creased when the cells were incubated at the non-permissive temperature, while expression of β-actin did not change.

Addition of 0.5 µg/ml of herbimycin A for 6–30 h to cultures of ts/NRK cells at the permissive temperature increased fibronectin expression and also changed the cell morphology, as shown in figure 2 and figure 3, respectively.

Addition of herbimycin A for 30 h enhanced fibronectin expression to almost the same level as that in normal cells, and altered the cell morphology to that of normal cells, as shown in figure 2D and figure 3D, respectively. Addition of herbimycin A did not change the expression of β-actin, as shown in figure 2a–d. Thus, herbimycin A specifically increased the level of fibronectin mRNA and the time course of this increase was closely related to the morphological change. We also found that enhancement of fibronectin expression by herbimycin A was reversible, as was the morphological change.

Cell surface fibronectin is linked to intracellular microfilaments and plays a role in construction of the cytoskeleton and in cell morphology[12]. Addition of fibronectin to the tumor cells is known to change their cell shape to that of normal cells[13]. Therefore, it is likely that induction of morphological change by herbimycin A was due to its enhancement of fibronectin synthesis.

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Relationships of the soluble human A, B, and H antigens of blood group A_1B individuals

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Summary. Ouchterlony double diffusion reactions with precipitating antibodies and lectins provide visual evidence that in saliva of A_1B secretor individuals the A and B antigens are predominantly situated on the same molecule, while the H antigen is on a different molecule.

Key words. Human ABH antigens; soluble substance; precipitin reaction; lectins; antibodies.

The human A, B, and H antigens are not only present on red cells and most tissue cells, but in a soluble form they may be detected in serum, and according to a defined pattern (secretor, nonsecretor), may also be present in other body secretions including saliva. The double diffusion precipitin reaction has rarely been used in the study of the soluble A and B antigens, presumably because the human anti-A and anti-B are non-precipitating antibodies. Yet, the double diffusion precipitin reaction is a powerful technique for determining similarities and differences between antigens and between antibodies and lectins. Findings on double diffusion precipitin reactions against the soluble A, B, and H antigens of A_1B saliva are presented in this report.

Saliva samples were obtained from individuals of a previously described study[1]. Unstimulated saliva samples were collected throughout the study. Specific anti-A antibodies were produced in rabbits as previously described[2]. Selected rabbits which lacked the A antigen in their saliva were repeatedly injected with human group A red cells. The immune sera were inactivated, diluted with an equal amount of saline, and absorbed with pooled human group B as well as O cells. Five or more absorptions, each with equal volumes of washed packed erythrocytes, were required to remove the detectable traces of anti-B and nonspecific antihuman reactivity. The anti-A reagents were cleared by twice-repeated precipitation of the γ-globulin fraction with saturated ammonium-sulfate in a ratio of 2 parts of ammonium-sulfate to 3 parts of reagent. The final precipitate was re-dissolved in phosphate buffered saline, and the ammonium-sulfate was removed by dialysis against saline at 4 °C.

Attempts to produce anti-B in rabbits yielded unsatisfactory results, presumably because all rabbits possess a B-like antigen. However, goats produced an anti-B of high titer suitable for precipitin reactions. These antisera were purified by selective absorption with group O and A red cells.

The lectins of *Ulex europaeus* were purified by fractionation with increasing concentrations of ammonium sulfate, followed by column chromatography separation. At relatively low concentrations of ammonium sulfate (e.g. 40%) the precipitate was rich in L-fucose specific anti-H. These fractions were purified further with a DEAE Fractogel column followed by an L-fucose affinity column to obtain the Ulex I fraction[3].

Double diffusion precipitin reactions were carried out in agar plates as previously described[4]. However, in this study, 0.5% SeaKem HGT(P) agarose (FMC Corporation, 5 Maple Street, Rockland, Maine 04841) was substituted for agar-Noble. The agar plates were incubated at 4 °C in a moist box and inspected daily for precipitin bands. Optimal band formation was observed after 2–5 days. Photographs were taken against a dark background with indirect light from 4 high intensity bulbs at an angle below the agar plate.

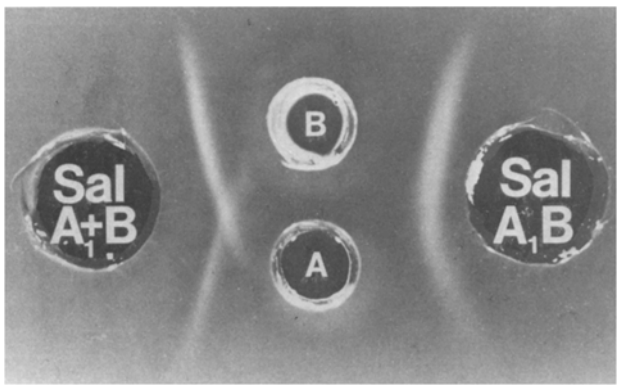

Figure 1. Double diffusion precipitin reactions of anti-A (A) and anti-B (B) antibodies against saliva (Sal) of an A_1B secretor individual and against a mixture of salivas of A_1 and B secretor individuals.

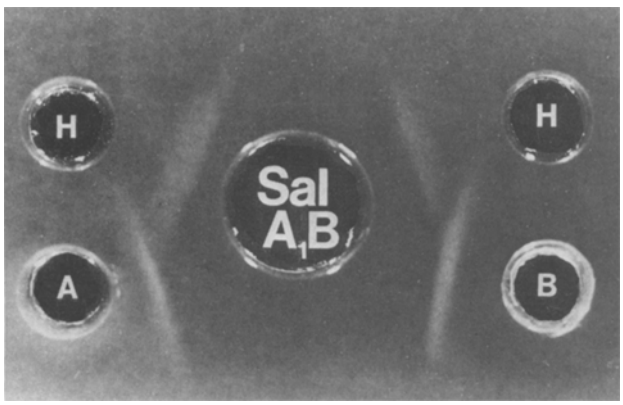

Figure 2. Anti-A and anti-B antibodies reacting besides anti-H (H) lectin against saliva of an A_1B secretor individual.

Figure 1 displays the precipitin pattern obtained from anti-A and anti-B antibodies reacting with saliva from a group A_1B secretor individual and a mixture of salivas from group A_1 and B individuals. The mixture of A_1 and B salivas exhibits bands of non-identity (bands crossing) while the A_1B saliva shows a pattern of identity (smooth continuation). These patterns provide visual evidence that in the A_1B saliva the A and B antigens are situated predominantly on the same molecule. All 7 A_1B salivas tested formed continuous precipitin bands between anti-A and anti-B antibodies.

Precipitin band formation of anti-A and anti-B antibodies placed in wells beside anti-H lectins is shown in figure 2. The patterns exhibit either partial identity or non-identity suggesting that in saliva of A_1B individuals the H antigen is primarily situated on molecules other than the A and B antigens. The figure shows relatively weak and diffuse bands against anti-H lectin, indicating relatively low concentration of H substance in the A_1B saliva. By hemagglutination inhibition tests, the mean concentration of H substance was reported to be lower in saliva of AB individuals than in those with blood group O, A_1, A_2 or B[5].

The band of identity formed against the A_1B saliva, displayed in figure 1, provides visual evidence that the A and B antigens are primarily situated on the same molecule. This may well be the first example of such a pattern of identity against soluble molecules with known different antigens, although it has been previously reported for the cucumber mosaic virus[6]. However, this virus is much larger than the soluble substances of saliva. Indirect evidence that the A and B antigens are on the same molecule has been previously reported[7,8]. The synthesis of soluble substances possessing predominantly both the A and B antigens requires a mechanism that adds regularly D-galactose and N-acetylgalactosamine to the same molecule. The previously reported finding of a hybrid glucosyltransferase in A_1B individuals[9], can explain the resulting band of identity (fig. 1). The foregoing figures further indicate that the soluble substances from A_1B individuals predominantly carry either the H or A and B, but not all three antigens.

Vitala et al.[10] reported that the A and B antigens of AB erythrocytes are located on different glycopeptide chains. This points to a possible difference in the arrangement of the A and B antigens of erythrocytes and the soluble substances. However, Vitala and coworkers digested erythrocyte membranes with pronase, obtaining blood group reactive glycopeptides with a mean mol.wt of 10,000. This is at least 20 times lower than the molecular weight of blood group substances[11]. Consequently, the question arises whether the glycopeptides after pronase digestion of membranes still represent the original antigen organization or whether this arrangement was broken.

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Genetic control of malate dehydrogenase in *Nicotiana suaveolens* and *N. glutinosa*

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Summary. The study of malate dehydrogenase patterns in leaves of *Nicotiana suaveolens, N. glutinosa* and their interspecific hybrid has been carried out, in order to propose a model of its genetic control. Two possible explanations for the genetic control of malate dehydrogenase have been postulated for *N. suaveolens,* and at least two loci appear to be implicated in the control of this system in *N. glutinosa.*

Key words. Nicotiana suaveolens; N. glutinosa; interspecific hybrid; malate dehydrogenase.