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ABO Blood Typing from Forensic Materials - Merits and demerits of detection methods utilized in our laboratories, and biological significance of the antigens

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ABSTRACT

We review the merits and demerits of the ABO blood grouping method developed and utilized in our laboratories during the past three decades according to the following 4methods. 1:Direct hemagglutination method with aged red blood cells derived from partially decomposed bodies. 2:Absorption elution method with blood stain, body fluid and hard tissues. 3:Histochemical method with hair, mummified skin and tissues, and including ABO grouping of the erythrocytes in the blood group incompatible case, blood group A sub-typing, determination of secretor status of ABO antigen and species identification. 4: ABO genotype from fresh blood and paraffin blocks. We would like to emphasize that the species identification prior to ABO grouping is an important procedure, because forensic materials such as from saliva, urine and seminal fluid might be contaminated with the fluid from indoor pets or animals expressing ABO antigens in their organs. The species identification is also important even in ABO genotyping, since DNA extracted from vertebrate might be amplified with the primer for ABO genotyping and the amplified DNA might be hybridized to those from humans. Although the biological significance of ABH antigens is not well elucidated

even at present, the presumptive roles of ABH antigens and Le x antigen that is one of the ABH-related antigens were suggested.

Keywords: Blood Group ABO System; Forensic Identification; Laboratory; Process Development; Hemagglutination; Erythrocyte; Decomposition; Absorption; Elution; Blood Stain; Body Fluid; Histochemistry; Hair; Tissue Preservation; Skin; Blood Group Incompatibility - Epidemiology; Blood Group A; Species Identification; Phenotype; Saliva Analysis; Urinalysis; Semen Analysis; Contamination; Domestic Animal; Antigen Expression; DNA Extraction; Gene Amplification; Dna Hybridization; Blood Group Lewis System; ABO Blood Group Antigens; Hemagglutination; Absorption Elution Method; Histochemical method; ABO Genotypes; Biological Significance; Human; Nonhuman; Review

Drug Index Terms

Blood Group Antigen - Endogenous Compound; Paraffin; Sialyl Lewis x Antigen - Endogenous Compound; Blood Group ABH Antigen - Endogenous Compound

EMBASE Classifications

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Introduction

The ABO blood antigens were discovered at the beginning of the last century[1]. The biochemical, molecular-biological and genetic nature of the ABH and related antigens is now quite well characterized and it can be said that they are involved in onco- and onto-developmental and/or cell recognition phenomena[2]. In the forensic science field the ABO system has been a major focus, since the record of this blood system is a very prevalent one and A, B and H antigens on erythrocytes are also associated with other cells and tissues throughout the body and are known to be considerably stable to the violent conditions such as heating or drying[3,4].

In this presentation we review the merits and demerits of the ABO blood-grouping methods utilized and developed in our units during the past three decades, and discuss the biological significance of the ABH and its related antigens on basis of the data showing the distribution of these antigens in the animal organs containing those from human.

Conventional serological methods

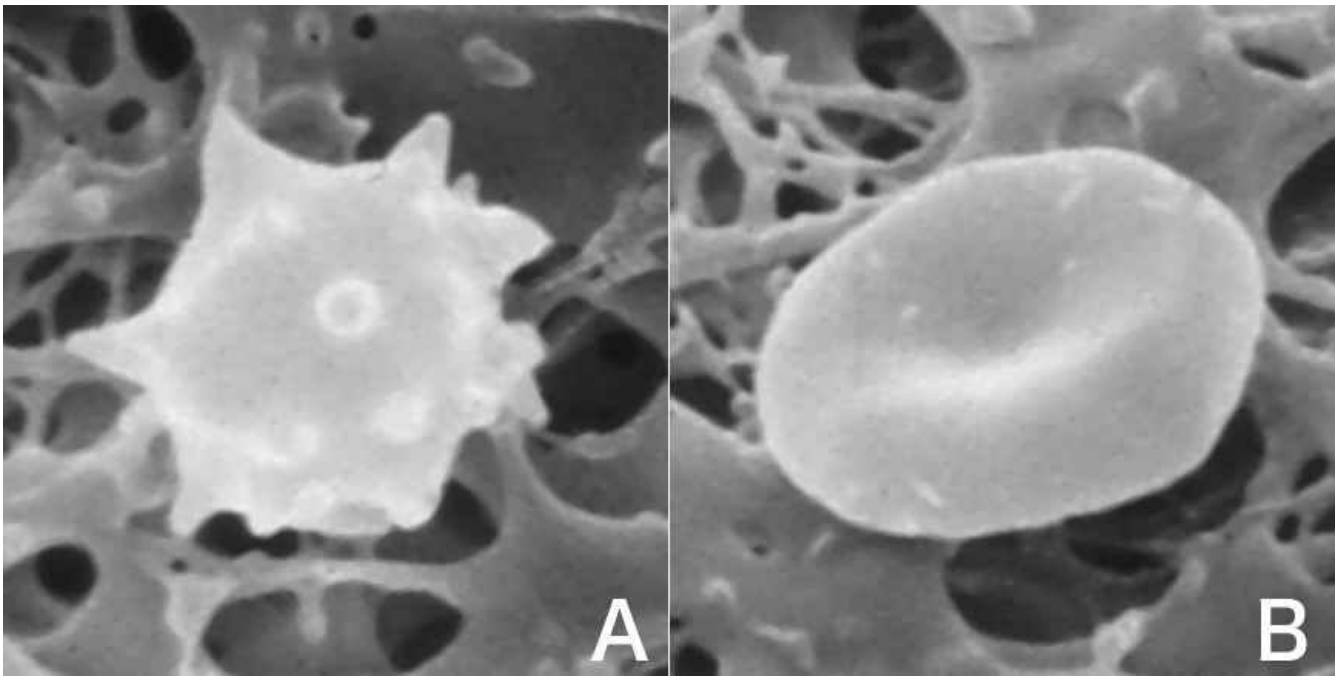


Figure 1. Scanning electron microscopic observation of erythrocytes. Erythrocytes stored for 8 weeks in blood bank condition were incubated in the solution containing adenine and inosine. A; before incubation, B; after incubation.

The conventional serological methods for blood grouping are the direct hemagglutination and antigen-antibody combined methods consisting of absorption, absorption-elution, absorption inhibition and some other modified method. These methods are have been used for ABO grouping in the forensic field. With developments in forensic serology, such as production of monoclonal antibodies, the modification and elaboration of these techniques had been accelerated. Enzyme linked immuno-sorbent assay (ELISA) has been used in forensic field since around in 1995[5] but the principle of this method is based on a modification of the absorption inhibition technique. Thus, the modification is more sensitive, easier to automate and requires much less amount of samples than traditional techniques, however, the underlying mechanism is basically the same as the conventional method.

Hemagglutination method

The hemagglutination technique is being utilized even to date as the indication of a positive or negative reaction in the serological reactions and the prevention methods of the control cells are now examined. * Agglutinability was recorded as the highest dilution at which the antiserum agglutinated the erythrocytes.

Table 1. Relationships between ATP content and agglutinability of erythrocytes stored 8 weeks[6].

Incubation period (min)	ATP content (mg/dl)	Agglutinability*	
anti B	Anti N	Anti D	
0	6.6	32	2 1
30	14.9	64	4 1
60	21.9	128	8 2
120	24.9	128	8 2

Although ABO grouping can be easily performed from corpses when fresh blood can be obtained, the agglutinability of red blood cells against homogeneous antibodies becomes progressively weaker as the storage periods of blood or in the postmortem time of corpses increases. Since the mechanism of hemagglutination of erythrocytes has not been well understood, we tried to make clear the mechanism by measuring the changes in the agglutinability, deformability, antibody binding capacity, osmotic fragility, morphology and ATP content after treatment with glutalaldehyde or heat, and storage in acidic-dextrose (ACD) solution, blood bank condition[6,7]. The shape of red blood cells was transformed from a discoidal into a crenated shape or a smooth shape after long-term storage up to 20 weeks under blood bank condition. Glutalaldehyde and heat treatment caused a dose-dependent decrease in agglutinability, deformability and osmotic fragility of erythrocytes, but had no effect on their antibody-binding capacity. When storing erythrocytes under blood bank condition, their ATP content, agglutinability, deformability and osmotic fragility gradually decreased as they change in shapes. Incubation of the aged erythrocytes in a saline solution containing 5 mM adenine, 20 mM inosine and 5 mM glucose for 60 min at 37^0 C is presumed to restored the ATP content, resulting in a recovery of the agglutinability and deformability of the erythrocytes as shown in Table 1 and Figure 1. The results indicated that the ATP content influences the shape and deformability of erythrocytes and consequently the membrane flexibility, which is presumed to be involved in hemagglutination of erythrocytes[6,7]. A scanning electron-microscopic observation revealed that the agglutinated erythrocytes were deformed in various shapes as shown in Figure 2.

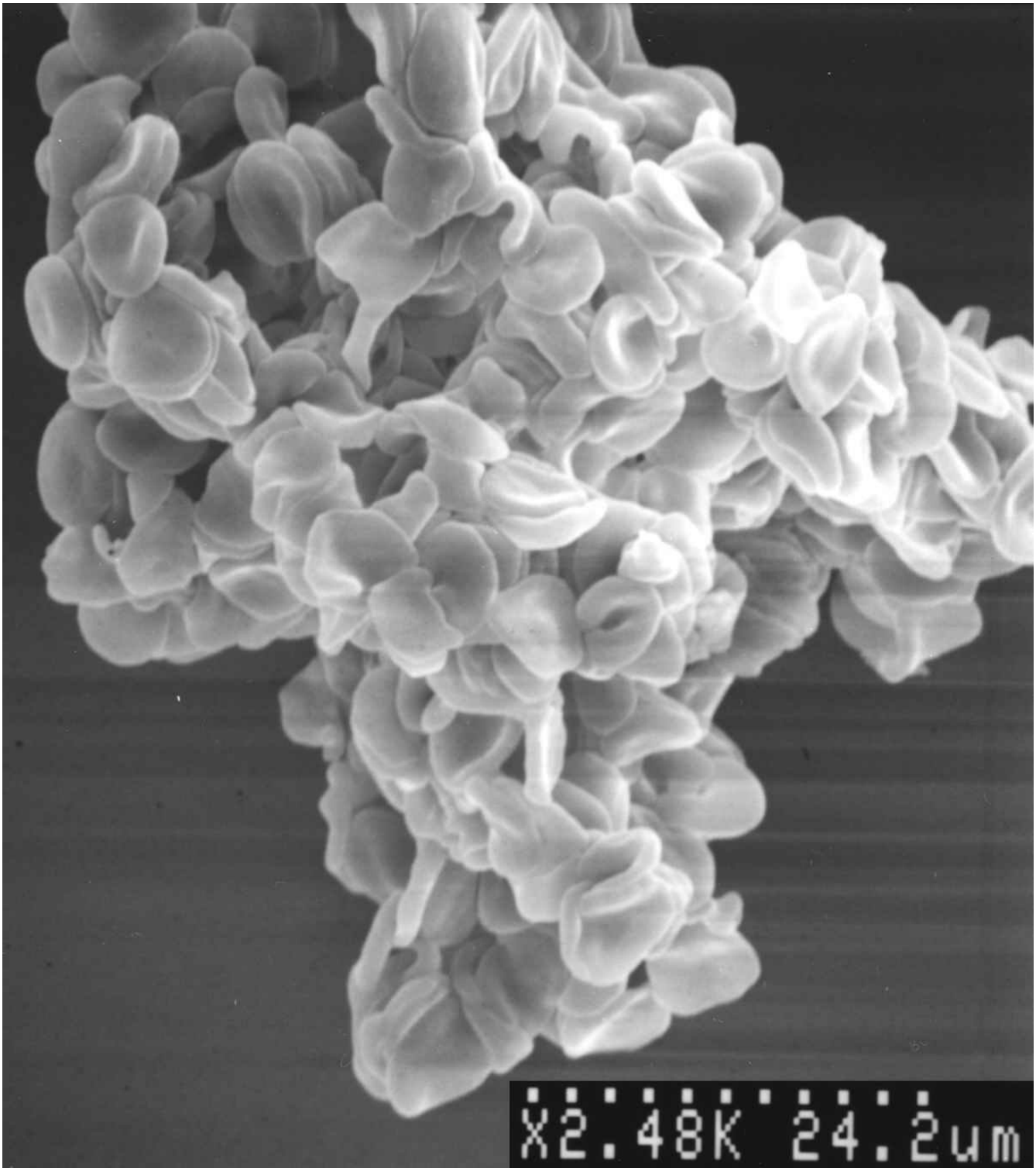


Figure 2. Scanning electron microscopic observation of agglutinated erythrocytes. Agglutinated erythrocyte were deformed and showed various shapes having a stick and a horn.

It is often seen that the hemolysis of aged erythrocytes obtained from partially decomposed bodies occurs during preparing the suspension of red blood cells with saline for the direct hemagglutination test. Michailow[8] successfully used partially hemolyzed blood in which the red blood cells had lost their agglutinability for the direct hemagglutination method by prior treatment with isotonic saccharose solution. In Michailow's method, 1 ml of blood sample was gently shaken for 5 min in 1ml of 40% saccharose solution adjusted to pH 6 with 25% citric acid, and then 5 ml of 7% saccharose solution (pH 6) was added. The sample was left standing for 5 min, then washed

three times with physiological serum (inactivated group AB human serum) and used to prepare a 2% red blood cell suspension in physiological serum for blood group testing. We verified that aged red blood cells increased their osmotic resistance after incubation in isotonic saccharose solution and his method proved to be more effective and reliable than the routine saline method[9]. The results obtained in our studies on the mechanism of agglutination of erythrocytes indicated that the ABH antigens of aged red blood cells derived from partially decomposed bodies could be detected with direct hemagglutination method by incubation the cells in a solution containing adenine and inosine or saccharose.

Absorption elution method

ABO typing of erythrocytes

Although the ABH antigens in blood stains on cotton cloth could be detected even after 42 weeks storage by means of absorption elution methods in a previous study[4], we have often encountered discrepant results when determining the blood group of wet stains or putrefied bodies by the elution method. This may be ascribed to the acquired antigens or destroying of antigenicity due to bacterial contamination and/or partial digestion of erythrocytes membrane by proteolytic enzymes which exist in erythrocyte membrane[10].

We compared previously the reliability of the direct hemagglutination method proposed by Michailow[8] and the absorption elution method for determining the ABO blood type of partially hemolyzed blood samples[9]. Grouping of blood samples stored at room temperature was reliable for 9 weeks by Michailow's method and for 7 weeks by the routine method. The blood-stains prepared from blood samples stored for more than 60 days at room temperature showed their native blood group, but they often cross-reacted with another antibody in the absorption elution method. The results indicated that mistyping could occur in aged blood samples and that blood, where blood group antigens could not be detected by the direct hemagglutination method, was not suitable for the blood-grouping test.

We have been often making autopsies of bodies who have died due to fire. We studied the serological thermo-stability of blood group active glycolipids and sialoglycoproteins partially purified from human erythrocytes membranes. Since the heating at 12^0c for 30 min or 14^0 c for 5 min in saline and solid state did not affect the A and B activity of the partially purified blood group substances, the ABH antigens were remarkably stable to heat[3] and the ABO blood grouping of severely burned bodies could be performed by means of absorption elution method using the burned blood or tissue samples.

ABO typing from body fluid

In a series of study we examined the distribution of the ABH antigens in human organ secreting ABH antigens as the body fluid such as saliva, urine, sweat and semen. We obtained the results described in Table 2-1 and 2-2[11].

Table 2-1. Types of organs and tissues expressing the ABH antigen

Organ	Tissue expressing ABH antigen
Skin	Stratum corneum, Sweat glands and Mammary glands
Hair	Medulla
Oral cavity	Squamous epithelium
Tongue(Squamous epithelium, Mucous cells of lingual glands and Serous cells of Ebner's glands)	
Mucous cells of Epiglottial glands	
Salivary glands	Mucous cells of salivary glands
Esophagus	Squamous epithelium
Stomach	Surface epithelium, Pyloric glands, Parietal cells and Chief cells
Small intestine	Surface epithelium, Goblet cells, Brunner's gland
Liver	Epithelium of gallbladder duct
Pancreas	Exocrinacinim, Duct
Kidney	Collection tubules
Urinary bladder	Urothelium
Prostate	Tubuloalveolar glands
Testis	Surface of germ cells, Rete testis, Effrentes duct,
Seminal vesicle	Epithelium
Thymus	Hassall's cprpuscles, Thymic epithelial cells
Trachea	Surface epithelium, Mucous cells, Goblet cells
Pituitary	Middle lobe
Blood vessels	Endothelial cells, Erythrocytes

Table 2-2. Types of organs and tissues in which the ABH antigens are not expressed

Organ	Tissue not expressing ABH antigens
Pituitary	Posterior lobe
Adrenal	Cortex, Medulla
Pancreas	Islets of Langerhans
Liver	Hepatocytes
Bone	
Cartilage	
Central nervous system	
Muscle	
Ovary	

Although the most tissues and organs express the antigens respective of the secretor status of the donor, in cells of some tissues such as striated and interlobular duct of the salivary glands, secretory coil cells of sweat glands and stratified epithelia of oral cavity, tongue, esophagus, respiratory tract, urinary tract and cervix. These results indicate that blood grouping from body fluid from secretor individuals can be made by absorption-elution method. Since Japanese non-secretor individuals express a small amount of the antigens in the secretory cells[12], the blood group antigens in the body fluid from Japanese may be detectable. In our previous study spermatozoa in the efferent duct showed no reactivity with anti bodies against ABO blood group antigens, indicating that the ABH antigens on the spermatozoa detected in the ejaculated semen are coating antigens such as alfa-2-seminoglycoprotein and similar to the Lewis antigens on erythrocytes[13].

It is widely accepted that ABH and related antigens are expressed in the secretory organs of many species of animals. We studied the distribution of the ABH antigens in some mammals[14]. The results indicated that salivary glands and taste bud cells of rat, cat, dog, rabbit and Japanese monkey interacted with antibodies against ABH antigens. The kidney and ductus epididymidis of cat and the prostate of rat showed good reactivity with these antibodies. The ABH and their related antigens were expressed in the salivary glands, kidneys and reproductive organs of animals as well as in those from humans. We must emphasize that the species identification prior to ABO blood grouping is indispensable, because of the forensic materials such as the stains from saliva, urine and seminal fluid might be contaminated with the fluid from indoor pets[15].

ABO typing from other tissue

Hair, skin, nail and bone tissue have been utilized for ABO group determination. It is well accepted that ABO typing from skin particle and nail with absorption elution method is relatively easily as an empirical knowledge.

The distribution of the ABH antigens in the skin was clearly revealed by histochemical technique[16]. Saito et al[17] analyzed the ABH active glycolipid extracted with chloroform- methanol from human nail and reported that the glycolipid contained blood group antigens consist of type 1,2 and 3 structures, and the amount of A, B and H active glycolipids were in the ratio of 1:4:1 in the nail from blood group AB individual.

On the other hand, the ABO grouping from hair and bone tissue often encountered difficulties. We attempted to detect the ABH antigen in the decalcified bone tissue by means of histochemical technique using bone sections sliced with a cryostat[18]. The reactivity with antibodies was only recognized on the endothelium or epithelial cells of the haversian canals. This may be reason for difficulty in ABO grouping from bone, since it contains small amount of ABH antigens compared with other tissues. Saito et al[17] reported that hair consisted of ABH active glycolipid in only type 2 structure, and expressional unbalance in the A, B and H antigens was observed in the hair from blood group AB individuals, that is, the amount of A, B and H active glycolipids were in the ratio of 1:20:4 by the dot blot analysis. They said that the dotting of the glycolipid onto PVDF membranes in a ratio of 10:1:4 for each anti A, B and H antibody was effective for well detection of ABH antigens with dot blot detection method.

Histochemical method

ABO Blood grouping from materials obtained from humans

ABH antigens are widely distributed in various tissues, being found not only erythrocytes, but also on other cells including dermal epithelium, endothelial cells of blood vessel Hassall's corpuscles[19], renal tubules, secretory cells of respiratory tract[20], male reproductive organs[13] and hair, as shown in Table 2[11]. The antigens are easily and certainly detected by means of immunohistochemical technique using antibodies against ABH antigens or lenctins specific to blood group A, B, or O[21]. It is a great merit that we can microscopically observe the distribution of antigens and reaction sites in the tissue by histochemical methods.

ABO blood group incompatible cases

In the ABO blood group incompatible cases the transfused erythrocyte could be easily detected inside the blood vessels with corresponding antibody and the blood group of individuals was also detected in the secretory cells, erythrocytes, endothelium and/or renal tubules by the antibody against their own blood group. In the blood vessels the reactivity of erythrocytes shows the mosaic pattern; that is positive and negative erythrocytes are observed in the blood vessels, since the transfused erythrocytes are stained only by correspomding antibody and the erythrocytes of the patient are not stained. The reverse reactivity by the antibody against blood group of patient was observed in the blood vessel. Figure 3 shows the result of the staining in the kidney in an incompatible transfusion case[22].

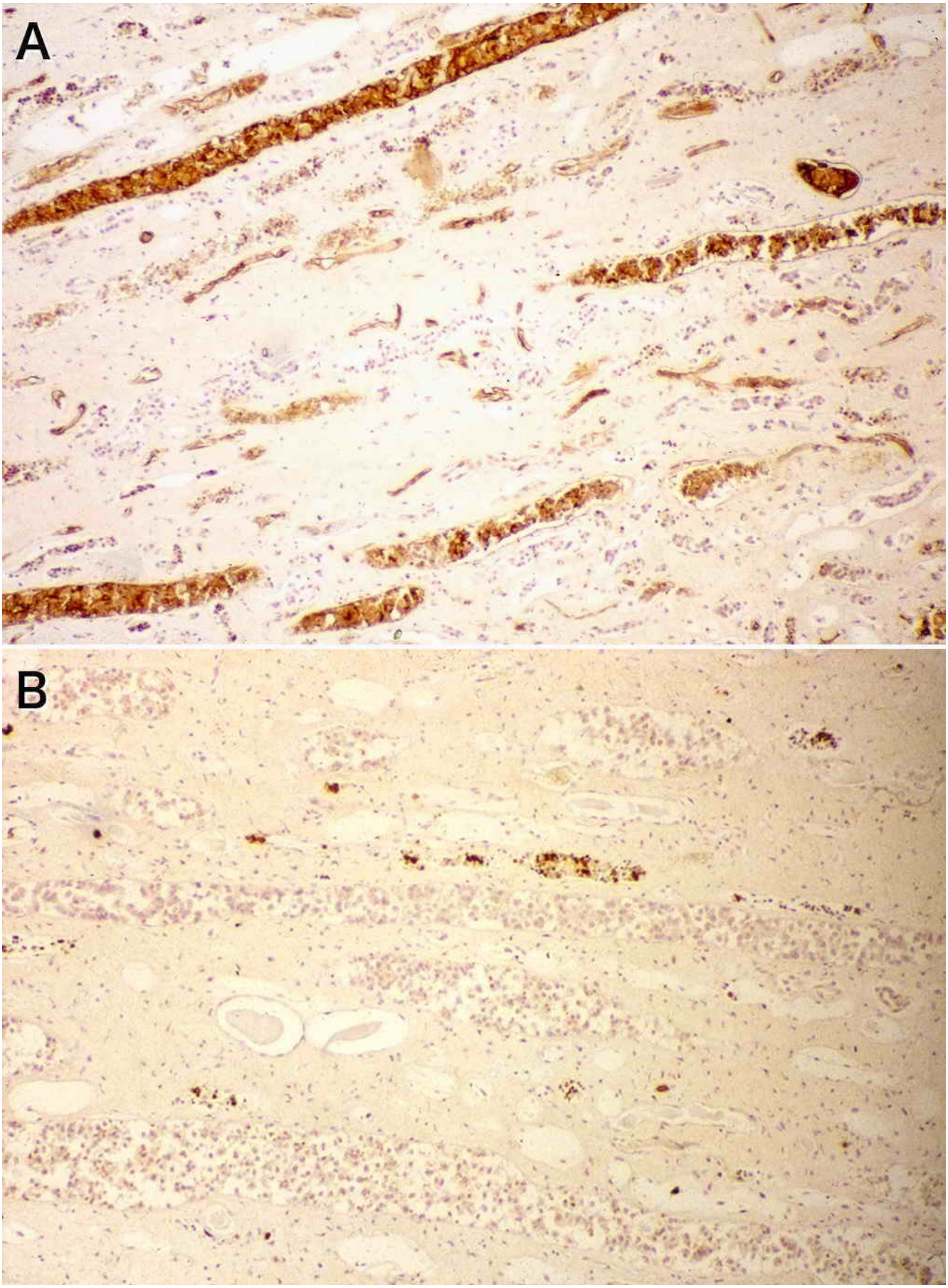


Figure 3. ABO group determination in the blood group incompatible case. The transfused A typed red blood cells indicated by arrows were easily detected inside blood vessels of the kidney with anti

A antibody (photo A), and a large amount of O typed red cells shown by arrow inside blood vessels and collecting tubules were stained by anti H antibody (photo B).

Hair

ABH antigens found in hair are extremely stable on storage, Yada et al[23] detected antigens from an 88-year-old hair rope by means of the absorption elution method. This result is a valid merit for forensic scientists. We also developed the detection method of ABH antigens in hair by an immuno-histochemical method using the Avidin-Biotin-Peroxidase technique[24]. The hairs were sliced longitudinally by hand under a 20-power microscope and cut into small pieces (each approx. 3 mm) before being subjected to the immunohistochemical staining procedure. This was carried out in small glass test-tubes by means of routine procedure. Since a positive reaction was recognized microscopically only in the medulla of the hair containing head, pubic and moustache hair, it is impossible to determine the ABO group from hair which has no medulla by this method. Although this result shows a gross shortcoming for the forensic practice, Miyasaka et al[25] examined the presence of blood group substances in the cortical part of hair using an immuno-electron-microscopical method and have suggested that blood group antigenicity is also located on the inner wall of the vacuole of medulla cells and in the interfibrillar matrix of cortical cells.

The medulla of hairs from blood group A or B type individuals reacted with anti A or B and H showing mosaic staining pattern as shown in Fig 4[24]. This indicates that longitudinal slicing of hair is necessary to obtain a correct result by an immunohistochemical method. A mosaic distribution of blood group antigens was first observed by Kent[26]. He compared the distribution of A and H antigen in Brunner's glands from blood group A individuals and found the reverse distribution of A and H antigens. We also reported independent expression of A, B and H antigens in acinar cells of the pancreas from blood group AB individuals[27]. The mosaic distribution pattern has been observed in other tissues such as mucous cells of human salivary gland, cervical glands of human uteri and mucous cells of salivary glands of rats[15].

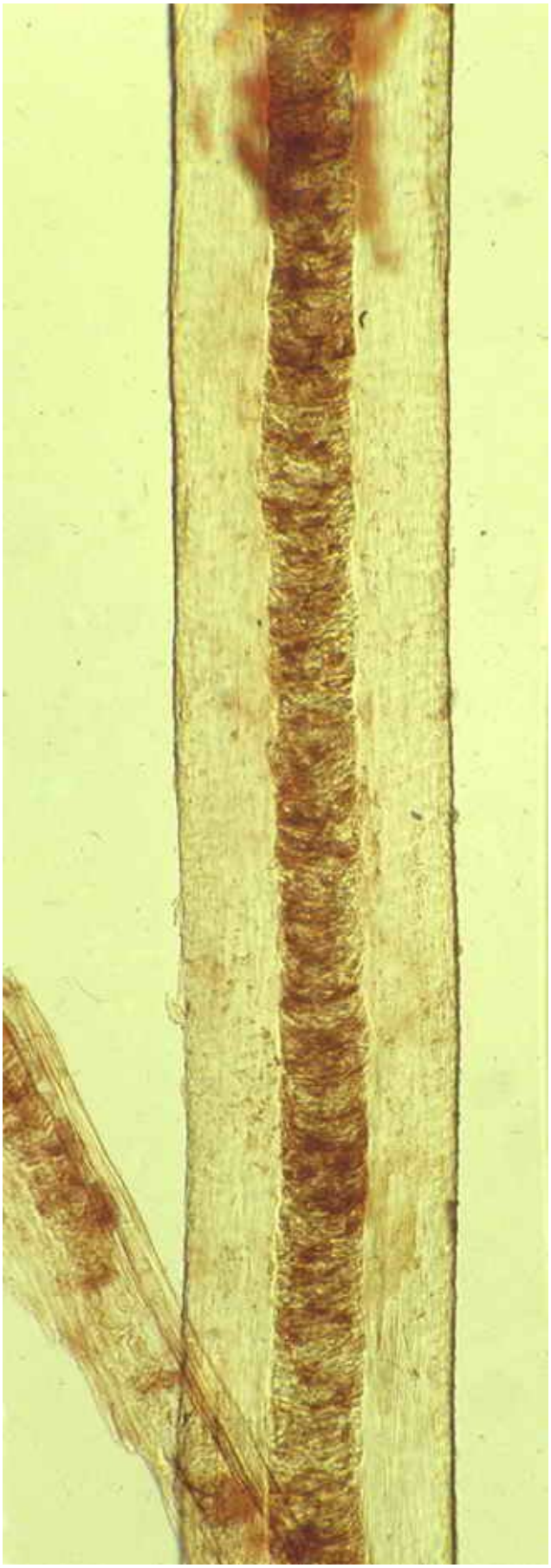


Figure 4. ABO grouping from hair. A-antigen was detected only in the medulla of the blonde hair showing mosaic pattern distribution. The positive and negative sits in the medulla are observed by turns

The expression of A and B antigens from Blood group AB hair is quantitatively different. Saito et al[17] reported that expression of A-antigen was quantitatively 10 to 5 % of that of B antigen from hairs of blood group AB individuals and type 2 chain of the antigens was only detected in the hair. For blood grouping of hair, type 2 chain specific antibodies are effective and the low diluted anti A-antibody might be used comparing with that of anti B-antibody[24]. We encountered two corpses who had been in the sea for about 6 months and blood group could not be detected from the hair samples. The identification of the bodies was made at autopsy and their blood groups were known to be group O. Although there were no noticeable microscopical changes in the structure of these hairs, the medulla of the hairs showed no reactivity with anti H monoclonal antibody. This revealed the necessity to use not only anti-A and B-serum but also anti-H serum for the blood group determination.

Typing of Blood Group A Subgroup

A qualitative difference in red blood cells of subgroup A1 and A2 has been clarified in terms of type 3 and type 4 based antigens[28], and the distribution of H type 1-4 chains of ABO(H) system in different cell types of human respiratory epithelium was also examined[29]. We revealed that a quantitative difference in A antigens has been observed in different tissues containing Hassall's corpuscles[19], respiratory secretory cells[20] and epithelial cells of blood vessel. In tissues from A2 individuals which constitutes about 20% of the European blood group A population and no of the Japanese population, H antigen is usually much more abundantly expressed than A antigen. Although monoclonal anti H antibody usually can not bind to the secretory cells, epithelium of Hassall's corpuscles or blood vessel of A1 individuals, these cells of A2 individuals reacted strongly with H antibody. A2 individuals may therefore be most clearly discriminated from A1 individuals by the difference in the reactivity of antibodies against A and H antigens in the tissue sections. The effects of other subgroups such as Ax or Bm on the expression of blood group antigens have not yet been examined in our unit.

Typing of secretor status

Since Le a antigen in the secretory cells of salivary glands was easily detected by immunohistochemical method, the secretor status of cadavers could be determined. In 1989 we reported the racial difference of expression of blood group antigens in salivary glands from non-secretor individuals[12].

We compared the histochemical properties of mucous cells of sublingual and submandibular glands from blood group A and AB Japanese non-secretors with those from German non-secretors, using conventional histochemical and immunochemical methods as well as lectin histochemical method[12]. In all the cases of blood group A and AB Japanese non-secretor individuals, monoclonal anti A-antibody, HPA and HAA lectins moderately or strongly reacted with mucous cells of the salivary glands. In contrast in the cases of German non-secretors, majority of mucous cells were not stained with these two lectins and monoclonal anti A antibody. No histological difference of mucous cells were observed between the Japanese and German non-secretors, except the staining with anti-A antibody and these two lectins. We revealed subsequently that type 1 chain of A, B or H antigens was expressed in the mucous cells from Japanese non-secretors[30]. In 1995 Rouquier et al[31] cloned the secretor gene (Sec 2) encoding a 1-2 fucosyl transferase and Kelly et al[32] demonstrated a G428A nonsense mutation of non-secretors from European individuals. Koda et al[33] demonstrated many

miss-sense or nonsense mutations in the non-secretors from many populations. Their workings proved the ethnical difference in the expression of blood group antigens.

ABO Blood grouping from materials obtained from criminal scene

In the forensic practice, various kinds of materials would be collected from the scene of crime, such as a mixture of body fluids containing buccal and sperm cells, tissue particles from animals or humans and materials contaminated by the bacteria, should be examined for the identification of the perpetrator. Since it is widely accepted that ABH antigens are expressed in the secretory organs of many species animals, it is important to exclude false positive reaction and misjudgment of species identification in the blood group determination, where the actual distribution of ABH antigens on organs and cells in each animal or human is revealed and the reaction sites on the tissue sections can be microscopically confirmed by a histochemmical method. This is a great merit of histochemical method. We have been studying the distribution of the ABH antigens in organs and tissues of several vertebrate species including humans, using the histochemical method[34,35]. The ABH antigens were expressed on vascular endothelium and erythrocytes as well as secretory cells of humans and anthropoid apes, while they were expressed on vascular endothelium and secretory cells but not on red blood cells of old world monkeys. In new world monkeys, non-primate mammals and non-mammalian vertebrate species, they were expressed only on secretory cells. Exceptionally and surprisingly, in frog, a reactivity against anti A or B antibody is observed in endothelial cells, secretory cells and red blood cells which possess a nucleus. UEA-I lectin specific to fucose stained vascular endothelium of humans and anthropoid apes. The vascular endothelium of new world monkeys and non-primate mammals showed good reactivity with GSAI-B4 lectin specific to galactose and not with UEA-I, and those from non-mammalian vertebrate species showed no reactivity with these lectins. G*; GSA-IB4 lectin, specific to galactose. U**; UEA -I lectic, specific to fucose -/+***; Cells from blood group B and O typed individuals reacted with B and H specific lectin, respectively.

Table 3. Expression patterns of ABH antigens and lectin binding in red cells, endothelial cells, and secretory cells of various animal species.

	Red Blood Cells	**Endothelian Cells**	**Secretion**				
Antibodies/Lectins	ABH	G*	U**	ABH	G*	U**	ABH
Human	+	-	+	+	-	+	+
Anthropoid apes							
Chimpanzee	+	-	+	+	-	+	+
Gibbon	+	-	-	+	-	+	+
Old world monkeys	+	-	-	+	-/+	-/ +***	+
New world monkeys	-	+	-	-	+	-	+
Prosimians	-	+	-	-	+	-	+
Non-primate mammals	-	+	-	-	+	-	+
Non-mammalian vertebrates							
Chickens	-	-	-	-	-	-	+
Saurian	-	-	-	-	-	-	+
Frog	+	+	-	-	-	-	+
Fish	-	-	-	-	-	-	+

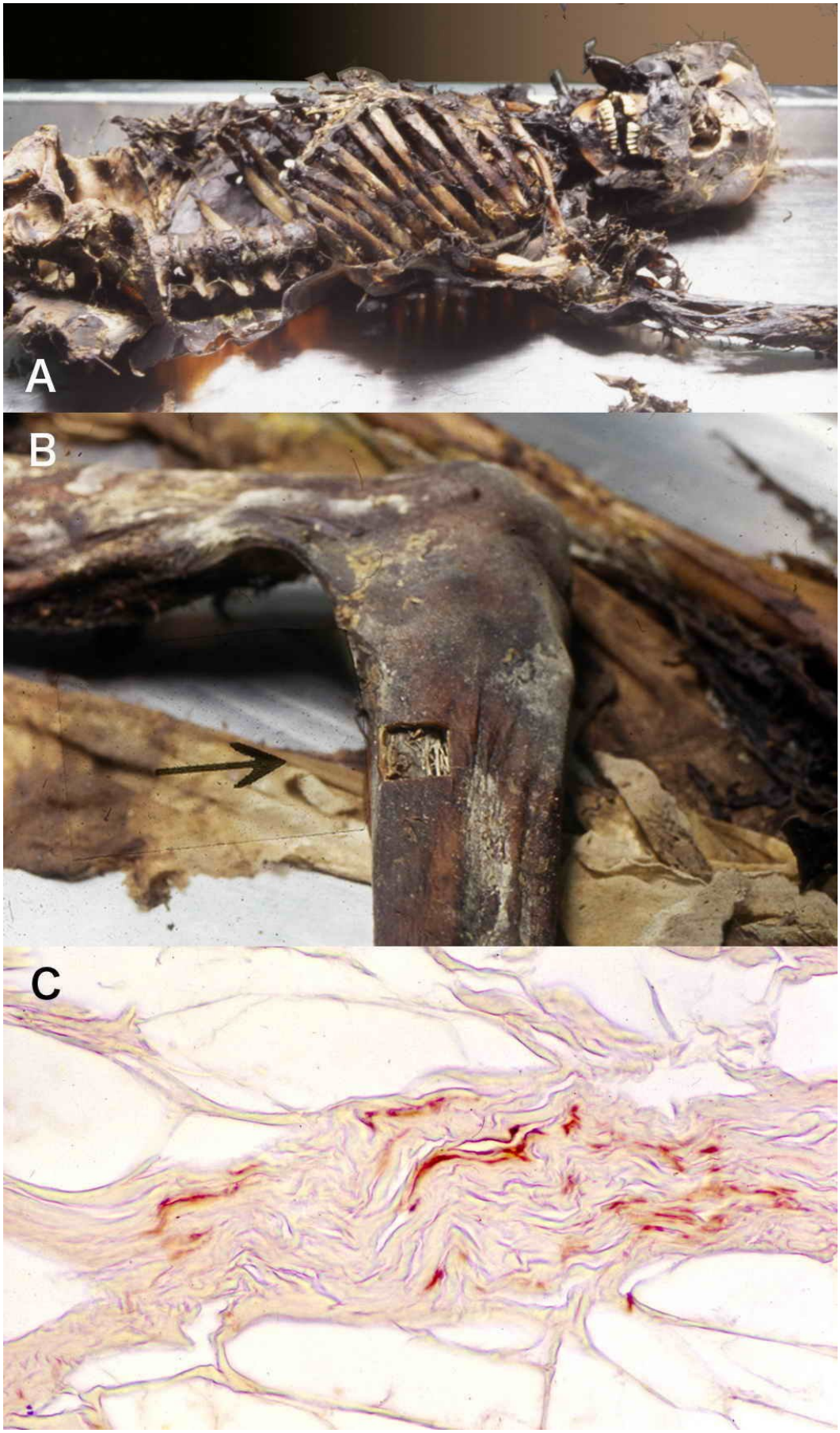


Figure 5. ABO grouping from mummified skin. Endothelial cells of blood vessel in the mummified skin obtained from the leg (photo B) of a skeleton body (photo A) showed reactivity with

corresponding anti body as shown in photo C. The PCR product of DNA obtained from the mummified skin could not be amplified

These results summarized in Table 3 indicate that both the changing of the carbohydrate epitope and the development of ABH antigen expression on red blood cells and endothelial cells are regarded as an important evolutionary event in vertebrate species and also showed that the species identification and blood grouping could be reliably performed by the histochemical observation of vascular endothelial cells and red blood cells, since the blood vessels are present in all tissues and are expected to be found in putrid and/or mummified tissues. In a previous study[36], investigations of forensic cases as well as experimental determinations were carried out on dried tissue samples to identify the ABH antigens and human hemoglobin using the immuno-peroxidase technique. Samples from various organs were stored at room temperature over a period of 1 year and periodically examined immnohistochemically. After the incubation of dried tissues in a rehydration medium, blood grouping and species identification were successfully performed in the whole experimental series. The availability of the rehydration medium was confirmed by Ogata et al[37]. We also use the histochemical method for blood grouping from skin of a mummified body, as shown in Figure 5.

ABO genotyping

Although the various methods for determining ABO types were modified and elaborated, especially after 1960's[38], they can detect only the ABO phenotype but not genotype. In 1990 Yamamoto et al[39] cloned and sequenced a complementary DNA(cDNA) encoding the A1 transferase, and subsequently cloned and analyzed both B and O allelic cDNA. After then, ABO genotyping using DNA samples from fresh blood, blood stains, fingerprint and other forensic materials has been reported[40,41,42,43]. We also determined the ABO genotypes with DNA extracted fresh blood[44] and formalin-fixed, paraffin embedded tissues[45]. We extracted DNA from paraffin blocks using proteinase K treatment followed by phenol/chloroform extraction. Approximately 1-10 ug of DNA was isolated from 20mg of a paraffin block. The DNA was degraded with a molecular weight ranging mostly from 100 bp to 2Kbp. The sequence of nucleotides for A, B and O genes was amplified by PCR using the primers according to Lee and Chang[46] (figure 6 [excel file] [htm file]). DNA fragment of 128bp and 200bp could be amplified in the second round of PCR, using an aliquot of the first round PCR product as template. Degraded DNA from paraffin blocks stored for up to 10.7 years could be successfully typed[45].

We further examined whether the sequence corresponding to human ABO genes existed in other species of animals[47], since it is well known that many vertebrate species express the ABH antigens in their secretory cells. DNA extracted from Japanese monkey, pig, rabbit, rat, Chinese hamster, mouse, mastomys and gold fish was amplified by PCR using three pairs of primers designed according to the sequence of human A1 gene[46]. DNA fragments from each animal could be amplified by the given primers used in the examination, as shown in Figure 5 where the electrophoresis patterns of PCR products were different among the species and similar within the same species. Amplified DNA fragment obtained from all animals were hybridized in the human ABO gene probe with washing at lower stringency. Even after washing at high stringency the amplified DNA fragment obtained from Japanese monkey, cat rabbit and gold fish were hybridized. The results indicate that a similar gene or pseudo gene encoding A or B transferases of the human may exist in these vertebrate species. According Yamamoto et al[39] there are seven consistent

nucleotide substitutions of A transferase leading to amino acid changes to form B transferase, B alleic cDNA. O allelic cDNA is formed due to the deletion of nucleotide position 261, which causes the loss of transferase activity. We determined the partial nucleotide sequence of DNA fragments obtained from the Japanese monkey[47], which was hybridized with the corresponding PCR product from human DNA. The amino acid sequence estimated by DNA sequence analysis resembled that from the human B transferase, as shown in Fig 6. Therefore, we must repeatedly emphasize that the species identification is an important procedure even in ABO genotyping by PCR using DNA materials because amplified DNA from vertebrate species appears to be hybridized to those from humans[48]. In such cases, a combination of the electrophoretic and hybridization analyses using amplified cDNA fragments might be a useful tool for species identification, as shown in Fig 7[47].

Biological significance of ABH and related antigens

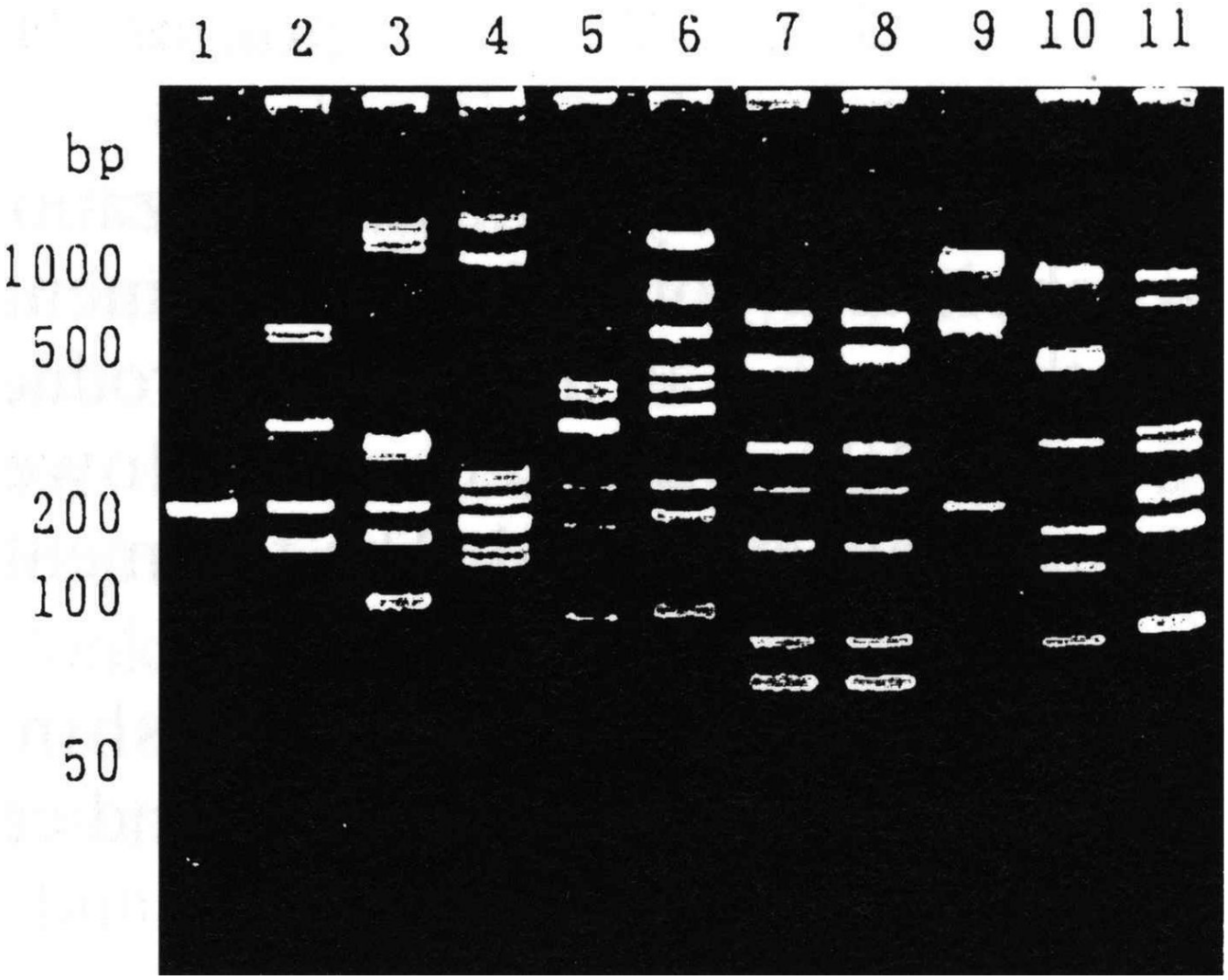


Figure 7. Electrophoretic pattern of the PCR product using pairs of primers for ABO genotyping. M; 174/Hae III, 1;Human, 2;Japanese Monkey, 3;pig, 4;Cat, 5;Rabbit, 6;Chinese Hamster, 7;Rat(Wistar), 8;Rat(Fischer), 9; Mastomys, 10;Mouse, and 11: Goldfish

Although detection methods and techniques of ABO phenotype and genotype have been completely established[17,23,25,36,38,43,49,50] , the biological significance of ABH and related antigens is not well elucidated even today. We have been studying the distribution of ABH and related antigens in salivary glands and reproductive organs of several mammals containing human, and the brain of

human in order to understand and explain the biological significance of the ABH and related antigens[51]. The taste bud cells and serous cells of von Ebner's gland showed good reactivity with blood group A, B, H, Le y or Le a antibodies corresponding to the blood group of individual animals[52]. The epithelial cells, secretory fluid and spermatozoa in the epididymal lumen of cat and rat were clearly stained with anti A, B and/or Le y antibodies. Anti A, H Le x and Le y antibodies stained the Golgi region of the cells in collecting tubules of cat kidneys[53]. Form these results it can be said that the ABH and related antigens may be associated with the chemo-receptor system in the taste buds cells and spermatogenesis in the reproductive organs of mammals. Although the expression of the ABH antigen in the organs relates to an important evolutionary event in vertebrate species, the expression on erythrocytes and endothelium of blood vessel occurred very late in evolutionary time, as described above. These results indicate that ABO polymorphism might appear in contact with the external environment and play a role in the relationships of the species with pathogens, although the antigen might be involved in cell-cell recognition in the embryo in lower vertebrate species[54].

The human fucosyltransferase gene constitute a multi-gene family, and nine genes encoding human fucosyltransferase(FUT1-FUT9) have been identified[33], showing the biological importance of these enzymes and genes. The FUT1 and FUT2 encode a(1,2) fucosyltransferase which adds a fucose to form H antigen, and the FUT 3-FUT9 encode alfa(1,3/1,4 or 1,3)fucosyltransferase which synthesize various Lewis antigens of type 1 and of type 2 chains.a(1,4)fucosyltransferase adds a fucose to a precursor to form the Le x antigen[33] which is also known as stage specific embryonic antigen I (SSEA-I) or CD 15 antigen. The possible involvement of Le x antigen, in the cell-adhesion and developmental process of vertebrate embryonic cells containing those from fish, frog, chick and turkey and of the mammalian brain are examined and discussed by many researchers[55,56,57]. We also examined the distribution and appearance of Le x antigen in human brain[58] as shown in Figure 8.

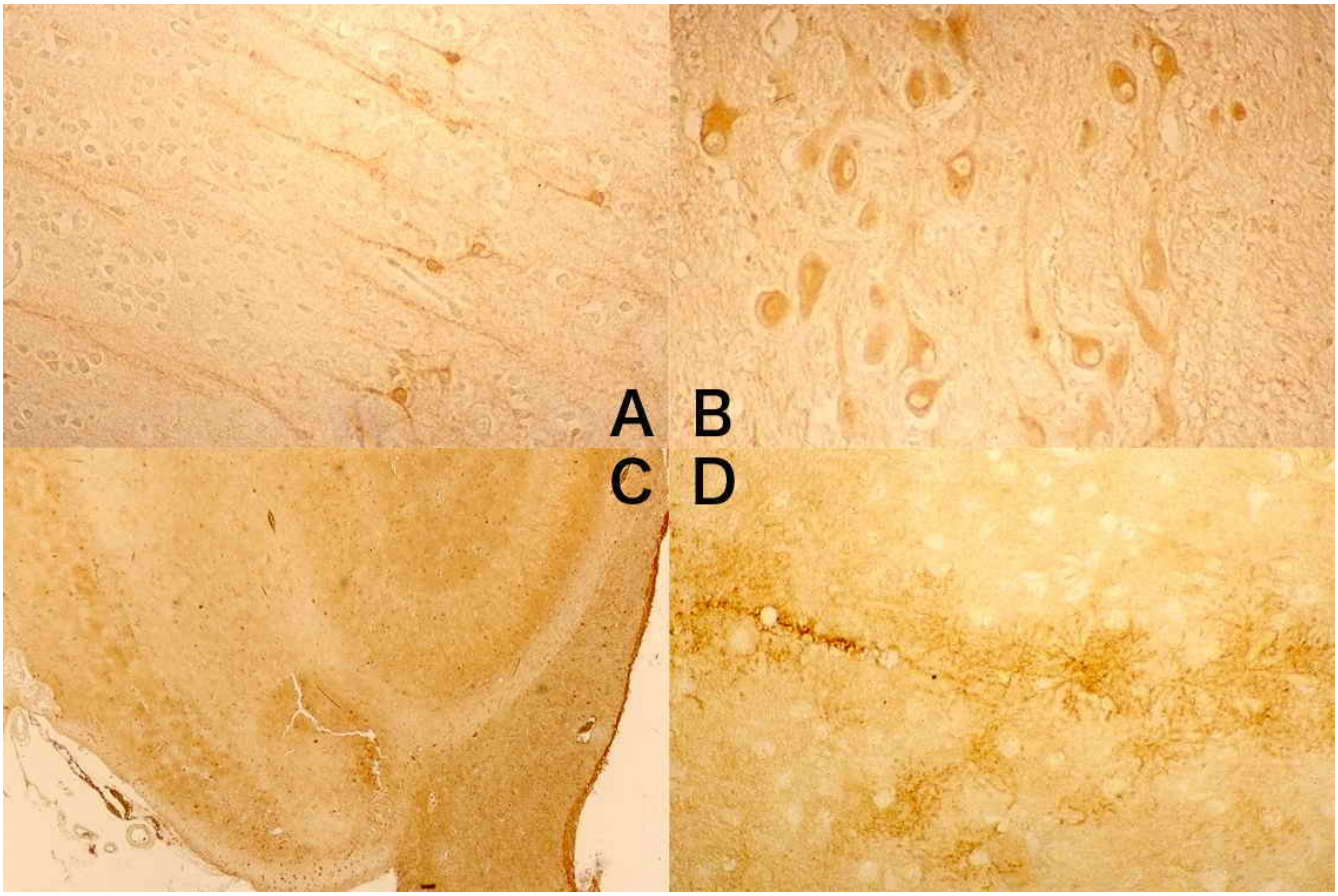


Figure 8. Expression of Le x antigen in the human brain. Neuronal cell surface in the hippocampus (A) and selected cells in the mid brain (B) from 2 months baby were stained by anti Le x. The boundary reactivity with anti Le x was observed and the Le x antigen was expressed on the outer surface of the unstained granular cells (C) in the granular cell layer of the dentate gyrus of the hippocampus from young adult. Astrocytes and microglia in the hippocampus from elderly were stained by anti Le x (D)

The anti Le x stained neurons in the cortex of the hipoccapmpal formation and also stained neurons in the selected nucleus of the mid brain from 2 months baby. While reactivity of the neurons with anti Le x disappeared around 3 months after birth, Le x positive astrocytes became concentrated in the cortex with month and age, and Le x positive micro-glia cells were remarkably detected and increased in the brain cortex from old adult. In the granular cell layer of the dentate gyrus of the hippocampus, the Le x antigen was expressed on the outer surface of the unstained granular cells which were stained by monnose specific PSA lectin in a basket-like form[58]. Eriksonn et al[59] reported that new neurons were generated from dividing progenitor cells in the dentate gyrus of adult human hippocampus, and indicate that the human hippocampus retained its ability to generate neurons throughout life. The Le x antigen among the ABH and related antigens might play a peculiar and important role in development and differentiation of the brain an/or embryonic cells and degenerative changes of the brain[60].

Conclusion

The merits and demerits of the ABO blood grouping methods utilized and developed in our unit during the past three decades were presented (Table 4). Although detection methods and techniques of ABO phenotype and genotype have been completely established, the species

identification prior to ABO typing is an important procedure in the detection of ABH antigens of blood stain, body fluid stain, tissue particle and even in ABO genotyping by DNA analysis.

Table 4. The comparison of ABO Blood Grouping Methods

ABO Blood grouping methods	Merits	Demerits
Red Cell Agglutination	Speedy and handy	Difficult preservation
Low cost	Putrefaction	
Direct observation	Bacterial contamination	
Basic of determination	Changing of antigenesity by bacteria	
Absorption Elution	Handy	Skilled technique
Low cost	Take time	
Easy preservation of sample	Contamination	
Easy reexamination	Indirect observation	
Histochemical method	Handy	Skilled technique
Direct observation	Take a lot of time	
Fewer-risk of contamination	Costly	
	Limited applications	
DNA	Genotyping	Skilled technique
Wide application	Expensive	
Micro-material	Take time	
	Contamination	

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