

IMMUNOFLUORESCENT EXAMINATION OF THE HUMAN
CHORIONIC VILLUS FOR BLOOD GROUP A AND B
SUBSTANCE*

BY H. A. THIEDE, M.D., J. W. CHOATE,† M.D., H. H. GARDNER, M.D.,
AND H. SANTAY

(From the Department of Obstetrics and Gynecology, University of Rochester School of
Medicine and Dentistry, Rochester, New York)

PLATES 82 AND 83

(Received for publication, March 8, 1965)

The placenta is a homograft of unusual tenure. Several theories have been proposed in an attempt to explain this apparent homograft immunity. Basic to this problem is the question of trophoblastic antigenicity. One facet of this question is the transportation of trophoblast into the maternal circulation in normal gestations. Since these cells are of fetal origin the possibility of ABO isoimmunization occurring secondary to their transportation is evident. Such an immunologic response might also contribute to the rapid destruction of these cells by the maternal host.

Of equal interest is a large body of statistical evidence which points to the selective intrauterine loss of gestations associated with fetal-maternal incompatibility within the ABO system.

These questions have led us to study human placentas of various gestational ages for the presence of blood group substances by means of immunofluorescent (IF) staining. This paper presents the first phase of this work, the search for A and B substance in the trophoblast of the human term placenta.

Materials and Methods

Tissue.—All tissue specimens were obtained at the time of delivery or surgical removal and were quick-frozen in egg albumin mounts in a dry ice-acetone mixture at -40°C as described by Rahman and Luttrell (1). These specimens include representative areas of term placentas, blocks of adult myometrium, cervix, stomach, and fetal kidney. These tissue blocks were stored at -20°C until sectioning in a cryostat immediately prior to incubation with the anti-serum.

Blood Grouping and Secretor Status.—The ABO blood group of each tissue donor was ascertained by hemagglutination tube typing employing standard human antisera.¹ The AB secretor status was determined with saliva by means of the hemagglutination inhibition method of Kabat (2).

* This investigation was supported by United States Public Health Service Grant HD 00509.

† Postdoctoral Trainee, United States Public Health Service Grant 2G-590.

¹ Dade Reagents, Inc., Miami, Florida.

Preparation of Antisera.—

Heterologous antiserum: Adult male albino rabbits (4 kg.) with naturally occurring anti-A were bled prior to immunization and the serum stored in small aliquots at -20°C . These rabbits were then inoculated intravenously with 3 ml of 3 times washed blood group A₁ Rh-negative human erythrocytes on alternate days for a total of 9 injections. The rabbits were bled either by ear vein or cardiac puncture 10 days after the last injection. In addition, rabbit antiserum to human A substance extracted from ovarian cyst fluid was generously provided by Dr. Harold Baer (3).² These crude heterologous antisera were stored at -20°C .

Absorption and characterization of the sera were performed immediately prior to their use in immunofluorescent studies. The crude immune rabbit serum was heat-inactivated at 56°C for 30 minutes. Absorption with guinea pig kidney suspension³ (5 ml/ml antiserum) was carried out for 20 minutes at 4°C and then at room temperature for 30 minutes. The resulting supernate was absorbed with blood group O amnion powder (100 mg/ml of antiserum) for 1 hour at room temperature. The supernate was recovered by high-speed centrifugation at 14,000 RPM for 20 minutes at 7°C . Using 4 times washed human blood group O and B Rh-positive MN erythrocytes (0.1 ml packed cells/ml of antiserum) the antiserum was absorbed for 15 minutes at room temperature and centrifuged at 4000 RPM for 10 minutes. This was repeated until the serum failed to agglutinate B and O erythrocytes on a slide test.

In addition to the above absorptions the crude preimmune serum was also absorbed with blood group A Rh-positive MN human erythrocytes.

Homologous antiserum: Naturally occurring human anti-A serum of high titer was obtained from a blood group O donor. Human anti-B serum was obtained from the Boston Blood-Grouping Laboratory, Boston, Massachusetts. Both these sera were heated at 56°C for 30 minutes prior to titering.

Characterization of Antibody.—

Heterologous antiserum: (a) Forssman activity (sheep cell hemolysis) was determined by incubating at 37°C 0.1 ml of a 5 per cent suspension of sheep red blood cells, 0.1 ml of a 1:20 dilution of guinea pig complement, and 0.1 ml of serial twofold dilutions of the absorbed antiserum. Unabsorbed antiserum served as a positive control. (b) ABO isoagglutinin titers were ascertained by titration of serial twofold dilutions of the absorbed antiserum using a 2 per cent suspension of washed human erythrocytes in saline and in fresh autologous serum. Following incubation for 1 hour at room temperature agglutination was checked both macroscopically and microscopically. Red blood cells which showed no agglutination were washed in saline and incubated for 1 hour at room temperature with sheep anti-rabbit globulin⁴ and once again examined for agglutination. The sheep antiglobulin used did not agglutinate human washed type O Rh-positive erythrocytes. (c) Hemagglutination inhibition was ascertained by neutralizing the antiserum with A and B substance⁵ using mixtures of 2:1, 1:1, and 1:2. The reaction was carried on for 15 minutes at room temperature. Serum alone served as a control. The neutralized serum was diluted 1:3 in both saline and in fresh human group A non-secretor serum. Serial twofold dilutions were then made in saline of the mixture diluted with saline, and in serum with the mixture diluted with serum. A 2 per cent suspension of washed A₁ and A₂ human erythrocytes from non-secretors was used as test cells for tube agglutination and/or hemolysis after incubation at room temperature for 1 hour. The indirect antiglobulin test was used as described above. (d) The absorbed antiserum was incubated at 37°C for 60 minutes with a panel of human reagent red blood cells for detection of antibody to other erythrocyte antigens according to the method outlined by the Ortho Pharmaceutical Corporation.

² Division of Biologic Standards, National Institutes of Health, Bethesda, Maryland.

³ Certified Blood Donor Service, Inc., Jamaica, New York.

⁴ Ortho Pharmaceutical Corporation, Raritan, New Jersey.

⁵ Merck, Sharp & Dohme, West Point, Pennsylvania.

Homologous antiserum: The ABO isoagglutinin titers and the hemagglutination inhibition titers were determined in the same manner as described for the heterologous antiserum except that rabbit anti-human globulin⁴ was employed for the indirect Coombs reagent.

Preparation of Fluorescein Conjugate.—The method used for coupling the isoagglutinin with fluorescein is described in detail by Goldstein, *et al.* (4). Briefly, the crude antiserum was initially fractionated with cold ammonium sulfate, and the gamma globulin portion then obtained by means of a DEAE cellulose column. After conjugation of this fraction with fluorescein isothiocyanate (1 mg/200 mg protein) the uncoupled fluorescein radicals and impurities were removed on a sephadex⁶ column. Conjugates with optimal fluorescein:protein (F:P) ratios were obtained by gradient elution from a DEAE cellulose column. The final F:P ratio was calculated using a Beckman DU spectrophotometer at 280 m μ and 495 m μ . The anti-A and the anti-B titers of the conjugated antisera were determined and the conjugate frozen in small aliquots and kept at -20°C until just before use.

Commercially prepared, fluorescein conjugated, goat anti-rabbit gamma globulin⁷ was absorbed with human liver nuclear sediment prepared according to the method of Hogeboom and Schneider (5). The conjugate and liver nuclear sediment were incubated at 37°C for 90 minutes followed by centrifugation at 10,000 rpm for 10 minutes. The absorbed conjugate was stored in 5 ml aliquots at -20°C .

Fluorescent Staining Procedures.—

Indirect: Immediately prior to staining the blocks of albumin-embedded tissue were sectioned at 8 m μ in a cryostat at -25°C . The sections were thawed and air dried at room temperature for 10 minutes. The sections were then fixed in acetone for 2 minutes, rinsed in 0.5 M phosphate-buffered saline, pH 7.2, and overlaid with rabbit anti-A. The slides were then placed in Petri dishes containing moistened filter paper for 90 minutes at room temperature. After 10 minutes of rinsing the excess buffer solution was removed from the slides and the sections covered with fluorescein conjugate for 45 minutes. The sections were again rinsed in phosphate-buffered saline for 10 minutes and placed in a 50 per cent solution of glycerol and phosphate-buffered saline. After 8 to 10 hours the IF preparations were covered with No. 1 coverslips and sealed with 90 per cent glycerol in phosphate-buffered saline. The preparations were examined for fluorescence with a Leitz ortholux microscope using an Osram HBO 200 mercury arc lamp with appropriate ultraviolet filters (3650 angstrom).

Direct: Sections of albumin-embedded tissue were air dried and fixed in acetone for 2 minutes and then rinsed in buffered saline for 10 minutes. The sections were then overlaid with the fluorescein-conjugated homologous antiserum and placed in Petri dishes containing moistened filter paper for 60 minutes at room temperature. The slides were then rinsed in 0.5 M phosphate-buffered saline, pH 7.2, for 10 minutes. The IF preparations were then covered with coverslips and examined immediately for fluorescence.

Routine immunofluorescent controls were run simultaneously under identical conditions. These controls are listed in Table I.

Photography.—Permanent records of the observed fluorescence were obtained with Kodak 35 mm high speed Ektachrome film (daylight type) with an exposure time of 4 minutes. The coordinates of the photographed area were carefully recorded so that the area could be rephotographed after histologic staining of the fluorescent sections.

Histologic Counterstaining.—Following photography of the fluorescent areas the slides were rinsed in phosphate-buffered saline and the coverslips floated off. The sections were then stained with either May-Grünwald-Giemsa (MGG) or with a PAS stain and then rephotographed.

⁶ Pharmacia Fine Chemicals, Inc., New York, N.Y.

⁷ Microbiological Associates, Bethesda, Maryland.

TABLE I
Immunofluorescent Staining Controls

Technique	Observed fluorescence* blood type			
	A	AB	B	O
Direct ‡				
Section only	0	0	0	0
Human anti-A	0	0	0	0
Conjugated human anti-A	+	+	0	0
Human anti-A, then conjugated				
Human anti-A (blocking)	0	0	0	0
Indirect				
Section only	0	0	0	0
Preimmune rabbit serum	0	0	0	0
Absorbed fluorescein conjugate	0	0	0	0
Rabbit anti-A (RBC)	0	0	0	0
Rabbit anti-A (cyst fluid)	0	0	0	0
Preimmune rabbit serum + conjugate	0	0	0	0
Rabbit anti-A (RBC) + conjugate	+	+	0	0
Rabbit anti-A (cyst fluid) + conjugate	+	+	0	0

* Myometrium.

‡ Identical controls were used with the conjugated human anti-B serum.

RESULTS

The absence of sheep cell hemolysis indicated that all anti-Forsman activity was removed from the crude rabbit antiserum following absorption with guinea pig kidney suspension. Table II summarizes the results of the direct erythrocyte agglutination studies of the absorbed homologous and heterologous antisera. The titers represent the last dilution at which agglutination was demonstrable microscopically. Table III demonstrates the inhibition of this agglutination in the homologous sera by heterospecific A and B substance. The evidence for inhibition of agglutination in the heterologous sera, as shown in the same table, is not as clearly defined. No hemagglutination was observed with the reagent red blood cell panel.

The results of the direct and indirect IF staining of chorionic villi, squamous epithelium of the cervix, and myometrial vessels are shown in Table IV. The staining results were identical with both the heterologous (cyst fluid and RBC's) and the homologous immune sera. Fig. 1 *a* illustrates the typical fluorescence of small vessels in the myometrium from a patient with blood group A after incubation with fluorescein conjugated human anti-A. Fig. 1 *b* is the identical section counterstained with PAS to provide histologic confirmation of the ob-

served fluorescence. IF staining was effectively blocked in the direct technique when the sections of myometrium and cervix were preincubated with unlabeled antiserum for 60 minutes or when the sections were preimmersed in ethanol for 20 minutes. The water soluble blood group antigens were readily detected in the gastric glands of A secretors (Figs. 2 *a* and 2 *b*). No staining was found in the specimens of stomach obtained from patients of blood group O. Similarly

TABLE II
Agglutination Titers of Absorbed Antisera

Antiserum	Medium	A ₁	A ₂	B	O
Rabbit anti-A ₁ (erythrocytes)	Saline	480	960	0	0
	FAS*	1920	480	0	0
Rabbit anti-A (cyst fluid)	Saline	1280	—	0	0
	FAS	1280	—	0	0
Human anti-A	Saline	480	—	0	0
	FAS	1920	—	0	0
Human anti-B	Saline	0	—	1920	0
	FAS	0	—	1920	0

* Fresh Autologous Serum.

TABLE III
*Inhibition of Agglutination of Group A₁ Erythrocytes by Specific Blood Group Substance**

Antiserum	Medium	I‡	II	III	IV
Rabbit anti-A	Saline	192 (48)	48 (0)	6 (0)	96 (768)
	FAS§	768 (1536)	384 (768)	192 (768)	192 (192)
Human anti-A conjugate	Saline	0 (3072)	0 (3072)	0 (96)	24 (3072)
	FAS	96 (96)	0 (96)	0 (48)	768 (768)
Human anti-B conjugate	Saline	0 (3072)	0 (96)	0 (48)	0 (768)
	FAS	192 (3072)	192 (384)	96 (192)	384 (1536)

Figures in parenthesis are the indirect Coombs titer.

* Human group B erythrocytes were used to demonstrate inhibition of the human anti-B globulin.

‡ I represents 2 parts test serum with 1 part A and B substance; II represents equal parts of test serum and A and B substance; III represents 1 part test serum with two parts A and B substance; IV represents test serum only.

§ Fresh autologous serum.

the blood group antigens were also demonstrated in the collecting tubules and endothelium of vessels in the fetal kidney (Table IV).

Fluorescence was absent in the villous trophoblast of blood group A, AB, B, and O term placentas incubated with type A and B antiserum regardless of the blood group of the mother or the secretor status of the baby and mother. The

TABLE IV
Observed Fluorescence

Tissue	Blood group	Fluorescent antiserum		
		Rabbit anti-A	Human anti-A	Human anti-B
Villous trophoblast	A	0	0	0
	B	0	0	0
	O	0	0	0
Blood vessels of chorionic villi	A	0	0	0
	B	0	0	0
	O	0	0	0
Cervix uteri	A	—	+	0
	B	—	0	+
	O	—	0	0
Stomach glands	A	+	+	—
	B	—	0	—
	O	—	0	—
Blood vessels of myometrium	A	+	+	0
	B	0	0	+
	O	0	0	0
Fetal kidney	A	—	+	—
	B	—	0	—
	O	—	0	—

+ Indicates specific fluorescent staining; 0 indicates *no* specific fluorescent staining; — indicates tissue was not studied.

ABO blood group and secretor status of both mother and fetus for the 21 term placentas examined are listed in Table V.

Of special interest is the twin gestation (specimen XXXV) in which the mother and one fetus were blood group A secretor, while the other fetus was blood group O. No specific fluorescence was observed in the vessels of the chorionic villi from this placenta but the endothelium of the maternal myometrial vessels revealed specific apple-green fluorescence. With rare exceptions the villous capillaries of all the other placentas examined were equally devoid of fluorescence.

TABLE V
Blood Group and Secretor Status of Mother and Baby of Placentas Studied

Specimen	Blood group mother/fetus	Secretor status mother/fetus	Antiserum	
			Rabbit	Human
VI	A/A	Non-secretor/non-secretor	×	
VII	A/A	Secretor/secretor	×	
IX	O/A	—/Non-secretor	×	
XXI	A/A	Non-secretor/non-secretor		×
XXXV	A/A (Twins)	Secretor/secretor	×	×
	A/O	Secretor/—	×	×
XXXVII	A/A	Secretor/non-secretor		×
	A/O (Twins)	Secretor/—		×
LXVI	A/A	Secretor/secretor		×
II	O/O	—	×	
VIII	O/O	—	×	
XII	O/O	—	×	
XV	A/O	Non-secretor/—	×	
XL	O/O	—		×
L	A/O	Secretor/—		×
XVI	B/B	Secretor/secretor		×
XXVII	O/B	—/Secretor		×
XXXII	O/B	—/Not obtained		×
LXXXVI	O/B	—/Non-secretor		×
XC	A/A	Secretor/non-secretor		×
XCI	A/A	Secretor/secretor		×
XCV	A/A	Secretor/secretor		×
XCVII	O/A	—/Non-secretor		×

DISCUSSION

Our interest in studying the distribution of the ABO antigens in the human placenta is not unique. In 1928 von Oettingen and Witebsky, (6) using group-specific antisera, investigated alcoholic extracts of the placenta. They concluded that "the placenta must be regarded as a neutral organ with respect to its group characteristics." Subsequent early investigators also relying upon extraction procedures have added conflicting reports to the literature. Reich (7), in 1932, confirmed the absence of blood group A antigen in the chorion frondosum but noted its presence in the decidua vera, decidua basalis, and amnion. Witebsky and Reich (8) also reported two cases of maternal-fetal heterospecificity in which the fetal blood group antigen was found in the decidua vera. Using saline extracts of placental homogenates and hemagglutination inhibition techniques, Freda (9) detected the *maternal* blood group antigen in the amniotic fluid, fetal membranes and decidua vera only when the *mother* was a secretor, and the *fetal* blood group antigen in the decidua vera only when the *fetus* was a

secretor. Blood group substances were noted in the placental villi of 7 of the 20 full term placentas Freda examined.

In more recent years several workers such as Glynn and Holborow (10), and Szulman (11) have employed the immunofluorescent technique of Coons (12) to identify blood group antigens in various tissues. This method provides a sensitive tool for the cellular localization of blood group antigens. The IF technique, however, is susceptible to numerous pitfalls unless the experiments are carefully designed to include extensive yet relevant controls. One of the foremost controls of this staining method is the demonstration of the specificity of the antibody. The agglutination titer and the hemagglutination inhibition test (Tables II and III) indicate the serological specificity of the antisera used in our experiments. In addition we have employed the fluorescence in endothelium, squamous epithelium, and glandular secretions as further indication of antibody specificity.

In contrast to the previously described extraction procedures with placental homogenates, the fluorescent antibody technique not only obviates the necessity of separating the maternal and fetal components of the placenta, but in fact permits the simultaneous study of both. This is important in the investigation of fetal-maternal incompatibility within the ABO system, a subject of widespread interest in both genetic and immunologic areas.

In the blood group heterospecific pregnancies the stimulation of maternal antibody production by the fetal blood group antigen may occur by several different routes. These include placental transmission of fetal erythrocytes and/or the direct passage of fetal blood group substance. The greatest increase in maternal antibody titer has been shown to occur 10 to 20 days following delivery (13). This rise may then be due to the passage of amniotic fluid containing blood group substance and/or fetal erythrocytes during delivery. Another possible explanation is that the placenta and fetus constantly produce sufficient antigens to neutralize the maternal antibody and create an equilibrium. When the pregnancy is terminated the source of antigen is removed and the maternal titer rises (14). There is, however, disagreement as to the passage of blood group substance across the placenta during pregnancy and also as to whether it plays any role in immunization at delivery. A potential source of antigen that has not been investigated is the trophoblast. The transportation into the maternal blood stream of these cells which are fetal in origin is known to occur throughout pregnancy. Our findings indicate that the villous trophoblast of the term placenta is devoid of A and B substance. Presumably, therefore, the villous trophoblast is not a direct factor in maternal immunization to the fetal ABO blood group antigens during the course of pregnancy.

First trimester abortion as a consequence of ABO incompatibility between mother and fetus appears to be more than just a possibility. Chung and Morton (15), analyzing 5414 families, showed that in children of type O mothers there

was a statistically significant decline in frequency of type A and B children with increasing parity. The frequency of incompatible children of A and B mothers did not apparently decline with increase in parity. In both cases there was a significant overall deficiency of incompatible children when compared with compatible matings. From their data they concluded that "isoimmunization by fetal antigens is a major factor in the outcome of pregnancies with type O mothers" and "that few gene loci can be as important a cause of fetal death as the ABO system." Pertinent to this question is the observation of Kochwa, *et al.* (16), that the 7S fraction of maternal ABO antibodies crosses the placenta without difficulty while the 19S fraction is retained. Hostrup (17) has demonstrated A and B substance in cord serum and believes that the water soluble blood group antigen is constantly produced by the fetus in order to neutralize the maternal isoantibody. He has shown *in vitro* that A and B substance can reduce the titer of homologous 7S antibody and inhibit the sensitization of erythrocytes exposed to anti-A (18). Szulman (19), on the other hand, using the IF technique, has studied early embryos for blood group substances and finds that while the epithelial and endothelial cell wall antigens are present in the earliest specimens available, the water soluble blood group antigens are not demonstrable before the 8th week postfertilization. Since fetal death in most abortions occurs before the 8th week postfertilization he believes that the lack of the protective buffering action provided by the water soluble blood group substances prior to this time directly exposes the fetal tissue to maternal isoantibody and leads to the death of the fetus.

Szulman also noted that the epithelial cell wall blood group antigens disappeared from many organs at a time that coincided with the maturation of the organ. Although A and B substances are not present in the trophoblast of the term placenta, it is possible that the trophoblast of early placentas may contain the epithelial cell wall blood group antigens. We are currently investigating this possibility.

Perhaps of more interest is our preliminary observation that the blood vessels of the term villi from the placentas of A or B newborns, regardless of secretor status, apparently contain little or no A and B substance. Szulman (19) has found that the endothelium of the cardiovascular system throughout the body contains blood group-specific substance from the earliest embryo (18 mm) throughout adulthood. The villous capillaries are thought to originate from the mesoderm of the placenta. Since the villous mesoderm and the villous trophoblast have a common origin, *i.e.*, the primitive trophoblast, perhaps it should not be surprising to find that the A and B blood group antigens are not expressed in both the villous trophoblast and the blood vessels of the chorionic villi of the term placenta. If our subsequent investigations of the endothelium support this observation, considerable weight will be added to the contention that the fetus and its placenta are immunologically dissimilar.

SUMMARY

The chorionic villi of term placentas were examined for A and B blood group substance using the IF technique with heterologous and homologous antisera. No specific fluorescence was found in either the villous trophoblast or vessels of the chorionic villi. The implications of these findings in relation to the question of trophoblastic antigenicity are discussed.

The authors gratefully acknowledge the advice of Dr. Scott N. Swisher and Miss Norma Trabold in management of technical problems. We also thank Dr. Scott N. Swisher and Dr. John P. Leddy for their assistance in preparation of this manuscript.

BIBLIOGRAPHY

1. Rahman, A. N., and Luttrell, C. N., Albumen embedding method for frozen sectioning of fresh tissues for histological fluorescent antibody and histochemical studies, *Bull. Johns Hopkins Hosp.*, 1962, **66**, 110
2. Kabat, E. A., *Blood Group Substances: Their Chemistry and Immunochemistry*, New York, Academic Press, 1956, 56.
3. Baer, H., and Rasmussen, U., Immunization of rabbits with purified blood group A and B substances, *J. Immunol.*, 1960, **85**, 1.
4. Goldstein, G., Slizys, I. S., and Chase, M. W., Studies on fluorescent antibody staining. I. Non-specific fluorescence with fluorescein coupled sheep anti-rabbit globulin, *J. Exp. Med.*, 1961, **114**, 89.
5. Hogeboom, C. H., and Schneider, W. C., The cytoplasm, *in* *The Nucleic Acids*, (E. Chargaff and J. N. Davidson, editors) New York, Academic Press, 1955, Vol. II, 212.
6. von Oettingen, K. J., and Witebsky, E., Plazenta und blutgruppe, *Münch. med. Wochenschr.*, 1928, Suppl. 385.
7. Reich, H., Die gruppenspezifische differenzierung der plazentarorgane, *Z. Immunitätsforsch.*, 1932, **77**, 449.
8. Witebsky, E., and Reich, H., Zur gruppenspezifischen differenzierung der plazentarorgane, *Klin. Woch.*, 1932, **11**, 1960.
9. Freda, V., ABO (H) blood group substances in the human maternal-fetal barrier and amniotic fluid, *Am. J. Obst. and Gynec.*, 1958, **76**, 407.
10. Glynn, L. E., and Holborow, E. J., Distribution of blood-group substances in human tissues, *Brit. Med. Bull.*, 1959, **15**, 151
11. Szulman, A. E., The histological distribution of blood group substances A and B in man, *J. Exp. Med.*, 1960, **111**, 785.
12. Coons, A. H., Creech, H. J., Jones, R. N., and Berliner, E., The demonstration of pneumococcal antigens in tissues by the use of fluorescent antibody, *J. Immunol.*, 1942, **45**, 157.
13. Smith, G. H., Iso-agglutinin titers in heterospecific pregnancy, *J. Path. and Bact.*, 1945, **57**, 113
14. Levine, P., Hemolytic disease due to antibodies other than anti-D, *Revue Hematol.*, 1955, **10**, 215
15. Chung, S. C., and Morton, N. E., Selection at the ABO locus, *Am. J. Human Genet.*, 1961, **13**, 9.

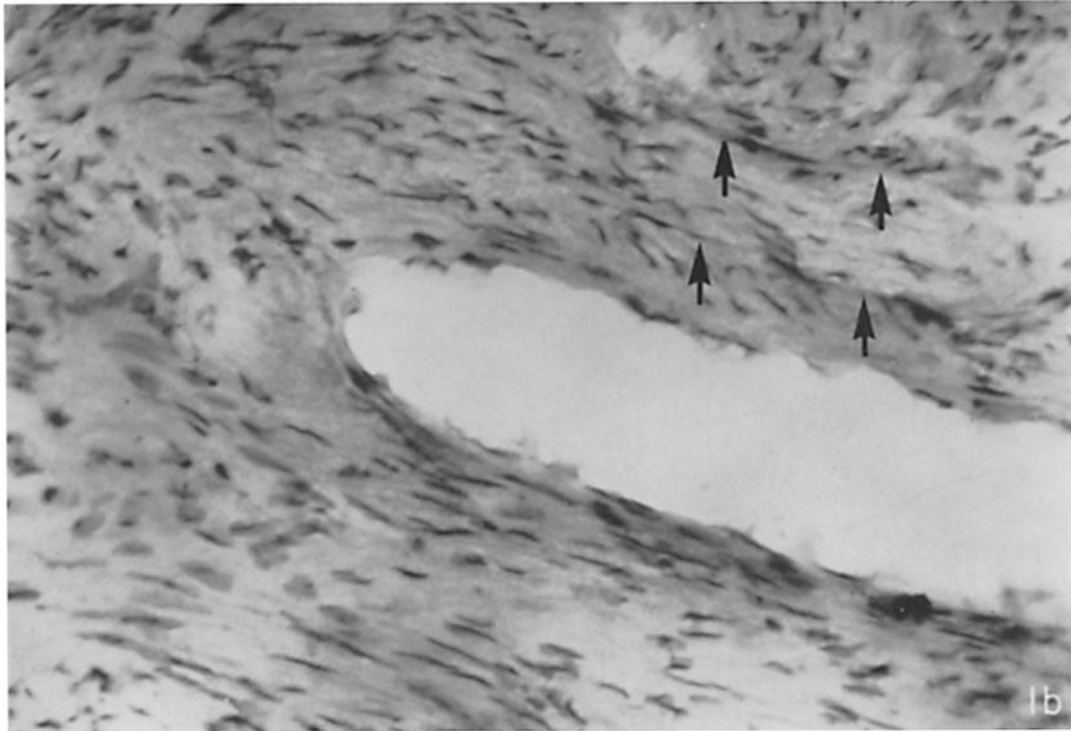
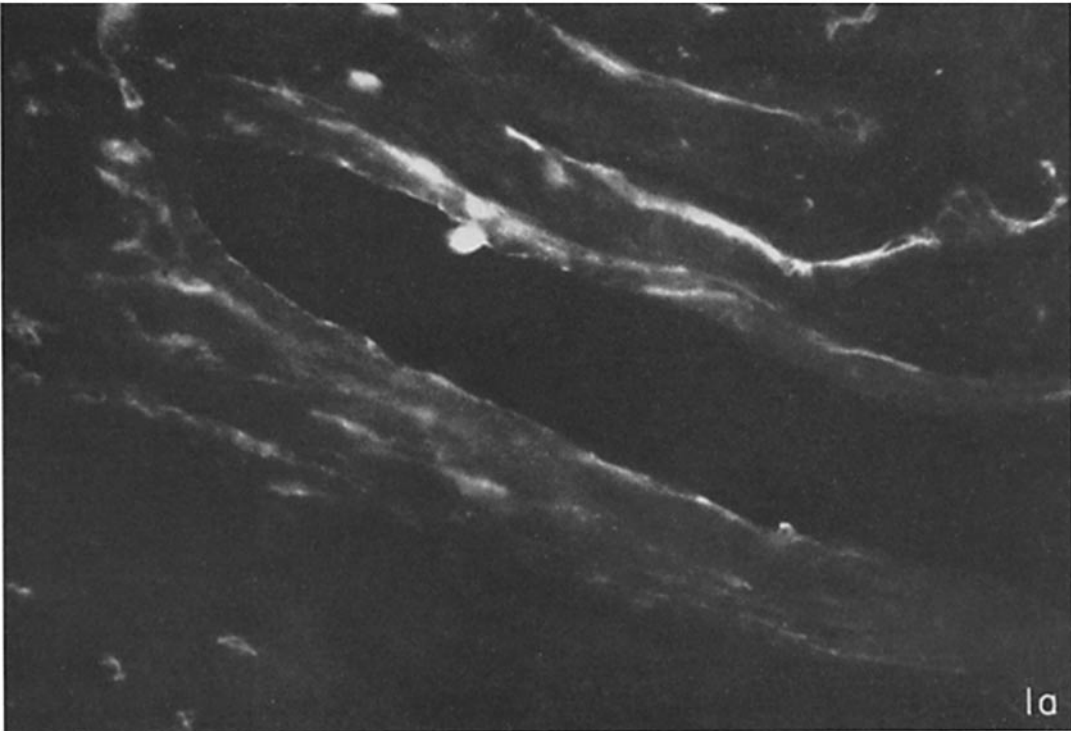
16. Kochwa, S., Rosenfield, R. E., Tallal, L., and Wasserman, L. R., Isoagglutinins associated with ABO erythroblastosis, *J. Clin. Invest.*, 1961, **40**, 874.
17. Hostrup, H., A and B blood group substances in the serum of the newborn infant and the foetus, *Vox Sanguinis*, 1963, **8**, 557.
18. Hostrup, H., The influence of A and B blood group substances on the 7S fractions of anti-A and anti-B. *Vox Sanguinis*, 1963, **8**, 567.
19. Szulman, A. E., The histological distribution of blood group substances A and B in man as disclosed by immunofluorescence. III. The A, B, and H antigens in embryos and fetuses from 18 mm in length, *J. Exp. Med.*, 1964, **119**, 503.

EXPLANATION OF PLATES

PLATE 82

FIG. 1 *a*. This frozen section of myometrium from a patient of blood group A was exposed to fluorescein-conjugated human anti-A serum. $\times 425$.

FIG. 1 *b*. The same section stained with PAS shows that the areas of fluorescence in Fig. 1 *a* correspond with the capillaries (some of which are indicated by arrows) and the intima of the large vein in the center of the photograph. $\times 425$.

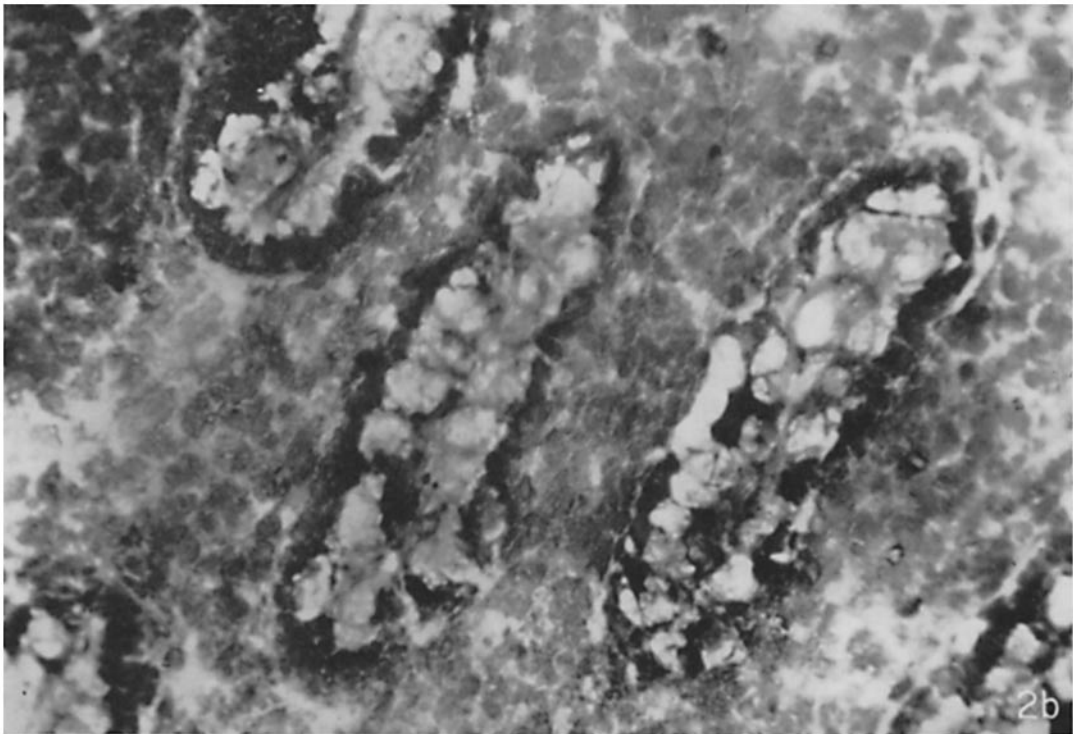
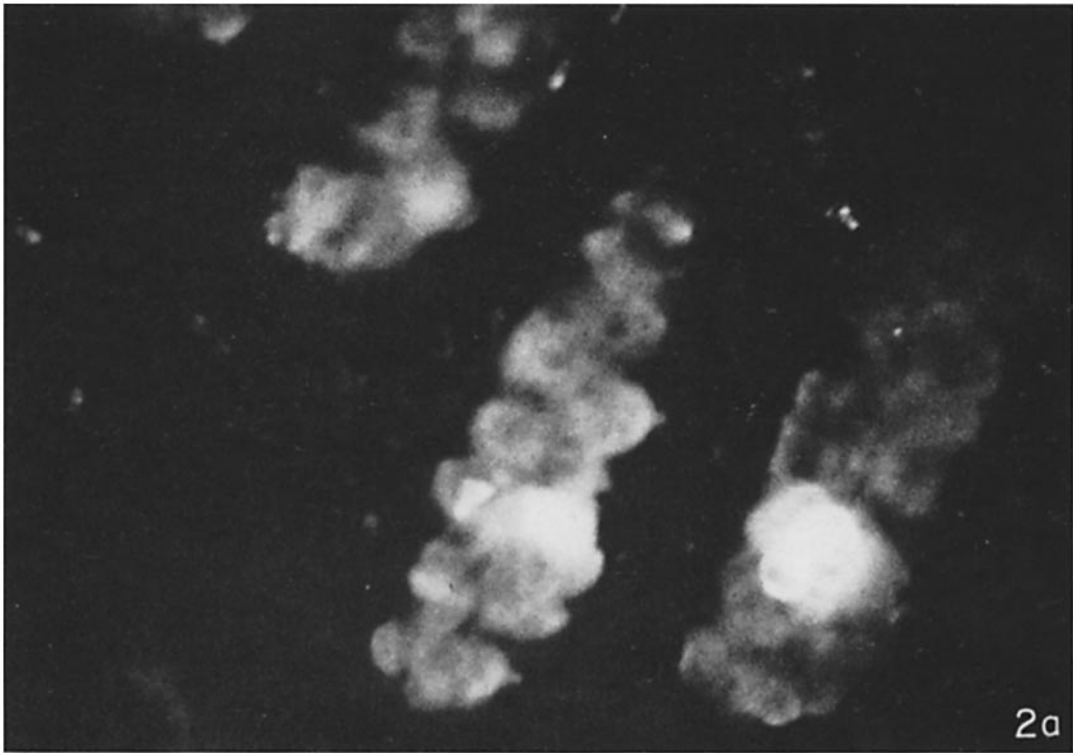


(Thiede *et al.*: Human chorionic villus)

PLATE 83

FIG. 2 *a*. This cryostat section of the gastric glands from a blood group A secretor patient shows bright fluorescence in the mucus after exposure to fluorescein-labeled human anti-A serum. $\times 425$.

FIG. 2 *b*. The same section stained with PAS to provide histological confirmation. $\times 425$.



(Thiede *et al.*: Human chorionic villus)