Lectin binding patterns in squamous epithelium in experimentally induced hamster buccal pouch carcinoma

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The distribution pattern of certain monosaccharides in the epithelial cells of the hamster buccal pouch was studied during carcinoma development induced by 9,10-dimethyl-1,2-benzathrancene (DMBA). An avidin-biotin-peroxidase complex (ABC) immunohistochemical technique with high affinity biotinylated lectins was employed to identify monosaccharides. Lectins used in this experiment included Concanavalin A (Con A), for identifying mannose or glucose, Ricinus communis agglutinin I(RCA-I), for identifying galactose, and Ulex europaeus agglutinin I(UEA-I), for identifying fucose. The results show that in normal buccal pouch epithelial cells, fucose or galactose were concentrated predominantly on the cellular membrane, while mannose and glucose were distributed in the cytoplasm. In the epithelial cells undergoing neoplastic transformation induced by DMBA, most cells showed decreased staining of the above-mentioned monosaccharides, while in other areas the cells were heavily stained. However, the most striking change which occurred was that galactose and fucose shifted from the cellular membrane to the intracytoplasmic area during the malignant transformation. Thus, the changes of anatomic location and intensity of staining of monosaccharides in the buccal pouch epithelium may be used as a criteria for early histochemical diagnosis of malignant transformation.

Key words: DMBA; hamster buccal pouch carcinoma; lectin pattern.

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Cell glycoconjugates are carbohydrate-rich molecules that are found intracytoplasmic and on the cell surface (1). The carbohydrate moieties of the cell coat are associated with surface membrane phenomena including cell adhesion, intercellular linkage, contact inhibition (2). Alteration in cell proliferation, morphology, and differentiation in some epithelia are accompanied by changes in these glycoconjugates (3, 4).

Lectins are proteins or glycoproteins that exhibit non-immune but selective binding to specific carbohydrate moieties within cells and their membranes (5, 6). Lectins have been utilized extensively to isolate glycoproteins, glycolipids, and polysaccharides and have been used as tools to investigate the sugar residues of the cell surface and cytoplasm (7, 8). They also have been used as histochemical markers to monitor developmental, functional, and pathologic changes in cell glycoproteins (9).

Changes in the lectin binding pattern have been noted in studies of tissue sections from various neoplastic and nonneoplastic pathologic conditions (10, 11). In addition, demonstration of altered lectin binding pattern by tumor cells has been used to predict the likelihood of both recurrence (12) and the invasive potential (13) of a neoplasm and to distinguish between benign and malignant tumors (14–16).

The mucosa of the hamster buccal pouch is a squamous epithelium composed of three to four cell layers. This epithelium is one of the most commonly used models for oral carcinogenesis (17). The histology of such experimentally induced squamous cell carcinoma has been studied in detail, and histochemical techniques have been used to localize enzymes in such tumors (18, 19). However, immunohistochemical techniques have not yet been applied to detect alterations in the distribution of sugar residues during carcinogenesis.

The purpose of this study is to identify changes of specific monosaccharides and to examine if a correlation exists between lectin binding and experimentally induced malignant transformation in the hamster buccal pouch epithelium by using immunohistochemical staining techniques. Changes in the lectin binding patterns may be of value for predicting and diagnosing early malignant development.

Material and methods

Forty-eight adult male hamsters (Cricetus auratus), obtained from commercial sources were divided into 16 groups, each group containing three animals.

The right pouches of all animals were painted three times per week for 16 wk with a 0.5% solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) (Sigma Chemical Co., St. Louis, Mo.) in heavy mineral oil (U.S.P.) (18). The left pouch of all animals remained untreated and served as a control.

Sacrifice of the animals started at the beginning of every week following a 3-day period free of DMBA painting. One group of three animals was randomly selected and killed by inhalation of an overdose of ethyl ether. The killing of all the animals was completed at the beginning of 17th wk by sacrificing one group each week. Specimens of the pouch mucosa obtained from the animals were fixed in 10% neutral buffered formalin and embedded in paraffin by routine procedures. Serial sections were cut at 5 microns.

Avidin-biotin-horseradish peroxidase technique (ABC) (20) was used for identifying a number of specific oligosaccharides (21). Biotinylated lectins purchased from a commercial source (Vector Lab.) were Concanavalin A (Con A), for identifying α-D-glucosyl or α-D-mannosyl residues, Ulex europaeus agglutinin (UEA-I), for β-D-fucosyl or α-D-fucosyl residues, and Ricinus communis agglutinin (RCA-I), for α-L-galactosyl residues (22).

Serial sections from the experimental and control groups of buccal epithelium were deparaffinized by xylene and cleansed with ethanol. The sections were dipped in 0.0075% HCl with ethanol for 15 min to remove endogenous peroxidase activity. The tissue was then washed in water and covered with 0.2% bovine serum albumin in 0.05M Tris-buffered saline (TBS/BSA) for 20 min. The sections were then incubated for 30 min in an appropriate dilution of biotinylated lectin (10 μg/ml for Con A; 50 μg/ml for RCA-I and UEA-I) in 10% albumin in Tris-buffered saline. (in the case of Con A, 1 mM each of $CaCl_2$ and $MnCl_2$ were added.) Sections were then washed in TBS/BSA, three changes.

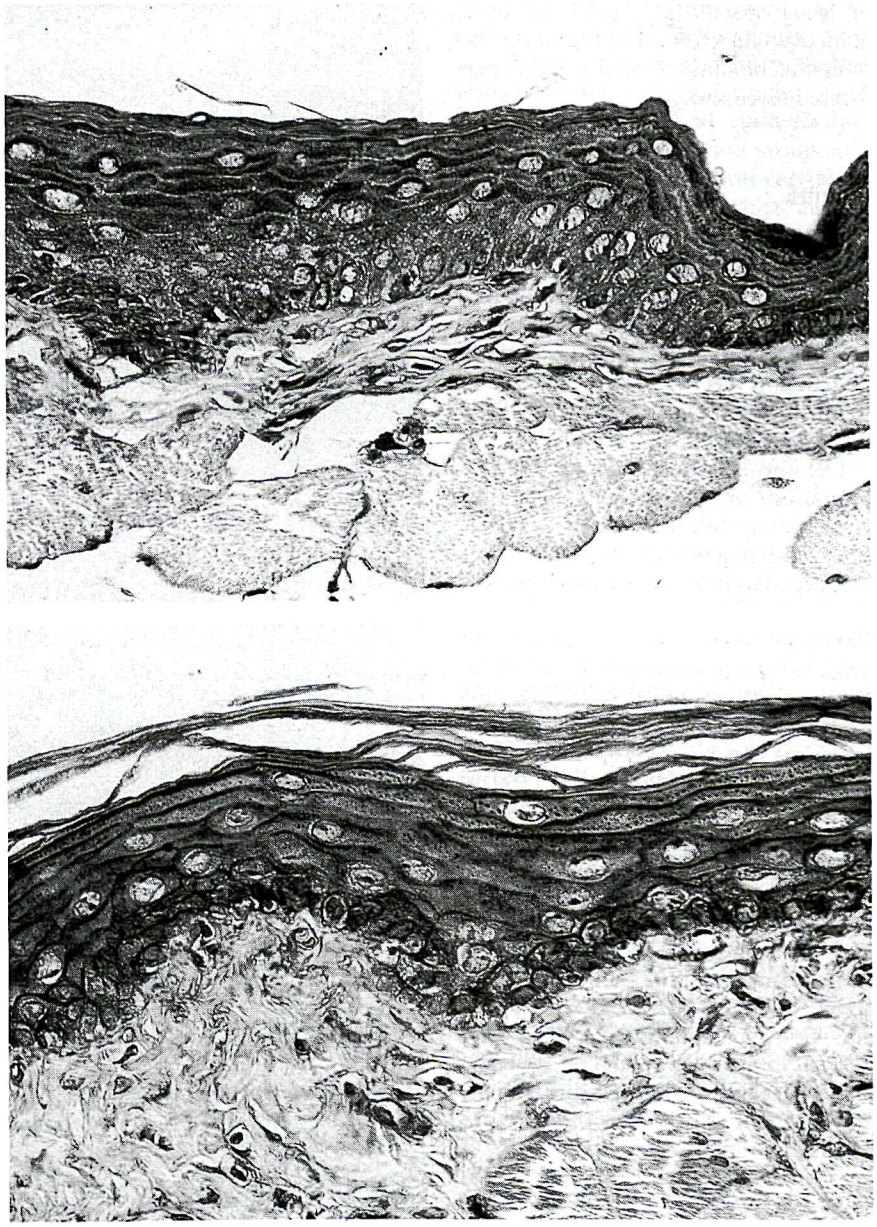

Fig. 1. A, positive staining for glucosyl/mannosyl residues in cytoplasm variably positive or negative staining occures on cell surface in normal hamster buccal pouch epithelium by Con A lectin binding. ABC stain, ×100. B, UEA-I, defining fucosyl residues, shows positive reaction on cell surface of hamster cheek pouch mucosal epithelium. Similar staining pattern is seen in RCA-I lectin binding. ABC stain, ×100.

Avidin-biotin-peroxidase complex was prepared 30 min before use, and applied to the sections for 1 h (Vectastain ABC kit #PK-4000), and then washed in three changes of TBS/BSA. Localization of the peroxidase labelled permanent brown coloration was visualized by adding diaminobenzidine tetrahydrochloride (0.04% in 0.05M ammonium acetate-citrate buffer, pH 5.5, containing 0.0075% hydrogen peroxide) for 2 min. Sections were then washed in five changes of water. Finally, sections were counterstained in hematoxylin for 3 min, washed, dehydrated, cleared and mounted in Permount before microscopic examination. Omitting the biotinylated lectins

Table 1. Distribution of monosaccharides defined by specific lectins.

Lectins	Normal mucosa		Tumor cells	
	Cell surface	Cytoplasm	Cell surface	Cytoplasm
Con A	−+	+	−+	+
RCA-I	+	−	−+	−+
UEA-I	+	−	−+	−+

−+ = Variably positive or negative reaction.
\+ = Positive reaction.
− = Negative reaction.

or using specific glycosides (glucose, mannose, galactose, and fucose) to inhibit the binding of lectins were performed as controls.

Results

The immunohistologic examination of sections of mucosal epithelium, stained by an avidin-biotin-peroxidase (ABC) method was performed. A reddish-brown deposit indicating a positive lectin reaction was found in the cytoplasm or the intercellular (cell surface) area of the cells (Table 1).

The normal hamster buccal pouch is composed of a thin squamous cell layer, connective tissue and a muscle layer. The mucosal epithelium consists of three to four layers of squamous cells exhibiting slight keratinization. The Concanavalin A (Con A) binding sites occurred predominantly in the cytoplasm of the epithelial cells (Fig. 1A) while the Ricinus communis agglutinin I(RCA-I) and Ulex europaeus agglutinin I(UEA-I) showed similar positive staining patterns on the cell surface (intercellular) (Fig. 1B).

During malignant transformation there were varying degrees of hyperkeratosis, basal cell hyperplasia, cellular pleomorphism and nuclear hyperchromatism observed at 3–8 wk after application of DMBA. Intracytoplasmic Con A binding sites were decreased in areas of malignant transformation (Fig. 2A). RCA-I and UEA-I also lost their intercellular binding sites primarily in middle and upper parts of the spinous cell layer (Fig. 2B).

In areas of papillary projections overlying squamous cell carcinomas, following DMBA application for ten to twelve weeks, staining for Con A, RCA-I, and UEA-I was either completely absent or showed a very weak reaction. An abrupt change of these staining patterns could be seen in the junctional areas of the normal epithelium adjacent to the dysplastic areas (Fig. 3A).

In the final stages after 12 wk application of DMBA, invasive squamous cell carcinoma were found. Tumor cells had infiltrated into the connective tissue, forming nests or small islands. The staining for Con A, RCA-I, and UEA-I was generally decreased. However, very intense staining was present in a few tumor cells scattered throughout the tumor nests in a very irregular pattern (Fig. 3B).

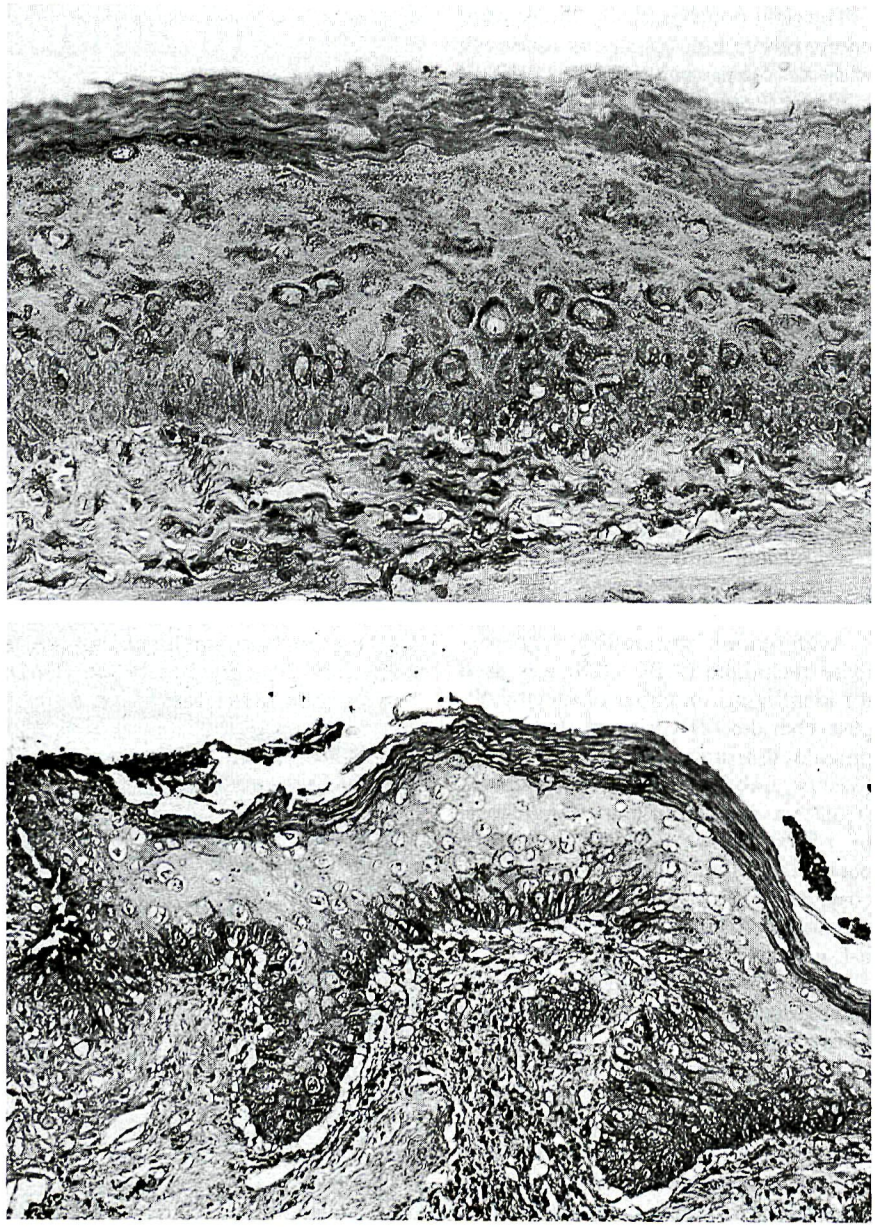

Fig. 2. A, glucosyl/mannosyl residues are markedly decreased in cytoplasm compared to normal mucosal epithelium (Fig. 1A) during malignant transformation. Con A ABC stain, ×100. B, less positive staining for RCA-I binding is shown during malignant transformation of mucosal epithelium mainly in upper portion of spinous cell layer. Similar pattern is also seen in UEA-I lectin binding. ABC stain, ×100.

Discussion

Considerable evidence indicates that changes in cell products occur during transformation of a normal cell into a malignant cell and that these changes determine some of the important properties of tumor cells (23–25). These properties include loss of contact inhibition, a decrease in cell adhesion, increased growth, prolonged survival, expression of new antigens, and an ability to escape from immune destruction by the host. Cell surface carbohydrates, glycoproteins and intracytoplasmic proteins are believed to be involved in many of these properties (10–16).

Studies have been done to identify different cellular products comparing normal and neoplastic cells by using immunohistochemical techniques (26). However, very few studies have been focused on time related changes of neoplastic transformation during experimentally induced carcinogenesis in animals especially in the hamster cheek pouch (27, 28). This is the first report to identify alterations in the levels and

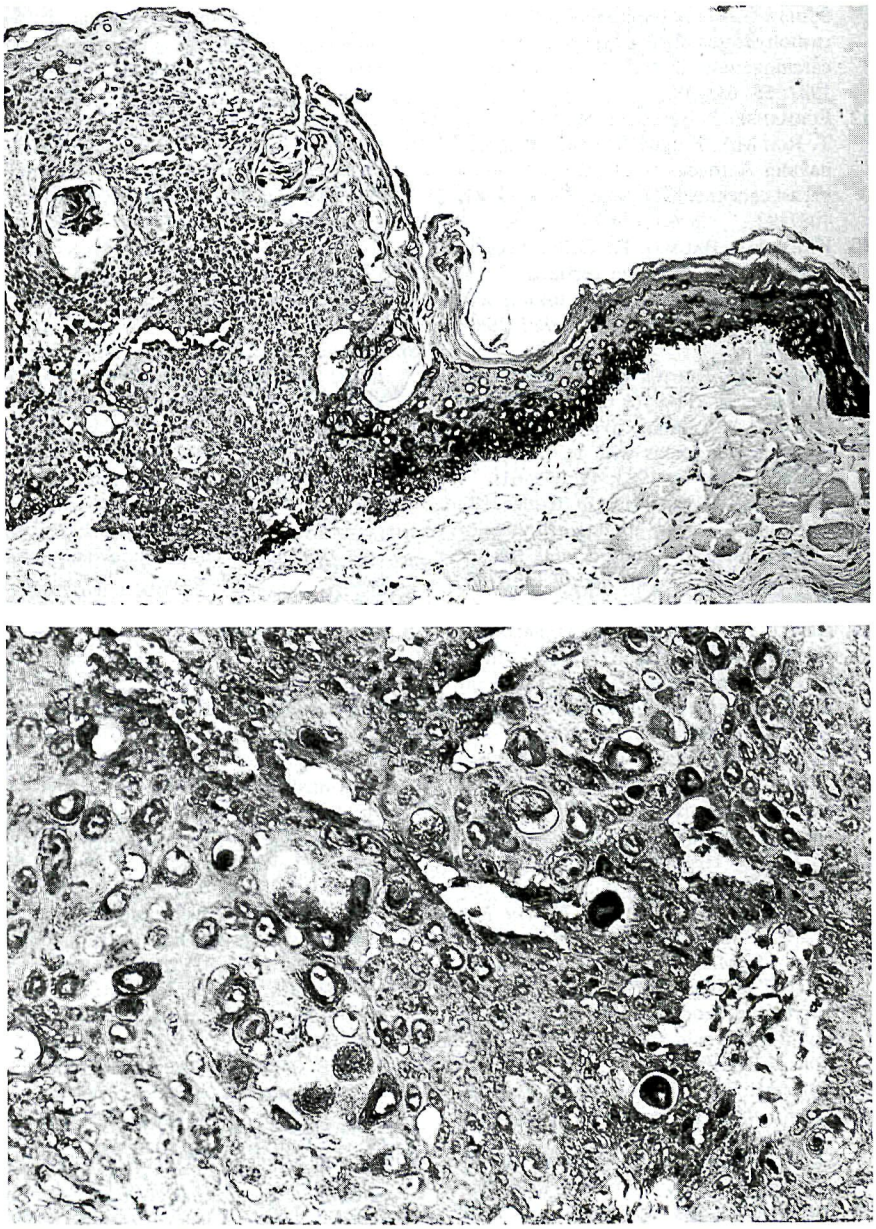

Fig. 3. A, continuity of the Con A staining of the epithelium is disrupted at junctional area where squamous cell become malignant and exophytic in growth. ABC stain, ×100. B, strongly positive staining of RCA-I receptors in perinuclear area is observed in invasive squamous cell carcinoma. ABC stain, ×100.

distribution of sugar residues during the process of carcinogenesis in the mucosa of a hamster buccal pouch.

In this study, the expression of monosaccharides during experimentally induced carcinogenesis in the buccal pouch mucosa of hamsters was investigated by using lectins along with ABC immunohistochemical techniques. In the normal squamous cell epithelium of hamster buccal pouch, mannosyl/glucosyl residues were immunohistochemically detectable in the cytoplasm of the cells whereas galactosyl and fucosyl sugar residues were primarily observed on the cell surface.

In the epithelial cells undergoing neoplastic transformation after DMBA application for 3 wk, most of the cells showed reduced staining of the above-mentioned monosaccharides. Other cells, however, were stained with high intensity in some areas. However, the most striking change was that galactose and fucose had shifted from the cellular membrane to an intracytoplasmic location during the malignant transformation.

The pattern of a reduced staining reaction for sugar residues on epithelial cells during neoplastic tranformation agrees with the concepts of modulation of cell surface structures which are associated with tumor cell mobility of the membrane component. This pattern is also consistent with many studies of cell surface changes in oral epithelia that have shown a loss of blood group antigen activity in cases of oral carcinoma (11, 16). Thus, the tissue which shows a loss of reaction for Con A, RCA-I, and UEA-I receptors on epithelial cells during carcinogenesis may be an indication of an increased potential for cell mobility. Besides this, the proteolysis of surface receptors (29) or masking of cell membrane receptors by other cell surface components such as sialic acid (30) may also be one of the causes of decreased staining of Con A, RCA-I, and UEA-I.

One possible explanation for the strong intracytoplasmic or perinuclear deposits of staining with Con A, RCA-I, and UEA-I, especially those staining only at one pole of the nucleus, may be due to the monosaccharide accumulation or glycosylation in the Golgi apparatus (31). In squamous cell carcinoma it may be that the protein in the Golgi apparatus cannot be delivered to the cell membrane after glycosylation, and thus it accumulates in the Golgi apparatus.

Genetic control for glycosylation is disturbed in squamous cell carcinoma (32). This may explain why some cells show negative staining while the others are strongly positive appearing phenotypically different. Furthermore, this may explain why, even in the same area of squamous cell carcinoma, the cells show individual differences in sugar residue identification.

The positive staining of Con A in the cytoplasm of neoplastically tranformed cells by the ABC method used herein was contrary to the findings reported previously by Louis *et al.* (15), in which a more grossly diminished level of staining was found in normal epithelium than in squamous cell carcinoma.

The observation of RCA-I localization in cells in this experiment was consistent with those reported by Kim *et al.* (33), but contrasts, however, with the findings reported by Dabelsteen & Mackenzie (34). The latter authors stated that in human tissue the RCA-I receptors could not be demonstrated in neoplastic cells of invading islands. However, cell surface carbohydrates in epithelia are expressed in a different way in human and rodents (35).

Thus, the immunohistochemical demonstration of galactosyl and fucosyl

residues in the cytoplasm as opposed to residues in the cellular membrane of epithelial cells may be helpful as a criterion for early histochemical diagnosis of malignant transformation.

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References

1. SHARON N. Lectins. *Sci Am* 1977; **236**: 108–19.
2. YAMAKAWA T, NAGI Y. Glycolipids at the cell surface and their biological functions. *Trends Biochem Sci* 1978; **3**: 128–35.
3. JAMIESON JD, INGBER DE, MURESAN V, *et al.* Cell surface proteins of normal, differentiating, and neoplastic pancreatic acinar cells. *Cancer* 1981; **47**: 1516–22.
4. ZIESKE JD, BERNSTEIN IA. Modification of cell surface glycoprotein addition of fucosyl residues during epidermal differentiation. *J Cell Biol* 1982; **95**: 626–34.
5. ALROY J, UCCI AA, PEREIRA MA. Lectins: Histochemical probes for specific carbohydrate residues. In: DELELLIS RA, ed. *Advances in immunohistochemistry*. New York: Masson Co., 1984; 67–88.
6. GOLDSTEIN IJ, HUGHES RC, MOUSIGNY M, OSAWA T, SHARON N. What should be called a lectin? *Nature* 1980; **286**: 66–70.
7. BROWN JC, HUNT RC. Lectins. *Int Rev Cytol* 1978; **52**: 277–84.
8. PEREIRA MEA, KABAT EA. Immunohistochemical studies on lectins and their application to the fraction of blood group substances and cells. *Crit Rev Immunol* 1979; **1**: 33–40.
9. RAEDLER A, RAEDLER E. The use of lectins to study normal differentiation and malignant transformation. *J Cancer Res Clin Oncol* 1985; **109**: 245–51.
10. ORGAD U, ALROY J, UCCI A, MERK FB. Histochemical Studies of epithelial cell glycoconjungated in atrophic, metaplastic hyperplastic, and neoplastic canine prostate. *Lab Invest* 1984; **50**: 294–302.
11. PRIME SS, ROSSER TJ, DAVIES LS, SCULLY C. Loss of epithelial cell surface carbohydrates during experimental oral carcinogenesis in the rat. *Br J Cancer* 1987; **55**: 633–38.
12. FURMANSKI P, KIRKLAND WL, GARGALA T, RCH MA. Prognostic value of concanavalin A reactivity of primary human breast cancer cells. *Cancer Res* 1981; **41**: 4087–92.
13. KAHN HJ, BAUMAL R. Differences in lectin binding in tissue sections of human and murine malignant tumors and their metastases. *Am J Pathol* 1985; **119**: 420–9.
14. COOPER HS, REUTER VE. Peanut lectin-binding sites in polyps of the colon and rectum: Adenomas, hyperplastic polyps, and adenomas with in situ carcinoma. *Lab Invest* 1983; **49**: 655–61.
15. LOUIS CJ, WYLLIE RG, CHOU ST, SZTYNDA T. Lectin-binding affinities of human epidermal tumors and related conditions. *Am J Clin Pathol* 1981: **75**: 642–7.
16. REIBEL J, WALLENIUS K, DABELSTEEN E. Blood group antigen staining pattern during experimental carcinogenesis in rat palate. *Acta Pathol Microbiol Immunal Scand* 1988; **96**: 161–7.
17. GARDNER AF. Buccal pouch of the Syrian hamster (Cricetus auratus). Importance in experimental oral biology. *J Dent Res* 1964; **43**: 1211–21.
18. SALLY JJ. Histologic changes in the hamster cheek pouch during early hydrocarbon carcinogenesis. *J Dent Res* 1957; **36**: 48–55.
19. MORI M, MIYAJI T, MURATA I, NAGASUNA H. Histochemical observations on enzymatic processes of experimental carcinogenesis in hamster cheek pouch. *Cancer Res* 1962; **22**: 1323–6.
20. HSU SM, RAINE L, FANGER H. The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. *Am J Clin Pathol* 1981; **75**: 816–21.
21. HSU SM, RAINE L. Versatility of biotin-labeled lectins and avidin-biotin-peroxidase complex for localization of carbohydrate in tissue sections. *J Histochem Cytochem* 1982; **30**: 157–61.
22. DEBRAY H, DECOUT D, STRECKER G, MONTREUIL J. Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. *Eur J Biochem* 1981; **117**: 41–55.
23. LÖNING T, STAQUET MJ, THIVOLET J, SEIFERT G. Keratin polypeptides distribution in normal and diseased human epidermis and oral mucosa. *Virchows Arch A Pathol Anat Histol* 1980; **388**: 273–88.
24. GOLD P, GOLD M, FREEDMEN SO. Cellular location of carcinoembryonic antigens of the human digestive system. *Cancer Res* 1968; **28**: 1331–4.
25. SMITH HS, RIGGS JL, MOSESSON MW. Production of fibronectin by human epithelial cell in culture. *Cancer Res* 1979; **39**: 4138–44.
26. PRIMUS FJ, GOLDENBERG DM. Functional histopathology of cancer: a review of immunoenzyme histochemistry. *Meth Cancer Res* 1982; **20**: 139–82.
27. TATEMOTO Y, FUKUI S, OOSUMI H, HORIKE H, MORI M. Expression of keratins during experimentally induced carcinogenesis in hamster cheek pouch visualized polyclonal and monoclonal antibodies. *Histochemistry* 1987; **86**: 445–52.
28. REIBEL J, PHILLIPSEN HP, FISKER AV, DABELSTEEN E. The distribution of blood group antigens in experimentally produced carcinomas of rat palate. *J Oral Pathol* 1986; **15**: 547–51.
29. HYNES RO. Role of surface alteration in cell transformation: The importance of protease and surface proteins. *Cell* 1974; **1**: 147–56.
30. VAN BEEK WP, SMETS LA, EMMELOT P. Increase sialic acid density in surface glycoprotein of transformed and malignant cells – a general phenomenon. *Cancer Res* 1973; **33**: 2913–22.
31. HAKOMORI S. Glycolipids of tumor cell membrane. *Adv Cencer Res* 1973; **18**: 265–315.
32. ROTH S, WHITE D. Intercellular contact and cell-surface galactosyltransferase activity. *Proc Natl Acad Sci USA* 1972: **69**: 485 (only).
33. KIM YS, ISSAACS R, PERDOMO JM. Alteration of membrane glycopeptides in human colonic adenocarcinoma. *Proc Natl Acad Sci USA* 1974: **71**: 4869–73.
34. DABELSTEEN E, MACKENZIE IC. Expression of ricinus communis receptors on epithelial cells in oral carcinomas and oral wounds. *Cancer Res* 1978; **38**: 4676–80.
35. REIBEL J, DABELSTEEN E, HAKOMORI S, YOUNG WW, MACKENZIE IC. The distribution of blood group in rodent epithelia. *Cell Tissue Res* 1984; **237**: 111–6.