gives a better resolution of nerve terminal and acetylcholinesterase localizations than that obtained by conventional combined techniques on the same material.

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Localization of human A, B and H isoantigens in Cynomolgus monkey tissues

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Summary. The presence and distribution of human A, B and H isoantigens were demonstrated in Cynomolgus monkey *(Macaca fascicularis)* by means of red cell adherence test. Although no human antigens were found on primate erythrocytes, various epithelial tissues revealed the presence of A, B or H antigenic substance. The distribution and localization was similar to that found in human tissues. Majority of specimens from each individual animal possessed only 1 human type isoantigen with the exception of the salivary and sweat glands, where all animals showed the presence of H antigen in addition to other specificity, and of Brunner's gland, where all sections reacted positively also for A antigen.

Blood group isoantigens A, B and H are present in human body fluids and tissues other than erythrocytes and their distribution has been well established[1,2]. In non-human primates, the presence of these antigens on red blood cells was also extensively described[3–5]. However, most of the Old-World monkeys do not possess the A-B-H factors on their erythrocytes, but the factors appear as soluble blood group substances in some of their secretions, as described by Wiener et al.[5], who were able to demonstrate all 4 A-B-0 groups in *Macaca fascicularis* by using the inhibition test on saliva. Franks et al.[6] showed the presence of the B antigen on buccal and kidney cells, using the mixed agglutination technique.

In the present study we have tried to establish the presence and distribution of human A, B and H antigens in various tissues obtained from Cynomolgus monkeys *(Macaca fascicularis)*, using the red cell adherence (RCA) test.

Materials and methods. RCA technique. The principle of the red cell adherence test is a modification of mixed cell agglutination reaction adapted for use in paraffin-embedded histologic sections[7]. Briefly, the tissue sections are de-paraffinized, transferred into tris-buffered saline and reacted with human anti-A or anti-B agglutinin for type A or B antigen, or with an extract of *Ulex europeus* seeds for type 0. After incubation at room temperature, the unfixed antibody or lectin is washed off and the sections are covered with erythrocyte suspension of the appropriate blood group. Following the incubation, the sections are inverted on supports in a layer of tris-saline in a petri dish. The non-attached red blood cells fall to the bottom of the dish and the erythrocytes adhering to the section show the localization of the antigen.

The RCA reaction is evaluated as: a positive reaction (+), where the attached erythrocytes demonstrate the presence of an antigen; a negative reaction (−), which indicates the absence of an antigen; and a weak positive reaction (±), where only some cells within a microscopic field demonstrate small amounts of antigenic material.

After the RCA test, each histologic section is stained with hematoxylin and eosin and the exact localization of the antigen is established.

Results. Several examples of a red cell adherence test on histologic sections of Cynomolgus monkey tissues are presented in the following photographs. Figure 1 demonstrates

Distribution of human type A-B-H antigens in Cynomolgus monkey

Animal No.	1			2			3			4			5			6		
Antigenic type	A	B	H	A	B	H	A	B	H	A	B	H	A	B	H	A	B	H
Esophagus	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−		NA	
Stomach	+	−	±	−	−	+	−	+	±	−	−	+	−	+	±	−	+	−
Duodenum	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−		NA	
Brunner's gland	+	−	−	+	−	+	+	+	−	+	−	+	+	+	−		NA	
Small intestine	+	−	−	−	−	−	−	+	−	−	−	+	−	+	−		NA	
Large intestine		NA		−	−	−	−	+	−	−	−	+	−	+	−	−	+	−
Pancreas	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−		NA	
Salivary gland	+	−	+	−	−	+	−	+	+	−	−	+	−	+	+	−	+	+
Oral epithelium	+	−	±		NA		−	+	±		NA			NA			NA	
Bronchus	+	−	−	−	−	+	−	+	−		NA			NA			NA	
Skin (keratin)	+	−	−	−	−	+	−	+	±	−	−	+	−	+	−		NA	
Sweat gland	+	−	+	−	−	+	−	+	+	−	−	+	−	+	+		NA	
Urinary bladder	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	+	−

NA, not available for evaluation; +, positive RCA, antigen is present; −, negative RCA, antigen is absent; ±, weak positive RCA, antigen is present in very small quantity.

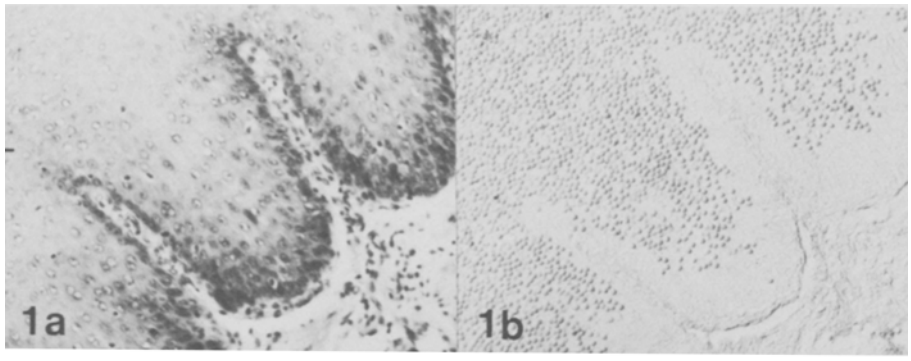

Fig. 1. Monkey No. 4, type H. Esophagus. *a* H & E; *b* RCA with *Ulex europeus* extract and 0 rbc is positive in squamous epithelium, basal layer and connective tissue are negative. × 100.

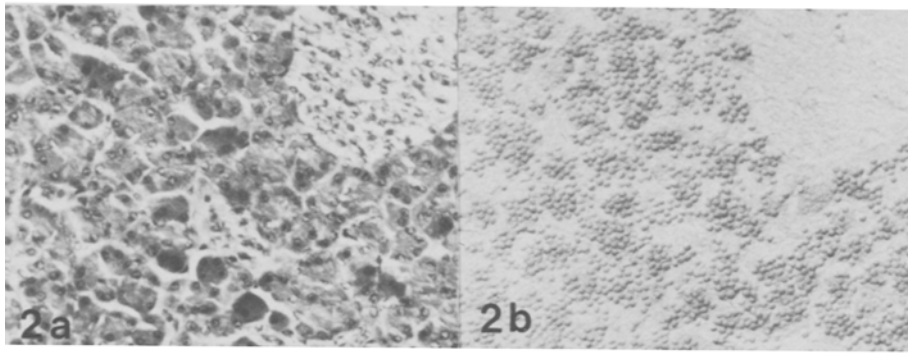

Fig. 2. Monkey No. 1, type A. Pancreas. *a* H & E; *b* RCA with anti-A serum and A_1 rbc is positive in exocrine glands, negative in the islet of Langerhans. × 120.

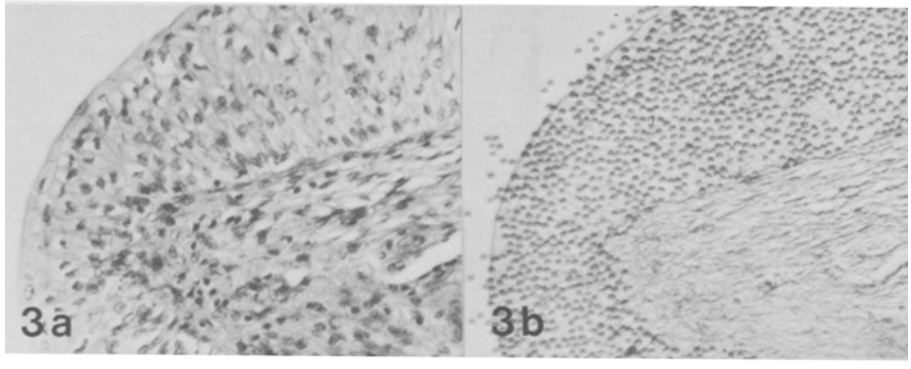

Fig. 3. Monkey No. 6, type B. Urinary bladder. *a* H & E; *b* RCA with anti-B serum and B rbc is positive in the transitional epithelium, negative in connective tissue and smooth muscle. × 120.

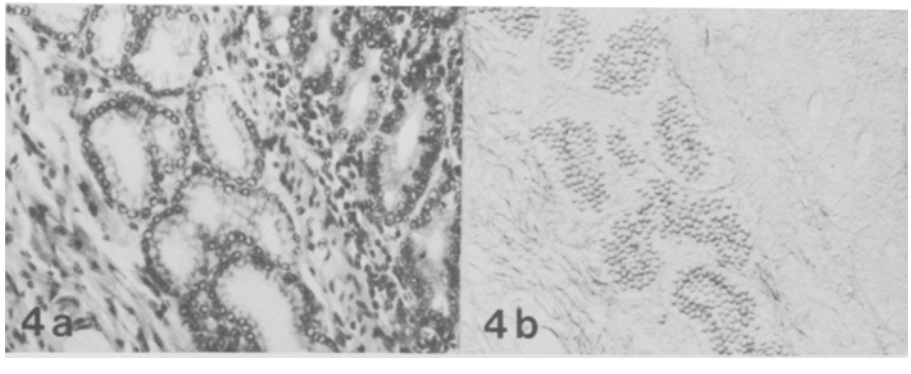

Fig. 4. Monkey No. 2, type H. Duodenum. *a* H & E; *b* RCA with anti-A serum and A_1 rbs is positive in Brunner's glands, the columnar surface epithelium is negative. × 100.

the positive reaction on the squamous epithelium obtained from the esophagus of a monkey. The antigenic substance is in all epithelial layers with the exception of the basal layer, which is always negative, as well as in human tissues. In figure 2, the attached indicator erythrocytes show the presence of an antigen in parenchymal cells of a pancreas, while the island of Langerhans is negative, again repeating the identical reactivity of a human pancreas. Figure 3 shows the reaction in the transitional epithelium of the urinary bladder. Similarly, in all available tissues from Cynomolgus monkey, the red cell adherence test showed almost identical localization of A, B and H antigens when compared with human tissues, with 1 exception; the fixed human erythrocytes, contained in lumina of vessels are always positive, while in the monkey this reactivity was never observed, confirming the results of previous studies that red cells of Old-World non-human primates do not contain A, B or H specific antigens.

The distribution of antigens is presented in the table. In all the organs listed, only the epithelial cells contained the antigenic material while smooth muscle and connective tissues were always negative.

Also, as in the human, the squamous epithelium of the skin contained the antigen only on the surface keratin layer.

An interesting phenomenon occurred in the Brunner's glands where all animals exhibited the presence of A antigen in addition to their own specific antigen (figure 4). Again, this shows certain similarity to human tissues, except that human Brunner's glands in persons of all blood types contain the H antigen[8]. Another unusual reaction was observed in salivary glands and sweat glands which contained in all animals the H antigen. (The weak or ± reaction with H antigen seen in the oral epithelium and stomach mucosa of monkeys Nos 1 and 3, and in the skin of monkey No. 3 could be possibly due to the passive absorption of an H antigenic material produced by salivary or sweat glands, respectively.)

Although not all the tissues were always available for our examination, the table shows clearly that monkey No. 1 was of A type, monkeys Nos 3, 5 and 6 of B type, and monkeys Nos 2 and 4 of 0 type. The lack of positive reaction in the intestinal mucosa of monkey No. 2 in repeated tests could not be explained.

Discussion. This work has attempted to demonstrate the presence of human A, B and H antigens in tissues of Cynomolgus monkey *(Macaca fascicularis),* and to compare the distribution and localization of these antigens with the results obtained on human material. The majority of tissue specimens from each individual animal possessed only 1 human type isoantigen with 2 exceptions. The salivary and sweat glands of all animals showed the presence of H antigen in addition to other specificity. This phenomenon could possibly support the hypothesis established in human tissues, that the production of H antigen is a necessary prerequisite for a subsequent transformation into A or B specificities by the action of genetically controlled enzymes which add the terminal sugars N-acetyl-galactosamine or D-galactose, thus converting the precursor to A or B active substances[8]. However, this explanation does not support the other exception in our study, i.e. the presence of A antigen in Brunner's glands of all examined animals.

The demonstration of the presence of A, B and H antigens in Cynomolgus monkey tissues can serve as a useful model system for the future studies about basic functions and localizations of tissue-associated polysaccharide materials such as blood group substances, and the basic differences in activity or function between the glycolipid and glycoprotein counterparts. Furthermore, the mechanism of the loss of isoantigens during malignant transformation of human cells[9] could be established.

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The effect of *Listeria monocytogenes* lipids on immune response to T-dependent and T-independent antigens

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Summary. The administration of *Listeria monocytogenes* lipids augmented the humoral immune response to ovalbumin (OVA), polyvinyl pyrrolidone and *E. coli* lipopolysaccharide, but failed to support the induction of delayed hypersensitivity to OVA.

Our previous studies have indicated that injection of *Listeria monocytogenes* lipids (LML) results in a significant increase of the humoral immune response to sheep erythrocytes and in an enhancement of resistance to some bacterial and fungal infections[2,3]. In the present work the effect of LML on the immune response to some T-dependent, ovalbumin (OVA)[4], and T-independent polyvinyl pyrrolidone (PVP) and lipopolysaccharide (LPS)[5] antigens was studied.

Materials and methods. Male Swiss albino mice weighing 16–18 g were immunized by i.v. injection of 250 µg OVA or 0.25 µg PVP, or 50 µg LPS in 0.2 ml of saline. Other groups of mice received injections of a mixture of antigen and 100 µg LML. The method of Cunningham and Szenberg was applied for detecting plaque-forming cells (PFC) in spleen[7]. Sheep erythrocytes (SE) coated with OVA[8], PVP[5,9] and LPS[10] were used both for hemolytic plaque assay and for estimation of specific antibodies in heat-inactivated and SE-absorbed sera. A method of passive microhemagglutination was used for determination of the specific antibody titer in native and 2-mercaptoethanol (2-ME)-treated sera. The selective removal of IgG, IgM and IgA was achieved