

THE ABH ANTIGENS IN HUMAN TISSUES AND SECRETIONS DURING EMBRYONAL DEVELOPMENT¹

ARON E. SZULMAN

Departments of Pathology, Magee-Womens Hospital, and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

The A, B, and H blood group antigens are present not only on the red cells, as their name would suggest, but also on the cell surfaces of many epithelial and apparently of all endothelial cells. Associated with soluble mucopolysaccharides they appear in the mucous secretions throughout the body, and, in water-soluble forms, in the secretions of the pancreas and in the sweat. Of all these substances, only the mucopolysaccharide produced by the mucus-secreting apparatus and the glycolipids isolated from the RBC envelope have been probed into and partially unravelled in chemical terms through the endeavors of Kabat (1956), Morgan (1964), Watkins (1964) and others. The nature of the other macromolecules still remains to be elucidated. So far, it would seem probable that we are dealing with several different species of molecules identical only with respect to relatively small side-chains that are responsible for their serological identity. Thus, it has been demonstrated that the mucus-borne and the erythrocyte antigens, respectively, are carried by differing macromolecules which, however, give a reaction of identity on an Ouchterlony plate employing an antiserum obtained by immunization with red cells (Watkins, Koscielak and Morgan, 1962). It is fair, then, to point out that this presentation deals with *antigens* rather than with whole substances. Similarly, the scheme devised for the genetically controlled biochemical sequences giving rise to the blood group substances A, B and H and Lewis proposed by Watkins (1959) and Ceppellini (1959) apply to the soluble mucoids and largely by inference to the erythrocytic substance.

This presentation encompasses human embryos and fetuses from 5–60 mm crown-rump length. The smallest specimens are 5, 7 and 8 mm, respectively, and represent the stage of 32–35 days ovulation age (Szulman, to be published); the larger specimens (Szulman, 1964) mark the

end of the first trimester of intrauterine life (10-weeks ovulation age).

METHODS

The immunofluorescence technique demands fresh specimens, rapidly frozen and cut at 4 μ in the cryostat (Szulman, 1960). It generally requires extremely careful handling, especially of the smaller specimens which were cut serially, with varying degrees of success. The reagents were hyperimmune sera obtained from human volunteers, immunized with A, B (Szulman, 1960) and H (Szulman, 1962) specific substances. Whereas it is a simple matter to immunize an O or a B group person in order to obtain an anti-A serum, and equally simple to produce an anti-B reagent, special circumstances were required to produce a hyperimmune anti-H serum, since all humans possess the H antigen, save for a few exceptional individuals mainly to be found in Bombay. One of such "Bombay" persons was accordingly immunized, and the serum was used in parallel with the anti-A and anti-B reagents. All sera had titers of at least 1000 as tested with 2% erythrocyte suspensions. All three were conjugated with fluoresceine or used unconjugated with a master conjugate such as horse anti-human globulin. In addition, rabbit anti-A and chicken anti-H sera were available. It can be stated at once that no discrepancy was obtained in using these various reagents; for example, human anti-A and rabbit anti-A sera gave the same results whether used as conjugates or as simple sera to be employed in a "sandwich" method.

Controls (Szulman, 1960; 1962) consisted of a battery of experiments designed to give inhibition of staining by (a) absorption of a serum or a conjugate with the homologous group erythrocytes, (b) absorption with secretor saliva or purified group substance of human or animal origin, (c) pretreatment of the tissue section with nonconjugated antiserum of human or animal origin of appropriate specificity, for example, human or rabbit anti-A serum followed by human anti-A conjugate.

In addition, no cross-reactions were observed between the anti-A or anti-B sera and non-A or non-B tissues, respectively; thus, no staining was

¹ This investigation was supported by Grant AI3554 and AI6443 from the National Institutes of Health, U. S. Public Health Service.

elicited with anti-A reagents in B and O group tissues, and similarly, none with anti-B reagents in A and O tissues.

The case of the "Bombay" anti-H reagent is somewhat more involved since the mucus-borne or erythrocytic H antigen seems invariably associated with the ABO group specificity; it is overshadowed by the A and B antigens, according to the dosage of the latter. Thus, in the absence of both A and B, that is in a group O person, the H antigen comes to full expression and is accordingly associated with phenotype O. In group A₂ the H antigen is partially, and in group A₁ more fully overshadowed by the A antigen. In AB individuals the overshadowing of the H antigen becomes more complete, but even there it remains detectable. It follows that the morphologic patterns of the mucus-borne ABH antigens are virtually identical and that in their developmental history there is a close parallel in space and time; (Szulman, 1962) in fact, as will be seen presently, the parallel obtains also for the endothelium and the epithelium as well.

RESULTS AND DISCUSSION

The ABH antigens are richly represented in the smallest embryos available, 5-8 mm, that is from

the 5th week after fertilization (Szulman, to be published). They are found on the cell membranes of the endothelium throughout the cardiovascular system and on the cell membranes of the epithelium of the integument, the digestive tube (Fig. 1), the mesonephric and (later) Mullerian ducts, in short, in all epithelial elements with the exception of those of the central nervous system, adrenals and liver. The erythrocytes, first encountered in the liver in this study at 18 mm are endowed with the antigens which, of course, persist throughout life; the same applies to endothelial antigens.

The epithelial antigens are at their maximal distribution at 35 mm, after which time they begin to wane. This they do in a remarkable manner (Szulman, 1964) since their disappearance coincides with recognizable steps of morphologic advancement and often with an ascertainable token of function, for example, secretion of mucus in the gastrointestinal tract (Fig. 2); trapping of radioactive iodine by the thyroid, which has just begun to form recognizable acini in the hitherto solid epithelial crescent; production of growth hormone by the pituitary, etc. Only the stratified

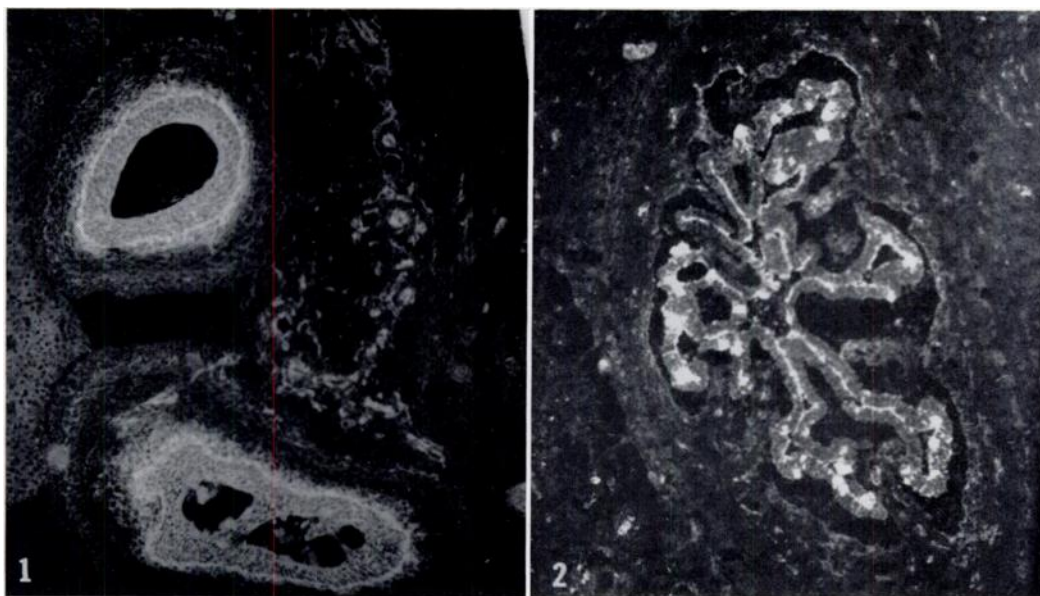


FIG. 1. Cross-section of a loop of small intestine from an embryo 24 mm, Group A, stained with rabbit anti-A serum followed by goat anti-rabbit conjugate. Observe the bright staining of the primitive mucosa and the rich vascularity of the mesentery, visualized by virtue of specific endothelial staining.

FIG. 2. Cross-section of small intestine from an embryo 40 mm, Group B, stained with the Bombay (anti-H) conjugate; similar staining obtained with an anti-B conjugate. Observe the disappearance of the epithelial antigen; the mucus-borne antigen is being produced by scattered goblet cells and coats the lumen with a thin layer.

epithelia of the integument, esophagus and lower urinary tract (including the simple epithelium of the renal collecting tubules) show the persistence of the antigen into and throughout extrauterine life. As emphasized before, the endothelial antigens are also permanent.

The mucus-secreting apparatus begins to function at 35–40 mm, *i.e.*, about 9-weeks ovulation age. The primitive salivary gland cords acquire lumens in which there are blebs of secretion rich in antigen. Similarly, the pylorus and the small intestine begin to secrete the antigens, with other segments of small and large bowel coming of age at fixed intervals. These antigens are to be produced permanently once their secretion is established. In nonsecretors (a problem still to be more thoroughly investigated for intrauterine life) the salivary glands produce no ABH antigens while small amounts are secreted by the gastrointestinal tract. In larger fetuses, say from the latter half of pregnancy, the production of the antigens in the deeper parts of the gastrointestinal mucosa can be shown (Szulman, 1962). Also, the production of other water-soluble ABH group substances in the pancreas and in the sweat go on independently of the secretor status (Szulman, 1960).

As can be seen from the foregoing, any speculation as to the biological significance of the so-called blood group substances must take into account their chemical diversity. The mucoids, the glycolipids of the erythrocytic envelopes, constitute two established species; the nature of the pancreatic secretion-borne substance has not been investigated, nor has the substance of the endothelial or the epithelial cell-wall been tackled. The last one promises to be especially interesting for the wide distribution of the antigens in minute embryos and fetuses, and their orderly disappearance when certain morphological stations are reached must needs have a meaning. Yet, it is also true that the ABH antigens per se cannot be essential in originating or continuing essential processes, for their absence, partial or total (as in "Bombay" persons devoid of all three: A, B and H) seems to be without significance (Szulman, 1964). Perhaps, they are rather to be regarded as

the immunologically ascertainable representatives of parent macromolecules that determine (wholly or in part) the active profile of the epithelial cell surface and thus participate in cell movement and in mutual cell recognition operative in embryologic processes. The antigens may thus be themselves only innocent bystanders thrown into notoriety by the curious property of Man to make antibodies against them, and may, in fact, owe their biologic significance, notably in transfusion and in ABO incompatible pregnancies, to that circumstance.

REFERENCES

- Ceppellini, R.: Physiological genetics of human blood factors. In *Ciba Foundation symposium on biochemistry of human genetics*, edited by G. E. W. Wolstenholme and C. M. O'Connor, p. 217. J. and A. Churchill Ltd., London, 1959.
- Kabat, E. A.: *Blood group substances: their chemistry and immunochemistry*. Academic Press, Inc., New York, 1956.
- Morgan, W. T. J.: 1964. Some aspects of immunological specificity in terms of carbohydrate structure. *Bull. Soc. Chim. Biol.* 46: 1627.
- Szulman, A. E.: 1960. The histological distribution of blood group substances A and B in man. *J. Exp. Med.* 111: 785.
- Szulman, A. E.: 1962. 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. *J. Exp. Med.* 115: 977.
- Szulman, A. E.: 1964. 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. *J. Exp. Med.* 119: 503.
- Szulman, A. E. *The A, B, and H antigens in three embryos, 5, 7 and 8 mm. (fifth week fertilization age)*, to be published.
- Watkins, W. M.: Blood group substances: their nature and genetics. In *The red blood cell*, edited by C. Bishop and D. M. Surgenor, Ch. 10. Academic Press, Inc., New York, 1964.
- Watkins, W. M.: Some genetical aspects of the biosynthesis of human blood group substances. In *Ciba Foundation Symposium on biochemistry of human genetics*, edited by G. E. W. Wolstenholme and C. M. O'Connor, p. 217. J. and A. Churchill Ltd., London, 1959.
- Watkins, W. M., Kościelak, J. and Morgan, W. T. J.: The relationship between the specificity of the blood group A and B substances isolated from erythrocytes and from secretions. In *Proceedings of the 9th Congress on Blood Transfusion*, p. 230. Karger AG, Basel, 1962.