strain (as well as its hybrids) is a carrier of a latent leukemogenic virus that can be released or activated by various disturbances of the cellular environment —for example, following irradiation of a parabiotic partner, neonatal thymectomy, or the induction of a GVHR.

From these considerations, it appears that the (SB)F_1 hybrid mouse, the host animal in the above experiments, is a carrier of tumor-inducing virus or viruses derived from both parents. Furthermore, it seems likely that both viruses are involved in lymphoma induction by the GVHR. Unlike the SJL/J strain, (SB)F_1 mice have a low incidence of spontaneously occurring lymphomas. However, the very fact that tumors were induced in 40 days after injection of small numbers of SJL/J spleen cells into (SB)F_1 mice, but only after 120 days after injection of massive doses of C57BL/1 cells, may indicate that the part played by the virus of SJL/J mice is more important in terms of tumor inducibility than that played by the virus of C57BL/1 mice. The latter virus may, on the other hand, be more important in the determination of the histocompatibility characteristics of the induced tumors (as indicated below).

Comparative experiments, in which various murine strain combinations were used, have demonstrated the immunological vigor of SJL/J spleen cells in the GVHR, with respect to F_1 hybrid morbidity and mortality (*13*). While this may be an expression of cellular immunity peculiar to this strain, it is quite possible that the virus of the SJL/J strain (*7*) released or activated, as a result of the cell damage associated with the GVHR, may at least be partly responsible. Thus the short latent period for tumor induction, 40 days after the initial injection of SJL/J parental spleen cells into (SB)F_1 mice, as well as the small number of cells required for tumor induction, could well be a reflection of the quantity of oncogenic virus in the spleen cell inoculum. This time interval is far shorter than that observed for GVHR induction of tumor with other strain combinations (*1–3*).

The induction of tumors in (SB)F_1 hybrid mice—which on transplantation grew in syngeneic (SB)F_1 and C57BL/1 mice, but not in SJL/J mice, the parental strain used for induction of the GVHR—is an interesting problem in the relation between viral oncogenesis and cell surface antigenicity. (It must be conceded that tumor acceptance in the SJL/J hosts might be demonstrable with a period of observation longer than 5 months; if this were so, the longer interval for tumor acceptance in this group would still pose questions relating to tumor histocompatibility.) At present, little is known about the exact relation between murine leukemia virus particles and the expression of virus-induced cell surface antigens (*14*). Antigenic changes in tumor cells may be considered in terms of antigenic loss or gain (*15*). Mitcheson (*16*) and Klein and Klein (*17*) have provided evidence of uniparental preference of heterozygous F_1 lymphomas and sarcomas which was interpreted as being due to loss of antigens derived from the other parent. In the experiments reported here, all tumors induced in F_1 mice were uniformly accepted by only one parental strain. This contrasts with the variable results in the above studies and implies a highly specific mechanism in the determination of their antigenic composition. Since C57BL/1 $\rightarrow$ (SB)F_1-induced tumors were also accepted by C57BL/1 mice, a common mechanism in the determination of the tumor cell surface composition may be operative in both GVHR's involving the same F_1 hybrid. Cell culture studies have demonstrated that the C57BL radiation leukemia virus could rescue a defective murine sarcoma virus genome; the resultant infectious virus particle carried the envelope of the C57BL virus (*18*). Thus in GVHR-induced tumors in (SB)F_1 mice, the C57BL/1 virus may be acting as a helper to the SJL/J virus.

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Concanavalin A Agglutination of Intestinal Cells from the Human Fetus

Abstract. *Concanavalin A markedly agglutinated isolated epithelial cells from the intestine of the human fetus but not from the intestine of the adult. Wheat germ agglutinin only moderately agglutinated cells from the intestine of an adult. These results extend the studies of concanavalin A agglutination of embryonic cells to human tissue, and they suggest that concanavalin A may be reacting with a common antigen on the fetal cell membrane.*

A major new approach in the study of cell surface membranes has been the use of phytoagglutinins, which bind to specific carbohydrate-containing sites on the cell surface, and which cause some cells to agglutinate (*1*). Although the exact role of these binding sites in the agglutination process is not understood, there appears to be a strong association between cell agglutination and changes in the cell surface properties associated with malignancy, such as decreased cell adhesion and contact inhibition (*2*, *3*). Fundamental changes in glycoproteins and glycolipids on the cell surface membrane accompany the changes in cell behavior that are involved in malignant transformation (*4*), and it has been suggested that cell surface glycoproteins serve as determinants of cell behavior (*5*). Moscona (*6*) extended these observations to embryonic differentiation by demonstrating agglutination of chick embryonic tissue cells by concanavalin A (Con A), and suggested that the unmasking of agglutinating sites on neoplastic cell membranes might reflect a return to the embryonic state with resultant increased cell mobility. This theory is consistent with recent clinical studies showing the appearance of fetal cell membrane factors in the serum of

patients with gastrointestinal neoplasms (*7*). Furthermore, Hakamori *et al.* have demonstrated agglutination of gastric and colonic carcinoma cells by wheat germ agglutinin (WGA) (*3*), and Yang and Hakamori have isolated an unusual fucosyl glycolipid from an adenocarcinoma of the stomach (*8*).

In view of these previous studies, we examined the agglutination, by Con A and WGA, of isolated intestinal epithelial cells. This tissue has a high turnover rate and graded areas of differentiation, and, therefore, is an excellent tissue for studying cell surface membrane changes associated with mitosis, differentiation, and the fetal state.

Tissues were obtained from adult human patients at operation, and from four human fetuses obtained by hysterotomy; fetal crown-rump length varied from 5 to 10 cm. Intestinal tissue was also obtained from 170-g female Sprague-Dawley rats. Preparations of isolated intestinal cells were made according to the method of Stern (*9*) using citrate for cell dissociation. Cells were collected and further treated with 1.5 m*M* ethylenediaminetetraacetate (EDTA) in phosphate-buffered saline (PBS); no proteases or other enzymes were used. This method isolates only epithelial cells, and excludes serosal and interstitial cells. After dissociation, cells were washed repeatedly with PBS containing no Ca^{2+}, Mg^{2+}, or EDTA, and finally suspended in PBS at an approximate cell concentration of 2×10^6 cells per milliliter. Wheat germ agglutinin was prepared by heat treatment as described by Burger and Goldberg (*10*), and was resuspended in PBS (1 mg/ml). Concanavalin A was also resuspended in PBS (1 mg/ml) (*11*). Protein was determined by the method of Lowry *et al.* (*12*).

For the agglutination assay (*10*), equal volumes of agglutinin solution and cell suspension were mixed in plastic test tubes to a final volume of 0.2 ml or 0.4 ml; final concentration of agglutinin was 0.5 mg/ml. A control mixture, containing cell suspension and PBS, was always included, and agglutination in the experimental mixtures was graded from + to ++++ by comparison with this control. The assay mixtures were incubated at 37°C in a rotary shaking water bath (70 to 80 rev/min). At specific intervals portions were removed with a Pasteur pipette, and a drop was placed on a glass slide for microscopic evaluation of agglutination.

Epithelial cells from the small intestine of adult humans were moderately agglutinated when exposed to WGA (Table 1). The agglutinated cells were in small clumps of five to ten cells, and their microscopic appearance was similar to that reported by Moscona for chick embryo retinal cells that had been dissociated by trypsin (*6*). Similar results were obtained with epithelial cells from the small intestine of the adult rat, but not with cells from the stomach or colon of the rat. In contrast to these findings, epithelial cells from the intestine of the human fetus were markedly and immediately agglutinated by Con A, but not by WGA (Table 1). This agglutination induced by Con A was visible within 5 minutes, and the clumps contained about 100 to 200 agglutinated cells, similar to those described by Moscona for chick embryo retinal cells that had been dissociated by EDTA (*6*). The Con A agglutination of intestinal cells from the human fetus was reversed by α-methylglucoside (3 m*M*).

Table 1. Agglutination of epithelial cells from the small intestine of human adults and fetuses by WGA and Con A.

Tissue	Time of incubation (minutes)	Cell agglutination	
		WGA	Con A
Adult	5	+	+/−
Adult	10	+	+/−
Adult	20	++	+/−
Adult	30	+++	+/−
Fetus	5	+	+++
Fetus	10	+	++++
Fetus	20	+	++++
Fetus	30	+	++++

Attempts were made to expose membrane sites reactive to WGA or Con A by treatment of intestinal cells from the adult rat with trypsin (*1*). However, it was observed that trypsin, even at low concentrations (2 mg/ml), and at 0°C, agglutinated intestinal cells and caused more than 50 percent of the cells to be destroyed. Papain and ficin also agglutinated cells, but pepsin did not.

These results on human small intestine are consistent with Moscona's findings with neural retinal and liver cells of the chick embryo (*6*). However, rather than comparing embryonic tissue to nonembryonic cells derived from tissue culture lines, the present studies compared both adult and fetal tissue prepared identically and derived from the same species. These results, therefore, answer some of Sivak's objections to Moscona's findings (*13*) concerning the comparison of embryonic tissue to true adult tissue, and, in so doing, support the concept that cells of embryonic or fetal origin have carbohydrate-containing sites on the surface membrane which are available for agglutination by Con A. The presence of Con A–reactive components on membranes of intestinal cells from the fetus raises the question as to whether these surface sites or factors are similar to the carcinoembryonic antigen described by Gold (*7*). This antigen, found in serums of patients with gastrointestinal neoplasms, appears to be associated with the surface membranes of neoplastic cells as well as fetal intestinal cells. However, regardless of whether these surface sites or factors are the same, the present results suggest that the cell membrane changes observed in virally transformed tissue culture cells, in chick embryonic cells, and in human fetal cells may be very similar. Whether these membrane changes are incidental expressions of the undifferentiated state or are actual cell behavior determinants is not known. However, there is increasing evidence that glycoproteins in the cell membrane play a role in cell adhesion, cell differentiation, and cell mobility (*4*)—factors that are important in neoplastic growth and metastasis. Whether or not the surface membranes of human neoplastic cells also have exposed sites reactive to Con A similar to those present in normal human fetal intestine, remains to be determined.

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