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

II. The H Antigen and Its Relation to A and B Antigens

By ARON E. SZULMAN

(From the Departments of Pathology, Harvard Medical School, and the Boston Lying-In Hospital, Boston)

Plates 106 to 110

(Received for publication, December 13, 1961)

In a previous communication the distribution of blood group antigens A and B in human tissues was described (42). The immunofluorescence technique (10) was employed in staining frozen tissue sections by human hyperimmune sera conjugated with fluorescein. Herein, an analogous investigation of the H antigen is reported; its separate character lies in the nature of the antigen investigated and the serologic reagent employed.

The problem of the identity of the product of the gene O has defied solution since the formulation of Bernstein's theory of the inheritance of ABO groups in 1924 (1). The O trait can be unequivocally established only by the negative reactions of erythrocytes (or secretions) with anti-A and anti-B sera. Thus determined, it is found to behave as an inheritable recessive character in a simple Mendelian system. The product of the O allele, however, has not been identified in a positive fashion for lack of a specific, authentic serum.

Various heterologous native (38) and immune (11, 29) animal sera regarded at first as true anti-O reagents were found to react with homozygous A and B or A_1B erythrocytes or secretions (37, 23, 46, 32) and were thus clearly not specific for the product of the O gene. Similarly, other, mostly human, sera purporting to be true anti-O reagents (3, 30) are no longer considered as of anti-O specificity by many observers (34, 45, 8), mainly on the grounds that they fail to distinguish consistently between homozygous (AA; BB) and heterozygous (AO; BO) bloods (45).

The receptor discovered in the quest for the O antigen was regarded by Hirszfeld as a species antigen appearing in quantities reciprocal to those of A,B (16). This observation led to his theory of the "pleiades" (17), according to which the A_1, A_2, and B genes were incomplete mutations of gene O, thus accounting for the persistence of the O remnant in A_1B and homozygous A and B bloods. The antigen was designated H by Morgan and Watkins (30) to acknowledge its heterogenic nature (21, 23) and to

* This investigation was supported by Research Grant E-3554 from the National Institutes of Health, United States Public Health Service.

emphasize its independence from gene O; the sera were accordingly designated as of anti-H specificity. At the same time, these investigators modified the theory of the pleiades by proposing the derivation of the genes ABO from a basic gene H.

An elegant comprehensive working hypothesis concerning the H, ABO, Lewis, and secretor genes and their products or effects has been formulated recently by Ceppellini (7–9), Morgan (27, 28), and Watkins (44). According to it, the H substance serves as a substrate from which the A and B substances originate by a process which is usually incomplete, thus allowing a certain degree of H specificity to remain. The gene O is assumed to be an amorph and accordingly, in individuals of group O, the H substance remains in its pristine state. Traced back in its genesis, the H substance is itself derived from a precursor through the action of an appropriate gene, H, independent of the ABO locus. In the production of the mucus-bound antigens, the precursor substance is acted on and possibly competed for by the genes H and Lewis. The production of the H substance (and hence of A,B) is favored by the dominant secretor gene (S), while its suppression and the enhancement of production of Le^a substance is associated with the recessive, non-secretor alleles (ss).

TABLE I

Characterization of Bombay Serum

Erythrocytes	O	A_2	A_1	B	A_2B	A_1B
Unabsorbed Bombay	1024	512	128	128	256	128
Absorbed with O secretor saliva	0	64	16	16		8
Absorbed with O erythrocytes	0	32		16		32
Absorbed with A_1 erythrocytes	256	128	0	64		8
Absorbed with A_1B erythrocytes	256		16	32		0

Titers as obtained with 2 per cent suspension of appropriate erythrocytes.

At the erythrocytes' surface the H and A,B genes exercise their action uninfluenced by the secretor and the Lewis genes, the products of the latter being acquired by the red cells secondarily from the plasma.

"Bombay" Bloods.—These were originally found in Bombay (2, 15) but are not confined to India (25, 33). The erythrocytes and secretions of Bombay persons lack the antigen H and its presumed derivatives A,B thought to be due to a block in the formation of the former (25, 44, 8). The absence of the H antigen allows for the appearance of the corresponding antibody which could be used as an anti-H reagent.

The mapping out of the H antigen in human tissues, the elucidation of its relationship to A and B and of its behavior with respect to the secretor status were the aims of this investigation. The use of anti-H reagents of other origin than the Bombay serum was, at the same time, believed desirable in the hope of buttressing the results and concepts ensuing from its application.

Materials and Methods

Antiserum.—Several reasonably accessible preparations were tried in search of a suitable anti-H reagent. Thus seed extracts (lectins) from *Ulex europaeus* (gorse) (6) and *Lotus tetragonolobus* (winged pea) (35) proved too weak for immunofluorescence even when tried in a double layer method with rabbit anti-lectin conjugate as the staining layer. Eel sera (20) although occasionally powerful enough, invariably evinced non-specific "sticking" to tissue sections. A rabbit anti-H conjugate had already been tried by Glynn *et al.* (13) on the stomach and duodenum, which warranted yet another approach.

A suitable serum was finally obtained in a manner similar to that employed in the investigation of A and B antigens (42). With the generous cooperation of Dr. H. M. Bhatia of the Indian Cancer Research Centre, Bombay, and of Prof. W. T. J. Morgan of the Lister Institute, London, one of the three original donors (Donor Z) of Bombay blood (2) was immunized with purified human H substance. The serum thus obtained showed a rise of titer from 16 to 1000 (4000 by Coombs test) with human group O erythrocytes. The globulin fraction was conjugated with fluorescein isothiocyanate (36). The loss of titer was entirely due to dilution requisite for conjugation, although further slight deterioration was apt to occur on storage at 4°C. Merthiolate was used as a preservative, in a final dilution of 1:100,000. Freezing and thawing of the conjugate markedly reduced its staining power.

Full characterization of the serum is given in Table I. It will be seen that the high titer antibody against group O and A_2 erythrocytes is absorbed out by type O secretor saliva, or by packed O erythrocytes. Although, judging from previous experience with anti-A and anti-B sera (42) the residual anti-A and anti-B agglutinins of the present serum were by far too weak to stain homologous antigens and thus interfere with its anti-H specificity, additional controls were employed in staining of tissues of groups A, B, and AB.

Chicken Anti-H Serum.—This is described together with its controls for specificity of staining at the end of this section.

Anti-A and Anti-B Sera.—These were hyperimmune, human sera described previously (42). An anti-A_1 serum was supplied by the Blood Grouping Laboratory of Boston.

Tissues.—Specimens were examined from subjects of groups O, A_1, A_2, B, and AB, both secretors and non-secretors, obtained at autopsy or at surgical operations. Included are 8 fetuses varying from 15 to 24 cm crown-heel length.[1]

The details of rapid freezing, cutting, fixation, staining, mounting, and photography as well as the determination of secretor status were exactly as described previously (42). An improvement in fixation of mucus-secreting glands and surfaces consisted of a quick dip in 10 per cent formalin solution followed by several changes of acetone; this maneuver resulted in abolition of staining of the endothelial and epithelial cell walls.

Eel or rat liver powder were used for purification of the conjugate.

The secretor status of fetuses and deceased infants could not be determined by conventional means; in such cases, the distribution of the mucus-bound antigen in appropriate organs, *e.g.* salivary glands, stomach, allowed for assignment to one of the two patterns (42). One of the fetuses, 24 cm crown-heel length, group O, showed a distribution of the mucus-bound antigen characteristic of a non-secretor.

H Antigen.—In preparation of control conjugates the following sources of H antigen were used: group O erythrocytes from donors of all three Lewis phenotypes; saliva from group O secretors; bovine O(H) substance kindly supplied by Dr. E. A. Kabat; *Taxus cuspidata* preparation (extract from twigs of yew trees), kindly supplied by Dr. G. F. Springer (39).

---

[1] The length of 15 cm as the lower limit was chosen as representing a stage of development at which the antigen assumes an adult distribution. The period of development from 1.8 cm to 15 cm is to be described separately.

Controls.—Preliminary experiments established that staining was due to globulin-bound fluorescein as it could be abolished by pretreatment of sections with unconjugated Bombay globulin, or by mixing the conjugate with the latter in a ratio of 1:1 or 1:2 (14).[2]

Neutralization with porcine O(H) substance grossly decreased the intensity of staining without totally abolishing it. The *Taxus cuspidata* preparation did not affect the conjugate's staining ability in agreement with previous experience (39). For routine use a convenient control reagent was provided by absorption of the conjugate with group O secretor saliva. One ml aliquots of serum were mixed with 0.1 to 0.2 ml of boiled, high titer saliva, sufficient to neutralize the power of agglutinating group O erythrocytes. Non-secretor saliva was without effect. In an alternative method, washed, packed O cells (of any Lewis phenotype) were used with equally good results. Conjugate neutralized in either way gave satisfactory, consistently negative results with A, B and AB tissues.

Additional Controls for Staining of Groups A, B and AB Tissues.—The weak anti-A and anti-B components of the Bombay serum were unlikely to play any part in immunofluorescence. That staining of A, B and AB tissues could not be due to their activity was readily proven by consistent and undiminished staining elicited by the conjugate absorbed with appropriate A_1 or A_1B cells. In practice, aliquots of the conjugate were twice or thrice absorbed with packed A_1B erythrocytes after which the cells were not further agglutinable by the supernatant conjugate.

The above results were further confirmed by "blocking" of the A and B tissue receptors with homologous antibody: Whether the Bombay conjugate was preceded by or mixed[3] with unconjugated anti-A(B) serum (14) its behavior did not alter with respect to the structures stained or the intensity of fluorescence. These and reverse experiments in which the anti-H serum would not block staining by anti-A and anti-B conjugates incidentally served to show that there were no steric hindrance effects with the reagents here employed.

Estimation of Relative Amounts of A, B and H Antigens.—Although the immunofluorescent technique employed herein does not lend itself easily to a sensitive quantitative study, an attempt was made to estimate roughly the relative amounts of the H and A, B antigens in non-O tissues. Two consecutive sections were mounted on one slide inside the cryostat, thus allowing for identical treatment during subsequent processing except for the application of different appropriate conjugates. At examination of the preparations individual histologic structures (*e.g.* glands, vessels, selected sectors of epithelia) could be compared and gross, unequivocal differences scored separately for the various forms of antigens[4] using a scale from 0 to 4+.

Chicken anti-H Serum.—Towards the end of this investigation an equivalent of 1 ml of this reagent in lyophilized form was kindly supplied by Dr. H. M. Bhatia of Bombay. It had been obtained by immunizing chickens with human, group O erythrocytes and absorbed with Bombay cells. On reconstitution, it proved to be of a titer of 1,000 against O erythrocytes and was diluted 1:2 for use. Absorbed with O secretor saliva or O erythrocytes, it gave minimal titers (2 to 4) with A_1 and B cells. Because of the small amounts available, the serum was employed

---

[2] Dilution *per se* of the conjugate in these and all subsequent experiments herein described was always well below that to influence the staining power of the conjugate, as checked by accompanying dilution experiments. In this case:

Tissue section A: 1(2) parts Bombay globulin + 1 part Bombay conjugate;

Tissue section B: 1(2) parts saline (or AB serum) + 1 part Bombay conjugate.

[3] Tissue section A: 1 (2) parts anti-A globulin + 1 part Bombay conjugate.

Tissue section B: 1 (2) parts saline (or AB serum) + 1 part Bombay conjugate.

[4] The term "form" refers here to the various guises of the blood group antigens in the various sites, *e.g.* bound in the cell walls of endothelia and epithelia or free in mucus and other watery secretions.

TABLE II

Organs Examined—Autopsy Material

Size or age	15 cm*	17 cm*	17 cm*	19 cm*	20 cm*	22 cm*	23 cm*	24 cm*	NB	NB	NB	NB	NB	NB	1 yr.	1 yr.	5 yrs.	5 yrs.	6 yrs.	50 yrs.
Group	0	0	A	0	0	0	0	0	0	0	0	A_1	A_1	B	A_1	A_1	AB	0	0	0
Secreton Status	S‡	S‡	S‡	S‡	S‡	S‡	S‡	NS§	S‡	NS§	S‡	S‡	S‡	S‡	S‡	S‡	NS§	NS	S‡	S
Adrenal				X											X				X	X
Bladder								X			X				X		X	X		
Brain				X						X				X				X		
Colon		X	X	X		X	X	X	X	X	X	X	X		X	X				
Esophagus			X	X				X							X				X	
Heart				X				X							X			X		X
Kidney	X		X	X	X	X		X	X		X		X		X	X	X	X	X	
Lung				X				X	X						X					
Liver				X		X		X	X				X		X	X	X	X	X	
Lymph node															X					X
Muscle				X				X							X			X		X
Pancreas	X	X	X	X		X			X	X	X		X	X	X	X	X	X	X	X
Parotid		X		X			X									X			X	
Pituitary										X				X				X		X
Skin						X	X									X	X	X		
Small bowel	X		X	X		X	X			X		X	X		X	X		X		X
Stomach	X	X		X	X	X	X	X	X	X		X	X	X	X			X		
Submaxillary/ sublingual			X	X		X	X	X	X	X	X	X		X		X	X	X	X	X
Spleen				X	X	X		X	X	X	X	X	X		X		X	X	X	X
Thymus				X	X				X				X	X	X	X				
Thyroid		X	X	X				X							X					X
Tongue		X	X	X		X		X			X			X			X	X	X	
Trachea and bronchi			X	X				X	X						X			X	X	

NB, Newborn; S, Secretor; NS, Non-secretor; ND, Secretor status not determined.

* Fetus; crown-heel length as indicated.

‡ Secretor status not determined conventionally; distribution of water soluble antigens that of secretor.

§ Secretor status not determined conventionally; distribution of water soluble antigens that of non-secretor.

in a double layer method with a commercial rabbit anti-chicken conjugate as the staining layer. The reagents were absorbed with rat liver powder prior to use. The rabbit conjugate alone gave no staining with human tissues.

Control reagents were prepared by absorbing aliquots of the chicken serum with group O cells. Neutralization with O secretor saliva left slight residual staining but the problem could not be further investigated since the supply came to an end.

TABLE III

Organs Examined—Surgical Material

Group	O			A_1			A_2			B			AB		
Secretor status	S	NS	ND	S	NS	ND	S	NS	ND	S	NS	ND	S	NS	ND
Appendix	1	1	1	2	1	2				1				1	
Breast	2		3	2		5				2		1			
Active		1	1	1			1			1		1			
Colon															
Ascending	3	1	1	2	1	1				1					
Descending	3	1		1	1		2					1			
Epididymis	1			1		1									
Gall bladder	6	1	2	5			1				1		3		
Ovary	1	1		1		1							1	1	
Ovarian cyst, mucinous		1		1				1		1			1		
Prostate	3	1	2	1	1					1					
Skin		1	4						1						
Small bowel	2		2	4	1	3	1			1					
Stomach	7	3	3	6	3	2	1			3	1		1	1*	
Testis	1	1	2	1		1						1			
Vesicle (seminal)	1				1										
Uterus:															
Proliferative endometrium	4	1		3	1					2	1		1		1
Secretory endometrium	2	2	2	4	1	2	1			2	1	2	1	1	
Endocervis	6	2	6	9		4	1	1	1	4	3	2	2	1	1
Colpocervix	3	1		1	1	1		1	2	1	1	2		1	
Fallopian tube	2	2	2	5	1	2		1		3	2	2	2		
Vagina	1	1				1					1				

Figures denote numbers of specimens examined.

S, secretor; NS, non-secretor; ND, secretor status not determined.

* Group A_2B

Staining with the "Bombay" conjugate could be inhibited by mixing it with an equal volume of the chicken serum. In the case of mucus, the inhibition was rendered complete if application of the *mixed* reagent was preceded by application of chicken serum alone.

RESULTS

The results are based on the examination of autopsy (Table II) and surgical specimens (Table III) to provide material from organs and tisses as listed,

TABLE IV

Quantitative Comparison of the H and A, B Antigens

Blood group	A_1		A_2		B		AB			O
Staining for antigens	H	A	H	A	H	B	H	A	B	H
Endothelium	0	4	tr.	2	0	4	0	3	3	4
	0	3	1	tr.	tr.	4	0	3	2	4
	0	2	2	4	tr.	3	0	3	1	4
	1	4	2	1	tr.	2	0	2	3	4
	1	3	2	1	1	4	1	3	2	3
	1	2	3	4	2	4	1	1	3	3
	2	4	3	3	2	3				2
Stratified epithelia										
Esophagus	3	4								4
Thymus	3	2								3
Thymus	3	1								3
Exocervix	3	1	3	3 NS	0	2				4
Exocervix	2	4			0	3				3
Exocervix					4	2				3
Tongue							0	3	3	4
Tongue							1	4	1	4
Renal calcyces	1	3			1	3				2
	2	1								4
Mucus-bound antigens										
Stomach	4	4 NS	4	4	4	4 NS	3	4	3	4 NS
	3	4			3	3				4
	2	4 NS			3	2				4
	2	3			2	3				4 NS
	1	4								3 NS
Gall bladder	0	4	1	tr.			0	4	3	4
	1	4					0	3	2	3
	3	3					tr.	2	2	3
	3	2								2
Small bowel	1	4	2	1	3	3				4
	1	2								3
	2	4								2
	3	4								
Ascending colon	tr.	4			4	4				4
	3	3								3
Endocervix	1	4	1	0	0	3	0	3	1	4
	2	4	1	0	1	4	0	3	tr.	4
	3	4	2	3	3	0				3
Mucinous ovarian cyst	4	4			1	4	3	4	2	

TABLE IV—*Continued*

Blood group	A_1		A_2		B		AB			O
Staining for antigens	H	A	H	A	H	B	H	A	B	H
Secreted and Excreted Antigens										
Pancreas	1	2			2	3	0	3	2	3*
	2	3								2*
	3	3 NS								2 NS
	3	3‡								2
Endometrium	2	3*			2	0*	1	3	1	3
	1	2			1	2	0	2	0	2
Fallopian tube	0	4			2	4	tr.	4	1	4
	1	4§					1	4	3	3
	2	3								1
Breast	tr.	1	2	3	1	0				4
	2	4			3	0				1
	3	±‖			4	±‖				
Kidney	1	4			1	3				4
	1	2								3
	4	3								3
Sweat Glands	3	2					tr.	4	2‡	4 NS
							1	1	1	2*

Numbers denote intensity of staining on a scale from 0 to 4. tr., trace.
Tissues bearing water soluble antigens are from secretors unless otherwise marked.
NS, non-secretor.
± Staining patchy.
* Secretor status not determined conventionally: distribution pattern that of secretor.
‡ Secretor status not determined conventionally: distribution pattern that of non-secretor.
§ Tubal pregnancy.
‖ Lactating.

from both secretors and non-secretors. The study demonstrates that there is a consistent parallel in the occurrence of antigen H and antigens A and B in their respective forms and locations, including the behavior of the water-soluble forms in secretors and non-secretors. While in group O tissues H constitutes the sole antigen of the ABH system, it generally appears also in non-O tissues in amounts varying from zero to those apparently equivalent to, or exceeding, A or B. The results are presented under six convenient headings.

(*a*) *The intima of vessels* of all calibers throughout the body, stains well by virtue of the antigen in the cell envelope, much as described for A or B group vessels (42). The

endothelium of the endocardium and of the pulmonary capillaries stains poorly, but has been satisfactorily demonstrated in fetal material (Figs. 1 and 2). An example of a subcutaneous hemangioma (Fig. 3) demonstrates bright staining of the cells lining the blood spaces. The vessels supplying malignant tumors are also positive.

Pretreatment with 95 per cent ethyl alcohol for 15 minutes abolishes the specific staining.

The amounts of antigen in vessels in group A_1, A_2, B, and A_1B tissues exemplify well the quantitative aspects of the H antigen in non-O tissues and are illustrated in Table IV. It will be seen that in group A_1 the H antigen is either present in small amounts or cannot be at all demonstrated. In group A_2, on the other hand, it is easily demonstrable and often in large amounts. B group endothelium seems to partake of the features of both A_1 and A_2. Finally, A_1B specimens akin to group A_1 show only very small amounts, down to negative results. In such cases application of another layer of anti-antibody conjugate (horse anti-human globulin) gave no result; however, in two cases of A_1 tissues, vessels negative with the Bombay reagent could be visualized with the chicken serum followed by anti-chicken conjugate.

(*b*) *The stratified epithelia* show the antigen in the envelope of the cells, the distribution resembling that of A and B antigens.

Included in the study are the skin, the lining membranes of the mouth and esophagus (Fig. 14), uterine exocervix (Fig. 12) and vagina, Hassall's bodies of the thymus and the transitional epithelium of the lower urinary passages (Fig. 16). The antigen is confined to the Malpighian layer in stratified squamous membranes with a predilection for the granular layer in the skin. Presumably due to its exposed situation the antigen in the latter tends to be small in amount and somewhat unpredictable; it stains much more consistently and uniformly in fetuses. In either case, it conforms to the general rule of having a basal layer which remains negative in all circumstances. The "transitional" epithelia of the urinary tract (Fig. 16) and of the upper respiratory passages (Fig. 15) demonstrate full staining of the basal layer with frequent attenuation towards the lumen. A well differentiated carcinoma of the skin included in the study was found to contain antigen in the walls of neoplastic, recognizable squamous cells (Fig. 17). Similarly, a case of cervical carcinoma–*in situ* demonstrated the antigen in full amounts with occasional sectors of the basal layer staining brightly (Fig. 13). In areas of squamous metaplasia, as seen in the cervix, the basal layer is positive but the staining of the whole membrane tends to be patchy. Pretreatment with alcohol results generally in attenuation of staining.

As the distribution of the group antigens in the skin is apt to be erratic, the comparison of H and A,B antigens was effected by examining stratified epithelia principally from the exocervix, esophagus, and tongue. As anticipated, (Table IV) the staining of the H antigen in A_2 tissues tends to be brighter than in those of group A_1; the B tissues present a mixed pattern, while AB tissues have amounts not rising above a mere glimmer. The epithelium of Hassall's corpuscles in the thymus demonstrates plentiful H antigen not only in O subjects but also in two A_1 infants.

(*c*) Some *simple epithelia* show various degrees of completeness of outlining of the cell walls, independent of secretor status. To this group belong the collecting tubules of the kidney in which the cell walls are consistently brightly outlined, independently of the luminal accretions of antigen; the H-A,B relationships follow those of the

calyceal epithelium. The tracheobronchial epithelium and those of the endometrium, endocervix (Fig. 6), Fallopian tube (Fig. 24), and breast (Figs. 18–23) gave outlining of cells which was inconstant in intensity within the same specimen, and at times altogether absent. Because of the erratic nature of the staining, no estimation of the relative amounts of H and A,B antigens was attempted. Pretreatment of sections with alcohol results in attenuation or abolition of staining.

The *parenchymal cells* of endocrine glands: adrenal cortex and medulla, thyroid (Fig. 26), pituitary, and pancreatic islets were consistently negative. The neurones, glia, and ependyma of the brain likewise show no antigen.

(*d*) In the *mucus-secreting apparatus* the close agreement of the distribution of the mucus-bound H antigen with that of A and B is again amply demonstrated for both secretors and non-secretors. Table IV lists some representative findings. In *secretors*, the following sums up the histological locations of antigen H.

Salivary glands: Mucous acini (Fig. 4) of mixed glands, with occasional serous acini showing slight and inconstant stippling. *Stomach:* The surface epithelium and the foveolae in the corpus, pyloric glands (Fig. 7). Goblet cells of *small intestine* (villi and crypts) (Fig. 11) and of the *large intestine* (surface and crypts) up to the level of the transverse colon. The antigens H, A, and B rapidly disappear from the mucus of the transverse and descending colon during the neonatal period; all fetuses and newborns examined showed large amounts of ABH antigens in ascending and descending segments of the large bowel. *Upper respiratory passages:* mucus glands and goblet cells of the larynx, (Fig. 15), trachea, and large bronchi. *Gall bladder:* abundant amounts in secretion of the surface epithelium and glands. *Uterine cervix:* surface epithelium and glands (Fig. 6). *Pseudomucinous ovarian cyst:* large amounts of mucus-borne antigen.

In *non-secretors* the antigen is not to be found (Fig. 5) except in certain special locations: the deep parts of the gastric foveolae (Fig. 8, 10) and of the pyloric glands, varying small numbers of goblet cells in the crypts of the small (Fig. 9) and large bowel. In one case several positive acini in a mucinous ovarian cystadenoma were found.

In non-O tissues, the H antigen is almost invariably detected and is found to conform in its distribution to the respective patterns of secretion and non-secretion. Table IV, based on surgical material, illustrates the findings.

(*e*) Among other *organs of secretion and excretion* the *pancreas* secretes the H antigen in the exocrine elements of the gland. This secretion becomes established in fetuses of about 15 cm crown-heel length and is found irrespective of the secretor status in both intrauterine and extrauterine life. The relative amounts of the antigens are illustrated in Table IV. The *parotid*, a serous salivary gland, secretes the ABH antigens in the fetus while in the adult only occasional stippling of the acini can be detected. In the *kidney* the antigen present at the luminal aspects of the lining of the collecting tubules and calyces (Fig. 16) is found in secretors and is absent in non-secretors. This correlation holds also for the sole non-secretor fetus of this series. The *merocrine sweat glands* show delicate outlining of the cells of the coils with the secretion-borne antigen staining brightly in the lumens, independently of secretor status. In the *endometrium* there is capping of the luminal aspects of the cells of the glands and deposition of antigen in the lumen, confined to the second week of the proliferative phase and in secretors only. No antigen is found in non-secretors, and it was missing in a few cases of secretors. The

epithelium of the rugae of the *Fallopian tubes* shows a similar picture (Fig. 24), but the antigen tends to persist into the secretory phase in some cases beyond the 20th day of the cycle. Two cases of tubal pregnancy showed abundant antigen at the tips of the cells and in the lumen, away from the site of the embedding ovum.

From the foregoing it will be seen that the table in the preceding study (42) summarizing the distribution of the A,B antigens in secretors and non-secretors is equally valid for the H antigen.

The *breast* proved an interesting organ as it secretes the antigen irregularly, the picture varying sometimes within one lobule, where some ductules or acini would con-

TABLE V

Tissues Investigated with Chicken Anti-H Serum and Rabbit Anti-Chicken Conjugate

Group	A			B			O		
Secretor status	S	NS	ND	S	NS	ND	S	NS	ND
Appendix		×							
Brain									×
Breast	×						×	×	×
Pancreas							×	×	
Prostate									×
Spleen			×			×			×
Stomach		×		×			×	×	
Submaxillary				×			×		
Testis								×	×
Uterus									
Endocervix	×								
Endometrium	×								
Endocervix	×						×		

Identical results were obtained with the Bombay conjugate.

×, denotes tissue or organ sampled; S, Secretor; NS, Non-secretor; ND, Secretor status not determined.

tain antigen side by side with similar non-antigen-containing structures (Fig. 18). "Cystic disease" tends to depress the production of antigen, although by no means invariably so. Prolactational and lactational changes give rise to plentiful antigen and much greater uniformity of its secretion (Fig. 19).

In A and B subjects there is constant, good staining obtained for H (Figs. 20, 22) with variable results for the A and B antigens. Of these, the B antigen appeared essentially absent in a majority of the glands. In the two lactating glands available from secretors, of group A and B respectively, patchy staining of small groups of acini was obtained with the appropriate anti-A and anti-B conjugates (Fig. 21) in individuals secreting both H and A,B antigens in the saliva. The relative amounts of H and A,B are illustrated by examples given in Table IV. It can be stated that the production of the H antigen by the resting and the lactating organ is more uniform and plentiful; that of A remains patchy and unpredictable, while that of B often virtually absent. The

atter constitutes the only instance in which the parallel between the antigens A and B does not hold.

No antigen was found in non-secretors (Fig. 23).

In the *male genital organs*: epididymis, seminal vesicles, and prostate, there is occasional luminal capping in the glandular epithelium in secretors. The large amounts of ABH antigens in the spermatic fluid would seem therefore to originate in some of the small auxiliary glands of the genital tract, not included in this study.

(*f*) In the *miscellaneous group* the testes show occasional feeble outlining of cells in the pre-spermatozoal levels; this finding is of dubious specificity and cannot be confirmed with chicken serum. Spermatozoa could not be stained. *Ova*, as seen in *Graafian follicles*, show no ABH antigen (Fig. 25). The *parietal cells* (Fig. 27) of the gastric glands are uniformly negative, even when stained by a two layer technique.

Results Obtained with the Chicken Anti-H Serum.—These are essentially identical with those obtained with Bombay conjugate (Table V). The two layer technique results in appreciably brighter staining and in a few cases of non-O tissues it brought to view the endothelial H antigen not visualized with the Bombay conjugate alone. Several non-secretors were included and gave characteristic results (Fig. 8).

DISCUSSION

Comparison with Data from Earlier Investigations.—The relative paucity of investigations which include the H(O) antigen and the occasional discrepancies in the findings may be ascribed, respectively, to the lack of an authentic standard reagent and the heterogenicity of the sera employed. Nevertheless, the results obtained from the present study generally agree with the data from other investigations in which antigen H was identified primarily in mucous secretions or extracts of mucous membranes. With the aid of hemagglutination inhibition or similar techniques, the "O" antigen was found in saliva, gastric juice, milk, and sperm with native cattle sera (37); in gastric juice with native beef sera (46); in mucinous ovarian cysts with native cattle sera (29); in saliva of newborns with a goat anti- *S. dysenteriae* serum (12); in meconium with a human serum (5) and a variety of human and animal sera (43); in a wide variety of organs and secretions with eel and rabbit anti-H sera (40).

The rabbit anti-H conjugate used recently in an immunofluorescent study of the mucus-borne antigen in the stomach and duodenum (13) yielded certain surprising histologic results; it is however no longer regarded as a true anti-H serum (4).

The Relationships of the ABH Antigens.—The outstanding point emerging from the current study is the virtually complete parallel of the antigens A and B on the one hand, and the antigen H, as revealed by the Bombay and chicken anti-H sera, on the other. The view according to which the blood group antigens A and B are derived from H, and may, indeed, be associated with one and the

same mucopolysaccharide molecule (8, 27, 44) gains thereby a further circumstantial confirmation. This holds not only for the mucus-bound forms, hitherto the only ones exhaustively investigated, but also for those borne in other secretions or excretions (pancreas, endometrial glands, Fallopian tubes, sweat and mammary glands, kidneys), as well as for those forming an integral part of the cell wall (endothelium, epithelia). The same intimate association of A,B and H antigens in tissues of non-secretors deserves emphasis, since it holds for the water-soluble forms through all their vagaries: the absence from mucous (with the noted exceptions), mammary, and endometrial secretions and their presence in the mucus of the deeper reaches of the gastrointestinal mucosa, exocrine pancreatic and sweat secretions. The table summarizing the distribution of the A,B antigens in secretors and non-secretors in the previous communication (42) is thus equally valid for the H antigen.

Ordinarily there is no steric hindrance caused by the various specific groupings studied, as proved by negative results of blocking experiments. Similar results were reported with respect to the H and A antigens on A_2 erythrocytes by the mixed agglutination technique (19).

The few instances in A_1, B, and A_1B tissues where negative results were obtained with the anti-H reagent are interpreted as due to complete overshadowing of H specificity by that of A,B sufficient to cast it below the threshold of immunofluorescence. A reference to Table IV will show that in A and B tissues this is most commonly encountered in endothelium and stratified epithelia, where the concentration of the group substance is low. Conversely, in the mucous or pancreatic secretions, both rich in the specific substance, the H antigen can be practically always detected.

In A_2 tissues there is an unequivocally richer complement of the H antigen with some instances of extreme depression of A stainability. These results are well in keeping with the central hypothesis, which embodies the idea that the A_2 substance differs only quantitatively from A_1. The few instances of absence of A staining (accompanied by weak staining of the H antigen) occurred in endocervical mucus which is prone to stagnation and perhaps loss of its full serologic characteristics. The only example of stratified squamous epithelium available from an A_2 *non-secretor* was of exocervix and showed large amounts of the A antigen, in contradistinction to reported findings on buccal mucosal cells examined by the mixed agglutination technique (41).

The relations of H and B specificity in group B tissues present a pattern consistent with the existence of various degrees of efficiency on the part of the B gene to modify the H substance, thus producing features shown by group A_1 and A_2 tissues. The findings in AB tissues reflect the combined action of the the two modifying genes A and B, which overshadows the H specificity completely, or nearly so, in all forms of the group substance.

The only exception to the parallel was found in the parietal cells of the

gastric glands, which while staining brilliantly for A or B antigens were consistently negative for H.

Cases regarded as genuine examples of "aberrant secretion" (26, 31) include (Table IV) specimens of endocervix, endometrium (from a different uterus), and three non-lactating mammary glands,[5] all of group B and lacking the latter antigen. This extraordinary deficiency has also been noted in previous investigations of milk (31, 24) and no explanation for it can now be offered.

Secretor: Non-secretor Status.—In the light of the histologic findings the most economic hypothesis would assign a supressor role to the non-secretor genes. While the information and equipment for production of the water soluble antigens is clearly available in the body, the actual manufacture is inhibited with various degrees of completeness. This quantitative aspect of non-secretion can take on a specific topographical pattern in normal organs notably the gastro-intestinal tract, while in the neoplastic mucinous ovarian cystoma occasional random acini are found to escape the suppression effect.

Comments on Histologic Findings.—The wide distribution of the blood group antigens has been amply demonstrated in a variety of tissues and secretions, including those of fetuses. In the case of water-soluble antigens, their source is seen in the cytoplasm of the secreting cells, and their course can be followed in the lumens of the glands and/or over surfaces of mucous membranes. The urinary excretion of these antigens, in secretor individuals (including fetuses) can be followed in the lower urinary tract, where they are found accreted on the luminal aspect of cells of the collecting tubules and transitional epithelium. This interpretation is strengthened by an observation (18) that cells from the latter could readily adsorb the group substance when suspended in secretor saliva.

Tracing of the group substances in the large intestine showed their rapid disappearance distal to the hepatic flexure, a sequence previously described for the A antigen in extraction studies (47). This condition differs from that found in the fetuses and newborns in whom the whole length of the colon produces abundant amounts of ABH substances. The change to adult status supervenes during the neonatal period with the establishment of a bacterial flora, presumably responsible for the enzyme(s) degrading the blood group substances (22).

The problem of origin of the antigens in cell walls of epithelium and endothelium is complicated by the fact that the adsorption of antigens from the surrounding fluids onto cell surfaces can be accomplished experimentally (18, 41). It would seem doubtful whether the concentration of group substances in the plasma and tissue fluids is sufficient to be a factor, although in the salivary glands and in the breast the secreted antigen may contribute to the outlining

[5] The exclusive finding of "aberrant secretors" in gynecological surgical material is no doubt a reflection of its preponderance within the total material available for this study.

of the walls of the glandular cells. The most convincing circumstance arguing for the generally autochthonous character of the cell-wall antigens, however, is their appearance in embryos, long antedating that of the water-soluble forms, and their presence in non-secretors in locations devoid of the water-soluble substances. Under this heading can be included: the vascular endothelium; skin; buccal, esophageal, exocervical, respiratory, calyceal, and vesical epithelia.

With regard to the problem of alcohol solubility, it has been surmised, if not proven, that abolition of staining of endothelium and the various epithelia obtained by pre-exposure of sections to alcohol is due to dissolving of the antigens off the wall of the cells (42). An alternative suggestion to be considered is based on the idea that serologic potency of the purified blood group substance may be reduced by changes in spatial orientation of the specific groups of the macromolecule (28). Could alcohol promote such reduction in endothelial and epithelial cells? Such explanation would seem to be in keeping with reports (18) that there is residual antigen demonstrable after alcohol treatment by the mixed agglutination technique.

SUMMARY AND CONCLUSIONS

The H antigen was mapped out by immunofluorescence in human tissues (including those of fetuses from 15 cm crown-heel length) from individuals of the various groups within the ABO system, both secretors and non-secretors. The distribution of the antigen can be summarized under the following headings:

Cell walls of endothelium: present throughout the cardiovascular system;

Cell walls of stratified epithelia: in skin, non-cornifying squamous stratified membranes, transitional epithelia;

Mucus: occurring wherever the latter is produced in secretor individuals and confined to a few special topographical areas in non-secretors;

Secretions and excretions: the pancreatic and sudoriferous (independent of secretor status), and mammary and uterine (governed by the secretor makeup) all contain it.

The distribution of the H antigen is most fully represented in tissues of group O. It follows an over-all universal pattern, characteristically modified in non-secretors, equally valid for antigens A and B described in a preceding study. Within this pattern, in tissues of the non-O groups, the complement of the H substance in its various forms wanes in a manner consistent with the hypothesis that it serves as a substrate for the A_1, A_2, B genes, exerting their action with different degrees of efficiency.

The secretor:non-secretor phenomena can be most simply interpreted by viewing the non-secretor, recessive gene (in the homozygous, ss condition) as inhibiting the production of some of the water-soluble forms of the blood group

substances. Since the gene was never found responsible for dissociation of the H and A, B antigens its inhibitory action is thought to be wrought at the point of formation of the basic H substance or its precursor.

The author wishes to thank Dr. H. M. Bhatia of the Indian Cancer Research Centre, Bombay, and Prof. W. T. J. Morgan of the Lister Institute of Preventive Medicine, London, for their cooperation in the production of the hyperimmune Bombay serum.

Thanks are due to the chiefs of the Departments of Pathology and their staffs at the Beth Israel, Boston Lying-In, Peter Bent Brigham, and Veterans Administration Hospitals in Boston for making available autopsy and surgical material.

Great indebtedness is acknowledged to Dr. F. H. Allen, Jr. of the Blood Grouping Laboratory of Boston for advice and helpfulness during the serologic part of the investigation and for the Lewis typing of numerous samples, and to Dr. Angelyn A. Konugres of the same Laboratory for many erythrocyte samples and help with some absorption experiments on the Bombay serum. Dr. Harold Baer of the Microbiology Department, School of Medicine, Tulane University, and Dr. C. McNeil of the Holy Cross Hospital, Salt Lake City, kindly supplied chicken sera and *Lotus tetragonolobus* extract, respectively, employed in some preliminary experiments.

Mrs. Barbara J. Kelly, B.S. and Mrs. Josephine H. Bristol, M.A. are to be thanked for highly competent technical assistance.

BIBLIOGRAPHY

1. Bernstein, F., Ergebnisse einer biostatistischen zusammenfassenden Betrachtung über die erblichen Blutstruksturen des Menschen, *Klin. Woch.*, 1924, **3,** 1495.
2. Bhende, Y. M., Dsehpande, C. K., Bhatia, H. M., Sanger, R., Race, R. R., Morgan, W. T. J., and Watkins, W. M., A "new" blood group character related to the ABO system, *Lancet*, 1952, **1,** 903.
3. Boorman, K. E., Dodd, B. E., and Gilbey, B. E., A serum which demonstrates the co-dominance of the blood group gene O with A and B, *Ann. Eugenics*, 1948, **14,** 201.
4. Brown, P. C., Glynn, L. E., Holborow, E. J., and Johnson, G. D., Differences between secretors and non-secretors in the distribution of blood group substances in the alimentary tract, *in* report on International Symposium on Immunology and Biochemistry of Human Blood, *Vox Sanguinis*, 1961, **6,** 232.
5. Buchanan, D. J., and Rapoport, S., Composition of meconium: Serological study of blood-group-specific substances found in individual meconiums, *Proc. Soc. Exp. Biol. and Med.*, 1951, **77,** 114.
6. Cazal, P. and Lalaurie, M., Recherches sur quelques phyto-agglutinines spécifiques des groupes sanguins ABO, *Acta Haematol.*, 1952, **8,** 73.
7. Ceppellini, R., Immunogenetica 1. Analisi fenotipica al livello submolecolare: Le specificità serologiche multiple dei mucoidi gruppospecifici salivari, *Folia Hered. et Path.*, 1959, **8,** 201.
8. Ceppellini, R., Physiological genetics of human blood factors, *in* Biochemistry of Human Genetics, Ciba Foundation Symposium jointly with International Union of Biological Sciences, (G. E. W. Wolstenholme and C. M. O'Connor, editors), London, J. & A. Churchill Ltd., Boston, Little, Brown and Co., 1959, 242.
9. Ceppellini, R., Dunn, L. C., and Innella, F., Immunogenetica 2: Analisi genetica

formale dei caratteri Lewis con particolare riguardo alla natura epistatica della specificità serologica Le^b, *Folia Hered. et Path.*, 1959, **8,** 261.

10. Coons, A. H., and Kaplan, M. H., Localization of antigen in tissue cells, *J. Exp. Med.*, 1950, **91,** 1.
11. Eisler, M., Über ein gemeinsames, Antigen in den Zellen des Menschen und in Shigabazillen, *Z. Immunitätsforsch.*, 1930, **67,** 38.
12. Formaggio, T. G., Development and secretion of the blood group factor "O" in the newborn, *Proc. Soc. Exp. Biol. and Med.*, 1951, **76,** 554.
13. Glynn, L. E., Holborow, E. J. and Johnson, G. D., The distribution of blood group substances in human gastric and duodenal mucosa, *Lancet*, 1957, **2,** 1083.
14. Goldman, M., Staining *Toxoplasma gondii* with fluorescein-labelled antibody, *J. Exp. Med.*, 1957, **105,** 557.
15. Hakim, S. A., Vyas, G. N., Sanghvi, L. D. and Bhatia, H. M. Eleven cases of "Bombay" phenotype in six families: Suppression of ABO antigen demonstrated in two families, *Transfusion*, 1961, **1,** 218.
16. Hirszfeld, L., Hauptprobleme der Blutgruppenforschung in den Jahren 1927–33, *Ergebn. Hyg., Bact., Immunitätsforsch., u. exp. Therap.*, 1934, **15,** 54.
17. Hirszfeld, L. and Amzel, R., Sur les pléiades "isozériques" du sang. Contribution à l'étude de sous-groupes sanguins, *Ann. Inst. Pasteur*, 1940, **65,** 251.
18. Holborow, E. J., Brown, P. C., Glynn, L. E., Hawes, M. D., Gresham, G. A., O'Brien, T. F., and Coombs, R. R. A., The distribution of the blood group A antigen in human tissues, *Brit. J. Exp. Pathol.*, 1960, **41,** 430.
19. Jones, A. R., and Kaneb, L., The relationship of the A and H antigens of erythrocytes of sub-group A_2, *Vox Sanguinis*, 1961, **6,** 110.
20. Jonsson, B., Blutgruppenstudien mit Japanischen und Schwedischen Aalseren, *Acta. Pathol. Microbiol. Scand.*, 1944, suppl. 54, 456.
21. Kabat, E. A., Blood group substances. Their chemistry and immunochemistry, New York, Academic Press, Inc., 1956, chapter 3.
22. Kabat, E. A., Blood group substances. Their chemistry and immunochemistry, New York, Academic Press, Inc., 1956, 88.
23. Lambert, J., L'antigène O. Sa répartition et ses rapports avec les antigènes de groupe, *Rev. Belge des Sciences Médicales*, 1941, **13,** 1.
24. Lawler, S. D., Blood group substances in human milk, *Proc. 7th Congr. Europ. Soc. Haematol.*, Part II, Basel, S. K. Karger, 1960, 1219.
25. Levine, P., Robinson, E., Celano, M., Briggs, O., and Falkinburg, L., Gene interaction resulting in suppression of blood group substance B., *Blood*, 1955, **10,** 1100.
26. McNeil, C., Trantelman, E. F., Kreutzer, V. O. and Fullmer, C. D., Aberrant secretion of salivary A, B and H group substances in human beings, *Am. J. Clin. Path.*, 1957, **28,** 145.
27. Morgan, W. T. J., Some immunochemical aspects of the products of human blood group genes, *in* Biochemistry of Human Genetics, Ciba Foundation Symposium jointly with International Union of Biological Sciences, (G. E. W. Wolstenholme and C. M. O'Connor, editors), London, J. & A. Churchill Ltd., Boston Little, Brown and Co., 1959, 194.

28. Morgan, W. T. J., A contribution to human biochemical genetics; the chemical basis of blood group specificity, (Croonian lecture), *Proc. Roy. Soc.*, London, Series B, 1960, **151,** 308.
29. Morgan, W. T. J., and Waddell, M. B. R., A specific blood group O substance, *Brit. J. Exp. Path.*, 1945, **26,** 387.
30. Morgan, W. T. J., and Watkins, W. M., The detection of a product of the blood group O gene and relationship of the so-called O substance to the agglutinogens A and B, *Brit. J. Exp. Path.*, 1948, **29,** 159.
31. Morganti, G., Cresseri, A., Serra, A., Beolchini, P. E., Barbaini, S., and Gianotti, G. A., Comparative studies on the A, B, O(H) blood substances in the saliva and the milk, *Vox Sanguinis*, 1959, **4,** 267.
32. Moureau, P., L'Antigène O dans ses rapports avec la génétique et la sérologie, *Rev. d'Hématol.*, 1946, **1,** 266.
33. Pottenkofer, H. J., Luboldt, W., Lanoun, H., and Niebuhr, R., Über genetische Suppresion der Blutgruppen ABO. Untersuchungen an eine Familie, bei der die Unterdrückung nicht das Blutgruppenmerkmal B betrifft, *Z. Immunitätsforsch.*, 1960, **120,** 288.
34. Race, R. R., and Sanger, R., Blood groups in man, Oxford, Blackwell Scientific Publications, 2nd edition, 1950, 39.
35. Renkonen, K. O., Studies on hemagglutinins present in seeds of some representatives of family *Leguminoseae, Ann. Med. Exp. et. Biol. Fenniae*, 1948, **26,** 65.
36. Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., and Metcalf, T. G., Isocyanate compounds as fluorescent labelling agents for immune serum, *Am. J. Pathol.*, 1958, **43,** 1081.
37. Sasaki, H., Über das Vorkommen gruppenspezifischer Eigenschaften im Speichel und anderen Körperflüssigkeiten und den Nachweis zweier Ausscheidungstypen, *Z. Immunitätforsch*, 1932, **77,** 101.
38. Schiff, F., Über den serologischen Nachweis der Blutgruppeneigenschaft O, *Klin. Woch.*, 1927, **6,** 303.
39. Springer, G. F., Relation of blood group active plant substances to human blood groups, *Acta Haematol.*, 1958, **20,** 147, and personal communication.
40. Springer, G. F., Rose, C. S., and György, P., Blood group mucoids: Their distribution and growth-promoting properties for *Lactobacillus bifidus* var. Penn., *J. Lab. and Clin. Med.*, 1954, **43,** 532.
41. Swineburne, L. M., Frank, B., and Coombs, R. R. A., The A antigen on the buccal epithelial cells of man, *Vox Sanguinis*, 1961, **6,** 274.
42. Szulman, A. E., The histological distribution of blood group substances A and B in man, *J. Exp. Med.*, 1960, **111,** 785.
43. Watkins, W. M., The O and H blood group characters in the newborn, *Brit. J. Exp. Path.*, 1952, **33,** 244.
44. Watkins, W. M., Some genetical aspects of the biosynthesis of human blood groups substances, *in* Biochemistry of Human Genetics, Ciba Foundation Symposium jointly with International Union of Biological Sciences, (G. E. W. Wolstenholme and C. M. O'Connor, editors), London, J. & A. Churchill Ltd., Boston, Little, Brown and Co., 1959, 217.

45. Watkins, W. M., and Morgan, W. T. J., Some observations on the O and H characters of human blood and secretions, *Vox Sanguinis*, 1944, **5**, 1.
46. Witebsky, E., and Klendshoj, N. C., The isolation of an O specific substance from gastric juice of secretors and carbohydrate-like substances from gastric juice of non-secretors, *J. Exp. Med.*, 1941, **73**, 655.
47. Witebsky, E., and Neter, E., Distribution of blood group properties and blood group property destroying factors in the intestinal tract of man, *J. Exp. Med.*, 1935, **62**, 589.

EXPLANATION OF PLATES

PLATE 106

FIG. 1. Endothelium of lung capillaries in a fetus, 19 cm crown-heel length (*ca.* 4½ months gestation), group O. Bombay conjugate. Pulmonary capillaries show feeble immunofluorescence and are rarely visualized after birth. Note staining of occasional bronchial epithelial cells. × 170.

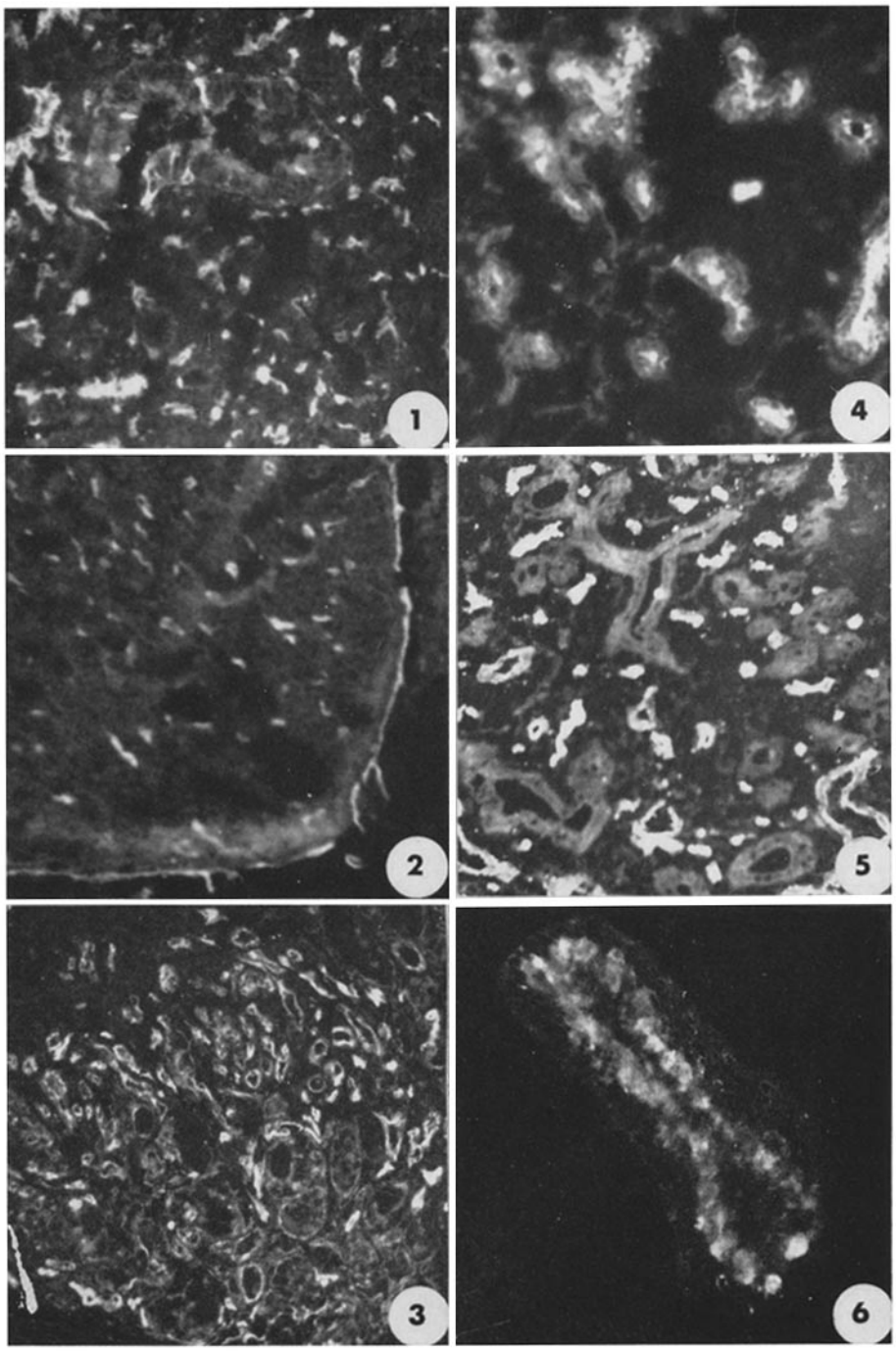
FIG. 2. Endothelium of endocardium and myocardial capillaries in the same fetus as in Fig. 1. Bombay conjugate. × 170.

FIG. 3. Hemangioma of subcutaneous tissue, child, group O. Bombay conjugate. Note clear outlining of endothelium of most vessels. × 170.

FIG. 4. Submaxillary gland, newborn, group O, secretor. Bombay conjugate. Mucus-borne antigen in ductules and acini. × 170.

FIG. 5. Submaxillary gland, newborn, group O, non-secretor. Bombay conjugate. Outlining of glandular elements due to autofluorescence; clear specific staining of vessels. × 170.

FIG. 6. Uterine endocervix, medium power view of a gland, group B, secretor. Bombay conjugate. Outlining of glandular epithelium mainly due to autofluorescence with mucus-bound antigen visualized at the luminal aspect of cytoplasm of cells and in the lumen of the gland. × 300.



(Szulman: Blood group antigens H and A, B in man)

PLATE 107

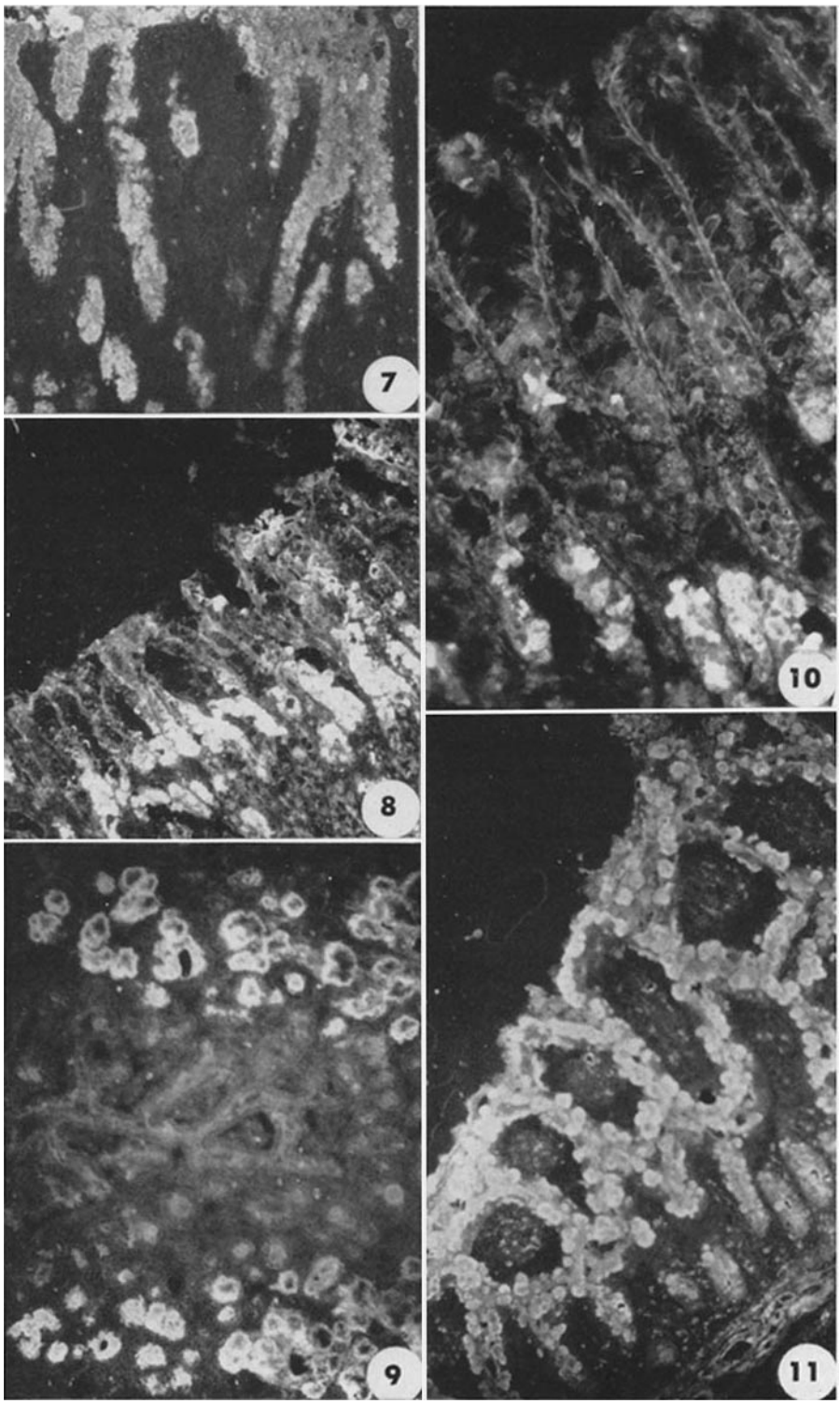
FIG. 7. Stomach (pre-pylorus), group A, secretor. Bombay conjugate (identical picture obtained with anti-A conjugate). Abundant mucus-borne antigen at all levels of mucosa including surface. × 100.

FIG. 8. Stomach (body): general view of mucosa, group O, non-secretor. Chicken anti-H serum and rabbit anti-chicken conjugate (identical picture obtained with Bombay conjugate). Mucus-bound antigen confined to deep parts of foveolae. × 70.

FIG. 9. Small intestine, general view of mucosa, newborn, group O, non-secretor. Bombay conjugate. Secretion of mucus-bound antigen in the deeper parts of crypts; negative villi seen in the lumen of the bowel, center of photograph. × 70.

FIG. 10. Stomach (body), group O, non-secretor. Bombay conjugate. Mucus-borne antigen in deep parts of foveolae; note its absence from the superficial levels. × 170.

FIG. 11. Ileum, mucosa, group O, secretor. Bombay conjugate. Abundant secretion of mucus-bound antigen at all levels; individual goblet cells are distinguishable.



(Szulman: Blood group antigens H and A, B in man)

PLATE 108

FIG. 12. Uterine exocervix, group O. Bombay conjugate. Clear outlining of cells, excluding the basal layer; section cut somewhat obliquely, showing two dermal papillae. × 170.

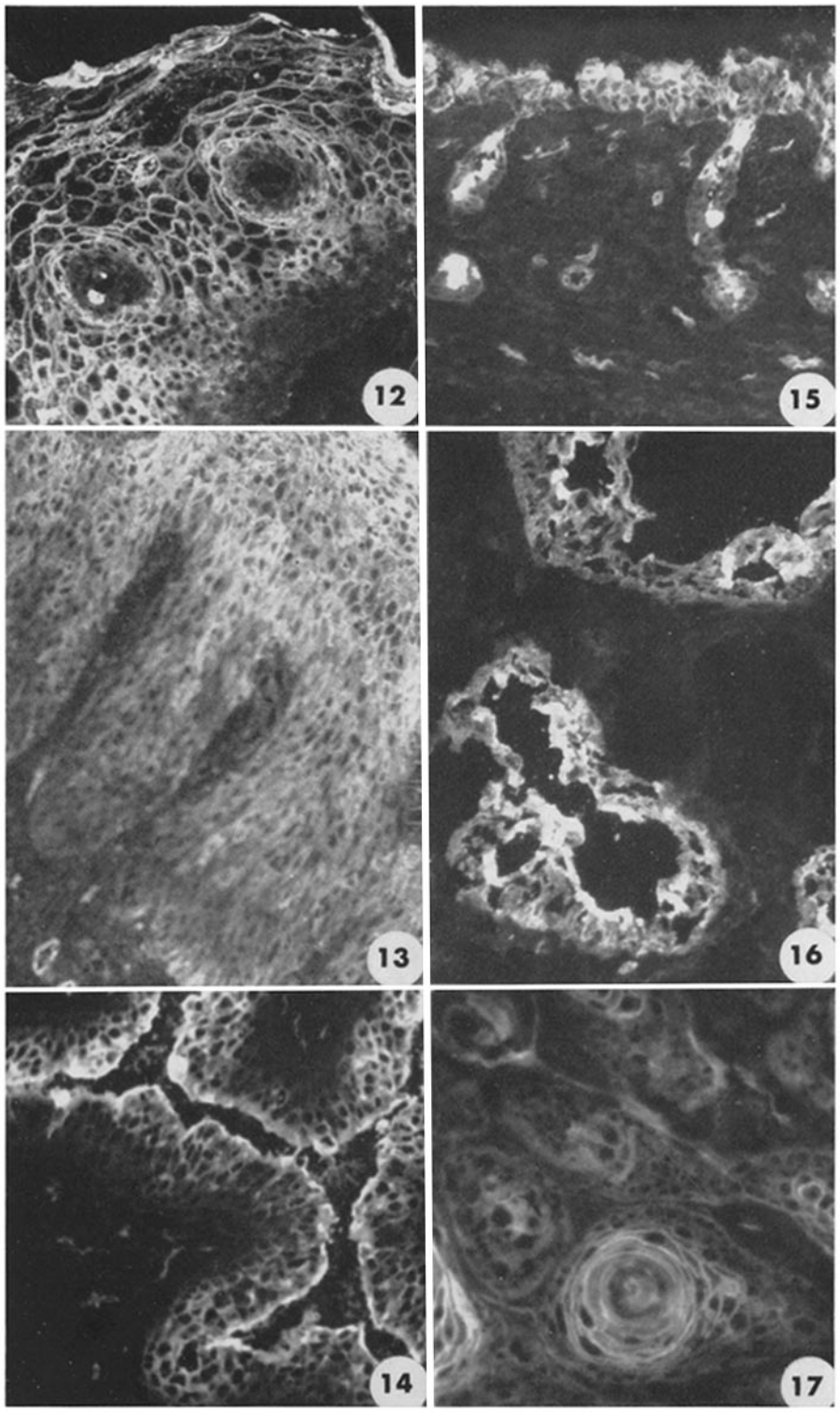
FIG. 13. Uterine cervix, carcinoma in situ, group A_2. Bombay conjugate (virtually identical picture obtained with anti-A conjugate). Good specific outlining of cells including stretches of basal cells. × 300.

FIG. 14. Esophagus, mucosa, fetus *ca.* 4½ months gestation (same as in Figs. 1 and 2), group O. Bombay conjugate. Specific outlining of stratified squamous cells excepting the basal layer; a continuous rim of mucus-borne antigen lines the lumen. × 170.

FIG. 15. Larynx, mucosa, fetus *ca.* 4½ months (same as in Figs. 1, 2, and 14), group O, secretor. Bombay conjugate. Specific outlining of stratified cells, including basal layer; mucus-borne antigen seen in the glands, ducts, and on the lining surface. × 170.

FIG. 16. Renal pelvis and calyx, fetus 15 cm crown-heel length, *ca.* 4 months gestation, group O, secretor. "Bombay" conjugate. Specific outlining of transitional epithelium, including basal layer; accretion of antigen on luminal aspect of membrane. × 170.

FIG. 17. Carcinoma of skin, well differentiated, group O. Bombay conjugate. Clear outlining of neoplastic squamous cells; the core of the "pearl" is visualized due to bright non-specific autofluorescence. × 170.



(Szulman: Blood group antigens H and A, B in man)

PLATE 109

FIG. 18. Mammary lobule from a non-pregnant woman, group O, secretor. Bombay conjugate. Note the haphazard specific staining: antigen present in some ductules (or acini) and absent in others; good outlining of epithelial cell walls and capillaries. × 170.

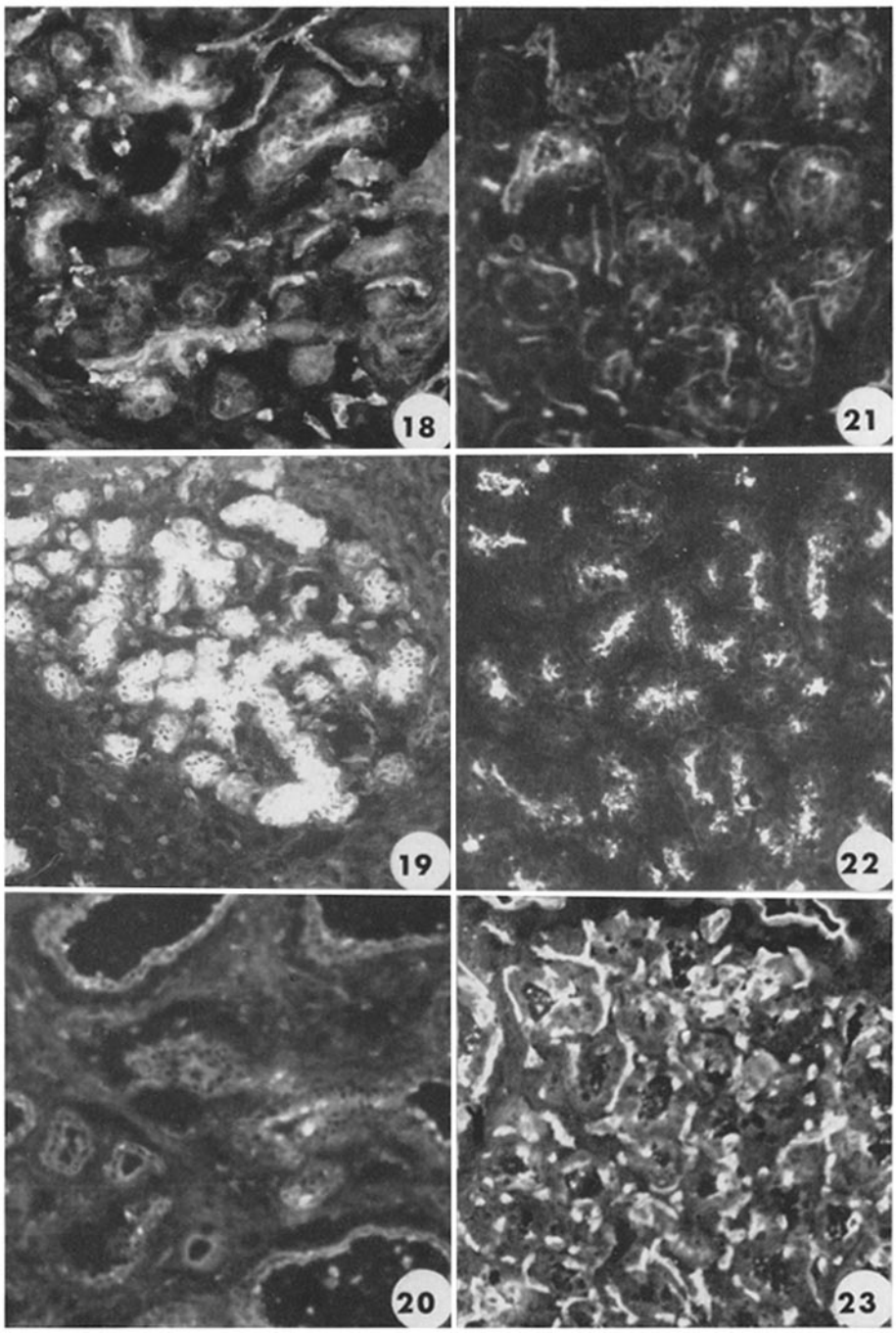
FIG. 19. Mammary lobule from a woman in latter half of pregnancy, group O, secretor. Bombay conjugate. Large amount of secretion uniformly rich in antigen; outlining of gladular epithelium. × 170.

FIG. 20. Cystic disease of breast, group B, secretor. Bombay conjugate (virtually no staining of secretion obtained with anti-B conjugate). Luminal "capping" of epithelial cells and occasional droplets in the lumen; no vessels visualized. × 170.

FIG. 21. Lactating breast, group B, secretor. Anti-B conjugate. A part of a lobule with several acini showing antigen, while others (left lower corner) are completely negative; periacinar capillaries. × 170.

FIG. 22. Section from the same organ as Fig. 21, stained with Bombay conjugate showing abundant secretion-borne H antigen. × 170.

FIG. 23. Lactating breast, group A, non-secretor. Anti-A conjugate. No secretion-borne antigen visualized with either anti-A or Bombay conjugate. Stained with the former, this section demonstrates periacinar capillary network only. × 170.



(Szulman: Blood group antigens H and A, B in man)

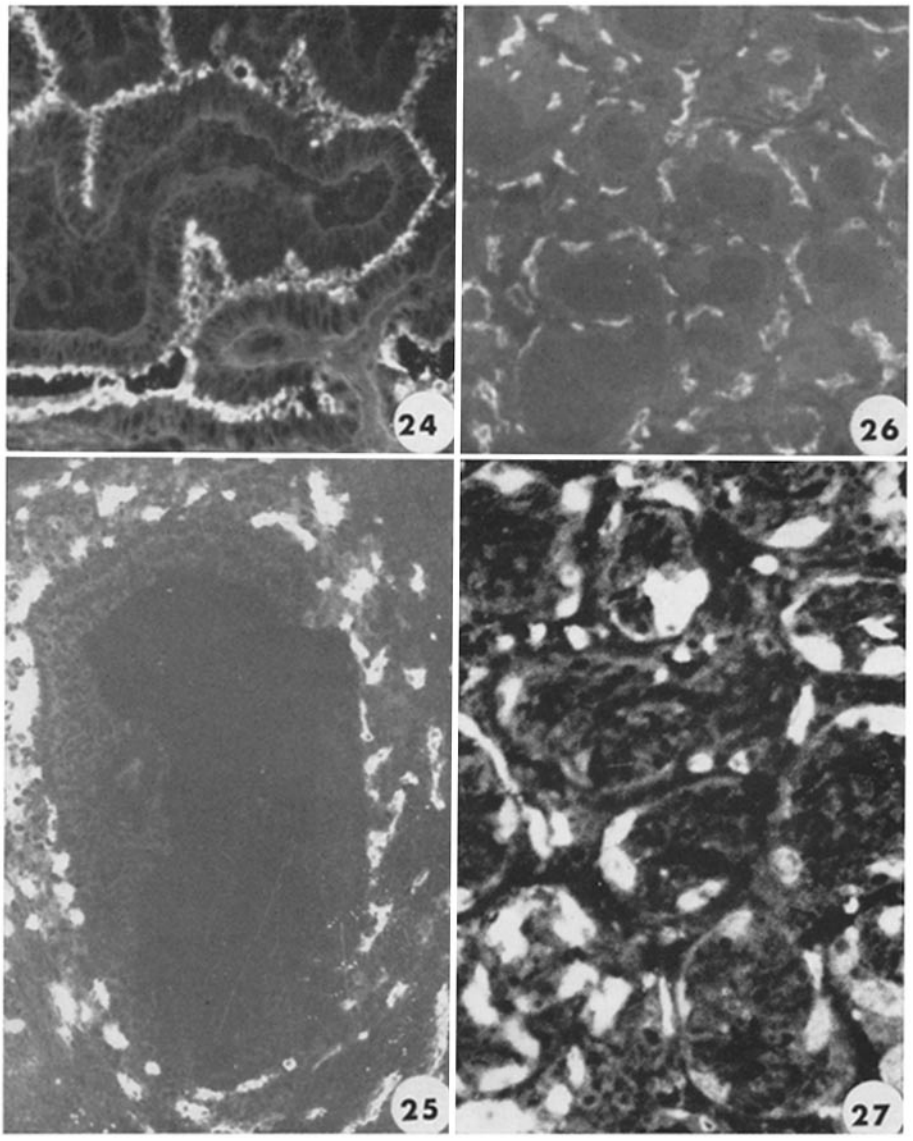
PLATE 110

FIG. 24. Fallopian tube, group O, secretor. Bombay conjugate. Strong luminal capping of epithelium of rugae. × 170.

FIG. 25. Graafian follicle, group O. Bombay conjugate. The ovum and granulosa cells are visualized due to weak autofluorescence only. The follicle is outlined by strong staining of stromal capillaries. × 170.

FIG. 26. Thyroid, group O. Bombay conjugate. The epithelium of the gland is negative; the acini are surrounded by a dense capillary network which stains as usual. × 170.

FIG. 27. Parietal cells of gastric glands, group B. Anti-B conjugate. Specific staining of parietal cells (and capillaries). These cells cannot be stained for the H antigen. × 480.



(Szulman: Blood group antigens H and A, B in man)