# Demonstration of Blood Group A and B Antigens in Human Tissue Using an Immunoperoxidase Staining Method

Joachim Lötterle and Wolf-Dieter Heine (Institut of Pathology, Leopoldina Hospital, D-8720 Schweinfurt, Federal Republic of Germany)

The distribution of A and B blood group substances and antigens in secretions and tissues was extensively studied by Putkonen (1930) and Hartman (1941), using the agglutination inhibition method. It was found that the strongest blood group activity was associated with mucous secretions and that non-secretors also produce small amounts of A and B substances. Later the distribution of blood group substances in human tissues was explored in more detail, using immunofluorescence microscopy (review in Szulman, 1966). It became possible with this method to demonstrate the actual histological localization of the antigens.

With the introduction of the peroxidase-anti-peroxidase technique by Sternberger et al. (1970), a new and more versatile method was offered, which was easier to work with than the immunofluorescence technique. Recently the peroxidase-anti-peroxidase method has been used to demonstrate the distribution of the ABH antigens in fresh and decomposed human kidney and placental tissue (Pedal and Hülle, 1984; Pedal and Becker, 1985; Pedal et al., 1985).

In our study the peroxidase-anti-peroxidase method was used to show the distribution of blood group antigens in human salivary glands and other tissues of particular interest for forensic medicine.

## Materials and Methods

Tissue specimens were taken from the autoptic material of 40 corpses with blood groups $A_1$, $A_1B$ and B. The tissue was fixed in unbuffered 10% formalin and routinely dehydrated, cleared and embedded. Serum glycerine fixative was used as an adhesive for the 4-5µ sections. Deparaffinization took place in a 60° C oven for at least one hour, followed by routine hydration. After rehydration the sections were submerged in PBS for only a few minutes and stained immediately afterwards, using the peroxidase-anti-peroxidase technique. All the steps were done at room temperature except for incubation with the primary antibody.

The slides were laid flat and incubated with 3% hydrogen peroxide for five minutes. After washing with PBS the tissue was incubated with normal swine serum (DAKO X 960) diluted 1:10 for 20 min. After tapping off the excess serum, an undiluted monoclonal anti-A or anti-B serum (Biotest, Seraclone) was applied and the slides were incubated at +4° C for 4 to 6 h. This method yieldet an intense specific stain with practically no background. Then the slides were gently rinsed with PBS and incubated for 20 min with peroxidase-conjugated rabbit antibody to mouse immunoglobulin (DAKO P 161) diluted 1:100. The slides were again gently rinsed with PBS and incubated for 20 min with peroxidase-conjugated swine antibody to rabbit immunoglobulin (DAKO P 217) diluted 1:100. After rinsing with PBS, a commercially available AEC (3-amino-9-ethylcarbazole) substrate solution was applied and the slides were incubated for 20 to 40 min. After a final washing in PBS the slides were counterstained with haematoxilin for 3 min.



Advances in Forensic Haemogenetics 1
Edited by B. Brinkmann and K. Henningsen

Several tissues, especially salivary glands and epididymitis, were stained in parallel, using the PAS (periodic acid schiff) reaction as described elsewhere (Romeis, 1968). Counterstaining was done with haemalaun.

## Results

**Blood-vessels.** The blood groups were found in all preparations - regardless of the secretor status - on the endothelia of the blood vessels. The erythrocyte membranes did not generally react as strongly as the blood vessel endothelia. A negative staining of capillary endothelia was always observed in the salivary glands of secretors whenever the mucous glands reacted with a particularly strong positive stain.

**Epididymis.** In epididymis neither the efferent duct nor the epididymid duct are stainde by the PAS reaction. With the peroxidase-anti-peroxidase technique, blood group substances were distinctly stained in the secretion of the efferent ducts, but not in the epididymid duct. It cannot be said whether or not this would be the case in non-secretors too, owing to the narrow scope of the study. The sperms themselves were not stained, or if so, only very lightly: thus, the seminal plasma is responsible for the large amount of blood group substance in the seminal fluid and not the sperms themselves.

**Sweat glands.** The sweat glands of the skin are PAS negative and produce blood group substances. According to Szulman (1962) this production is not under the control of the secretor genes.

**Hair.** The blood groups of hair with intact roots were determined in the hair papillae, because these are supplied with blood by the capillaries. Yet, most attempts to determine the blood group in a single hair with this method have failed, since most teared out hairs do not contain vascular elements. The inner and outer parts of the hair shaft occasionally stain with a false positive stain and are therefore not suitable for demonstrating the ABO-bloodgroup. If the hair follicle has been preserved, the blood group can also be seen here in the capillaries.

**Salivary glands.** In the study of the salivary glands of 26 persons (19 A secretors, 3 A non-secretors, 3 B secretors, 1 B non-secretor; for glandula sublingualis, glandula submandibularis and Parotis), the results obtained for the mucous endpieces and connective pieces, the epithelia of the excretory ducts and the capillary endothelia can be seen in Table 1. The information **pos**/neg means that in the greater number of cases the reaction was positive, but was also negative in a small number of cases. Of particular note is that in the glandula submandibularis in some of the secretors, individual cells of the mucous end and connective pieces produce a strong reaction, whereas others produce practically no reaction at all with anti-A or anti-B. Similar behaviour was found by Kent (1961) in the Brunner's glands of the duodenum.

**Placenta.** Studies were carried out in five mature placentas. The fetal erythrocytes always stained weaker than the maternal ones. These results agree nicely with those of Pedal et al. (1985) and are due to the fact that the fetal erythrocytes have just a relatively small number of blood group antigens (Hakomori, 1981).

**Table 1**

Staining of different elements from salivary glands of secretors (Se) and nonsecretors (Ns) with the peroxidase-anti-peroxidase (PAP) and PAS staining method

| | Staining | Parotis | Submandibularis | Sublingualis |
|---|---|---|---|---|
| mucous end-pieces and connective pieces | PAP Se | - | pos | **pos**/neg |
| | PAP Ns | - | neg | **neg**/pos |
| | PAS | - | pos | pos |
| epithelia of excretory ducts | PAP Se | **neg**/pos | pos | pos |
| | PAP Ns | **neg**/pos | pos | pos |
| | PAS | neg | neg | neg |
| capillary endothelia | PAP Se | pos | **neg**/pos | neg |
| | PAP Ns | pos | pos | pos |
| | PAS | neg | neg | neg |

## Discussion

The studies in the salivary glands confirmed the earlier findings that the Parotis has nearly no mucous ("water soluble") blood group substance, as opposed to the glandula sublingualis, which has extremely large amounts of the substance (Lötterle and Scheithauer, 1984). In salivary glands the antisera appear to have far greater affinity for the water soluble blood group substances than for the alcohol soluble blood group substances in blood vessel endothelia and erythrocytes. For that reason, the vessel endothelia remained unstained in the glandula sublingualis in secretors.

The conventional subdivision into water soluble and alcohol soluble blood group substances should be further investigated, keeping the PAS reaction in mind, in order to determin if the PAS negative water soluble blood group substance is controlled by the secretor gene. Our preliminary findings in

the salivary glands, the sweat glands and the epididymis suggest in the light of Szulman's results (1962) that this is not the case.

The studies, however, also showed that the PAP method is an effective technique for demonstrating blood group antigens, even in very small tissue specimens. Through improved preparation techniques it should be possible by using this method to demonstrate ABH blood groups in, for example, blood stains and vaginal epithelium.

## References

Hakomori SI (1981) Blood group ABH and Ii antigens of human erythrocytes: chemistry, polimorphism and their developmental change. Semin Hematol 18: 39-62

Kent S P (1961) J Histochem Cytochem 12: 591 cited from Szulman (1966)

Lötterle J, Scheithauer R (1984) Sekretoreigenschaft bei frischen und fäulnisveränderten Leichen. Beitr gerichtl Med 42: 251-255

Pedal I, Hülle J (1984) Immunenzymatische Bestimmung des ABO- und Sekretorstatus an paraffineingebettetem Autopsiematerial. Z Rechtsmed 94: 289-300

Pedal I, Baedeker C (1985) Immunenzymatische Darstellung der Isoantigene A, B und H in fäulnisverändertem Nierengewebe. Z Rechtsmed 94: 9-20

Pedal I, Kuhn K, Hülle J (1985) Immunhistochemische Bestimmung von mütterlicher und kindlicher Blutgruppe (ABO) an reifem Placentagewebe Z Rechtsmed 94: 145-153

Romeis B (1968) Mikroskopische Technik. Oldenburg Verlag, München S. 275

Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG (1970) The unlabeled antibody-enzyme method of immunohistochemistry.Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-horseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18: 315-333

Szulman A E (1962) The histological distribution of the blood group substances in man as disclosed by immunofluorescense. II. The H antigen and its relation to A and B antigens. J Exp Med 115: 977- 995

Szulman AE (1966) Chemistry, distribution and function of blood group substances. A Rev Med 17: 307-322