells of transitional epithelium. The desquamated cells in the lumen tend to show cytoplasmic staining. × 70.



(Szulman: A, B, and H antigens in human embryos)