Regulation of arylsulphatase A and sulphogalactolipid turnover by cortisol in myelinogenic cultures of cells dissociated from embryonic mouse brain

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Myelinogenic cultures of cells dissociated from embryonic mouse brain were used to study the regulation of myelination-associated molecules by cortisol. Cortisol in physiological concentrations ($0.03\ \mu\text{M}$) caused an increased accumulation of myelination-associated sulphogalactolipids. It also stimulated the myelin- and oligodendroglia-specific cyclic nucleotide phosphohydrolase. The increase in sulphogalactolipid content was caused by a cortisol-concentration-dependent inhibition in arylsulphatase A activity and not by an increase in either cerebroside sulphotransferase activity or an increase in availability of adenosine 3'-phosphate 5'-phosphosulphate. Of several steroid hormones tested only the glucocorticoid types brought about these changes. The relationship between net sulphogalactolipid accumulation and arylsulphatase A inhibition induced by cortisol was confirmed by sulphogalactolipid turnover studies. Depending on whether a single-phase or a two-phase decay calculation is used, the turnover of sulphogalactolipid with cortisol present was decreased at 22 days in culture by either 62% or 65% respectively of that without cortisol. This decrease in turnover can be attributed completely to the decrease of arylsulphatase activity by cortisol to 63% of the value for normal cells grown under the same conditions.

Attempts to measure the effect of cortisol (hydrocortisone) on myelination in whole animals have been limited to the use of the hormone in relatively high non-physiological concentrations (Walravens & Chase, 1969; Howard & Benjamins, 1975; Noguchi *et al.*, 1982). A number of interesting studies have shown that the administration of pharmacological doses delays myelination in young animals (Field, 1955; Howard, 1965; Noguchi *et al.*, 1982) and causes a decrease in the number of brain cells (Howard, 1965, 1968; Cotterrell *et al.*, 1972). The studies made by Noguchi *et al.* (1982) indicate that at high concentrations cortisol may be bringing about these deleterious results by preventing the release of growth hormone (somatotropin) from the pituitary gland. However, it is uncertain if this indirect action by cortisol on myelination is its only mechanism of action or if it can act directly with the myelin-producing oligodendroglial cells, especially at physiological concentrations.

In contrast with the intact animal, the growth of animal cells in culture offers the possibility of controlling the concentration of a hormone and of measuring a direct interaction between a hormone

and a target cell. We have adapted the primary culture system of Sensenbrenner (1977) and Yavin & Yavin (1974), in which cells dissociated from the cerebral hemispheres of embryonic mice are grown as surface-adhering re-aggregating cultures. The efficacy of using these cultures to study myelogenesis is based on the observations that the cultures possess all of the known myelination-associated molecules, that the expression of the ontogenic patterns of these molecules closely mimics that observed *in vivo* (Bhat *et al.*, 1979, 1981a,b; Sarlieve *et al.*, 1980; Shanker & Pieringer, 1983) and that multilamellar myelin-like membranes are produced (Sarlieve *et al.*, 1983). These cultures have proved to be particularly useful for studying regulation. The differentiation of oligodendroglia as indicated by the initiation of the synthesis of myelination-associated molecules and enzymes is highly dependent on thyroid hormone (Bhat *et al.*, 1979, 1981a,b; Shanker *et al.*, 1981; Shanker & Pieringer, 1983), whereas cellular proliferation is dependent on insulin but not on thyroid hormone (Bhat *et al.*, 1983). Using this culture system, which has been adapted to grow in a chemically defined medium to eliminate the

problem of interfering hormones in serum, we have investigated the effect of cortisol on myelination-associated biochemical properties. In the present paper we report our results on sulphogalactolipid metabolism.

Materials and methods

Preparation of cell cultures

Cultures were prepared as previously described (Sarlieve *et al.*, 1980). Briefly, embryos were removed from 15–16-day-pregnant CD-1 mice (Charles River, Wilmington, MA, U.S.A.) and placed in Dulbecco's modified Eagle's medium (Gibco Laboratories) supplemented with glucose (6.0 mg/ml), NaHCO_3 (2.3 mg/ml), penicillin (90 units/ml), streptomycin (90 $\mu\text{g}/\text{ml}$) and Fungizone (0.225 $\mu\text{g}/\text{ml}$) and adjusted to pH 7 (DME medium). Maintenance of high glucose concentrations is especially important during stages of rapid growth and myelinogenesis (Zuppinger *et al.*, 1981). To dissociate to single cells, cerebral hemispheres were dissected and forced through an 88 μm nylon mesh (Small Parts, Miami, FL, U.S.A.) into 15% (v/v) heat-inactivated calf serum in DME medium. Viability was 80–90% as judged by the Trypan Blue-exclusion technique carried out in the absence of serum. The cell suspension was then plated into flasks that had been wetted inside with 0.02% polylysine (although polylysine did not appear to be absolutely required), at a density of one brain (approx. 3×10^6 cells) per 25cm^2 . Cultures were incubated at 37°C under air/ CO_2 (9:1) at 95% relative humidity. Most cells were attached to the surface by 4–6 days *in vitro*, at which time the growth medium and any unattached cells were removed. The attached cells were washed twice with serum-free defined medium (see below) before adding a final 3 ml of the serum-free defined medium. Thereafter 50% of the medium was exchanged for fresh defined medium every 5–7 days.

The defined medium was a modification of the N2 medium of Bottenstein & Sato (1979). It was based on the DME medium described above and also contained gentamicin (10 $\mu\text{g}/\text{ml}$), insulin (5 $\mu\text{g}/\text{ml}$), transferrin (50 $\mu\text{g}/\text{ml}$), NaSeO_3 (50 nM), putrescine (100 μM) and bovine serum albumin (1 mg/ml) as a lipid carrier and an aid in cell attachment and survival (Zuppinger *et al.*, 1981; Bottenstein *et al.*, 1980). The defined medium also contained a supplement of lipids, vitamins and minerals as used by Honegger *et al.* (1979). Triiodothyronine (usually 20 nM) and steroid hormones were added as specified, and were present throughout the period of culturing in defined medium unless otherwise specified. Cultures could be maintained on this medium for at least 50 days.

The cultures switched to the serum-free defined medium from defined medium containing 15% (v/v) heat-inactivated calf serum at day 5 were morphologically similar to the cultures grown in the presence of calf serum throughout. The major difference was that the neuron-like cables projecting from the surface-adhering aggregates were retained to a greater extent in the cultures grown on the serum-free defined medium. Biochemically, the cultures grown in the defined medium displayed the usual myelination-associated molecules and developmental patterns. For example, the developmental pattern of cyclic nucleotide phosphohydrolase, an oligodendroglia- and myelin-specific enzyme, showed no significant qualitative or quantitative differences when grown in the presence and in the absence (after the fifth day) of serum up to 36 days in culture. Glutamine synthetase, an astroglia-associated enzyme, had essentially the same activity in both media (9.4 and 7.6 $\mu\text{mol}/\text{h}$ per mg from media with and without serum respectively at 21 days in culture). The incorporation of $^{35}\text{SO}_4^{2-}$ into lipids was the same (approx. 19×10^3 c.p.m./mg) from cells grown to 17 days in culture with and without serum. However, in relatively old cultures (50 days) the amount of $^{35}\text{SO}_4^{2-}$ incorporated into the sulphogalactolipid of cells grown on the defined medium did fall to 10×10^3 c.p.m./mg, whereas the serum-containing medium supported extents of incorporation as high as 70×10^3 c.p.m./mg. Sulphotransferase activity also showed some difference; cells grown to 22 days in the presence and in the absence (after 5 days) of serum had activities of 22.3 and 7.2 pmol/h per mg respectively. Arylsulphatase A was about as active in cells grown in either type of medium at 15 days (27 and 21 nmol/min per mg in the presence and in the absence of serum after 5 days respectively), but showed a somewhat greater difference as the cultures aged to 36 years (82 and 52 nmol/h per mg with and without serum respectively). Also, the myelination-associated biochemical properties of these cultures responded to thyroid-hormone treatment in the same manner as did the same parameters of the cultures grown in the presence of serum (see data of the present paper and of Bhat *et al.*, 1979, 1981a,b). These findings suggest that the cultures grown on defined medium after day 5 are as suitable as those grown on calf-serum-containing medium for studying the regulation of myelinogenesis, especially during the first 3 weeks in culture.

Protein and DNA synthesis

Cells were incubated with 1 μCi of $[2\text{-}^{14}\text{C}]$ -methionine per flask (final specific radioactivity 1.6 $\mu\text{Ci}/\mu\text{mol}$) for 2 h, and protein synthesis was assayed by incorporation of ^{14}C label into the

trichloroacetic acid-insoluble fraction (Bollum, 1968). Total protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. Total DNA was determined by the method of Giles & Myers (1965).

Sulphogalactolipid accumulation and turnover in vitro

Cultures were incubated for 16 h with 200–250 μCi of $\text{H}_2^{35}\text{SO}_4$ (final specific radioactivity 40 $\mu\text{Ci}/\mu\text{mol}$) and then washed with saline (0.9% NaCl) and harvested. Incorporation was linear for 20 h with or without cortisol present at all ages of culture studied from 15 to 48 days *in vitro*. Lipids were extracted from the cell homogenate by the method of Bligh & Dyer (1959). After the extract had been washed twice with theoretical Folch upper phase, a sample of the chloroform fraction was taken and its radioactivity counted in a liquid-scintillation spectrophotometer.

In turnover studies, cultures grown for 21 days were incubated with $\text{H}_2^{35}\text{SO}_4$ as above, then the medium was removed and the cultures were washed twice with fresh medium containing relatively large quantities (0.25 mM) of Na_2SO_4 for 5 min. This wash was then replaced with fresh medium and the cultures were re-incubated. At various times after removal of $^{35}\text{SO}_4^{2-}$ from the medium, flasks were washed five times with saline, harvested and homogenized in distilled water. Lipids were extracted, and a sample of the chloroform fraction of the extract was taken and its radioactivity counted. The amount of radioactive lipid remaining was plotted on a semi-logarithmic scale and the rate of turnover was expressed in terms of the half-life ($t_{1/2}$) of the labelled sulphogalactolipid or as percentage turnover of the label in 24 h by the formula:

$$(\text{cpm}_{t=0} - \text{cpm}_{t=24})/\text{cpm}_{t=0}$$

where cpm represents c.p.m./mg of protein). Lines were drawn by the least-squares method, and correlation coefficients (r) were calculated as an evaluation of linearity and best-curve-fitting.

Enzyme assays and other procedures

Adenosine 3'-phosphate 5'-phosphosulphate: cerebroside sulphotransferase activity was determined as described by Bhat *et al.* (1981a). Cells were homogenized in 0.32 M-sucrose with the Polytron homogenizer (Brinkman Instruments). The homogenate was used fresh or reconstituted from a freeze-dried powder in 0.5% Triton X-100. Approx. 400 μg of homogenate protein was incubated in imidazole/HCl buffer, pH 7.0, with 0.5 μCi of adenosine 3'-phosphate 5'-phospho[^{35}S]-sulphate (specific radioactivity 2.1 Ci/mmol) in a final volume of 0.2 ml for 30 min at 37°C. The reaction was stopped and lipids were extracted by the method of Bligh & Dyer (1959).

A sample of cell homogenate was used to assay arylsulphatase A activity by the method of Baum *et al.* (1959). Glutamine synthetase activity was assayed by the method of Kirk & Moscona (1963). 2',3'-Cyclic nucleotide phosphohydrolase activity was assayed by the method of Prohaska *et al.* (1973).

Glycosaminoglycan (mucopolysaccharide) synthesis was determined by the method of Frantantonio *et al.* (1968). Cells were incubated for 16 h with 200–250 μCi of $\text{H}_2^{35}\text{SO}_4$ (final specific radioactivity 40 $\mu\text{Ci}/\mu\text{mol}$), washed five times with saline and homogenized in distilled water. Lipids were extracted into the Bligh & Dyer (1959) single phase, and the [^{35}S]glycosaminoglycan remaining in the cell pellet was dissolved in 0.5 M-NaOH and its radioactivity counted in a liquid-scintillation spectrophotometer. Although this method measures intracellular glycosaminoglycans, it is representative of total glycosaminoglycan because, as demonstrated by Wiesmann *et al.* (1975), extracellular glycosaminoglycans do not contribute much to the total (14–18%) and do not vary over a rather extensive period of growth (8–28 days *in vitro*) in culture.

Fatty acid and sterol synthesis were assayed by the method of Volpe & Marasa (1975). Cells were incubated for 1 h with [^{14}C]acetic acid (0.25 mCi/6.8 mg) (final radioactivity 0.7 $\mu\text{Ci}/\text{ml}$), then washed with saline and harvested, and the cell pellet was digested in 0.5 M-NaOH. The digest was then saponified in 20% (w/v) NaOH in methanol at 60°C for 1 h and extracted twice with light petroleum (Naphtha B) to remove sterols. The digest was then acidified and extracted again twice with light petroleum to remove fatty acids. Sterol and fatty acid fractions were collected and dried and their radioactivities counted.

Chemicals

Non-radioactive chemicals associated with culture preparations were purchased from Gibco Laboratories (Grand Island, NY, U.S.A.). Other non-radioactive chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All radioactive chemicals were obtained from New England Nuclear (Boston, MA, U.S.A.).

Results

Effect of cortisol on cyclic nucleotide phosphohydrolase activity and sulphogalactolipid accumulation

Previous studies (Bhat *et al.*, 1979, 1981a,b) have shown that myelination-associated biochemical properties such as cyclic nucleotide phosphohydrolase activity and sulphogalactolipid accumulation in primary cultures of re-aggregating surface-adhering cells derived from embryonic mouse

Fig. 1. Effects of cortisol and tri-iodothyronine on (a) the rate of ${}^{35}\text{S}$ incorporation into lipids and (b) cyclic nucleotide phosphohydrolase activity in myelogenic cell culture at 21 days in vitro

Concentrations of cortisol were as indicated on the abscissa, and those of tri-iodothyronine were as follows: $\circ$, 0 nM; $\blacksquare$, 2 nM; $\blacktriangle$, 10 nM; $\bullet$, 20 nM. In (a) all points were significantly different ($P < 0.05$) from no controls without cortisol. In (b) all points except zero and 2 nM-tri-iodothyronine at 0.03 μM -cortisol were significantly different from controls without cortisol. Each point represents the mean for three flasks.

cerebral hemispheres are regulated by thyroid hormone. The normal developmental expression of both these properties is completely dependent on the presence of physiological concentrations of thyroid hormones. In the present study we tested their response to cortisol. Both cyclic nucleotide phosphohydrolase activity and sulphogalactolipid accumulation are stimulated by cortisol in a concentration-dependent manner up to 0.3 μM , and this stimulation is augmented additively by increasing concentrations of thyroid hormone (Fig. 1). Half-maximum stimulation by cortisol occurs at 0.02–0.03 μM , a concentration well within the physiological range.

It is important to recognize that, because the data of Fig. 1 are expressed as activity per mg of total protein, it is possible to obtain a false apparent stimulation of cyclic nucleotide phosphohydrolase activity and sulphogalactolipid accumu-

lation if the cortisol caused a decrease in total protein content. This possibility was tested in cultures grown on calf-serum-containing medium until day 5 and thereafter on chemically defined medium with or without 0.3 μM -cortisol for a total of 10, 15, 21, 36 and 48 days. The presence or absence of cortisol did not alter the total protein or DNA content of any of these cultures.

Effect of cortisol on the synthesis of sulphogalactolipids

The increased ability of cortisol-treated cells to accumulate and maintain the concentration of sulphogalactolipids could be explained by a stimulation of one or more of the anabolic reactions involving sulphogalactolipid synthesis. For example, we have shown (Bhat *et al.*, 1981a) that thyroid hormone induces the adenosine phosphate phosphosulphate: cerebroside sulphotransferase.

Fig. 2. Effects of cortisol and tri-iodothyronine on arylsulphatase A activity, assayed *in vitro*, in myelinogenic cell culture at 21 days *in vitro*

Concentrations of cortisol were as indicated on the abscissa, and those of tri-iodothyronine were as follows: ○, 0 nM; ●, 20 nM. All points for cortisol-treated cultures were significantly different ($P < 0.05$) from controls without cortisol. The presence of tri-iodothyronine led to no further significant differences. Each point represents the average for three flasks.

When this enzyme was examined for its response to cortisol a slight inhibition rather than stimulation was observed. Cells grown from day 5 to day 21 on the defined medium containing $0.3 \mu\text{M}$ -cortisol had a sulphotransferase activity of 5.9 ± 0.5 pmol transferred/h per mg of protein, and cells grown under the same conditions but in the absence of cortisol had an activity of 7.3 ± 0.04 pmol/h per mg of protein. Each of these values is the average of assays carried out on 14 flasks, and they are statistically different ($P < 0.05$).

Degradation of sulphogalactolipid

Increased sulphogalactolipid accumulation could be explained by a decrease in its degradation. As shown in Fig. 2, arylsulphatase A is inhibited in a concentration (physiological)-dependent manner, by cortisol, but is unaffected by tri-iodothyronine. A study of the developmental course of arylsulphatase A showed that cortisol ($0.3 \mu\text{M}$) prevents the normal increase in arylsulphatase A activity with age of culture (Fig. 3). At 22 days *in*

Fig. 3. Development of arylsulphatase A activity in myelinogenic cell culture with (●) and without (○) $0.3 \mu\text{M}$ -cortisol in a defined medium beginning at 5 days *in vitro*. A composite of 12 experiments is shown. Each point represents the mean $\pm$ S.E.M. for six to 32 flasks. * $P < 0.05$.

vitro the arylsulphatase A activity in cortisol-treated cells is 63% of the activity in untreated cells. An even greater disparity is observed at older ages, because the activity of treated cells remained unchanged whereas the activity in untreated cells increased further.

The inhibition of arylsulphatase A and stimulation of sulphogalactolipid accumulation appears to be somewhat specific for glucocorticoid steroid hormones, in that several other steroid hormones had no effect (Fig. 4). Glutamine synthetase was used as a control in these studies, since it had been demonstrated to be specifically stimulated by glucocorticoids in other culture systems of the central nervous system (Piddington & Moscona, 1967; Moscona *et al.*, 1980; Juurlink *et al.*, 1981). Cortisol, corticosterone and dexamethasone (three derivatives of glucocorticoid steroid hormones), but not oestrogen, testosterone or progesterone (three non-glucocorticoids), stimulated glutamine synthetase, and sulphogalactolipid accumulation, and inhibited arylsulphatase A (Fig. 4).

Since it seemed possible that the use of a cell homogenate as an enzyme preparation could obscure a possibly important relationship between arylsulphatase A compartmentation in lysosomes and its accessibility to the substrate sulphogalactolipid in a microsomal or membrane compartment in live cells, we measured the effect of cortisol on the turnover of sulphogalactolipids in living cells in culture. Cultures of cells were divided into two groups. On day 5 one half were switched to a chemically defined medium containing no cortisol, and the other half to a defined medium containing $0.3 \mu\text{M}$ -cortisol. At 21 days *in vitro* the cells were exposed to $^{35}\text{SO}_4^{2-}$ for 16 h (as explained in the

Fig. 4. Specificity of glucocorticoid-hormone action on myelinogenic cell culture

Key to hormone added: HC, cortisol ($0.3 \mu\text{M}$); CA, corticosterone ($0.3 \mu\text{M}$); DEX, dexamethasone ($0.3 \mu\text{M}$); E, oestrogen ($0.2 \mu\text{M}$); T, testosterone ($0.2 \mu\text{M}$); P, progesterone ($0.2 \mu\text{M}$). Cultures were harvested at 21 days *in vitro*. Each bar represents the mean $\pm$ S.E.M. for four to eight flasks. * $P < 0.05$.

Materials and methods section) and then to non-radioactive sulphate for the indicated times (Fig. 5). An exponential decay was observed in both cortisol-treated and control cultures over the 5-day period studied (Fig. 5). When these data were represented as a first-order decay process, the half-life of the sulphogalactolipid was 5.3 days in cortisol-treated and 2.1 days in control cultures. In order to compare the decay rates with those obtained in arylsulphatase A studies, we have calculated a relative percentage of initial label turned over in 24h. With use of the first-order approximation, control cultures turned over 20.3% and cortisol-treated cultures turned over 12.6% of the initial label in 24h. Thus decay in cortisol-treated cultures was only 62% of controls. This inhibition of decay by cortisol agrees very favourably with the data of Fig. 3 in which the arylsulphatase A activity of cortisol-treated cells at 21 days *in vitro* was 63% of the controls. When represented as a two-step decay process, one fast and one slow, which was a better fit to the

exponential-decay data than the first-order approximation, the half-life of the fast-decay pool was 3.0 days in the cortisol-treated and 1.9 days in control cultures, and the half-life of the slow-decay pool was 27 days in the cortisol-treated and 11 days in the control cultures. It has been pointed out that an accurate representation of the fast-decay phase must include a correction for slow turnover occurring on during this time (Hennacy & Horrocks, 1978). (In Fig. 5 the corrected t_1 values are shown.) When these corrections were made and turnover was calculated as percentage of initial label degraded in 24h, fast turnover was 22.8% in cortisol-treated compared with 35% in control cultures, and slow turnover was 1.4% in cortisol-treated compared with 5.1% in control cultures. Thus fast decay in cortisol-treated cultures was only 65% of that in controls, and slow decay was 28% of that in controls. The fast decay, which was decreased to 65% and accounts for most of the total decay, is again in close agreement with the 63% arylsulphatase A activity remaining in the cortisol-treated cells at 22 days in culture.

Effect of cortisol on adenosine phosphate phosphosulphate availability

Although the inhibition of arylsulphatase A by cortisol is sufficient to account for all of the decreased turnover of sulphogalactolipids in cortisol-treated cells, there are several other potential regulatory points where the hormone could be exerting an effect. One potential point is the availability of the substrate adenosine phosphate phosphosulphate. Since the sulphation of glycosaminoglycans also uses this compound as a substrate, this reaction provides a convenient means of indirectly assaying for the amount of adenosine phosphate phosphosulphate in cells (Dawson & Kernes, 1979; Bhat *et al.*, 1981a). It also has been used successfully to demonstrate that thyroid hormone does not stimulate glycosaminoglycan synthesis from adenosine phosphate phosphosulphate but does stimulate sulphogalactolipid synthesis from the same substrate (Bhat *et al.*, 1981a). The rates of incorporation of ${}^{35}\text{SO}_4^{2-}$ into glycosaminoglycans by cells treated and untreated with cortisol was measured and found not to be significantly different (Fig. 6), which suggests that the availability of adenosine phosphate phosphosulphate was not altered by cortisol. These data also imply that cortisol had no influence on the ability of the cells to take up the ${}^{35}\text{SO}_4^{2-}$ from the medium.

Effect of cortisol on fatty acid and sterol synthesis

The lack of sterol and fatty acid has been implicated as a possible cause of decreased synthesis of sulphogalactolipid (Siegrist *et al.*,

Fig. 5. Turnover of ^{35}S -labelled lipids in myelogenic cell culture at 21 days *in vitro*

At 21 days *in vitro* cells were incubated with $^{35}\text{SO}_4^{2-}$ for 16 h and then with non-radioactive sulphate for the time indicated. Assays were carried out as explained in the Materials and methods section. The broken line represents an approximation of first-order kinetics; the continuous line represents an approximation of second-order kinetics. r is the correlation coefficient of the least-squares curve approximations. The summary of turnover rates include the correction of fast turnover by the method of Hennacy & Horrocks (1978). Key: CDMT, cultures switched on day 5 to defined medium containing 20 nM-tri-iodothyronine; CDMTH, cultures switched on day 5 to defined medium containing 20 nM-tri-iodothyronine and 0.3 μM -cortisol.

1976; Volpe & Marasa, 1976; Kim & Pleasure, 1978). This possible relationship was checked as described in the Materials and methods section by measuring the incorporation of [^{14}C]acetate into sterol and fatty acid in untreated cells, and in cells treated between 5 and 21 days in culture with 0.3 μM -cortisol or 20 nM-tri-iodothyronine or both. The incorporation into sterol expressed as c.p.m./mg of protein in control cells was 2480 ± 60 , in cortisol-treated cells 1710 ± 100 , in tri-iodothyronine cells 1840 ± 120 and in cortisol + tri-iodothyronine-treated cells 1410 ± 120 . The incorporation into fatty acid expressed as c.p.m./mg of protein in control cells was 22600 ± 3700 , in cortisol-treated cells 18300 ± 980 , in tri-iodothyronine-treated cells 22900 ± 6500 and in cortisol + tri-iodothyronine-treated cells 10400 ± 970 .

Hence cortisol did exert a slight inhibitory effect on sterol synthesis. Cortisol did not inhibit fatty acid synthesis significantly except when tri-iodothyronine was also present. This inhibition might be related to the small inhibition of the sulphotransferase, but it would not explain the observed stimulation by cortisol of sulphogalactolipid accumulation.

Discussion

One of the interesting features of these results is that, like thyroid hormone (Bhat *et al.*, 1979, 1981a,b; Shanker *et al.*, 1981; Shanker & Pieringer, 1983) cortisol brings about the net accumulation of sulphogalactolipid, an oligodendroglia-produced myelin-specific molecule. However,

Fig. 6. Time course of incorporation of $^{35}\text{SO}_4^{2-}$ into cellular glycosaminoglycans of myelinogenic cell culture with (●) and without (○) $0.3 \mu\text{M}$ -cortisol in a defined medium beginning at 5 days *in vitro*

Cultures were harvested at 21 days *in vitro*. A composite of four experiments is shown. Each point represents the mean for two to ten flasks. Differences were not significant at 8, 12, 16 or 20h.

unlike thyroid hormone, cortisol and other glucocorticoids accomplish this by inhibiting the degradative enzyme, arylsulphatase A, rather than by stimulating the anabolic sulphotransferase. The involvement of cortisol in regulating the degradative process was confirmed by the turnover studies in culture. It appears that all of the decrease in turnover can be attributed to its inhibition of arylsulphatase A by cortisol.

Although the cells were exposed to the cortisol on day 5, inhibition of arylsulphatase A was not observed until after day 15 (Fig. 3). This relatively long latent period suggests that cortisol does not inhibit the enzyme through a direct interaction with native arylsulphatase A. Rather, the data of Fig. 3 indicate that the hormone exerts its effect perhaps by inducing the synthesis of an inhibitor or by modifying the structure of newly synthesized enzyme to a less-active form.

The data from the turnover studies were best approximated by dividing the decay curve into fast and slow segments. This procedure has been used by other investigators (Hennacy & Horrocks, 1978) to show that the turnover of sulphogalactolipids (Davison & Gregson, 1966; Jungalwala, 1974) and phospholipids (Smith, 1968; Miller *et al.*, 1977; Miller & Morell, 1978) was more rapid in the microsomal than in the myelin fraction. Our faster turnover rate of ^{35}S -labelled lipid ($t_1 = 2-3$ days) compares well with that found by others for the microsomal fraction (Davison & Gregson, 1966; Jungalwala, 1974) and with a rapidly degradable

sulphatide pool described by Burkart *et al.* (1981a). The slower sulphogalactolipid turnover ($t_1 = 11$ days for control without cortisol, 27 days for cortisol-treated culture) is considerably faster than those of sulphatide (Jungalwala, 1974) or phosphatidylcholine and phosphatidylethanolamine (Miller *et al.*, 1977) of myelin fraction *in vivo*. Perhaps this indicates a greater instability of the slow-turnover sulphogalactolipid pool in these dissociated cell cultures than in the intact animal.

The synthetic enzyme, cerebroside sulphotransferase, has been localized in the microsomal fraction specifically associated with the Golgi membranes (Siegrist *et al.*, 1979). Sulphatide synthesized at this subcellular location is then thought to become associated with a lipoprotein for transport to the myelin membrane (Herschkowitz *et al.*, 1968). The degradative enzyme, arylsulphatase A, is contained in lysosomes (Farooqui, 1981) and appears to be enriched in neuronal fractions (Freysz *et al.*, 1974), although this location may be dependent on age (Raghaven *et al.*, 1972). Several elegant studies have demonstrated that, during development, sulphatide synthesis precedes and is in excess of sulphatide degradation (Burkart *et al.*, 1981a,b) and that during this time newly synthesized sulphatide turns over 40–70% in 24h. Further, it has been suggested that sulphatide progresses from its microsomal site of synthesis, to a lysosomal compartment before entering myelin (Burkart *et al.*, 1982). Thus it is possible that lysosomes serve as a transport compartment from Golgi apparatus to myelin and that the rapidly degraded pool represents sulphatide before entry into myelin. Although cerebroside has been accepted as the best marker for oligodendrocytes during development, it has been suggested that sulphatide may be associated with more mature oligodendrocytes (Zalc *et al.*, 1981). Arylsulphatase A may be an important factor in regulating the balance of cerebroside and sulphatide in the oligodendrocyte membrane during myelinogenesis. In the extreme case, the complete lack of this enzyme and its regulation leads to the devastating fatal results observed in metachromatic leucodystrophy.

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