H Substance - A Precursor of A and B Tissue Isoantigens

The aspects of previous genetic studies on the ABO system were reviewed by WATKINS[1] and ANDERSON[2]. Information gained from work on the inheritance of human blood groups has led to the conclusion that the formation of H substance is controlled by the allelic genes H and h. The H substance is then acted upon by genetically controlled enzymes which add the terminal sugars N-acetyl-galactosamine or D-galactose, thus converting the precursor to A or B active substance, respectively. The Hh and ABO systems are inherited independently. However, if H substance is truly the only precursor for the production of A and B substance, the phenotypic expression of the two systems is closely related. In our work we attempted to detect the presence of precursor H substance in normal tissues of individuals of A, B, and AB blood groups, using the specific red cell adherence (SRCA) test[3,4].

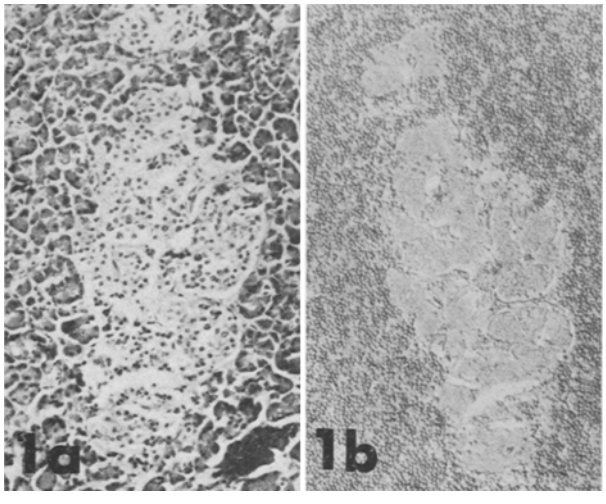

Fig. 1. Pancreas; blood group A. a) H & E; b) SRCA with anti-A serum and A_1 erythrocytes is positive in exocrine glands, islet of Langerhans is negative. ×100.

Materials and methods. Paraffin sections of formalin fixed tissues obtained from autopsies or surgical specimens were used throughout the study. Commercially available pooled human anti-A and anti-B sera with agglutinin titers of 512 were used for the detection of A and B antigens by the SRCA test as described previously[3]. The extract of *Ulex europeus* seeds[5] with an agglutinin titer of 1024 was used for the detection of H antigen. Human red cells of groups A_1, B ,and O were used in 1% suspension in *Tris*-buffered saline. A positive built-in control[4] was the ubiquitous endothelial lining of vessels, while the connective tissue served as a negative built-in control.

Results and discussion. The presence of only H antigen in fetal pancreatic exocrine glands from individuals of all 4 blood groups was shown in our previous study[6]. This phenomenon was observed up to 20 weeks of gestation, when the exocrine glands contained both H antigen and that present in the individual's red blood cells. Similar reaction was observed in fetal Brunner's glands of the duodenum, where the appearance of H substance preceded that of A or B specificity. Our study of adult tissues showed similar reactivity. All examined pancreatic tissue sections exhibited the presence of individual's own blood group isoantigen in exocrine glands, while the islets of Langerhans were negative (Figure 1). When the identical or subsequent sections were treated with *Ulex europeus* extract, some acini always showed the presence of H antigen (Figure 2). The perpetual presence of either isologous or H antigenic substance in all sections suggests the independence of pancreatic parenchyma on the Sese secretory status. Tissue sections from duodenum of A or B blood group adults demonstrated the presence of

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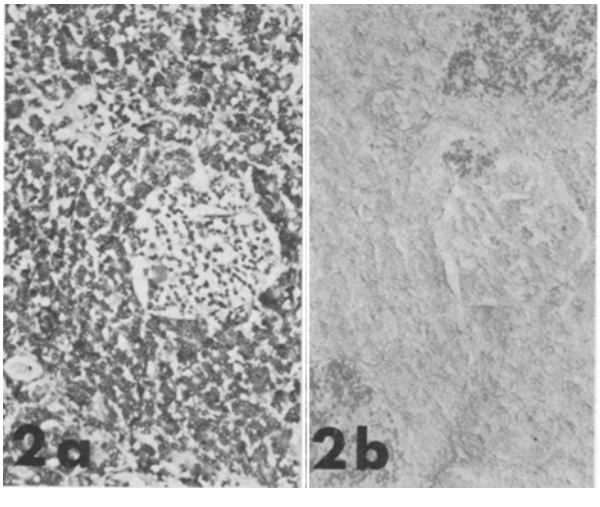

Fig. 2. Same tissue specimen as in Figure 1. a) H & E; b) SRCA with *Ulex europeus* extract and O erythrocytes. Some exocrine glands are positive. ×100.

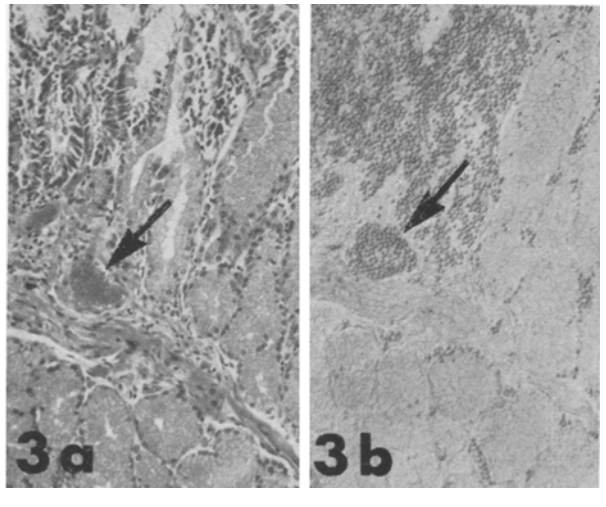

Fig. 3. Duodenum; blood group A. a) H & E; b) SRCA with anti-A serum and A_1 erythrocytes is positive in mucosal epithelium, host erythrocytes (arrow) and endothelium; Brunner's glands are negative. ×130.

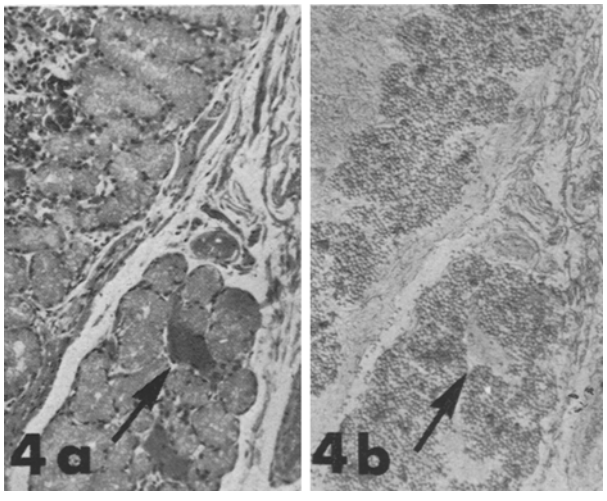

Fig. 4. Same tissue specimen as in Figure 3. a) H & E; b) SRCA with *Ulex* extract and O erythrocytes; mucosa, host erythrocytes (arrow) and endothelium are negative; Brunner's glands show positive reaction. ×130.

isologous antigen in mucosal epithelium in about 80% of examined cases which would correspond to the general distribution of secretors. Brunner's glands reacted positively for the isologous blood group substance in some acini, while others were negative (Figure 3). When sections of A or B blood group were reacted with *Ulex* extract, the mucosal epithelium was always negative but acini of Brunner's glands showed positive SRCA (Figure 4). Glands from the breast or uterine cervix showed great similarity in reactivity. In both organs only some glands contained the antigens while others were negative, being probably in a 'resting' phase. From all examined cases of these two organs approximately 60% had both the isologous and H antigen, 20% had either isologous or H antigen, and the remaining 20% were completely negative, obtained possibly from non-secretors. Other tissues containing the H substance in individuals of all blood types were mucosa of the stomach and sweat glands. SZULMAN[7] described the H antigen in other tissues such as endothelium, stratified epithelium or tracheobronchial epithelium using immunofluorescence on frozen sections. These tissues showed in our experiments the presence of only isologous antigen. We believe that the differences in results were probably due to various techniques used, the SRCA test being more sensitive and specific.

The early appearance and continuous presence of H substance in some secretory glands of A, B, and AB individuals support the hypothesis that its production is a necessary prerequisite for a subsequent transformation into A or B specificities. The presence of H substance in glands which do not contain the individual's own isoantigen suggests that this H antigen might be transported from site of production to other areas. In these sites the glycosyl transferase enzymes could then add the specific sugar units to the transported glycoprotein molecule to complete the synthesis of the blood group antigen.

Zusammenfassung. Das Vorhandensein der H-Substanz im normalen Gewebe von Personen mit den Blutgruppen A, B und AB wurde mit dem spezifischen Blutkörperchen- Anhaftungstest, «specific red cell adherence» (SRCA), festgestellt. Das frühe Erscheinen und die dauernde Anwesenheit der H-Substanz in manchen Exokrindrüsen bekräftigen die Hypothese, dass die Erzeugung der H-Substanz für die folgende Umwandlung in A oder B Spezifitäten notwendig ist.

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Effect of Cholinesterase Inhibition by Eserine and Phospholipase D on Human T Lymphocyte Rosetting[1]

A large majority of human peripheral blood lymphocytes form rosettes spontaneously with sheep red blood cells (SRBC) and apparently belong to the thymus dependent (T) subpopulation[1]. The exact basis of this phenomenon is not established. Rosetting with red cells of several species such as the pig, dog, burro, horse and goat supports the antigen-nonspecific nature of the phenomenon. It is likely that the physicochemical properties of the cell membranes involved are significant factors in rosette formation. There are striking biophysical and biochemical differences in surface membranes of T and B (thymus independent, Bursa processed lymphocytes[2]; the latter cell type does not form rosettes with SRBC spontaneously but would do so through immune binding of immunoglobulin and complement surface receptors.

We have looked at the effect of cholinesterase inhibition or depletion by eserine and phospholipase D respectively on SRBC rosette formation by human T lymphocytes.

Peripheral blood lymphocytes were isolated from heparinized venous blood of a healthy adult donor by Ficoll-Hypaque density centrifugation. The leukocyte suspension was washed twice in Medium 199. The absolute yield of lymphocytes was greater than 85% with 99–100% viability judged by dye exclusion and greater than 95% purity. These lymphocytes or SRBC were incubated with pharmacologic agents at 37 °C for 1 h, washed and mixed for rosetting assay[3]. The cell pellet was fixed in glutaraldehyde before counting. Cell viability judged by trypan blue exclusion was more than 95%. 200 cells were examined. There was a marked reduction in the number of cells forming rosettes when either human lymphocytes or SRBC had been pretreated with eserine or phospholipase D (Figure). Preincubation of lymphocytes with these chemicals had a significantly greater inhibiting effect on rosette formation.

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