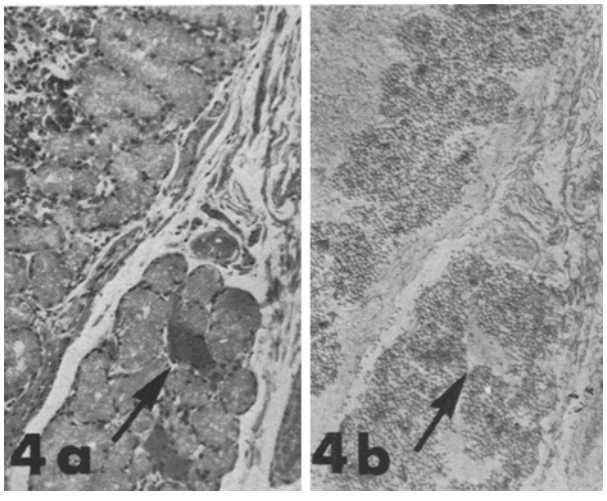

Fig. 4. Same tissue specimen as in Figure 3. a) H & E; b) SRCA with *Ulex* extract and O erythrocytes; mucosa, host erythrocytes (arrow) and endothelium are negative; Brunner's glands show positive reaction. ×130.

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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Treatment of T lymphocytes with a variety of agents inhibits rosetting. These include trypsin[3], iodacetate[3], azide[4], phospholipase A[5], neuraminidase[6], sodium cyanide[7], iodoacetamide[8], azathioprine[9], cytochalasin B[10], and antilymphocyte serum[7]. All these agents have widespread membrane damaging properties or are metabolic inhibitors and cell poisons. Our observation of a similar effect with eserine and phospholipase D suggests that the integrity of membrane-bound cholinesterase is essential for the bond between SRBC and T lymphocytes. Eserine specifically inhibits cholinesterase, increases erythrocyte fragility[11] and cellular depletion of potassium[12]. Phospholipase D splits the link between phosphoric acid and choline in the lecithin molecule and releases cholinesterase with consequent depletion of membrane-bound enzyme. Blast cells from patients with acute lymphoblastic leukemia do not form rosettes with SRBC nor do these bear surface immunoglobulin determinants[13,14]. The failure of such neoplastic cells to form rosettes may possibly be due to their low cholinesterase activity[15,16].

There is a growing appreciation that membranes are dynamic structures that participate in regulatory mechanisms though the precise role played by them is unclear. The extremely heterogeneous lipid composition, chemistry and structure are very important to the properties and functions of membranes. Spin label techniques show that the lipids of most functional membranes under physiological conditions are largely in a fluid rather than a rigid state[17]. Based on calorimetry[18] and X-ray diffraction studies[19], the phospholipids seem to be arranged asymmetrically across the two halves of the lipid bilayer. The integral proteins, as opposed to the peripheral proteins, are directly involved with the lipids in determining the structure of the membrane matrix[20]. The carbohydrates apparently project from the outer membrane surface, where they may be involved in cell contact and interaction. Our studies have shown that cholinesterase exerts a stabilizing influence on lipoproteins, in the serum[21] as well as in cell membranes[22]. It is possible that lipid-cholinesterase-protein interaction contributes to the stability and function of surface membrane of cells, including SRBC and lymphocytes.

Inhibition of human T lymphocyte-SRBC rosetting by eserine and phospholipase D suggests that membrane-bound acetylcholinesterase exerts a modulating influence on the receptors involved in this phenomenon[23].

Résumé. La formation de rosettes entre les lymphocytes T humains et les globules rouges du mouton est inhibée par l'ésérine et la phospholipase D. Cela suggère que l'acétylcholinesterase liée aux membranes influence les récepteurs impliqués dans la formation des rosettes.

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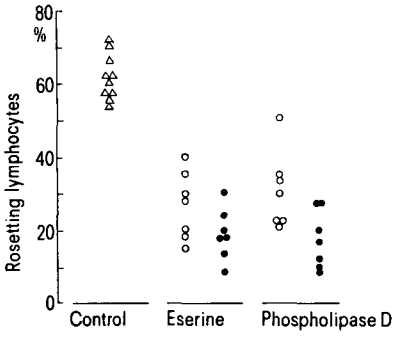

Percent of T lymphocytes forming SRBC rosettes. SRBC (○) or lymphocytes (●) were pretreated for 1 h at 37 °C with eserine (1×10^{-3} *M*, 1.25 mg/ml) or with phospholipase D (0.62 mg/ml). Each value is the mean of duplicate experiments set up on 1 day. Control figures are shown as △.

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Défaut d'incorporation du soufre dans le thymus des souris autoimmunes Swan âgées

Defect of Incorporation of S in Old Swan Autoimmune Mice Thymus

Clark[1] a montré qu'il existait un parallélisme net entre l'incorporation de soufre radioactif dans les cellules réticuloépithéliales du thymus, sous forme d'un complexe macro-moléculaire et le taux de multiplication des thymocytes dans cet organe. Aussi considère-t-il cette incorporation de soufre comme un test d'activité fonctionnelle thymique. C'est la raison pour laquelle nous avons utilisé cette exploration chez les souris Swiss normales et chez les souris autoimmunes Swan[2,3] pour étudier l'état fonctionnel du thymus de ces animaux. Nous avons en effet déjà pu mettre en évidence chez les

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