

# Estimation and comparison of the contents of blood group B antigens in selected human tissues by microphotometric quantification of *Griffonia simplicifolia* agglutinin I-B<sub>4</sub> staining with or without prior $\alpha$ -galactosidase digestion

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**Summary.** *Griffonia simplicifolia* agglutinin I-B<sub>4</sub> (GSAI-B<sub>4</sub>) has broader specificity for B antigen variants and can recognize the antigens in a wide variety of human tissues. Thus, the concentration range of GSAI-B<sub>4</sub> required for staining and the susceptibility of staining to  $\alpha$ -galactosidase digestion is presumed to correlate well with the density of B antigens in tissue sections. By microphotometric quantification of staining intensity at different concentrations of GSAI-B<sub>4</sub> with or without  $\alpha$ -galactosidase digestion, concentration of B antigens in selected tissues was evaluated and compared. Based on the present results and the previous ones of direct measurement of galactose of B antigens in sublingual glands and red blood cells (Ito et al., 1993), the order of concentration of B antigens in tissues examined was estimated as follows; mucous cells of sublingual glands from German nonsecretors < red blood cells and vascular endothelial cells ( $=2.7 \times 10^{-3}$  nmole/cm<sup>2</sup>), thyroid papillary carcinomas and Hassall's corpuscles from nonsecretors < mucous cells of sublingual gland from Japanese nonsecretors < pancreatic acinar cells from both secretor and nonsecretors, Hassall's corpuscles and kidney collecting tubules from secretors < mucous cells of sublingual gland from secretors ( $>8.5$ - $11.7$  nmole/cm<sup>2</sup>) and mucous cells of Brunner's gland from nonsecretors < mucous cells of Brunner's gland from secretors. From the above estimation, it is apparent that the expression of B antigen in Brunner's gland is partly dependent on the secretor status of individuals and that Japanese nonsecretors secrete substantial amounts of B antigens from sublingual gland while German nonsecretors do not. The present results also revealed an unexpected staining behavior of GSAI-B<sub>4</sub> in some tissues, i.e. in mucous cells of sublingual glands and collecting tubules of kidney from secretors, staining

intensity was markedly depressed at higher concentration of the lectin and this depression was recovered by prior  $\alpha$ -galactosidase digestion. In addition, the present method was successfully applied for the estimation of the content of B antigens neo-expressed in thyroid papillary carcinomas, showing that the content of B antigen had a similar level to that of red blood cells and vascular endothelial cells.

**Key words:** Blood group antigen, Histochemistry, Lectin, Microphotometry

## Introduction

It is well established that the blood group-related antigens are widely distributed throughout different human tissues (Szulman, 1960, 1962; Ito and Hirota, 1992). Until now, effects of blood group ABO and secretor status on the expression of these antigens in each tissue have been extensively examined. However, there is little information on the quantity of these antigens in tissues except for semiquantitative data obtained by the classical hemagglutination inhibition method (Anderson, 1969). Although numerous immunochemical and histochemical studies have shown that dramatic changes occur in the expression of blood group-related antigens during development and malignant transformation of cells (Feizi, 1985; Lloyd, 1987; Hakomori, 1989; Ito and Hirota, 1992), these findings have not yet been substantiated by quantitative estimation of the antigens. To make more precisely clear the mechanism of altered glycosylation, quantitative estimation of the carbohydrate antigens in both normal and malignant tissues is indispensable.

In the previous study (Ito et al., 1993), we intended to determine directly the terminal galactose residues of B antigens susceptible to  $\alpha$ -galactosidase digestion in

tissue sections by using high performance liquid chromatography (HPLC) and to correlate the results with the histochemical findings. From these experiments, B antigen sites of unit surface area of the mucous cells of sublingual glands were estimated to be more than 8.5–11.7 nmole/cm<sup>2</sup> which are over 1000-fold more numerous than those on erythrocyte membranes (2.7×10<sup>-3</sup> nmole/cm<sup>2</sup>) (Harpaz et al., 1975; Ito et al., 1993). Although α-galactosidase digestion eliminated B antigens and concomitantly, revealed H, Le<sup>b</sup> or Le<sup>y</sup> antigens in pancreatic acinar cells, only trace amounts of galactose were detected by HPLC and it was difficult to make a quantitative estimation (Ito et al., 1993). Thus, it is difficult to apply this method to estimate the contents of carbohydrate antigens in tissues other than sublingual glands and, at present, only a histochemical approach may provide a possible means for deducing the content of tissue antigens.

In a previous study, we also suggested that GSAI-B<sub>4</sub> exhibits broader specificity for B antigen variants and can react with type 1 [Galα1-3(Fuca1-2)Galβ1-3GlcNAc], type 2 [Galα1-3(Fuca1-2)Galβ1-4GlcNAc] and their difucosylated derivatives [Galα1-3(Fuca1-2)Galβ1-3GlcNAc (Fuca1-4) or 1-4GlcNAc(Fuca1-3)]. Thus, its staining intensity and susceptibility to α-galactosidase digestion is assumed to correlate well with the density of B antigens in tissue sections (Ito et al., 1990, 1993). In the present study, effects of different concentrations of GSAI-B<sub>4</sub> with or without prior α-galactosidase digestion on staining intensity of GSAI-B<sub>4</sub> in various human tissues were quantitatively evaluated by a microphoto-metric method. This makes it possible to compare the density of B antigens in different tissues from different blood groups. In addition, this method was applied for estimating the contents of B antigens in thyroid papillary carcinomas. It has been well known that in normal thyroid glands, the blood group antigens are not expressed, whereas these antigens are neo or oncofetally expressed in carcinomas of the thyroid glands (Szulman, 1960; Ito and Hirota, 1992). The mechanism of neo-expression of the antigens has not yet been fully understood (Ito et al., 1994, 1995, 1996a,b; Yokota et al., 1995).

## Materials and methods

### Tissue samples

Human tissues were obtained at autopsy or at surgery. The tissues examined in this study were as follows: cervic glands, pancreas, thymus, Brunner's glands, sublingual glands, kidney, and papillary carcinomas of the thyroid. They were fixed in 10% formalin and embedded in paraffin. Serial sections were cut at a thickness of 4 μm. The blood group ABO and Lewis of the individuals were determined by a routine hemagglutination method. The secretor status of the tissue donors was determined by the Lewis blood type or was deduced histochemically from the stainability with

*Ulex europaeus* agglutinin-I (UEA-I) in serous cells of the corresponding donor's submandibular glands (serous cells from secretors are positive with UEA-I and those from nonsecretors are negative) (Ito et al., 1989; Ito and Hirota, 1992).

### Reagents

*Griffonia simplicifolia* agglutinin I-B<sub>4</sub> (GSAI-B<sub>4</sub>) conjugated with horseradish peroxidase was purchased from E.Y. Laboratories (San Mateo, CA, USA). α-galactosidase (from green coffee beans) was purchased from Sigma Chemical Co. (St. Louis, Mo, USA) or from Boehringer Mannheim (Indianapolis, IU, USA). Monoclonal anti B antibody (MAb-B) H079 was obtained from Knickerbocker, S.E.A. (Barcelona, Spain).

### Histochemical procedure

Deparaffinized sections were treated with methanol containing 0.3% H<sub>2</sub>O<sub>2</sub> to block endogenous peroxidase, hydrated and treated with PBS (0.1M phosphate buffer, pH 7.2, containing 0.2M NaCl) three times, each time for 3 min. They were then incubated in PBS containing 1% bovine serum albumin (BSA) for 15 min. Sections were blotted dry with filter paper, and were then incubated for lectin staining with or without prior α-galactosidase digestion.

Series of stepwise 2-fold dilution of 80 μg/ml of labeled GSAI-B<sub>4</sub> were used for staining. Following incubation of the sections with different concentrations of GSAI-B<sub>4</sub> for 2 hr at 4 °C, tissue sites reactive with the lectin were visualized by incubating with a solution (pH 7.0, 0.1M phosphate buffer) containing 3,3'-diaminobenzidine tetrahydrochloride (0.1 mg/ml)-H<sub>2</sub>O<sub>2</sub> (0.2 μl/ml) medium. The detailed staining procedure has been described in a previous paper (Ito et al., 1986). Immunostaining with MAb-B was carried out according to the method reported previously (Ito et al., 1990). α-Galactosidase digestion of tissue sections was carried out at a concentration of 1U/ml in 50mM of citrate phosphate buffer (pH 6.5) at 37 °C for 15 hr (Ito et al., 1989, 1993).

### Quantitative estimation

Quantitative estimation of the lectin staining intensity was carried out by using an Olympus System Photometer (OSP-1, Olympus, Optical Co. LTD., Japan) which was equipped with a microscope. Percent of transmission (%T) of visible light (400–700 nm) was measured 500 times in the pinhole spots of 10 μm diameter and data were integrated.

Measurement was carried out in at least 5 different tissue sites in the same section at a magnification of x100–200.

Absorbance was calculated from the equation as follows. Absorbance = -logT.

## Results

GSAI-B<sub>4</sub> stained the cells from the tissues examined in this study of blood group B and AB individuals although the concentration of the lectin required for maximum staining was quite different from each other and dependent on the secretor status of the individuals in some cases. On the other hand, tissues from A and O individuals were not at all stained with GSAI-B<sub>4</sub>. MAb-B staining gave nearly identical staining patterns to those obtained with GSAI-B<sub>4</sub> staining in the tissues examined.

To evaluate the content of B antigens in vascular endothelial cells, connective tissues of cervic glands were mainly used. Comparative results were obtained with other tissues. As shown in Fig. 1, up to 80  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub> was required for obtaining maximum

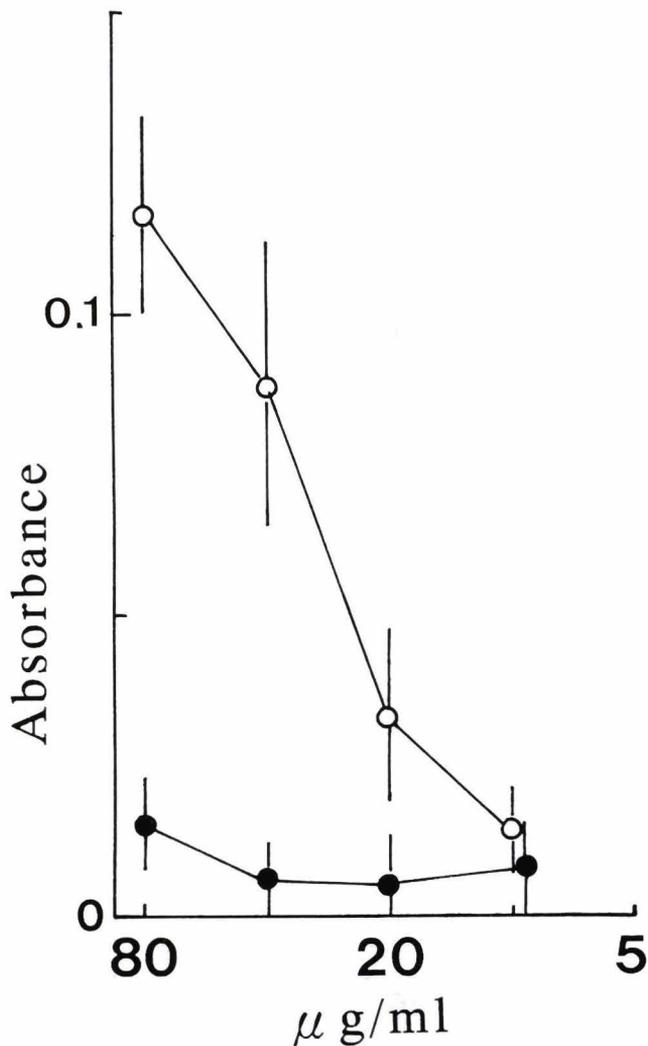


Fig. 1. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with vascular endothelial cells before (open circle) and after (filled circle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

absorbance of vascular endothelial cells. Usually, we used labeled GSAI-B<sub>4</sub> at a concentration of 20  $\mu\text{g/ml}$  in histochemical staining but at this concentration, endothelial cells were not visualized by the lectin staining as described in the previous paper (Ito et al., 1993).  $\alpha$ -Galactosidase digestion nearly completely eliminated the reactivity of GSAI-B<sub>4</sub> with endothelial cells. Similar results were obtained in red blood cells.

Fig. 2 shows effects of different concentrations of GSAI-B<sub>4</sub> and  $\alpha$ -galactosidase digestion on the staining intensity with the lectin in pancreatic acinar cells from

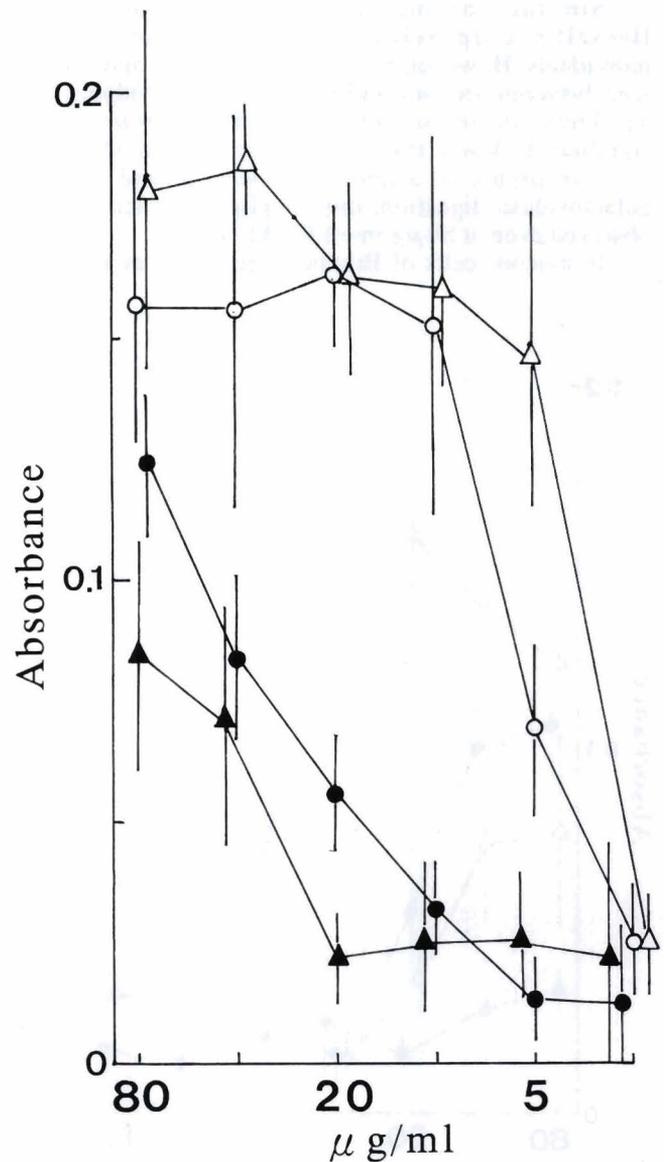


Fig. 2. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with pancreatic acinar cells from a secretor (circles), and a nonsecretor (triangles) individual before (open circle, open triangle) and after (filled circle, filled triangle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

## Quantitative estimation of B antigens

blood group B secretor and nonsecretor individuals. There is no essential difference in the staining patterns of acinar cells from secretors and nonsecretors in concentration range of GSAI-B<sub>4</sub> between 80  $\mu\text{g/ml}$  to 2.5  $\mu\text{g/ml}$  with or without  $\alpha$ -galactosidase digestion. Maximum absorption was obtained between the concentration range 80-5  $\mu\text{g/ml}$ . Below 10-5  $\mu\text{g/ml}$ , staining intensity was markedly reduced and in fact, at 2.5  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub>, stainability was almost completely abolished.  $\alpha$ -Galactosidase digestion markedly reduced the staining intensity and below 20-10  $\mu\text{g/ml}$ , no staining was observed.

Similar staining patterns were obtained with Hassall's corpuscles of thymus from secretor individuals. However, in this case, a clear difference was seen between secretor and nonsecretor individuals (Fig. 3). Thus, in nonsecretors, staining intensity was significantly lower than that of secretor individuals and at 20-10  $\mu\text{g/ml}$ , no staining was detected and after  $\alpha$ -galactosidase digestion, only negligible reactivity was observed even at 80  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub>.

In mucous cells of Brunner's glands from secretors,

maximum staining intensity was obtained between the concentration range 80-2.5  $\mu\text{g/ml}$  and even at 0.625  $\mu\text{g/ml}$ , about 50% of maximum absorbance remained (Fig. 4).  $\alpha$ -Galactosidase digestion did not affect the absorbance at 80-20  $\mu\text{g/ml}$  GSAI-B<sub>4</sub>. Below 10  $\mu\text{g/ml}$ , staining intensity was markedly reduced and at 1.25  $\mu\text{g/ml}$ , reactivity with the lectin was completely eliminated by enzyme digestion. In nonsecretor individuals, staining intensity was at a similar level to that of secretors at 80-20  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub>, but below 20  $\mu\text{g/ml}$  staining intensity in nonsecretors was gradually reduced and significantly lower than that in secretors at 5-2.5  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub> (Fig. 5).  $\alpha$ -Galactosidase digestion much more effectively reduced the staining intensity in nonsecretors than secretors and at 2.5  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub>, stainability was completely lost.

In mucous cells of sublingual glands from blood group B secretors, staining was suppressed at a higher concentration (80-40  $\mu\text{g/ml}$ ) of GSAI-B<sub>4</sub> and maximum

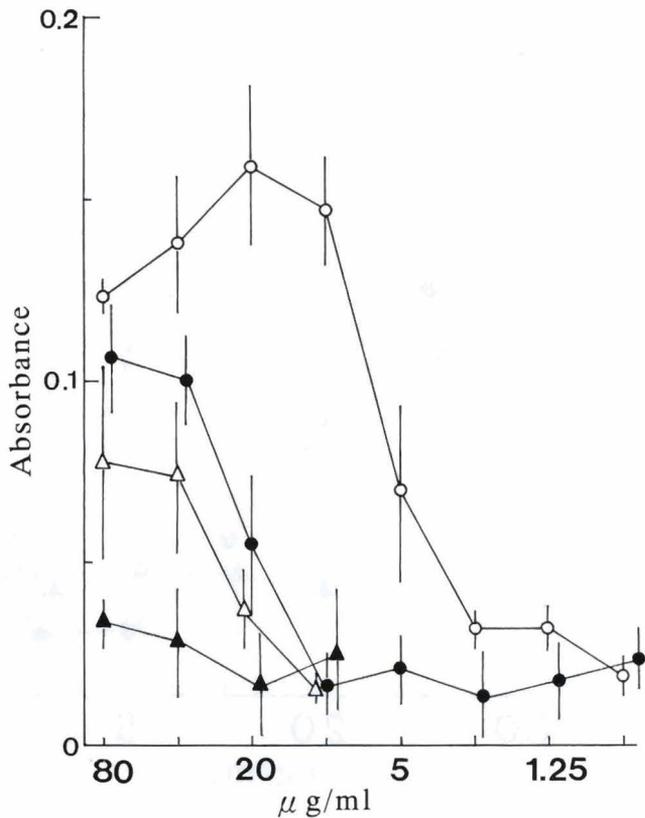


Fig. 3. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with thymic Hassall's corpuscles from a secretor (circles) and a nonsecretor (triangles) individual before (open circle, open triangle) and after (filled circle, filled triangle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

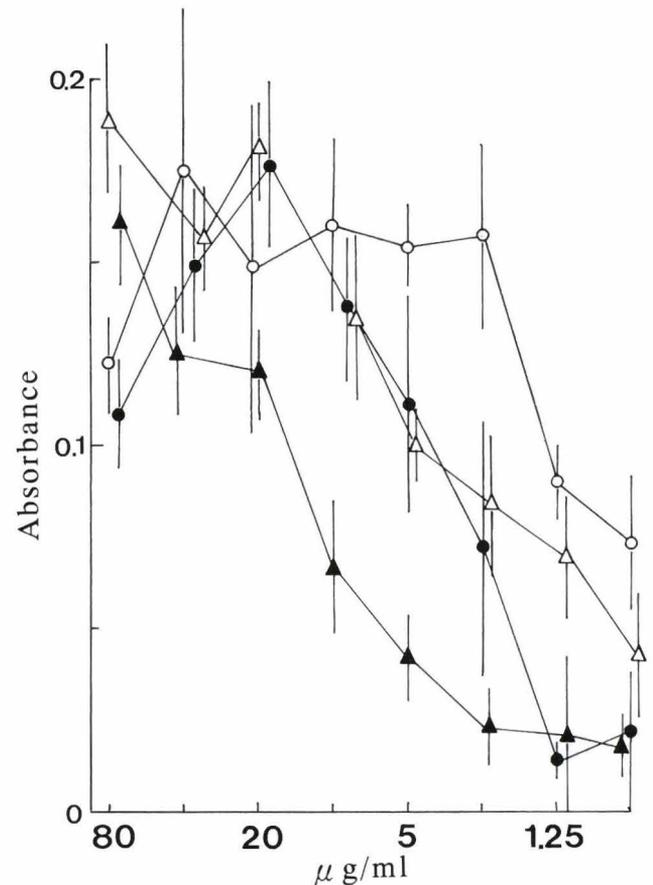


Fig. 4. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with mucous cells of Brunner's gland from a secretor (circles) and a nonsecretor (triangles) individual before (open circle, open triangle) and after (filled circle, filled triangle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

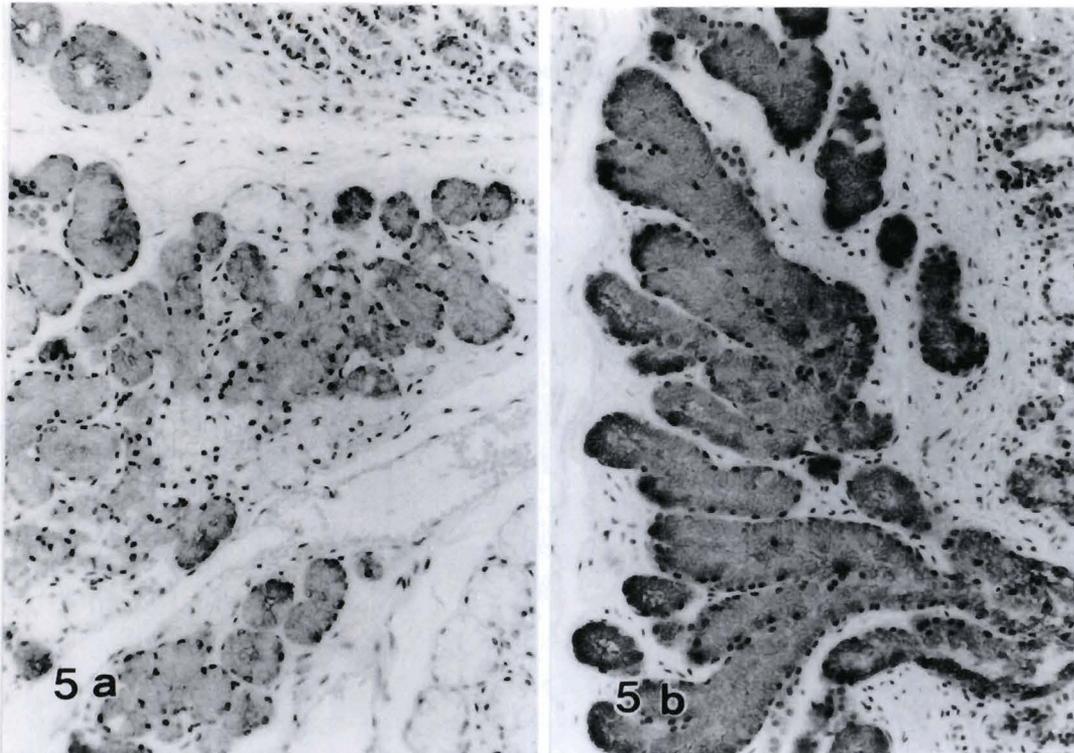


Fig. 5. Sections of Brunner's gland from a nonsecretor (a) and a secretor (b) stained with 2.5  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub>. Staining intensity in the nonsecretor is significantly lower than that in the secretor. x 200

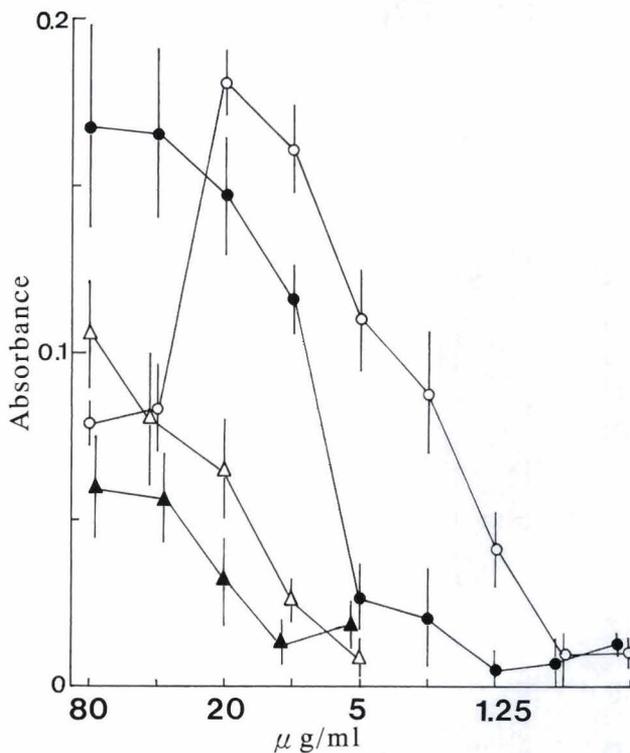


Fig. 6. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with mucous cells of sublingual gland from a secretor (circles) and a nonsecretor (triangles) individual before (open circle, open triangle) and after (filled circle, filled triangle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

absorbance was obtained at 20-10  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub> (Fig. 6). Below 10  $\mu\text{g/ml}$ , staining intensity linearly decreased as a function of decreasing concentrations of GSAI-B<sub>4</sub> and at 0.625  $\mu\text{g/ml}$ , staining was not observed. This pattern was fairly parallel to that in mucous cells of Brunner's glands from nonsecretors (Fig. 4).  $\alpha$ -Galactosidase digestion enhanced the staining intensity at 80-20  $\mu\text{g/ml}$  to recover the maximum level (Fig. 7a,b) but below 10  $\mu\text{g/ml}$ , marked reduction in staining intensity was observed. The pattern of reduction in staining intensity was also parallel to that in mucous cells of Brunner's glands from nonsecretors (Fig. 4). At 5  $\mu\text{g/ml}$ , stainability with the lectin was nearly completely abolished after  $\alpha$ -galactosidase digestion (Fig. 7c,d). Similar results were obtained with blood group AB secretors. In blood group B nonsecretors, about a half of maximum absorbance of secretors was obtained at 80  $\mu\text{g/ml}$  (Fig. 8a) and a consistent decrease in staining intensity was observed with decreasing concentrations of GSAI-B<sub>4</sub>. At 10-5  $\mu\text{g/ml}$ , no staining was observed.  $\alpha$ -Galactosidase digestion effectively reduced the absorbance between 80-20  $\mu\text{g/ml}$  of GSAI-B<sub>4</sub>.

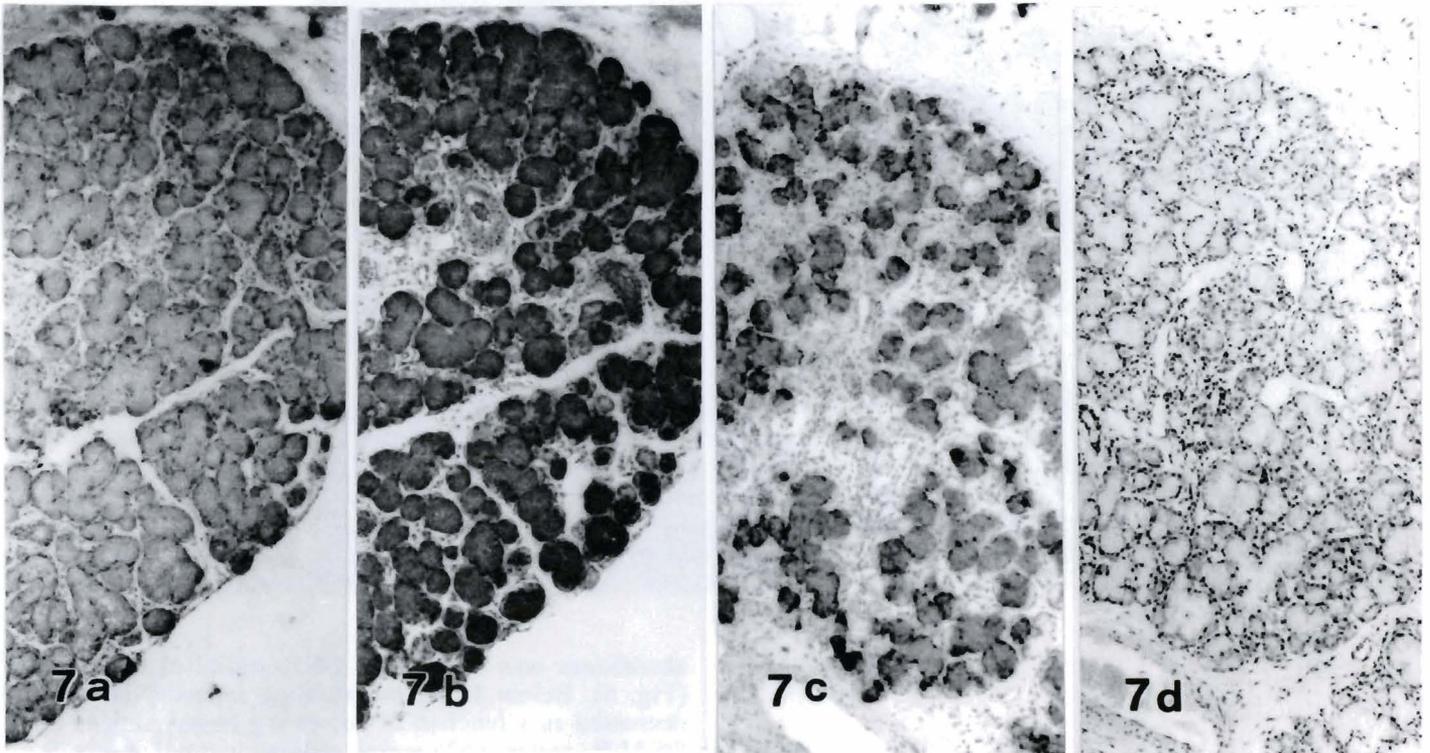
Suppression of staining intensity and recovery with  $\alpha$ -galactosidase digestion at a higher concentration of GSAI-B<sub>4</sub> was also observed in kidney collecting tubules from secretors (Fig. 9). However, in this case, reactivity of GSAI-B<sub>4</sub> with kidney tubules was much more sensitive to  $\alpha$ -galactosidase digestion than that with sublingual glands from secretors.

Fig. 10 shows the staining patterns of GSAI-B<sub>4</sub> with

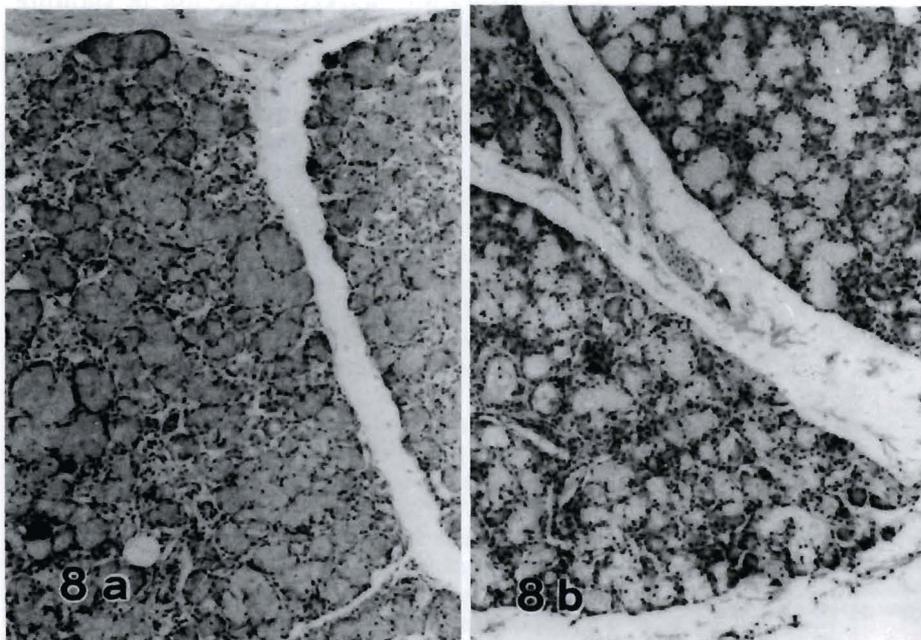
*Quantitative estimation of B antigens*

mucous cells of sublingual glands from German secretor and nonsecretor individuals. In secretors, essentially the same staining patterns were obtained as with Japanese

secretors. However, in the nonsecretor, there was little or no significant staining even at the highest concentration of GSAI-B<sub>4</sub> (Fig. 8b).



**Fig. 7.** Sections of sublingual glands from blood group B (a,b) and AB (c,d) secretor individuals stained with GSAI-B<sub>4</sub> at 80  $\mu$ g/ml before (a) and after (b)  $\alpha$ -galactosidase digestion and at 2.5  $\mu$ g/ml before (c) and after (d) enzyme digestion. At 80  $\mu$ g/ml of the lectin, enzyme digestion markedly enhances the staining intensity while at 2.5  $\mu$ g/ml, no staining is seen following enzyme treatment. x 100



**Fig. 8.** Sections of sublingual glands from a Japanese nonsecretor (a) and a German nonsecretor (b) stained with GSAI-B<sub>4</sub> at 80  $\mu$ g/ml. Moderate staining is observed in the cells from the Japanese nonsecretor but not from the German nonsecretor. x 100

### Quantitative estimation of B antigens

Fig. 11 shows the staining patterns of papillary carcinomas of the human thyroid glands from a blood group B individual before and after  $\alpha$ -galactosidase digestion. As in the case of vascular endothelial cells, a higher concentration of GSAI-B<sub>4</sub>, above 40  $\mu\text{g/ml}$ , was required for visible staining and GSAI-B<sub>4</sub>, below 20  $\mu\text{g/ml}$  did not stain the carcinoma cells at all.  $\alpha$ -Galactosidase digestion completely eliminated the reactivity with GSAI-B<sub>4</sub> throughout the concentration range used in this study (Fig. 12). Although follicular carcinomas and adenomas from the blood group B and AB individuals likewise expressed blood group B antigens, the staining was not consistent and usually weak, suggesting that the contents of B antigens in these neoplasms was significantly lower than that in papillary carcinomas.

### Discussion

By quantitative estimation of staining intensity at different concentrations of GSAI-B<sub>4</sub> with or without  $\alpha$ -galactosidase digestion, the results of the present study demonstrated that the concentration range of the lectin

required for staining and the susceptibility of staining to  $\alpha$ -galactosidase digestion is well correlated with the density of B antigens in tissue sections. Previous studies have suggested that GSAI-B<sub>4</sub> exhibits broader specificity for B antigen variants and can bind type 1, type 2 and their difucosylated derivatives (Ito et al., 1990, 1993). Such a property of the lectin may be indispensable for quantitative estimation and comparison of the antigens in a wide variety of tissues since the proportion of each variant structure to the total amount of the antigens may be quite different from each other among of the human tissues (Ito and Hirota, 1992). MAbs against the blood group antigens are likewise useful for detecting the antigens in tissue sections. However, their specificities for variant structures are usually highly strict and they are not suitable for quantitative estimation and comparison of the antigens in different tissues.

Although GSAI-B<sub>4</sub> reacts with  $\alpha$ -galactose residues other than B antigens such as Gal $\alpha$ 1-3Gal epitope in certain normal and malignant tissues (Castronovo et al., 1989; Petryniak et al., 1991; Lang et al., 1994; Ito et al., 1996), the tissues selected for the present study have been proven to produce blood group antigens (Szulman, 1960; Ito and Hirota, 1992) and the reactivity of GSAI-B<sub>4</sub> was observed exclusively in these tissues from B and AB individuals and in some cases dependent on the

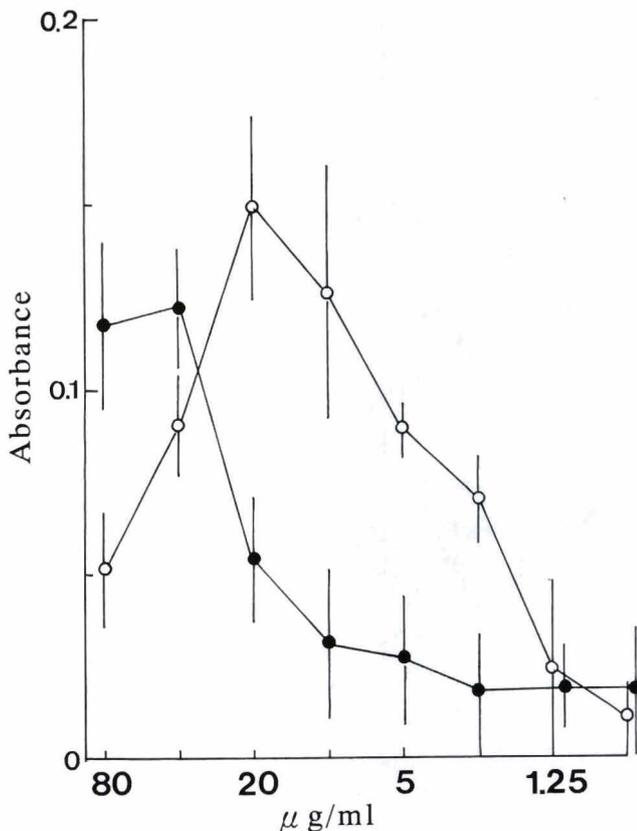


Fig. 9. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with kidney collecting tubule from a secretor individual before (open circle) and after (filled circle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

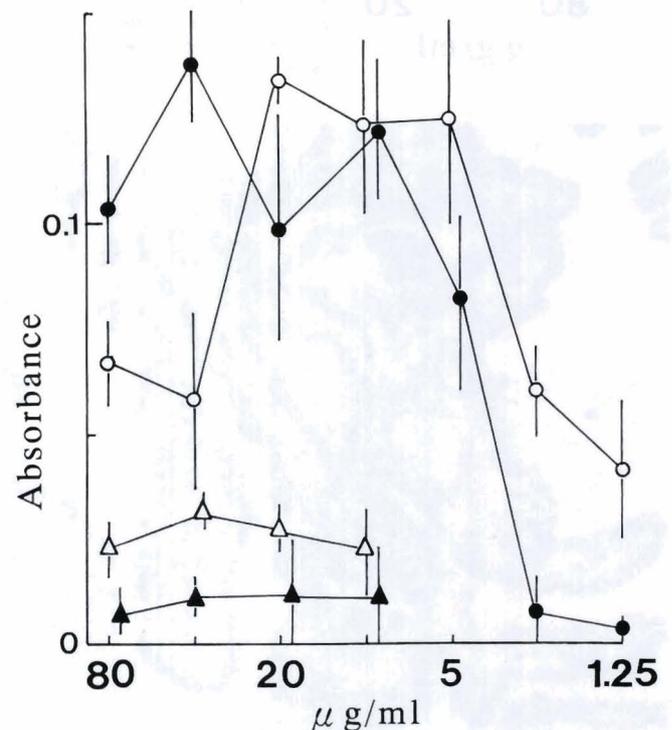


Fig. 10. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with mucous cells of sublingual gland from a German secretor (circles) and a nonsecretor (triangles) individual before (open circle, open triangle) and after (filled circle, filled triangle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

## Quantitative estimation of B antigens

secretor status of the individual as presented in this study. Thus, the reactivity of GSAI-B<sub>4</sub> with the tissues examined in this study is largely attributable to  $\alpha$ -gal

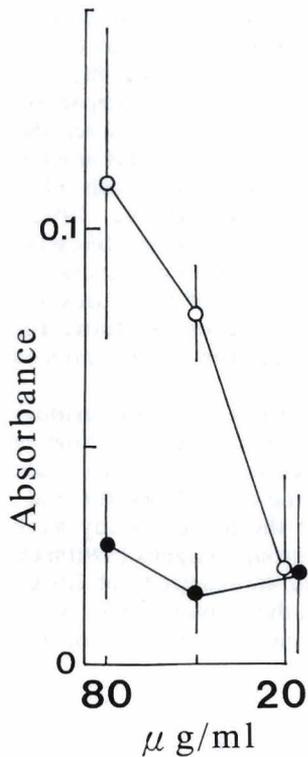


Fig. 11. Changes in staining intensity at different concentrations of GSAI-B<sub>4</sub> with thyroid papillary carcinoma cells before (open circle) and after (filled circle)  $\alpha$ -galactosidase digestion. Bars indicate standard error of the mean.

residues of B antigens.

On the basis of these considerations, it is reasonable to assume that stainability at a lower concentration of GSAI-B<sub>4</sub> reflects a higher concentration of B antigens in the tissues, while requirement of a higher concentration of GSAI-B<sub>4</sub> for staining and higher susceptibility to  $\alpha$ -galactosidase digestion is ascribed to a lower concentration of the antigens. On the basis of these assumptions and the previous results of direct estimation of B antigens (Ito et al., 1993), it is possible to rank the tissues in the order of the density of B antigens as follows; mucous cells of sublingual gland from German nonsecretors < red blood cells and vascular endothelial cells ( $2.7 \times 10^{-3}$  nmole/cm<sup>2</sup>), papillary carcinomas, and Hassall's corpuscles from nonsecretors < mucous cells of sublingual gland from Japanese nonsecretor < pancreatic acinar cells from both secretor and nonsecretors, Hassall's corpuscles and kidney collecting tubules from secretors < mucous cells of sublingual gland from secretor (>8.5-11.7 nmole/cm<sup>2</sup>) and mucous cells of Brunner's gland from nonsecretors < mucous cells of Brunner's glands from secretors.

The above quantitative estimation verifies some of the previous findings obtained by qualitative histochemical studies. Thus, the previous studies have shown that in pancreatic acinar cells, the expression of blood group antigens is not under the control of the Se gene (Szulman, 1960; Ito and Hirota, 1992) and in addition, in mucous cells of salivary glands, Japanese nonsecretors produce significant amounts of blood group A antigens while German nonsecretors do not (Nishi et al., 1990).

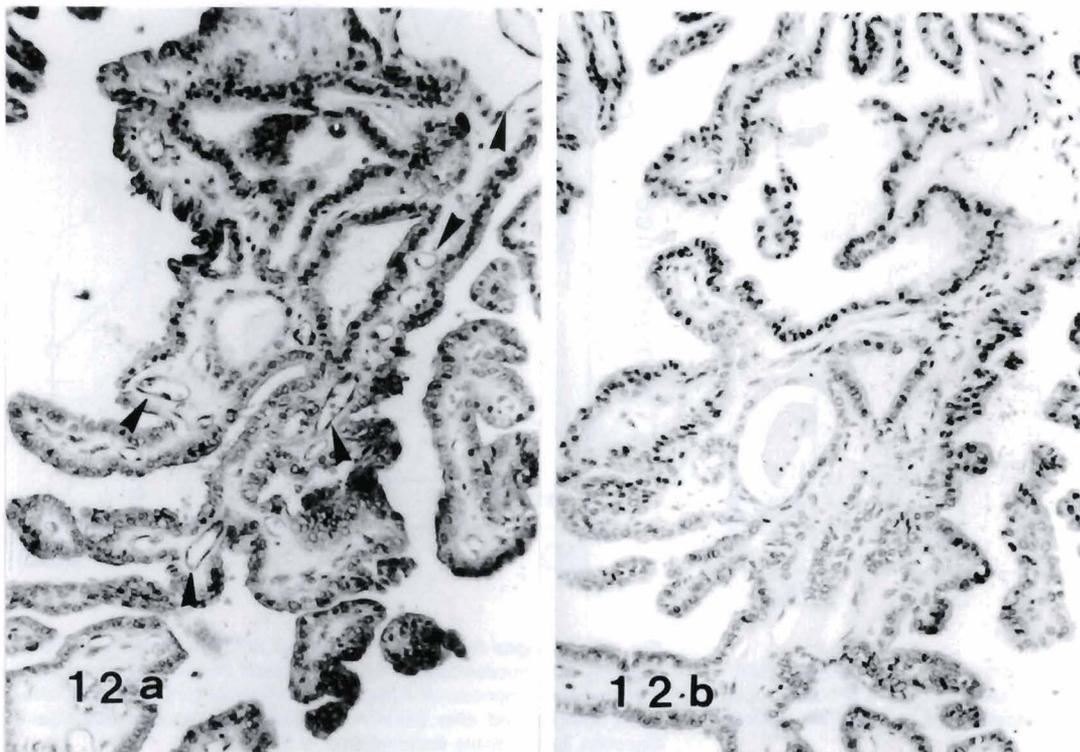


Fig. 12. Sections of thyroid papillary carcinomas stained with GSAI-B<sub>4</sub> (80 µg/ml) before (a) and after (b)  $\alpha$ -galactosidase digestion. Following enzyme digestion, stainability of the lectin with cell surfaces and cytoplasm of cancer cells is completely abolished. Staining with vascular endothelial cells and intravascular red blood cells (arrowheads) is also completely eliminated by enzyme digestion. x 200

Such phenotypical difference in the levels of blood group antigens between Japanese and German nonsecretors is quite consistent with the recent findings at the molecular genetic level. Kudo et al. (1996) have shown that all the Japanese nonsecretors examined had mutant Se gene (*sej*) homozygously (*sej/sej*) which differs from Caucasian nonsecretor gene (*pCDM8*) and  $\alpha$ 1-2 fucosyltransferase coded by the *sej* gene remains 2-3% of the activity coded by the Se gene whereas the enzyme coded by Caucasian nonsecretor gene is completely devoid of the activity.

Contrary to the generally accepted notion, the present estimation revealed a significant difference in the content of B antigens in Brunner's glands between secretors and nonsecretors. It has been believed that the blood group antigens found in Brunner's glands are exclusively of type 2 based antigens and their expression is not dependent on the secretor status of the individuals (Szulman, 1960, 1962; Oriol, 1990; Ito and Hirota, 1992). The present results suggest that the expression of the antigens in Brunner's gland is partly under the control of the Se gene.

Unexpectedly, at higher concentration of GSAI-B<sub>4</sub>, the staining intensity was markedly depressed in the mucous cells of sublingual glands and kidney tubules. It is also unexpected that  $\alpha$ -galactosidase digestion enhanced the staining intensity of GSAI-B<sub>4</sub> at a higher concentration to recover the maximum level. Although at present, it is difficult to explain the cause of such paradoxical results, configuration and arrangement of highly compacted B antigens and the neighboring other molecules is presumed to be responsible for such complex phenomena. Further biochemical and histochemical studies on the structural features of the antigens *in situ* is required for understanding the mechanism.

Although neo- or oncofetal expression of the blood group-related antigen has been reported in carcinomas of certain tissues such as liver, rectum and thyroid gland (Feizi, 1985; Lloyd, 1987; Hakomori, 1987; Ito and Hirota, 1992), little is known about the structure and amounts of these antigens. The results of the present study demonstrated that the content of B antigens neo-expressed in thyroid papillary carcinomas was at a similar level to that of red blood cells and vascular endothelial cells. Previous studies have demonstrated that the blood group ABH antigens found in papillary carcinomas are predominantly the type 2 based antigens (Ito et al., 1994) and are carried by linear long and highly branched poly-N-acetyllactosamine (Ito et al., 1995, 1996a,b; Yokota et al., 1995). Since the ABH antigens expressed on erythrocyte membrane are exclusively the type 2 based antigens and likewise carried by linear or branched poly-N-acetyllactosamine (Fukuda, 1994), it seems that the blood group ABH antigens neo-expressed in thyroid papillary carcinomas are quite similar to those expressed on erythrocyte membrane, both qualitative and quantitatively. Similar quantitative estimation of the antigens neo-expressed in

other carcinomas such as hepatic and distal colonic ones is required in order to clarify the mechanism of their neo-expression.

By applying a quantitative microphotometric method, the results of the present study provide a basis for quantifying the contents of carbohydrate antigens in tissue sections. Further extensive studies using this method may contribute to a better understanding of the control mechanism of biosynthesis of the blood group-related antigens in both normal and malignant cells.

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