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DEVELOPMENTAL STAGE-SPECIFIC CHANGES IN LECTIN BINDING TO MOUSE CEREBELLAR CELLS *IN VITRO*[1]

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Abstract

Eleven fluorescein isothiocyanate-conjugated (FITC) lectins, each with distinct carbohydrate-binding properties, were used to assess cell surface glycoconjugates of embryonic and early postnatal cerebellar cells *in vitro*. Fluorescence staining of embryonic day 13 (E13) cerebellar cells with FITC *Ricinus communis* agglutinin diminished markedly between 24 and 72 hr *in vitro*. No staining of postnatal day 0 (P0) or postnatal day 7 (P7) cells was observed with FITC *Ricinus communis* agglutinin. A similar, but less pronounced decrease in FITC concanavalin A, FITC *Lens culinaris*, and FITC wheat germ agglutinin was observed between embryonic day 13 and birth.

No specific staining of E13, P0, or P7 cultures was observed with FITC peanut agglutinin, FITC *Dolichos bifloris* agglutinin, FITC soybean agglutinin, FITC *Wistaria floribundis* agglutinin, FITC *Phaseolus vulgaris* agglutinin, FITC *Limulus polyphemus* agglutinin, or FITC *Ulex europaeus*$_I$ agglutinin.

Similar results were obtained with ^{125}I-lectin binding assays. *Ricinus communis* ^{125}I-agglutinin binding decreased dramatically between embryonic day 13 and birth. Less pronounced decreases were observed in ^{125}I-concanavalin A and wheat germ ^{125}I-agglutinin binding. Very low levels of soybean ^{125}I-agglutinin or *Ulex europaeus*$_I$ ^{125}I-agglutinin were bound by either embryonic or early postnatal cerebellar cells *in vitro*.

Changes in cell surface properties have been suggested to mediate specific cell contacts in the developing mammalian brain (Sidman, 1974; Sidman and Rakic, 1973). In the mouse cerebellum, lectins have been used to catalog alterations in carbohydrate-containing macromolecules (Hatten and Sidman, 1978; Hatten et al., 1979; Wood et al., 1974; Zanetta et al., 1978). Studies with anti-carbohydrate antibodies (Trenkner and Sarkar, 1978) and carbohydrate-specific toxins (Willinger and Schachner, 1980) also have revealed alterations in cerebellar glycoconjugates that are regulated developmentally.

Histochemical studies of lectin binding to developing mouse cerebellum have demonstrated that some alterations detected by lectins are restricted to particular cell types (Hatten et al., 1979). Although light microscopic histochemical studies allow precise localization of lectin binding from the cellular geometry of the tissue, lectin binding is not restricted to the cell surface. One way to visualize lectin labeling of the cell surface with light microscopy is to label dissociated cells maintained as a monolayer *in vitro* with fluorescein isothiocyanate-conjugated (FITC)[4] lectins.

In the present study, 11 FITC lectins, each with a different carbohydrate-binding specificity, have been used to define the lectin binding properties of cerebellar cells dissociated at embryonic day 13 and postnatal days 0 and 7 and maintained in microcultures. The results with FITC lectins have been compared to studies of ^{125}I-lectin binding to dissociated cerebellar cells *in vitro*.

Materials and Methods

Cerebellar cell cultures. All experiments were carried out with tissue from C57Bl/6J mice derived from a breeding colony in this department. Females were

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[4] The abbreviations used are: Con A, concanavalin A; DBA, *Dolichos bifloris* agglutinin; E0, embryonic day 0; E13, embryonic day 13; FITC, fluorescein isothiocyanate-conjugated; L-Fuc, L-fucose; D-Gal, D-galactose; D-GalNAc, *N*-acetyl-D-galactosamine; D-Glc, D-glucose; D-GlcNAc, *N*-acetyl-D-glucosamine; LCA, *Lens culinaris* A agglutinin; LPA, *Limulus polyphemus* agglutinin; D-Man, D-mannose; Me-α-D-Man, methyl-α-D-mannose; P0, postnatal day 0; P7, postnatal day 7; PBS, phosphate-buffered saline; PHA, *Phaseolus vulgaris* agglutinin; Plys, poly-D-lysine; PNA, peanut agglutinin; RCA$_I$, *Ricinus communis* agglutinin; RITC, rhodamine isothiocyanate-conjugated; SBA, soybean agglutinin; UEA$_I$, *Ulex europaeus*$_I$ agglutinin; WFA, *Wistaria floribundis* agglutinin; WGA, wheat germ agglutinin.

checked daily for the presence of vaginal plugs, with the day of impregnation being designated E0. Cerebellar tissue was removed at embryonic day 13 (E13) or postnatal days 0 (P0, birth) or 7 (P7).

Cells were dissociated from whole cerebellum as described (Hatten and Sidman, 1978; Hatten, 1981) and plated at a final cell density of 0.8 to 2 × 10^6 cells/ml on glass coverslips treated with poly-D-lysine (Plys, 25 μg/ml) in microcultures (Hatten, 1981). In brief, culture dishes were prepared by drilling a 4-mm hole in 50-mm Petri dishes (Falcon, No. 1006) and attaching a glass coverslip (20 mm diameter, Arthur H. Thomas Co., Philadelphia, PA) as a false bottom with a mixture of Vaseline and Tissue-Tek (Fisher Scientific, 3:1). Plates were sterilized by exposure to shortwave ultraviolet irradiation (30 to 60 min, 20°C), washed three times with H_2O (20°C), air dried, and treated with Plys (25 μg/ml) as described (Hatten and Sidman, 1978).

In total, cultures were prepared from cerebellar tissue from 14 litters of E13 embryos and more than 60 postnatal animals. More than 750 microcultures were assayed for FITC lectin binding. More than 5800 microwell cultures were assayed for ^{125}I-lectin binding studies.

Histochemical procedures: Binding of FITC lectins. The binding of FITC concanavalin A (FITC-Con A), FITC *Dolichos bifloris* agglutinin (FITC-DBA), FITC *Lens culinaris* A agglutinin (FITC-LCA), FITC *Limulus polyphemus* agglutinin (FITC-LPA), FITC peanut agglutinin (FITC-PNA), FITC *Phaseolus vulgaris* agglutinin (FITC-PHA), FITC *Ricinus communis* agglutinin (120,000 daltons, FITC-RCA_I), FITC soybean agglutinin (FITC-SBA), FITC *Ulex europaeus*$_I$ agglutinin (FITC-UEA_I), FITC wheat germ agglutinin (FITC-WGA), and FITC *Wistaria floribundis* agglutinin (FITC-WFA) to dissociated E13, P0, and P7 cells was assayed in microcultures.

Cultures were washed with phosphate-buffered saline (PBS, 0.2 M phosphate, pH 7.2) three times (5 min, 4°C or 20°C) and FITC lectin was added (10 to 500 μg/ml in PBS, 15 min, 4°C or 20°C). FITC lectin was removed and the cultures were washed three times with PBS (5 min, 20°C). In some experiments, FITC lectin solutions were prepared with hapten carbohydrate. The haptens were as follows: for Con A, methyl-α-D-mannose (50 mM); for LCA, D-Glc (50 mM); for WGA, *N,N'*-diacetyl chitobiose (50 mM); for RCA_I, lactose (50 mM); for PNA, D-Gal (50 mM); for SBA, DBA, WFA, and PHA, D-GalNAc (50 mM); for UEA_I, L-Fuc (50 mM); and for LPA, sialic acid (50 mM).

For FITC-LPA, all procedures were carried out in Tris-HCl (pH 7.4).

FITC lectins were purchased from E. Y. Laboratories (San Mateo, CA). We confirmed the carbohydrate specificity of each lectin by hemagglutination (Goldstein and Hayes, 1978). The ratio of OD_{495}/OD_{280} for the lectins were as follows: Con A, 1.25; WGA, 1.24; RCA_I, 1.6; PNA, 0.8; SBA, 0.93; UEA_I, 1.35; DBA, 1.05; PHA, 1.21; and LPA, 1.08.

As a positive control for FITC lectin staining of dissociated cerebellar cells in microcultures, erythrocytes from different species were labeled with FITC lectins by a modification of the procedure of Burger (1976). In brief, washed, packed, fresh cells were resuspended at 2 × 10^6 cells/ml in PBS (4°C). The suspension (90 μl) was transferred to a porcelain plate and 0.01 ml of FITC lectin solution of varying concentrations (10 to 1000 μg/ml) was added. The suspension was aspirated gently, and after 1 min, 20 μl was transferred as a hanging drop onto a microscope slide. After 4 to 15 min, FITC lectin binding was visualized by fluorescence microscopy. As a control for carbohydrate specificity, FITC lectin solutions with hapten carbohydrate (0.1 M) were used.

Rhodamine isothiocyanate-conjugated (RITC) lectins also were purchased from E. Y. Laboratories and used for some experiments. Methyl-α-D-mannose, D-glucose, lactose, D-galactose, *N*-acetyl-D-glucosamine, and sialic acid were purchased from Sigma (St. Louis, MO). *N,N'*-Diacetyl chitobiose and chitin hydrolysate were prepared by the method of Rupley (1964).

^{125}I-labeling of lectins. Con A, WGA, RCA_I, SBA, and UEA_I were iodinated by the chloramine T method (Freeman, 1967). Lectins were preincubated with hapten carbohydrate (100 mM) to protect the carbohydrate binding site. Briefly, 1 mCi (10 μl of carrier-free iodine in NaOH, New England Nuclear, Boston, MA) of ^{125}I-sodium was added to lectin (50 μl of 1 mg/ml of solution containing 100 mM hapten carbohydrate, pH 7.2). Chloramine T (10 μl of 3.5 mM solution in PBS) was added, and after 20 sec, the reaction was stopped by the addition of sodium metabisulfite (10 μl of 3.5 mM solution in PBS). Labeled lectins were applied to a Sephadex G-50 (Pharmacia, Piscataway, NJ) column (bed volume, 8 ml) and separated from free iodine and carbohydrate by elution with PBS. Labeled Con A was eluted with PBS containing methyl-α-D-mannose (50 mM) and dialyzed against PBS (4°C). The specific activities of ^{125}I-labeled lectins (given as counts per min per gm × 10^{-4}) were: Con A, 12.1; WGA, 6.38; RCA_I, 11.7; SBA, 8.14; and UEA_I, 9.09.

We assayed the carbohydrate-binding specificity of ^{125}I-labeled lectins by hemagglutination (Goldstein and Hayes, 1978). All lectins were purchased from E. Y. Laboratories. Chloramine T was purchased from Eastman (Rochester, NY) and sodium metabisulfite was obtained from Sigma.

Binding of ^{125}I-lectins to cerebellar cells. For ^{125}I-labeling experiments, E13, P0, and P7 cerebellar cells were plated at 2.5 × 10^6 cells/ml in flexible Microtest II plates (Cooke Engineering, Alexandria, VA). Cell viability, as measured by exclusion of the dye trypan blue (0.1%, GIBCO), was greater than 85% for the cell cultures used for ^{125}I-lectin binding assays. After 24 hr *in vitro*, cultures were rinsed three times with PBS (5 min, 4°C) and ^{125}I-lectin was added (50 μl of lectin, 10 to 100 μg/ml final concentration, 15 min, 4°C). The wells were rinsed three times with PBS (5 min, 4°C), after which, individual wells were cut out and transferred to a 12 × 75 mm tube (Falcon, No. 2054). Radioactivity was measured with a Beckman Gamma 4000 counter. All assays were carried out at 4°C.

Binding experiments were carried out in triplicate or in groups of six wells for each of the four lectin concentrations (10, 25, 50, and 100 μg/ml) tested. For each lectin concentration assayed, a paired well was labeled with ^{125}I-lectin with hapten carbohydrate (50 mM). Specific

binding was calculated by subtracting the counts in the presence of hapten carbohydrate from counts in the absence of hapten carbohydrate.

As a positive control, erythrocytes from different species were labeled with ^{125}I-lectins. Washed, packed, fresh erythrocytes were resuspended at 2×10^6 cells/ml in PBS (1.0 ml). ^{125}I-Lectin was added (50 μl of lectin, 10 to 100 μg/ml final concentration, 15 min, 4°C). The cells were washed three times by centrifugation (1000 rpm) and the final pellet was assayed for radioactivity with a Beckman Gamma 4000 counter. For each lectin concentration assayed, a paired sample was labeled with ^{125}I-lectin with hapten carbohydrate (50 mM) and specific binding was calculated as described. Cell lysis was assayed by measuring the absorption of the supernatant of washed cells at 520 nm. The results reported were from samples with absorptions less than 0.1.

Results

Histochemical studies

Carbohydrate-specific binding of FITC-RCA$_I$, FITC-LCA, FITC-Con A, and FITC-WGA was observed for cerebellar cells dissociated at E13, P0, or P7 and maintained in microcultures for 12 to 72 hr. Very weak or no specific binding of FITC-SBA, FITC-PHA, FITC-WFA, FITC-DBA, FITC-LPA, or FITC-UEA$_I$ was observed with E13, P0, or P7 cells in microcultures even at very high concentrations (500 to 1000 μg/ml) of lectin (Table I).

Over the concentration range tested (10 to 500 μg/ml), fluorescence intensity for cells labeled with FITC-RCA$_I$, FITC-Con A, FITC-LCA, or FITC-WGA did not increase significantly at concentrations above 50 μg/ml. At concentrations greater than 50 μg/ml, hapten carbohydrate inhibition of binding was sometimes incomplete. In studies of ^{125}I-lectin binding (see below), more than 90% of the maximal specific lectin binding was achieved at 50 μg/ml. Above that concentration, low affinity, nonspecific binding increased linearly. Therefore, 50 μg/ml of lectin was chosen as a standard concentration for histochemical studies. In cases where no FITC lectin binding was observed, concentrations up to 500 μg/ml were tested.

No differences in fluorescence intensity or distribution of FITC-RCA$_I$, FITC-Con A, FITC-LCA, or FITC-WGA were observed when cultures were labeled at 4°C instead of 20°C. For convenience, experiments were generally performed at 20°C. Identical results were obtained with RITC lectins.

FITC-RCA$_I$ binding to cerebellar cells. After 24 hr *in vitro*, cells dissociated from E13 cerebellum were stained intensely with low concentrations (10 to 50 μg/ml) of FITC-RCA$_I$ (Fig. 1*A*). Intense, diffuse staining of cell bodies and processes of glial, neuronal, and non-neuronal flat cells in the cultures was observed. No differences in staining intensity were observed among the cell types present. Complete, hapten-specific inhibition of FITC-RCA$_I$ binding occurred in the presence of 50 mM lactose (Fig. 2) but not in the presence of D-Glc, Me-α-D-Man, or L-Fuc (100 mM). Identical results were obtained when E13 cells were cultured in the absence of serum. After 72 hr *in vitro*, the specific binding of FITC-RCA$_I$ was re-

TABLE I

Binding of FITC lectins to cells dissociated from E13, P0, and P7 mouse cerebellum

Labeling was carried out with 50 μg/ml of lectin as described under "Materials and Methods." Fluorescence intensity is given from maximal intensity (++++) to no staining (—) after 24 hr *in vitro*.

Age of Tissue	Lectin	Con A	LCA	WGA	RCA$_I$	PNA	PHA	SBA	DBA	WFA	UEA$_I$	LPA
	Hapten	Me-α-D-Man	D-Glc	D-GlcNAc	D-Gal D-GalNAc	D-Gal	D-GalNAc	D-GalNAc	D-GalNAc	D-GalNAc	L-Fuc	Sialic Acid
E13		+++	+++	+++	++++	—	—	—	—	—	—	—
P0		++	++	++	—	—	—	—	—	—	—	—
P7		++	++	++	—	—	—	—	—	—	—	—

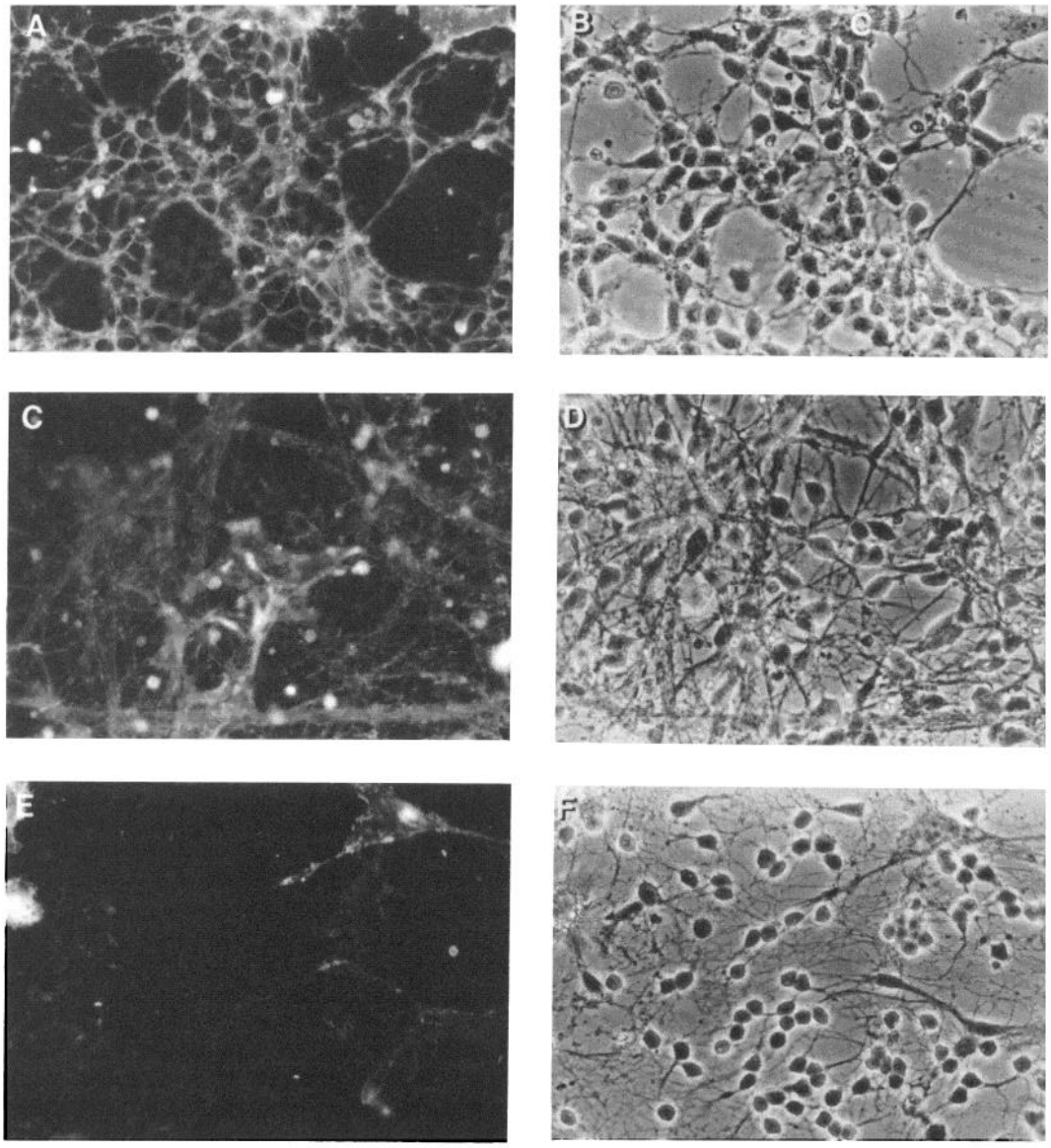

Figure 1. Fluorescence staining of E13 and P0 cerebellar cells with FITC-RCA_I (50 μg/ml) after 24 or 72 hr *in vitro*. Identical fields were photographed with fluorescence (*A*, *C*, and *E*) or phase contrast (*B*, *D*, and *F*) illumination. E13 cells stained after 24 (*A* and *B*) or 72 hr (*C* and *D*) *in vitro*; P0 cells stained after 24 hr (*E* and *F*) *in vitro*. Magnification × 210.

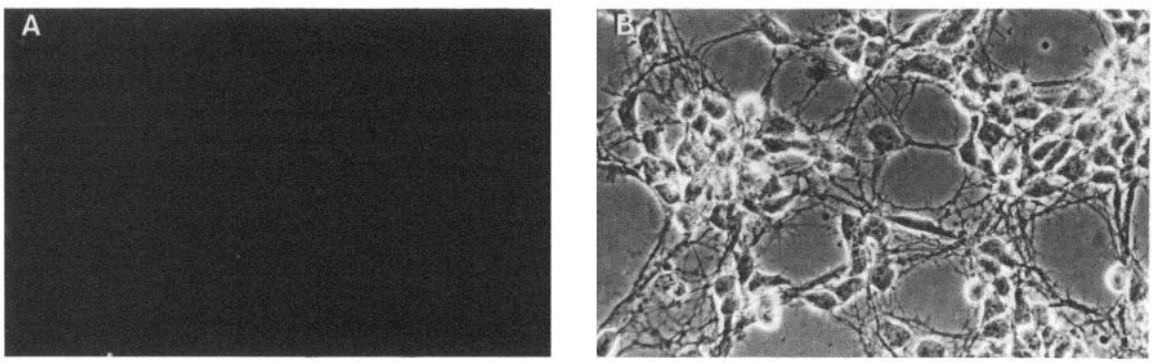

Figure 2. Inhibition of fluorescence staining of E13 cerebellar cells with FITC-RCA_I (100 μg/ml) in the presence of lactose (50 mM) after 24 hr *in vitro*. The identical field was photographed with (*A*) fluorescence and (*B*) phase contrast illumination. Magnification × 220.

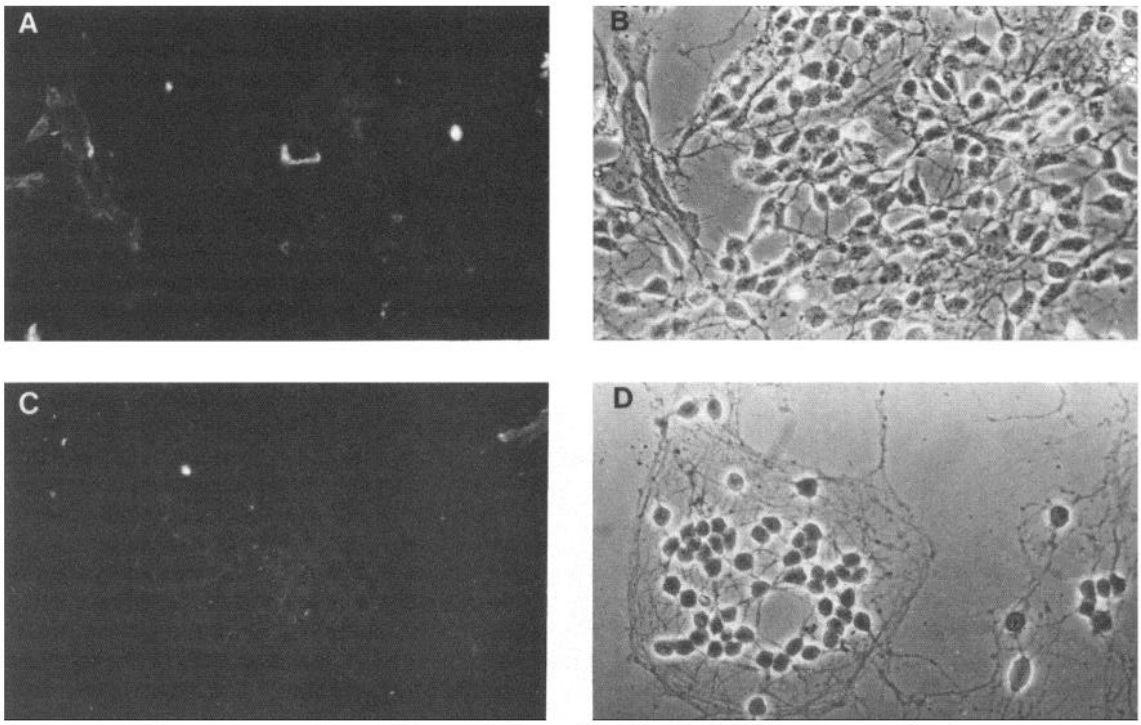

Figure 3. Fluorescence staining of P7 cerebellar cells with FITC-RCA_I (100 μg/ml) after 24 or 72 hr *in vitro*. The same field was photographed with fluorescence (*A* and *C*) or phase contrast (*B* and *D*) illumination. P7 cells stained after 24 (*A* and *B*) or 72 hr (*C* and *D*) *in vitro*. Magnification × 220.

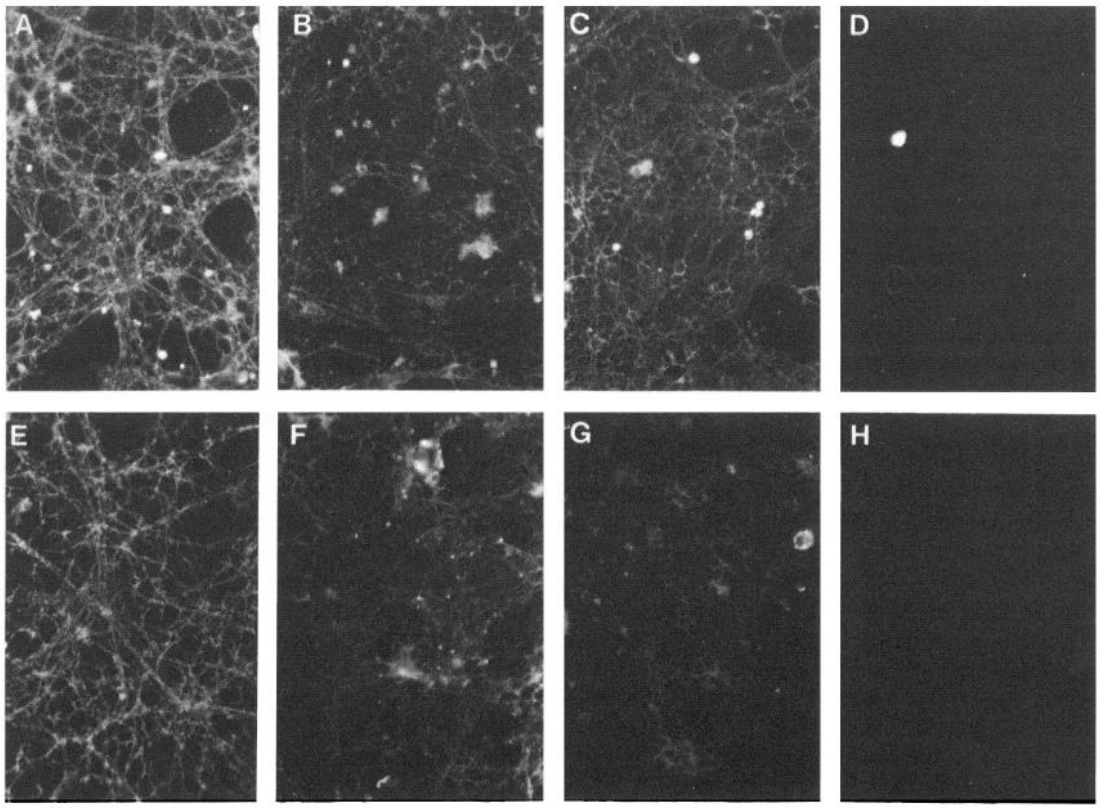

Figure 4. Fluorescence staining of E13, P0, and P7 cerebellar cells with FITC-Con A (50 μg/ml) and FITC-WGA (50 μg/ml) after 24 hr *in vitro*. The panels show FITC-Con A staining of (*A*) E13, (*B*) P0, or (*C*) P7 cultures; FITC-Con A staining of E13 culture in the presence of methyl-α-D-mannose (50 mM) (*D*); FITC-WGA staining of (*E*) E13, (*F*) P0, and (*G*) P7 cerebellar cells; and FITC-WGA staining of E13 cerebellar cells in the presence of *N,N'*-diacetyl chitobiose (50 mM) (*H*). Magnification × 220.

duced markedly. The decrease in FITC-RCA_I binding was especially pronounced in cell bodies. Weak staining of the network of processes present was still evident after 72 hr *in vitro* (Fig. 1*C*).

In contrast, very weak or no specific binding of FITC-RCA_I was observed with P0 (Fig. 1*E*) or P7 cultures (Fig. 3) after 24, 72, or 96 hr *in vitro* over the entire range of concentrations assayed (10 to 500 μg/ml). Some staining of non-neuronal flat cells persisted (Fig. 3).

Identical results were observed when postnatal cells were preincubated in medium without serum for 12 to 24 hr prior to staining.

FITC-Con A and FITC-LCA binding to cerebellar cells. After 24 hr *in vitro*, dissociated E13 cerebellar cells were stained intensely with FITC-Con A (50 to 100 μg/ml). Bright, hapten-specific staining of the cell bodies and processes of glial and neuronal cells was observed over the range of cell densities studied (Fig. 4*A*). Staining with FITC-Con A was inhibited completely by co-incubation with methyl-α-D-mannose (50 mM, Fig. 4*D*) but not by lactose, D-Gal, D-GalNAc, or L-Fuc (100 mM). The labeling of cell bodies was especially prominent with Con A. No differences in staining intensity were observed among the glial and neuronal cell types present. Identical results were observed when cells were cultured in the absence of serum. Fluorescence intensity was equivalent to that observed with FITC-RCA_I (Fig. 5).

After 72 hr in culture, slightly less intense fluorescence staining with FITC-Con A was observed. The difference was not as pronounced as that seen with FITC-RCA_I. FITC-Con A staining was inhibited completely by co-incubation with methyl-α-D-mannose (50 mM, Fig. 4*D*). Dissociated P0 and P7 cerebellar cells also were stained with Con A (Fig. 4, *B* and *C*). After 24 hr *in vitro*, the overall staining intensity of postnatal cells was lower than that of E13 cells. No differences in fluorescence intensity were observed among the glial and neuronal cell types present (Fig. 6). Identical results were obtained when cells were incubated in medium without serum for 12 to 24 hr prior to labeling with FITC-Con A.

Identical results were obtained with FITC-LCA (data not shown). The binding of FITC-LCA was inhibited completely in the presence of D-Glc (50 mM) but not in the presence of D-Gal, D-GalNAc, or L-Fuc (100 mM).

FITC-WGA binding to cerebellar cells. After 24 hr *in vitro*, bright staining of dissociated E13 cerebellar cells was observed with FITC-WGA (10 to 50 μg/ml). The cell bodies and processes of both glial and neuronal cells in the culture were stained brightly (Fig. 4*E*). The staining of the processes was prominent. The fluorescence intensity of staining was equivalent to that with RCA_I and Con A (Fig. 6). After 72 hr, the pattern of staining was unchanged. All staining of E13 cells with FITC-WGA was inhibited completely by co-incubation with *N,N'*-diacetyl chitobiose (50 mM) (Fig. 4*H*). Co-incubation with D-Glc, Me-α-D-Man, D-Gal, D-GalNAc, or L-Fuc (100 mM) had no effect on FITC-WGA staining. Identical results were obtained when E13 cells were cultured in the absence of serum.

Dissociated P0 and P7 cells also were stained intensely with FITC-WGA (Fig. 4, *F* and *G*). After 24 hr *in vitro*, the overall staining of P0 and P7 cerebellar cells was less intense than that of embryonic cerebellar cells. No differences in staining intensity were observed among the cell types present in cultures of P0 and P7 cerebellum (Fig. 7). Staining was inhibited by *N,N'*-diacetyl chito-

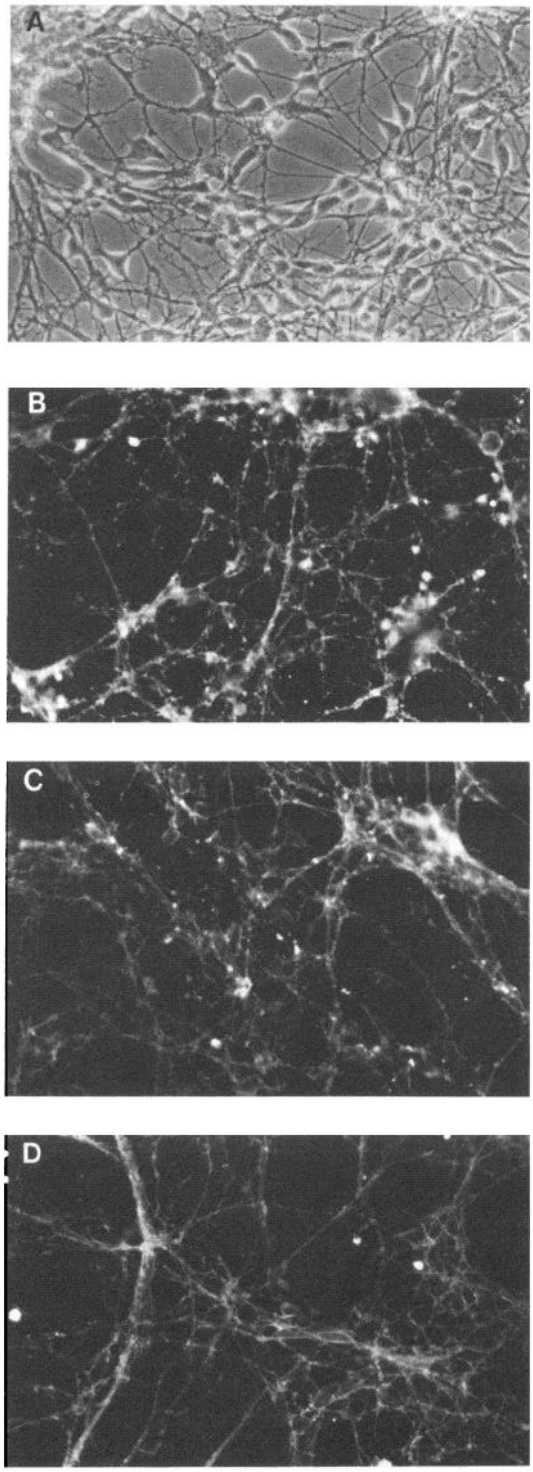

Figure 5. Relative fluorescence intensity of FITC-Con A, FITC-RCA_I, and FITC-WGA staining of E13 cerebellar cells after 24 hr *in vitro*. Cells were stained with 50 μg/ml of lectin, photographed, and printed under identical conditions. *A*, Phase contrast microscopy; *B*, FITC-Con A; *C*, FITC-RCA_I; *D*, FITC-WGA. Magnification × 220.

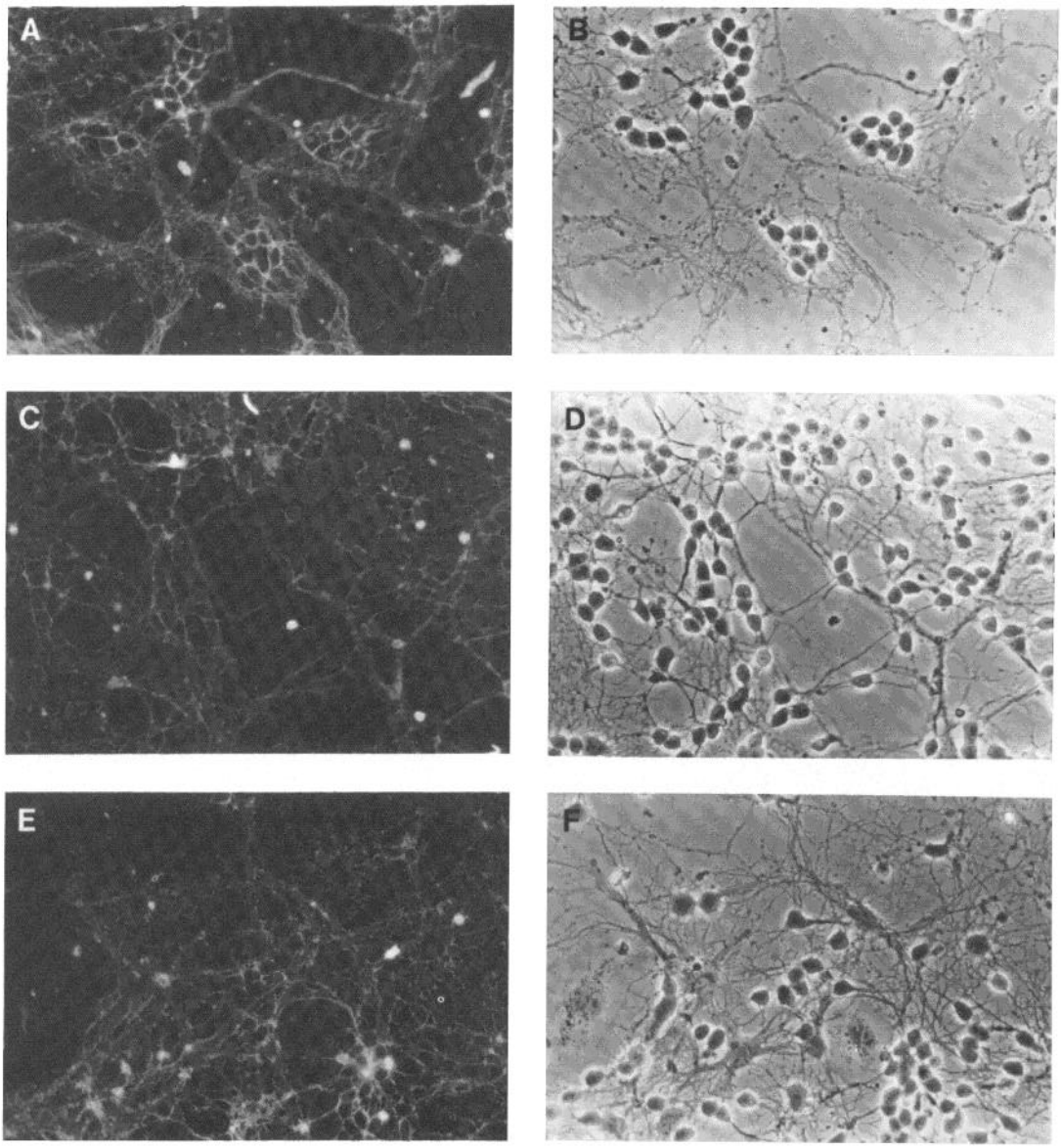

Figure 6. Binding of FITC-Con A (50 μg/ml) to dissociated P7 cerebellar cells after 72 hr *in vitro*. In three fields, all cell bodies and processes were stained. *A*, *C*, and *E*, Fluorescence microscopy; *B*, *D*, and *F*, phase contrast microscopy. Magnification × 220.

biose (50 mM) but not by D-Glc, Me-α-D-Man, D-Gal, D-GalNAc, or L-Fuc (100 mM). Identical results were obtained when cells were incubated in medium without serum for 12 to 24 hr prior to labeling with FITC-WGA.

FITC-DBA, FITC-LPA, FITC-PHA, FITC-PNA, FITC-SBA, and FITC-WFA binding to cerebellar cells. No specific staining of E13, P0, or P7 cerebellar cells was observed with any of these lectins, even at very high lectin concentrations (500 μg/ml), over the range of cell densities studied (data not shown). Weak, specific staining of some non-neuronal, flat cells in the culture was observed with FITC-SBA.

Identical results were obtained when E13 cells were grown without serum or when postnatal cells were incubated in medium without serum for 12 to 24 hr prior to lectin staining.

Intense staining of human erythrocytes (type A) was observed with FITC-DBA, FITC-PHA, FITC-SBA, and FITC-WFA (25 to 100 μg/ml). The staining with FITC-DBA and FITC-WFA was inhibited in the presence of 0.1 M D-Gal. The staining with FITC-PHA and FITC-SBA was inhibited by 0.1 M D-GalNAc.

Intense staining of horse erythrocytes was observed with FITC-LPA. The staining was inhibited by 0.1 M sialic acid.

Intense staining of rabbit erythrocytes was observed with FITC-PNA (25 to 100 μg/ml). This staining was inhibited by 0.1 M D-Gal.

FITC-UEA$_I$ binding to cerebellar cells. No specific staining of E13 or P0 cerebellar cells was observed with FITC-UEA$_I$ (10 to 500 μg/ml). Weak, specific staining was observed for P7 cells when very high concentrations

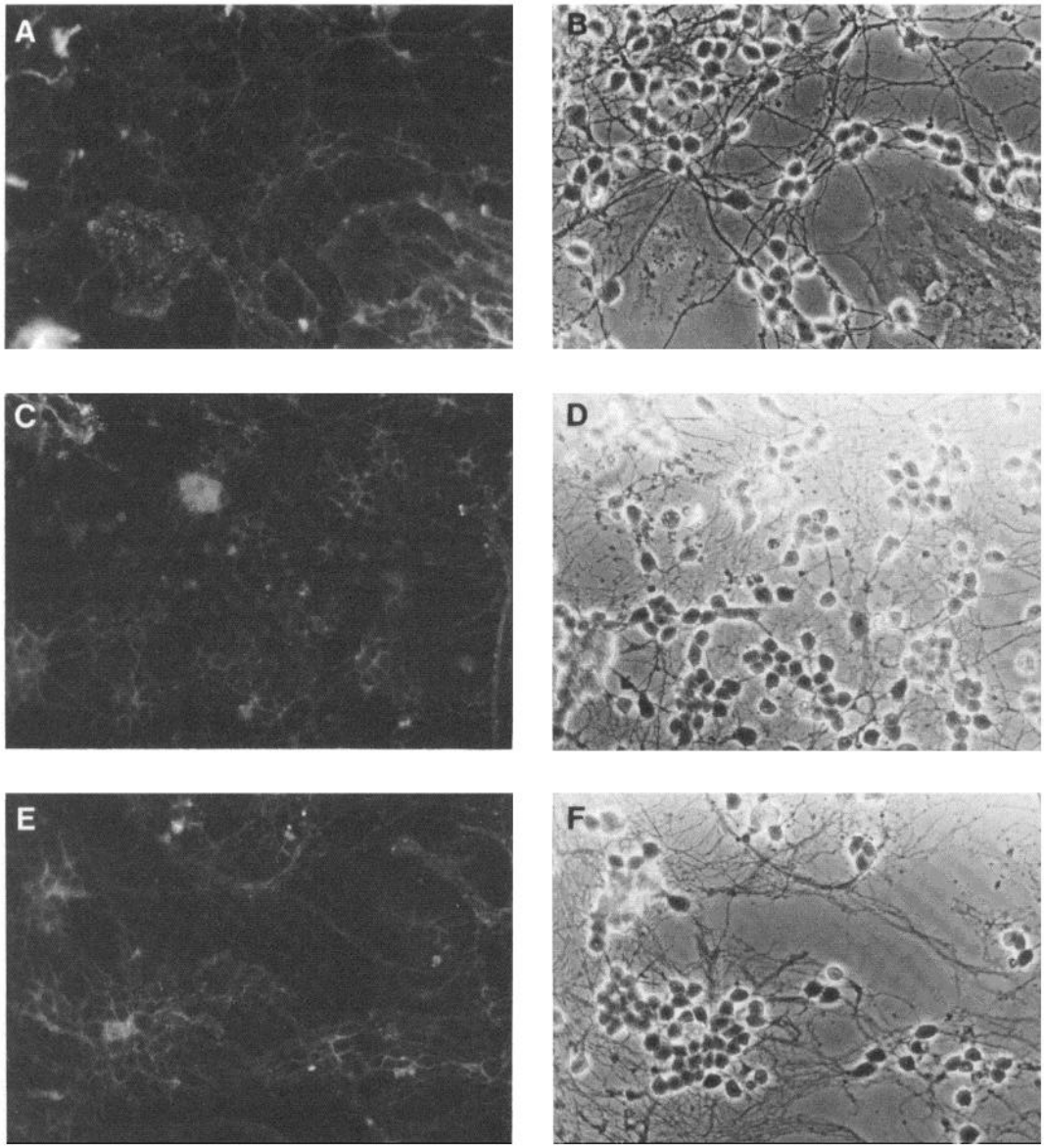

Figure 7. Binding of FITC-WGA (50 μg/ml) to dissociated P7 cerebellar cells after 72 hr *in vitro*. In three fields, all cell bodies and processes were stained. *A*, *C*, and *E*, Fluorescence microscopy; *B*, *D*, and *F*, phase contrast microscopy. Magnification × 220.

of lectin (500 μg/ml) were used (data not shown). The weak UEA_I binding at 500 μg/ml was inhibited in the presence of 0.1 M L-Fuc.

Intense staining of human erythrocytes (type O) was observed with FITC-UEA_I (50 μg/ml). The staining was inhibited in the presence of 0.1 M L-Fuc.

Binding of ^{125}I-lectins to E13, P0, and P7 cerebellar cells

^{125}I-Lectin binding assays revealed a dramatic decrease in ^{125}I-RCA_I binding between E0 and P0 (Fig. 8*a*). Similar, but less pronounced decreases were observed with ^{125}I-Con A and ^{125}I-WGA (Fig. 8, *b* and *c*). For all three of these lectins, binding approached saturation between 50 and 100 μg/ml. Concentrations above 100 μg/ml were not assayed, since the major source of binding at high concentrations was non-carbohydrate specific.

^{125}I-SBA and ^{125}I-UEA_I binding assays of E13, P0, and P7 cerebellar cells indicated that the binding of these lectins was approximately 10-fold lower than that of RCA_I, Con A, and WGA. Very low counts, approximately background, were obtained with these lectins. At concentrations above 100 μg/ml, some binding of ^{125}I-UEA_I was observed in P7 cultures (800 to 1000 cpm/10^6 cells).

Specific binding of ^{125}I-UEA_I was observed with type O human erythrocytes (5000 to 6000 cpm/10^6 cells). Specific binding of ^{125}I-SBA was observed with type A human erythrocytes (7000 to 8000 cpm/10^6 cells).

Discussion

These studies suggest that quantitative changes in lectin binding occur during early cerebellar development. The most dramatic change was a decrease in RCA_I-binding glycoconjugates, D-Gal- and D-GalNAc-contain-

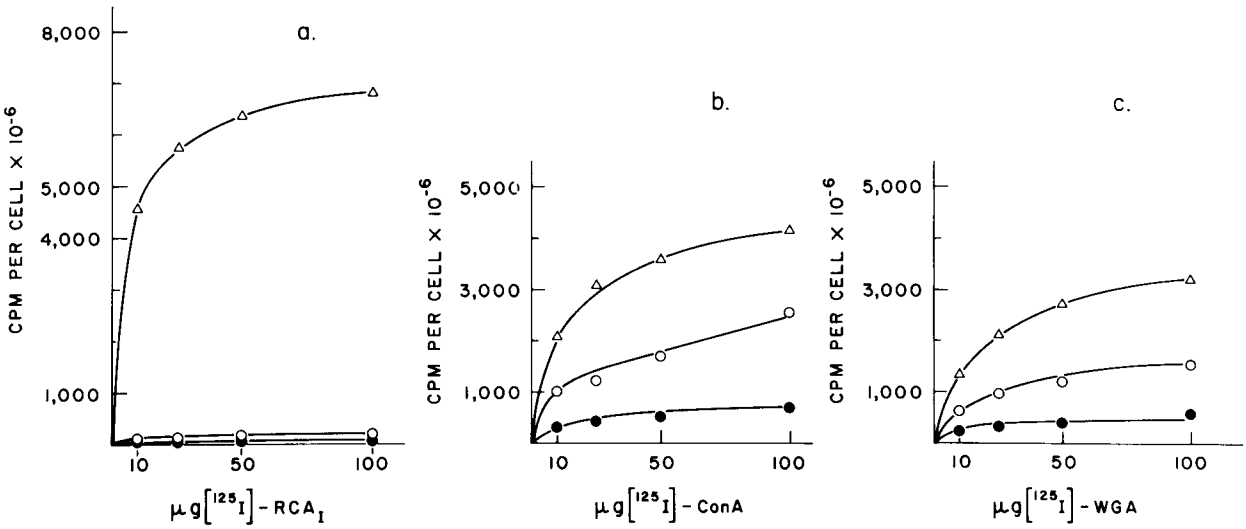

Figure 8. Specific binding of ^{125}I-labeled RCA$_I$ (*a*), Con A (*b*), and WGA (*c*) to E13 (△), P0 (○), and P7 (●) cells in microwells. Details of lectin binding are given under "Materials and Methods." ^{125}I-Lectin binding was assayed in the presence and absence of hapten carbohydrate (50 mM). Specific binding was calculated by subtracting counts in the presence of hapten from those in the absence of hapten. Six duplicates for each assay were carried out for each lectin concentration and for each hapten assay. Results reported are from counts within 10% of the mean. The typical standard deviation for six points was 150 to 500 cpm. As an example, nonspecific binding (in the presence of hapten) to E13 cells at a lectin concentration of 50 μg/ml was 800 cpm/10^6 cells for RCA$_I$, 1850 cpm/10^6 cells for Con A, and 1930 cpm/10^6 cells for WGA.

ing residues, between E13 and P0. A similar, but less pronounced decrease occurred with the lectins Con A, LCA, and WGA during the same time period. This suggests that some D-Glc-, D-Man-, and D-GlcNAc-containing residues have changes in number, exposure, or affinity between E13 and P0.

In experiments where lectin binding was carried out at 4°C, it is likely that all fluorescence or ^{125}I-lectin labeling was derived from surface binding sites. Identical results were obtained with FITC lectin labeling at 4°C and 20°C. Even at 20°C, after a 15-min incubation, it is likely that all FITC lectin binding would be either to cell surface receptors or to the small percentage of surface receptors that might have been internalized by receptor-mediated endocytosis (Willingham et al., 1979). Thus, the changes in RCA$_I$, LCA, Con A, and WGA binding probably related exclusively to cell surface alterations.

The decrease in RCA$_I$ binding between embryonic and postnatal cerebellum also was observed when E13 cells were maintained *in vitro* for 3 or 4 days. This finding supports other results that suggest that at least some aspects of cell differentiation occur in microwell cultures and on roughly the same timetable as is the case *in vitro*. Other properties that differentiate *in vitro* include astroglial development (Hatten and Liem, 1981), fiber outgrowth, and synaptogenesis (Hatten and Sidman, 1978; Trenkner and Sidman, 1977).

Recent studies suggest that, during the 1st week *in vitro*, nearly all, if not all, of the processes in cultures of dissociated embryonic and early postnatal mouse cerebellum are glial in origin (Hatten and Liem, 1981). The network of glial processes appears to set down a template for the positioning of developing cerebellar neurons on a polylysine-treated culture substratum. It is therefore likely that the FITC lectin staining of processes related primarily, if not exclusively, to glial processes. This fact suggests that FITC-RCA$_I$ bound to neuronal cell bodies and glial processes at E13 but that, after 72 hr *in vitro*, FITC-RCA$_I$ primarily stained glial processes.

These studies are in agreement with previously published reports of lectin-induced cell agglutination and lectin binding to tissue sections of mouse cerebellum (Hatten and Sidman, 1978; Hatten et al., 1979; Wood et al., 1974; Zanetta et al., 1978), although precise comparisons between these methods is not possible. The agreement between histochemical studies of lectin binding to mouse cerebellar tissue and the present work probably reflects the fact that the majority of neurons present in the cultures were granule cells.

The absence of specific histochemical or radiochemical binding of SBA, PHA, PNA, DBA, and WFA at both embryonic and early postnatal developmental stages suggests that D-Gal- and D-GalNAc-containing residues bound by these lectins were either absent, sterically blocked, or had a lower binding affinity. Other, more complex interpretations cannot be excluded. The finding that FITC-LPA did not bind to cerebellar cells at E13, P0, or P7 was unexpected, since sialic acid content is generally highest in embryonic cells.

Very weak binding of UEA$_I$ was observed in P7 cerebellar cells. This binding was probably less specific than that observed for RCA$_I$, Con A, LCA, and WGA, since much higher concentrations of lectin had to be used for it to be detected.

These studies do not address many of the complex features of lectin binding, such as heterogeneity, mobility, and distribution of binding sites, or the interaction of any subset of sites with the cytoskeleton (Ash and Singer,

1976; Nicolson, 1975; Rapin and Burger, 1974). Precise studies of the kinetics of lectin binding would be very difficult in microcultures and, indeed, would not be useful if a large number of different binding sites were present. Preliminary attempts to isolate glycoproteins from E13, P0, and P7 cerebellum with lectin affinity chromatography have indicated that a family of sites exists for RCA_I, LCA, Con A, and WGA, and that some of these are modified between E13 and P0 (M. E. Hatten, unpublished observations). It remains to be proved which of these glycoproteins are restricted to the cell surface and which are important to cell contacts.

Three aspects of this study are of special interest. First, lectin binding to cerebellar cells seemed to follow the group specificity of carbohydrate binding of the lectins (Goldstein and Hayes, 1978). Second, it is of interest that the most pronounced changes occurred with RCA_I, one of the lectins known to bind to glycolipids. RCA_I also has proved useful in the isolation of partially sialated glycoproteins (Surolia et al., 1975). Third, although a general decrease in lectin binding seems to be typical of developing brain cells, different regions of the immature nervous system bind different lectins (Hatten and Sidman, 1978; McLaughlin et al., 1980; Pfenninger and Maylie-Pfenninger, 1975; Sieber-Blum and Cohen, 1976; Schwab and Landis, 1981). This suggests the presence of different families of glycoconjugates in different brain regions.

The absence of discernible differences in lectin binding among the cell types present in embryonic and early postnatal cerebellum suggests that the lectins studied are not useful as routine, specific cell markers. Instead, they should prove useful in the isolation of specific membrane components that are under developmental regulation.

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