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Changes in galactosyltransferase activity in chick pectoral muscle during embryonic development

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The two major vertebrate galactosyltransferases have been investigated in developing chick muscle *in ovo* and *in vitro*, and in cultured chick fibroblasts. The two enzymes were UDP-galactose–*N*-acetylglucosamine galactosyltransferase (galactosyltransferase I) and UDP-galactose–*N*-acetylgalactosamine galactosyltransferase (galactosyltransferase II). Both activities fell during muscle development *in ovo*. Galactosyltransferase I activity was constant from day 7 to day 16, after which it declined 5-fold, whereas galactosyltransferase II activity fell markedly from day 9 to 13 and 16 to 20, displaying an overall 8-fold decrease. In primary muscle cultures, galactosyltransferase I activity fell slightly during 7 days in culture, whereas galactosyltransferase II increased 2-fold during the same period. No significant change in activity of either galactosyltransferase was observed during intercellular recognition and fusion. Analysis of muscle cultures treated with cytosine arabinoside and of fibroblast cultures revealed that the majority of galactosyltransferase I activity in primary muscle cultures is associated with fibroblasts, whereas the majority of galactosyltransferase II activity is muscle-associated. The addition of 5-bromodeoxyuridine to primary muscle cultures resulted in a 3-fold rise in activities of both transferases.

During skeletal-muscle myogenesis, mononuclear myogenic cells fuse to form multinucleated myotubes. Formation of myotubes can be divided into three phases: (1) cell multiplication; (2) cell–cell interactions; and (3) cell fusion (Bischoff, 1978). Since the latter two processes require cellular interaction via the cell surface, changes in plasma-membrane components during myogenesis have been investigated and changes have been observed in cell-surface proteins (Chen, 1977; Moss *et al.*, 1978; Cates & Holland, 1978) and glycoconjugates (Winand & Luzzati, 1975; Whatley *et al.*, 1976; McEvoy & Ellis, 1977).

Several lines of evidence suggest that cell-surface galactose-containing components are involved in myogenesis. The activity of a lectin specific for β-galactose increases concomitantly with intercellular adhesion and fusion (Den *et al.*, 1976; Kobiler & Barondes, 1977). It has been suggested that this protein plays a role in myoblast recognition and/or adhesion (Gartner & Podleski, 1975; Mac-Bride & Przybylski, 1978), but that role has been disputed (Den *et al.*, 1976). Alterations in levels of galactose-containing glycoconjugates have been observed during myogenesis (Winand & Luzzati, 1975; McEvoy & Ellis, 1977), but no direct involvement in myogenesis has been demonstrated.

To learn more about the role of galactose-glycoconjugates in myogenesis, it is necessary to study the biosynthesis and metabolism of these compounds during development. In particular, a basic understanding of the galactosyltransferases responsible for the transfer of galactose to these glycoconjugates is necessary. The major glycoprotein galactosyltransferases are galactosyltransferase I (EC 2.4.1.22) and galactosyltransferase II which catalyse the transfer of galactose to asparagine- and to serine (or threonine)-linked oligosaccharides respectively (Jentoff *et al.*, 1976). We have determined the activities of these two galactosyltransferases in both cultured and non-cultured embryonic pectoral muscle and in cultured muscle fibroblasts. The effects of the inhibitor of DNA synthesis β-arabinofuranosylcytosine and the DNA base analogue 5-bromodeoxyuridine on these enzymes were also investigated.

Abbreviations used: galactosyltransferase I, UDP-galactose–*N*-acetylglucosamine galactosyltransferase; galactosyltransferase II, UDP-galactose–*N*-acetylgalactosamine galactosyltransferase.

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Materials and methods

Materials

β-Arabinofuranosylcytosine, 5-bromodeoxyuridine, dithiothreitol, 4-morpholine-ethanesulphonic acid, *N*-acetyl-D-glucosamine, Triton X-100 and UDP-galactose were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. UDP-[1-^{3}H]galactose (sp. radioactivity 11.8 Ci/mmol) was purchased from New England Nuclear. Ovine submaxillary mucin was isolated and desialysed as previously described (Carlson *et al.*, 1966, 1973).

Muscle cell cultures

Fertile White Leghorn chicken eggs were obtained from the Indiana Farm Bureau Coop Hatchery, West Lafayette, IN, U.S.A. Eggs were incubated in a humidified egg incubator for the indicated period of time. Muscle cell cultures from 11 day embryos were prepared and cultured in 100 mm dishes as previously described (Kent, 1979), except that culture dishes were coated with either rat tail collagen or 0.01% gelatin. Cultures were given a half change of media on days 1, 3, 4, 5 and 6 after plating and a full change on day 2. At the time of harvest, the culture dish was washed three times with 8 ml of cold calcium/magnesium-free Earle's balanced salt solution (Grand Island Biological Co., Grand Island, NY, U.S.A.). The calcium/magnesium-free Earle's balanced salt solution was removed, an additional 1.5 ml of calcium/magnesium-free Earle's balanced salt solution was added and the cells were scraped off with a 'rubber policeman'. This suspension was then homogenized with 20 strokes of an ice-cold Dounce homogenizer, followed by sonication on ice with a Sonifer Cell Disruptor (model W200P; Heat Systems-Ultrasonics, Inc., Plainview, NY, U.S.A.) set at 40 with a 20% pulse for two 15 s pulses and 1 min cooling period between each pulse.

Non-cultured muscle

Pectoral muscle from 7 to 20 day-old chick embryos was dissected with removal of loose connective tissue and minced into 1–2 mm pieces. The minced muscle was then centrifuged at 75 *g* for 5 min in an IEC tabletop centrifuge and wet weight was determined. The muscle was then homogenized in 10 ml of calcium/magnesium-free Earle's balanced salt solution/g wet weight and sonicated as described for cultured muscle cells.

Fibroblasts

Pectoral muscle from 11 day embryos was trypsin-treated and plated in serum-free Leibovitz L-15 media for 2 days (Puri & Turner, 1978). After 2 days most myoblasts had detached from the substratum and the plates were washed three times with calcium/magnesium-free Earle's balanced salt solution and fed with the same media used for primary cultures. The fibroblast-enriched cultures were allowed to grow for 3 more days and were then trypsin-treated with 10 ml of 0.25% trypsin (Grand Island Biological Co., Grand Island, NY, U.S.A.) in phosphate-buffered saline per 150 mm plate for 30 min at 37°C. Trypsin digestion was stopped with 2 ml of foetal calf serum. The cells were harvested by centrifugation at 180 *g* for 4 min, suspended in minimum essential medium, and plated at a density of 1.6×10^6 cells/150 mm gelatin-coated culture plate in 20 ml of medium. Fibroblasts were harvested as described for primary muscle cultures.

Galactosyltransferase assay

Galactosyltransferase activity was measured as described by Carlson *et al.* (1973). The concentrations of substrates used were: 10 mM-UDP-[1-^{3}H]galactose and either 20 mM-*N*-acetylglucosamine or 2 mM-asialo ovine submaxillary mucin (with respect to *N*-acetylgalactosamine residues) as acceptors. Incubations were carried out in a shaking water bath for 1 h at 37% at a final volume of 50 μl and a protein concentration of 0.5–2 mg/ml. Incorporation of [^{3}H]galactose into product was measured by using high-voltage borate-buffer paper electrophoresis (Carlson *et al.*, 1973) on a Savant high-voltage electrophoresis apparatus at 2000 V for 45 min. Radioactivity was determined in either Beckman Ready-Solv NA (Beckman Instrument Inc., Fullerton, CA, U.S.A.) or toluene 'scintillation cocktail' [7 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene]. Radioactivity was measured in a Searle Mark III liquid-scintillation counter set on heterogeneous mode. Galactosyltransferase activity was obtained by subtracting the amount of [^{3}H]galactose incorporated without exogenous acceptor from the [^{3}H]galactose incorporated with acceptor. β-Galactosidase and nucleotide pyrophosphatase activities were monitored by examining the electrophoretograms for the appearance of [^{3}H]galactose and [^{3}H]galactose 1-phosphate.

Miscellaneous procedures

Protein was determined by a modified Lowry procedure (Schacterle & Pollack, 1973) and DNA by the method of Levya & Kelly (1974).

Analysis of data

Statistical significance was determined by the Student *t* test and differences were considered significant if $P \leq 0.05$ was found. Analysis of variance (F-test) or Mann–Whitney test was used to determine if a significant difference existed in a mean value between two different treatments or over a particular developmental period (Netter *et al.*, 1978).

Results

The assay conditions for the galactosyltransferases were examined with homogenates of chick pectoral muscle. Activities were linear with time up to 1 h and with protein from 0.5 to 2.5 mg/ml. β-Galactosidase and nucleotide pyrophosphatase, enzymes that would complicate the measurement of transferase activity, were not active under these assay conditions. Activities of the transferases in non-cultured muscle were found to have a broad optimum between pH 6.0 and 7.5. Transferase activity was not affected by the sonication used in preparation of the muscle homogenate.

Galactosyltransferase activities were examined in homogenates of chick pectoral muscle from day 7 of embryonic development, when the rudiments of pectoral muscle first appear, to day 20, one day before hatching (Fig. 1). Galactosyltransferase I activity was not significantly altered between days 8 and 16, but fell markedly between days 16 and 20. Galactosyltransferase II decreased 2-fold from days 8 to 13, levelled off until day 16 and then declined again between days 16 and 20. The first decline in galactosyltransferase II specific activity cannot be explained by an increase in cellular protein, since the amount of protein/mg of DNA remained almost constant between days 8 and 16 (Fig. 1). However, the decline in activity of both transferases after day 16 was partly offset by the doubling of the amount of protein per mg of DNA between days 17 and 20 (Fig. 1). Thus the initial fall in galactosyltransferase II between days 8 and 16 is due to an actual drop in the enzyme activity/unit of DNA. Muscle homogenates from days 10 and 16 were assayed simultaneously in the same reaction mixture and no inhibition of either galactosyltransferase II or galactosyltransferase I was observed, indicating that the decrease in activity of galactosyltransferase II was not caused by the appearance of an inhibitor.

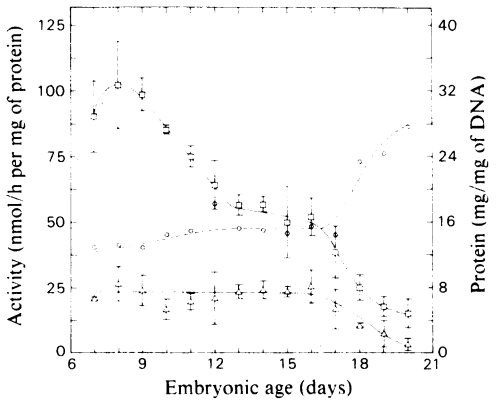

Fig. 1. *Galactosyltransferase activities in chick pectoral muscle*

Galactosyltransferase assays were performed as described in the Materials and methods section. △, Galactosyltransferase I activity; □, galactosyltransferase II activity; ○, protein content (mg/mg of DNA). The assay was performed in duplicate on two separate preparations. Each point represents the mean ± S.D. ($n = 4$). Analysis of variance of data for galactosyltransferases I and II between days 7 and 16 gave F(9,28) values of 1.2 and 18 respectively.

Whole embryonic muscle is a complex tissue, composed of muscle, connective, neural and vascular tissue in various stages of development and a relationship between changes in activities of galactosyltransferases and myogenesis is thus not easily ascertained. Primary cultured muscle cells are considerably more homogeneous with respect to myogenic cells. Transferase activities were therefore measured at different stages of myogenesis.

Eleven-day chick pectoral muscle was cultured for a period of 7 days and the activities of the two galactosyltransferases were measured each day (Fig. 2*a*). The specific activities of galactosyltransferases I and II in primary cultures were similar to that of non-cultured pectoral muscle from 11-day embryos. Activity of galactosyltransferase I increased 2.5-fold between days 1 and 2 from 4.75 ± 2.5 to 12.8 ± 3.5 (mean ± S.D.) nmol/h per mg of protein ($n = 6$, $P = 0.005$), and appeared to slowly decrease as the cultures aged. The mean value over 8 days in culture for galactosyltransferase I was 8.4 ± 3.0 nmol/h per mg of protein. Galactosyltransferase II showed no significant change, remaining steady at 69 ± 3.6 nmol/h per mg of protein over 8 days in culture (Fig. 2*a*). However, the amount of protein per mg of DNA showed a small but constant increase over the 8 day period (Fig. 2*b*). Therefore, when galactosyltransferase activity was expressed per mg of DNA, a steady linear rise in activity of galactosyltransferase II was found (Fig. 2*b*). The activity of galactosyltransferase II/mg of DNA doubled by day 7, whereas galactosyltransferase I showed the same apparent downward trend observed in Fig. 2(*a*). Comparison of galactosyltransferase activity between non-cultured (Fig. 1) and cultured muscle (Fig. 2) showed that galactosyltransferase II specific activity fell in non-cultured muscle and remained constant in cultured muscle.

Primary chick muscle cultures contain at least three different cell types: myoblasts, myotubes and fibroblasts. Fibroblasts initially represent 10–15% of total cells in primary cultures, but increase to 20–25% of total nuclei by day 6. To ascertain whether the data in Figs. 1 and 2 reflect processes occurring in myogenic cells, the following experiments were performed. First, pure fibroblasts from muscle tissue were cultured and galactosyltransferase activity measured over several days. Second,

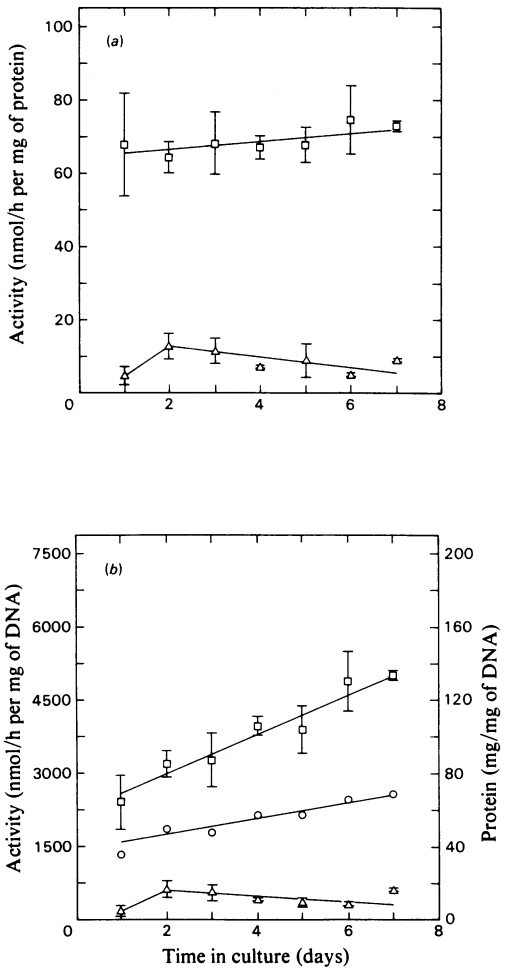

Fig. 2. *Galactosyltransferase activities in primary chick pectoral muscle cultures*

Embryonic muscle cells from day 11 embryos were prepared and cultured as described in the Materials and methods section. △, Galactosyltransferase I activity; □, galactosyltransferase II activity; ○, protein (mg/mg of DNA). Time in culture represents the number of days since the initial plating of the cells. Data were obtained from different cultures and the enzymic assays were usually performed in duplicate. Each point is the mean ± S.D., where $n = 5$ for day 1, $n = 6$ for days 2 and 3, $n = 4$ for day 5 and $n = 2$ for days 4, 6 and 7. (*a*) Transferase activities are expressed per mg of protein and are means ± S.D. (*b*) Transferase data are the same as in (*a*), except that they are expressed per mg of DNA. Linear regression analysis was used to fit lines for galactosyltransferase II and mg of protein/mg of DNA data. Analysis of variance of data for galactosyltransferases I and II between days 1 and 7 in Fig. 2(*a*) gave an F(6,15) value of 3.21 and an F(6,20) value of 0.51 respectively. In Fig. 2(*b*), analysis of variance for data for galactosyltransferases I and II gave an F(6,14) value of 5.7 and an F(6,20) value of 12.7 respectively.

galactosyltransferase activity was determined in muscle cultures treated with β-arabinofuranosylcytosine to decrease fibroblast contamination (Fischbach, 1972).

Table 1 shows activities of transferase in fibroblasts. The specific activity of galactosyltransferase I in fibroblasts was 2–4-fold higher than cultured muscle (Fig. 2*a*) depending on the days compared. The specific activity of fibroblast galactosyltransferase II ranged from 10 to 50% higher than cultured muscle (Fig. 2*a*). When fibroblast transferase activity was expressed per mg of DNA and compared with that of cultured msucle (Fig. 2*b*), galactosyltransferase I ranged from 2–10-fold higher in fibroblasts and galactosyltransferase II from no difference to 2-fold higher. If one assumes that the transferase activity in pure cultures of fibroblasts is comparable with the activity in fibroblasts in primary muscle cultures, then fibroblasts contribute the majority of galactosyltransferase I activity in cultured muscle (e.g. 85 and 80% on days 1 and 5 respectively). Galactosyltransferase II activity in cultured muscle is mainly muscle-cell-associated (e.g. 74 and 61% on days 1 and 5 respectively).

To reduce the contribution of fibroblasts in the later days of primary culture, β-arabinofuranosylcytosine was added on day 2. The number of fibroblasts by day 5 in β-arabinofuranosylcytosine-treated cultures fell to 8% of total nuclei compared with 26% in its absence. Average specific activities for galactosyltransferases I and II after addition of β-arabinofuranosylcytosine to muscle cultures were 5.8 ± 3.9 and 68.8 ± 1.6 (mean ± S.D., $n = 8$) nmol/h per mg of protein respectively. Comparison of these values with control cultures (days 3 to 6; Fig. 2*a*) showed no significant difference in galactosyltransferase II activity with β-arabinofuranosylcytosine treatment. However, galactosyltransferase I did

Table 1. *Galactosyltransferase activity in cultured chick fibroblasts obtained from embryonic pectoral muscle*

Fibroblasts were obtained as described in the Materials and methods section. Values are means ± range of two determinations except day 1, where $n = 6$. Enzymic activity is expressed as nmol/h per mg of protein. Values in parentheses are expressed as nmol/h per mg of DNA.

Time in culture (days)	Activity: Galactosyltransferase I	Activity: Galactosyltransferase II
1	21 ± 12	81.6 ± 14
	(1382 ± 790)	(5271 ± 920)
3	17.9 ± 2.6	107.9 ± 2
	(880 ± 128)	(5308 ± 98)
5	30.1 ± 2.5	108.6 ± 9.7
	(1869 ± 155)	(6744 ± 600)

show a significantly lower activity compared with controls after β-arabinofuranosylcytosine treatment (the Mann–Whitney test gave σ values of 1.813 and 0.273 for galactosyltransferase I and II respectively). Therefore, in agreement with the results with pure fibroblast cultures, the majority of galactosyltransferase II activity appears to be associated with muscle cells and the majority of galactosyltransferase I associated with fibroblasts.

The effect of 5-bromodeoxyuridine on galactosyltransferase activities of primary muscle culture was examined to elucidate a relationship between transferase activities and differentiation. 5-Bromodeoxyuridine prevents differentiation in muscle cultures and as a result mononucleated cells continue to proliferate and fail to form myotubes. At concentrations greater than 3 μM, 5-bromodeoxyuridine has been shown to block muscle-cell fusion after one round of DNA synthesis, and does not inhibit macromolecular biosynthesis (Bischoff & Holtzer, 1970). Levels of both transferases in primary muscle cultures grown in the presence of 33 μM-5-bromodeoxyuridine increase markedly over 6 days in culture (Table 2) to a specific activity 3-fold higher than that of control cultures (Fig. 2*a*). The activities of both transferases per mg of DNA on day 6 were 2–3-fold higher than controls (Fig. 2*b*). Thus 5-bromodeoxyuridine caused a significant increase in the activity of both transferases whether expressed on a protein or DNA basis.

Since 5-bromodeoxyuridine causes a marked increase in cell proliferation and density, the effect of the DNA synthesis inhibitor, β-arabinofuranosylcytosine, on 5-bromodeoxyuridine-treated cells was examined. Three slightly different experimental conditions were employed; two cultures contained 5-bromodeoxyuridine at all times and to one of these β-arabinofuranosylcytosine was added 24 h after plating. A third culture contained 5-bromodeoxyuridine for the first 24 h in culture and thereafter only β-arabinofuranosylcytosine (Table 3). Addition of β-arabinofuranosylcytosine did not prevent a rise in the transferases and both enzymes increased in activity to the same or higher values than observed in the 5-bromodeoxyuridine treated control. The effect of 5-bromodeoxyuridine on these transferases is therefore not related to the increased cellular proliferation and the resulting higher cell density. The results of experiment 3 (Table 3) show that the effect of 5-bromodeoxyuridine is initiated within 24 h after its addition to the cultures and the continued presence of 5-bromodeoxyuridine is not required in the absence of cell division. Since β-arabinofuranosylcytosine itself does not affect transferase activities, it is not the cause of the increased activities seen in 5-bromodeoxyuridine- and β-arabinofuranosylcytosine-treated cultures.

Mixing of untreated and 5-bromodeoxyuridine-treated culture homogenates resulted in additive enzymic activities for both transferases. Thus 5-bromodeoxyuridine treatment of primary muscle cultures resulted in an actual rise in galactosyl-

Table 2. *The effect of 5-bromodeoxyuridine on galactosyltransferase activity in primary muscle cultures*

Chick embryonic pectoral muscle was cultured as described in the Materials and methods section. 5-Bromodeoxyuridine was present throughout the culture period at a final concentration of 33 μM. Values are means ± range of two determinations and are expressed as nmol/h per mg of protein. Values in parentheses represent activity expressed as nmol/h per mg of DNA.

Time in culture (days)	Activity: Galactosyltransferase I	Activity: Galactosyltransferase II
2	2.8 ± 0.4 (106 ± 12)	55.4 ± 6.2 (2060 ± 97)
4	15.6 ± 2.3 (664 ± 127)	142 ± 6.2 (8010 ± 350)
6	26.5 ± 0.7 (1397 ± 34)	193 ± 9 (10160 ± 470)

Table 3. *The effect of β-arabinofuranosylcytosine and 5-bromodeoxyuridine on primary muscle culture galactosyltransferase after 6 days in culture*

Muscle was cultured as described in the Materials and methods section. β-Arabinofuranosylcytosine and/or 5-bromodeoxyuridine were added to primary cultures at concentrations of 10 and 33 μM respectively. 5-Bromodeoxyuridine was present in the initial media and present throughout the culture period except in experiment 3, where it was removed after 24 h. β-Arabinofuranosylcytosine was added in experiments 2 and 3 at 24 h and was present up until harvesting of the cultures on day 6. Results are means ± range of two determinations.

Expt. no.	Additions to medium: Day 1	Additions to medium: Days 2—6	Specific activity (nmol/h per mg of protein): Galactosyltransferase I	Specific activity (nmol/h per mg of protein): Galactosyltransferase II
1	5-Bromodeoxyuridine	5-Bromodeoxyuridine	23.7 ± 3.8	167 ± 1.1
2	5-Bromodeoxyuridine	5-Bromodeoxyuridine + β-arabinofuranosylcytosine	27.8 ± 1.3	178 ± 1.5
3	5-Bromodeoxyuridine	β-Arabinofuranosylcytosine	19.7 ± 10	221 ± 14

transferase activity and not an alteration in the concentration of an inhibitor of these activities.

Discussion

This is the first developmental study of galactosyltransferase activities in embryonic chick pectoral muscle and the effect of β-arabinofuranosylcytosine and 5-bromodeoxyuridine on these enzymes. Embryonic chick pectoral muscle contains these two galactosyltransferases at similar activities in cultured and in non-cultured muscle. Our results show that galactosyltransferase activities are higher at earlier stages of pectoral muscle development *in ovo*. During development, galactosyltransferase II in non-cultured muscle displays a biphasic decline in activity, whereas galactosyltransferase I activity is constant to day 16, after which its activity declined. Non-cultured muscle contains several different types of cells and the activities of transferases in muscle cells could not be directly determined. Galactosyltransferase assays of primary muscle and fibroblast cultures revealed that the majority of galactosyltransferase I activity (80–85%) was associated with fibroblasts and the majority of galactosyltransferase II activity (61–74%) was muscle-associated. No overt changes in transferase activities in muscle cells were observed during the time of developmental processes of intercellular recognition and fusion (Fig. 2, days 2 and 3). However, subcellular redistribution of transferase activity cannot be ruled out. Galactosyltransferase I activity increased in primary muscle cultures between days 1 and 2 (Fig. 2*a*). The higher amount of galactosyltransferase I activity in fibroblasts suggests that this increase in activity may not be associated with myoblast differentiation. A steady 2-fold rise in galactosyltransferase II activity was noted over the entire culture period. Since galactosyltransferase II utilizes a mucin-type glycoprotein as substrate [mucin-type glycoproteins are defined as having *N*-acetylgalactosamine *O*-glycosidically linked to serine or threonine (Zinn *et al.*, 1977)], this increase may reflect the biosynthesis of mucin-type glycoproteins in myotubes.

As muscle tissue develops, cultured and non-cultured cells showed opposite trends in galactosyltransferase II activity. This difference could result from several factors, such as a lack of neural, vascular or hormonal effects found *in ovo* but absent *in vitro*, or to the fact that cultured cells represent a relatively homogeneous cell type in synchronous development and that non-cultured muscle is more heterogeneous in composition and development.

The lack of innervation in cultured muscle may be an important factor. It is well documented that innervation has a profound effect on muscle metabolism and phenotype (Gutman, 1976; Weeds *et al.*, 1974). At 1 week after denervation of adult rat skeletal muscle, a 2–3-fold increase is found in the hexose, hexosamine and sialic acid content of muscle plasma membranes as well as a doubling of galactosyl- and sialyl-transferase activities associated with the plasma-membrane fraction (Jeffrey & Appel, 1978). Sialyltransferase activities in whole rat muscle homogenates were also found to increase after muscle denervation (McLaughlin & Bosmann, 1975). These results show that a lack of innervation raises the activity of galactosyltransferases in muscle and may explain the observed differences between galactosyltransferase II activities in cultured and non-cultured muscle cell.

Hormonal influences also have been shown to influence galactosyltransferase activities. In the parotid gland, galactosyltransferase I increases 5–7-fold on administration of the β-adrenergic agonist isoprenaline (Jentoff *et al.*, 1976). In a similar manner, hormones may influence muscle transferase activities *in ovo*.

A lack of correlation between enzyme activities in cultured and non-cultured tissue has been observed by others (Garfield & Ilan, 1976; Kent *et al.*, 1979). This difference in behaviour of cultured and non-cultured cells is similar to that seen with muscle galactosyltransferases and suggests that galactosyltransferase activities may be controlled by extracellular influences that are absent from standard culture media.

5-Bromodeoxyuridine has been shown to block differentiation and the appearance of tissue-specific proteins in many developing systems including muscle (Bischoff & Holtzer, 1970), cartilage (Abbott *et al.*, 1972), pancreas (Rutter *et al.*, 1968) and mammary epithelial cultures (Turkington *et al.*, 1971). In the presence of 5-bromodeoxyuridine, galactosyltransferase I and II activities increased in cultured pancreas and retinas respectively, but galactosyltransferase II in pancreas remained constant (Carlson *et al.*, 1973; Garfield & Ilan, 1976). Induction of lactose synthetase by prolactin was not inhibited in the presence of 5-bromodeoxyuridine (Turkington *et al.*, 1971). In our studies, 5-bromodeoxyuridine-treated primary muscle cultures showed a 3-fold rise in the activities of both transferases. Thus it appears that the effects of 5-bromodeoxyuridine treatment on galactosyltransferase activities depend on the tissue examined, suggesting a tissue-specific mechanism of 5-bromodeoxyuridine action. The effect of 5-bromodeoxyuridine on transferase activity in cultured muscle was shown to be initiated within the first 24 h after its addition and not to require cell division after the first 24 h. This is consistent with the suggested mechanism of action of 5-bromodeoxyuridine (i.e. direct incorporation into DNA; O'Neill & Stockdale, 1974). In muscle, the rise in galactosyl-

transferase II in 5-bromodeoxyuridine-treated cultures and its decrease in developing muscle *in ovo* is consistent with an anti-differentiation effect of 5-bromodeoxyuridine.

Recent evidence indicates that some tissues contain inhibitors of galactosyltransferase activity (Lau & Carlson, 1979; Shur & Bennett, 1979). Such inhibitors were not responsible for changes in galactosyltransferase activities in non-cultured muscle and on 5-bromodeoxyuridine treatment, and the change in these enzyme activities is due to an actual change in their cellular activities.

Since galactose-containing glycoproteins may be involved in muscle-cell differentiation, the activities of these two glycoprotein galactosyltransferases during myogenesis are of interest. The results of the present study indicate that no overt change in total galactosyltransferase activity occurs during muscle-cell recognition and fusion and perhaps changes in the activity of cell-surface galactosylglycoproteins and/or lectins may control these processes. Further examination of the muscle-cell surface will be necessary to elucidate the role of galactosyl-glycoproteins in myoblast recognition and adhesion.

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