respectively 76, 54 and 38% lower than in the control cells on days 1, 2 and 4.

For thymidine kinase (fig. 2,b), as for polymerase α, the control cells present an increasing activity especially between the 1st and the 3rd day. This activity equals 1 unit/10^8 cells at 4 h and reaches 39.6 units/10^8 cells on day 3. The thymidine kinase activity of the 20-OH-ecdysone treated cells, which is not detectable before 26 h of culture, increases slowly afterwards but remains at least 70% lower than in the control cells.

Discussion. In the present paper we have described the effect of 20-OH-ecdysone on the DNA synthesis and on the enzymatic activities of DNA polymerase α and thymidine kinase of a diploid clonal *Drosophila melanogaster* cell line.

The growth curve of the control clonal population shows a long lag phase followed by a very short growth phase (fig. 1,a). For the treated cells a growth inhibition is observed. An inhibition of growth initiation seems also to be involved; indeed, from 18 h on the DNA synthesis of the 20-OH-ecdysone treated cells is inhibited by 60% (fig. 1,b), An even earlier inhibition has been described for a Kc_0 *Drosophila* cell line[14]. Moreover our growth curves show that the treated cells do not go through their 1st cell cycle. This agrees with recent results showing an arrest of cell division in the G2 phase, 12 h after 20-OH-ecdysone treatment of *Drosophila* Kc_0 cells[15].

With respect to the major enzymes involved in DNA replication, DNA polymerase α[16,17] and thymidine kinase which catalyses the phosphorylation of thymidine to thymidine 5′-monophosphate[18], at 26 h of culture we measure low enzymatic activities while DNA synthesis is already high. Later on, up to 48 h the time course of the DNA synthesis in the control cells corresponds to the time course of the enzymatic variations. Afterwards, while the DNA synthesis declines, the DNA polymerase α and thymidine kinase activities remain high. Similar variations of DNA polymerase α activities occur in 20-OH-ecdysone treated cells, but these activities are at least 40% lower than in the control cells. Thymidine kinase activity remains 70% lower than in the control cells. These results suggest that TdR kinase, a component contributing to the value of DNA specific activity, and involving the salvage pathway, is unlikely to be the single limiting factor of DNA synthesis in our system as its activity remains very low at least up to 26 h while DNA synthesis takes place. It seems that de novo synthesis of pyrimidine nucleotides, involving important enzymes, like thymidilate synthetase, will be involved in the modifications of DNA synthesis observed. Salvage activities may also be affected as suggested by various authors, linking variations in TdR kinase activities and variations in DNA synthesis[19-21].

Besides, for the 20-OH-ecdysone treated cells, the specific activities of the acid-soluble fraction, which correspond in part to the (^{3}H) thymidine uptake across the cell membrane, are strongly inhibited (70%). It is to be noticed that the extent of thymidine uptake has been correlated in hamster cells with the level of thymidine kinase[22].

In conclusion, a lower acid-soluble specific activity, and a lower DNA polymerase α activity associated with a lowered DNA synthesis, 20-OH-ecdysone dependent, are compatible with an arrest in cell division which has been shown to take place in the G2 phase of the cell cycle[15].

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Glutamine synthetase activity during mouse brain development

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Summary. The specific activity of glutamine synthetase (GS) in mouse brain was 2-fold higher in the olfactory bulbs than in other regions. After birth, the specific activity of GS increased more rapidly in medulla oblongata and in olfactory bulbs, than in cerebral and cerebellar cortex. The activity of GS in primary cultures of brain hemispheres increased more slowly than in homogenates of whole brains. However, when astroblasts were treated in vitro with glucocorticoids or mouse brain extracts, GS activity reached 4 times the level measured in the homogenate of an adult mouse brain. We conclude that levels of GS activity may relate to the maturation of astrocytes, and propose that GS may be used as a marker of astrocytic maturation.

It is now well-estabished that glutamine synthetase (EC 6312) is implicated in the metabolism of glutamic acid, an excitatory transmitter. L-Glutamate ammonia ligase (ATP forming) also seems to play a role in the detoxification of brain ammonia. Its presence in glial cells has been demonstrated by immuno-histochemical procedures[1]. Using similar techniques, Norenberg has assigned the astrocyte glutamine synthetase (GS) location to the hippocam-

pus and cerebellum cortex of mouse brain[2]. In an attempt to confirm his observation, we determined the activity of GS in some specific areas of mouse brain during pre- and postnatal development, and compared it with that found in primary cultures of highly enriched mouse astrocytes, the latter having been previously characterized in our laboratory[3].

Biorex 70 and Dowex AG 1×8 (200–400 mesh chloride form) were obtained from Biorad (Richmond, Cal.), tricine, $MgCP_2$, KCl, 2-mercaptoethanol from Merck and Co. (Darmstadt, FRG), L-glutamate monosodium salt was obtained from Sigma Chemical Co. (Saint Louis, Mo.), readysolv TMMP from Beckman (Glenrothes Scotland), ATP sodium salt from Boehringer (Mannheim, FRG), L-[^{14}C]-glutamic acid from Amersham International Limited (Amersham, Buckinghamshire, England), and lyophilized hydrocortisone from Roussel (Paris, France).

Different regions were isolated from the brain of freshly sacrificed mice. Four areas were considered: Area I, olfactory bulbs (OB); area II, cerebral hemispheres (CH); area III, cerebellum cortex (CC); area IV, medulla oblongata (MO). Each sample was placed in cold tricine-buffered saline (1 M tricine, pH 7.5, 5 mM $MgCl_2$, 20 mM KCl, 5 mM 2-mercaptoethanol); 10 ml of this solution per g tissue were used. The tissue was then homogenized with a Potter Elvejhem homogenizer at 4 °C. Protein content was determined by the Lowry method (1951), using bovine serum albumin as standard. Primary cultures of astrocytes were prepared as described previously[4]. The cells were collected at different stages of culture with a rubber policeman and recovered in the same buffer as above. In each case, GS activity was measured by the procedure of Pishak and Phillips[5] with slight modifications. Unless otherwise stated, the assay contained the following components, in a final volume of 50 µl: 50 mM tricine at pH 7.5, 20 mM $MgCl_2$, 10 nM ATP, 10 mM [U^{14}C] L-glutamate (1.06 mCi/nmole), 4 mM NH_4Cl, 1 mM 2-mercaptoethanol, and the enzyme sample containing approximately 4×10^{-5} g protein. Mouse brain extracts were prepared from adult mice as described by Pettman et al.[6] and added to the nutrient medium in a proportion of 1 ml/10 ml. All samples were incubated at 37 °C for 10 min. The reaction mixture was transferred to a column of Dowex AG 1×8 (200–400 mesh, chloride form) mounted, above a Biorex column. The tandem column arrangement permitted separation of the product, [^{14}C]-glutamine, from unreacted [U^{14}C]-glutamate as well as from other labeled compounds which may be formed by a competitive reaction. The specific activity of GS is expressed as nmoles of glutamine, produced per min per mg protein.

At the 16th day in utero, GS activity was low in total brain homogenates and in cerebellum cortex, but was much higher in medulla oblongata. Activity could not be detected at this period either in olfactory bulbs or cerebral cortex. It increased progressively until the 19th day, the period where activity was measurable in olfactory bulbs as well as in cerebral cortex. A larger increase occurred 5 days after birth. Values in total brain homogenates attained levels of about 8 nmoles/mg protein 20 days after birth. Comparing the 4 separate areas, we observed that in 2, i.e. OB and MO, GS activity increased more rapidly than in cerebral and cerebellar cortex over a period of 20 days after birth (fig. 1). In OB and MO, GS activity began to rise significantly after the 10th day, whereas the same increase was observed in CH and CC only after 20 days. In the most active regions in 5-week-old mature male mice, GS activity reached levels 5–7 times greater than those found in newborn mice (fig. 2). In particular, olfactory bulbs contained twice as much activity as other areas.

When GS activity was measured in astroblasts isolated from dissociated areas enriched in cerebral cortex of newborn mice, its activity increased more slowly than in total brain homogenates. However when 3-week-old astroblasts were treated with mouse brain extracts as well as with glucocorticoids, astroblasts differentiated morphologically into astrocytes (fig. 3) and GS activity reached levels which

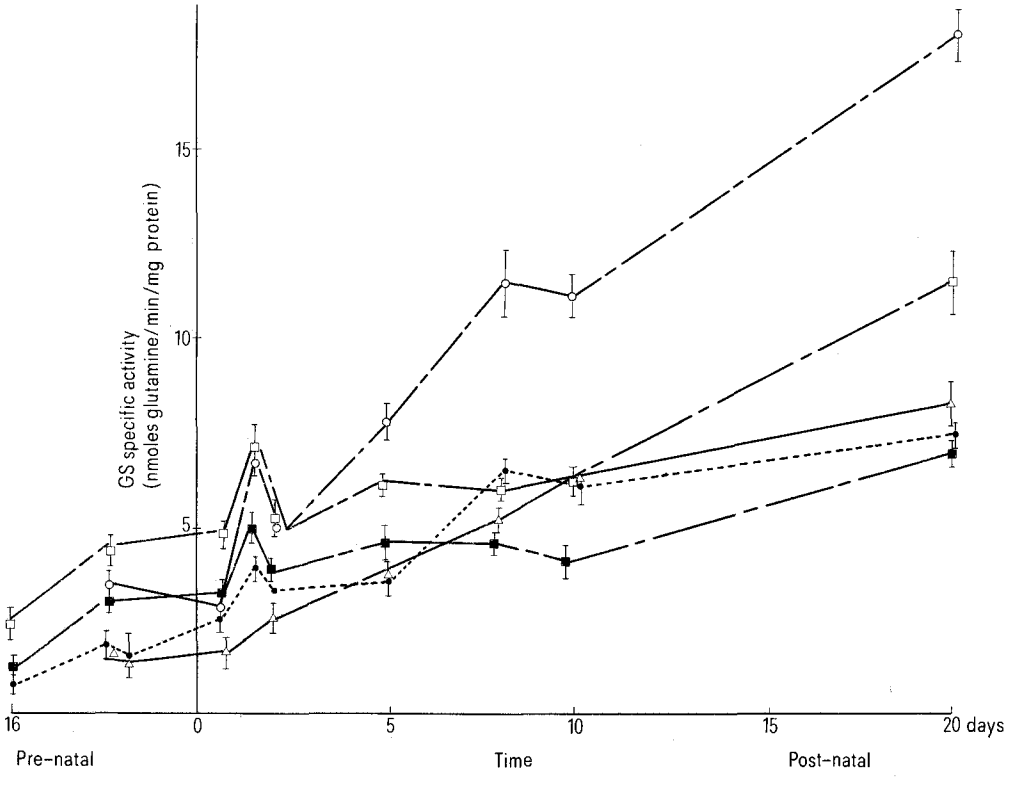


Figure 1. GS specific activity in total mouse brain (●), olfactory bulb (○), medulla oblongata (□), cerebral hemispheres (▽), and cerebellum cortex (■) during perinatal development. Each point is given as mean ± SEM (n = 3–6). Results are expressed as nmoles of glutamine produced per min per mg protein as a function of age.

were some 4-fold higher than those of the homogenate (fig. 4). The low levels of GS activity preceding birth in all regions of the brain probably result from the immaturity of all glial cells present in these regions. At this time, the precursors of glial cells, called radial glial cells[7] do not express any measurable GS activity. After birth, the rates of GS activity increased in all brain areas, parallel with astrocytic maturation. An early increase in the MO and OB GS activity may be related to their early ontogenetic development[8]. These areas are almost fully formed at birth, whereas glial cell proliferation occurs mainly during the first 12 days of postnatal development[9]. Accordingly, cerebral hemispheres and cerebellum constituted by neoformations such as cerebral neocortex and cerebellar neocortex develop mainly after birth[8], reaching a peak around the 20th postnatal day[9]. We consider that in all areas, the adult rate is reached around 5 weeks after birth.

Another finding can be correlated with the general growth in GS activity, i.e. the growing presence of glucocorticoids in brain after birth. The induction by these hormones of GS is well established in vitro[10-12] and recently Patel et al.[13] have shown that such an induction occurs in vivo. The increase in glucocorticoid receptors in the brain during development parallels that in GS activity[8]. GS activity increases rather slowly in vitro in glial cell cultures, but its activity may be greatly enhanced in these cells by addition of glucocorticoids to the culture medium, as well as by addition of brain extracts. The fact that GS activity is significantly increased in astrocyte-enriched cultures and that the rise in its activity in brain areas during development could be related to astrocyte maturity is consistent with the suggestion that GS may be used as an astrocytic marker to monitor astrocytic maturation. Astrocytes may

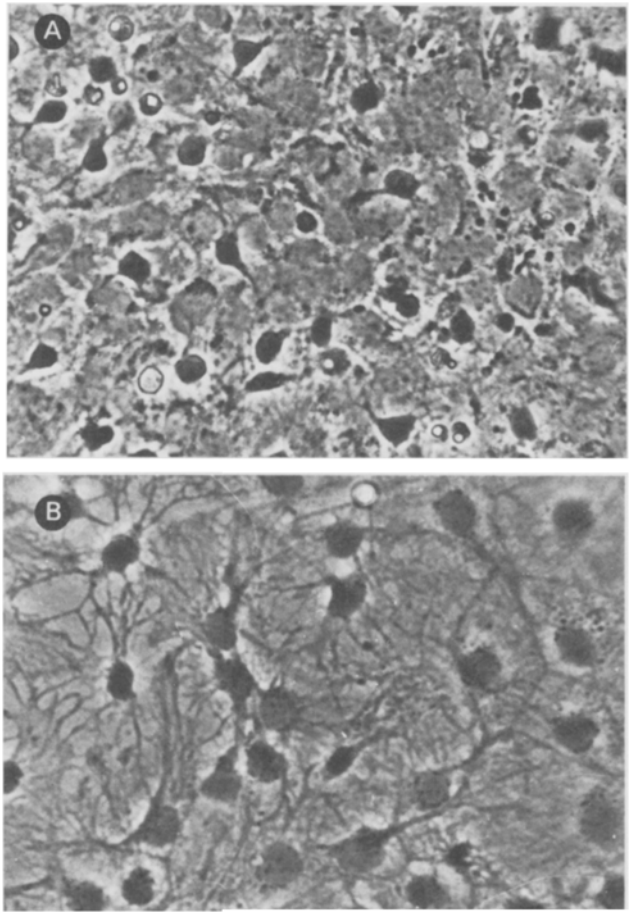
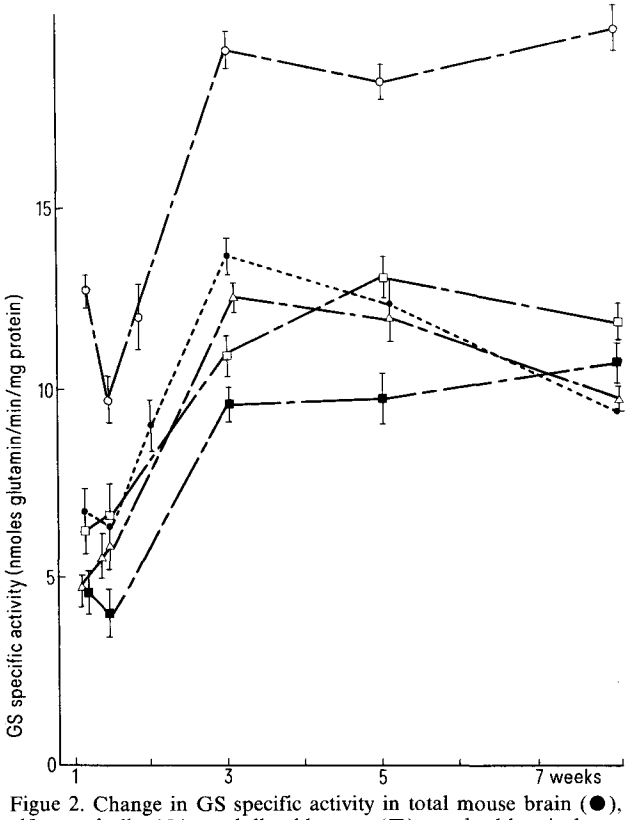


Figure 3. Glial cells obtained from dissociated newborn mouse brain hemispheres cultured for 3 weeks in vitro. *A* Cultures in standard nutrient medium. *B* Cultures in the presence of brain extract (10%) and glucocorticoids (1 μM for 48 h).



Figue 2. Change in GS specific activity in total mouse brain (●), olfactory bulbs (○), medulla oblongata (□), cerebral hemispheres (△), and cerebellum cortes (■). Each point is given as mean ±SEM (n=3-6). Results are expressed as nmoles of glutamine produced per min per mg protein as a function of age.

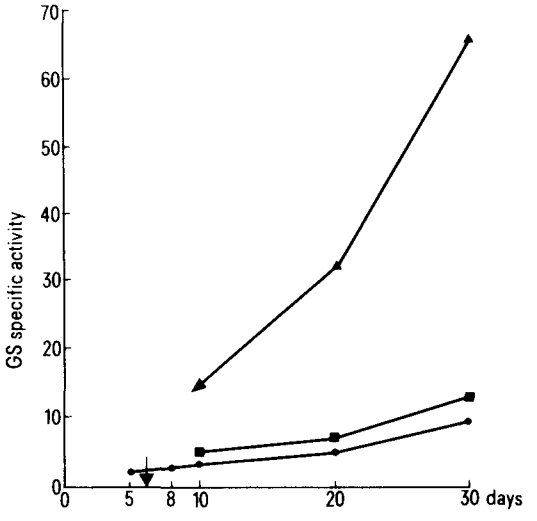


Figure 4. GS specific activity during astrocytic development as a function of growth in vitro. Cortex-enriched fractions of 3-day-old mice were dissociated and plated as described (methods). Control values (●) are compared to cells treated with hydrocortisone (1 μM for 48 h) (■) at the 10th, 20th and 30th day of culture and to cells treated with adult mouse brain extracts dring the growth period as of the 6th day in vitro and with hydrocortisone (1 μM, 48 h) administered at the same time (▲). Each point is the mean value obtained from 2-4 cultures.

need some environmental factors in order to attain maturation and the cooperative effect of glucocorticoids and brain extracts which we observed is in agreement with the recent finding of Kamatsu et al.[14]. These authors showed a marked increase in GS activity in a highly enriched K^+-containing medium, a condition which is found in the neuronal environment.

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Monovalent cation conductance in liposomes induced by ionophore A23187[1]

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Summary. In the absence of divalent cations, ionophore A23187 supports low rates of monovalent cations loss ($Na^+ > K^+$) from unilamellar liposomes containing the sulfate salts. Monovalent cation efflux is optimal when a pH gradient (interior alkaline) is applied. The maximum observed rate of 0.56 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$ A23187 is insufficient to account for the rates of K^+ efflux induced by the ionophore in mitochondria (150 ngion $K^+ \cdot min^{-1} \cdot nmole^{-1}$ A23187). These studies therefore support the concept that A23187 induces loss of K^+ from mitochondria by removal of regulating divalent cations from an endogenous K^+/H^+ exchanger.

The carboxylic acid ionophore A23187 has been shown to activate K^+/H^+ exchange in liver and heart mitochondria[2-6]. This ionophore is known to deplete mitochondrial Mg^{2+} and Ca^{2+} to very low levels[7-9]. It has been proposed that A23187 activates an endogenous K^+/H^+ exchange component present in the mitochondrial membrane which is normally maintained in an inactive form by matrix Mg^{2+}, Ca^{2+}, or both[2-4,10]. Other studies with mitochondria and model systems[5,6] have concluded that A23187 itself can promote K^+/H^+ exchange, especially in the presence of a transmembrane pH gradient. A strong argument against this interpretation is provided by the observation that once divalent cations have been removed from mitochondria, wide variations in the amount of A23187 present do not affect either the rate of net K^+ efflux[3,4] or the steady-state level of K^+ in liver mitochondria treated with valinomycin[3] However, as Dordick et al.[3] have pointed out, even a small direct contribution of A23187 to K^+/H^+ exchange would complicate the interpretation of the kinetics of K^+ efflux on the putative mitochondrial K^+/H^+ exchanger. For this reason we have used a liposome model to re-examine the question of whether A23187 promotes a direct K^+/H^+ exchange. This system is free of proteins which may contribute to uniport or exchange reactions in native membranes and it also permits imposition of an artificial pH gradient of known magnitude.

Materials and methods. Unilamellar liposomes were prepared and characterized essentially as described by Johnson et al.[11]. Asolection (Associated Concentrates, Inc., Long Island, N.Y.) was sonicated at 25 mg/ml in 0.1 M K^+ or Na^+ sulfate containing Tris (10 mM, pH 8.7), EGTA (25 μM), and EDTA (25 μM). All of the solutions and liposome preparations used in this study contained this level of divalent cation-chelators in order to avoid possible interaction of A23187 with adventitious Ca^{2+} or Mg^{2+}. The resulting suspension of vesicles was passed over Sephadex G-50 equilibrated with tetramethylammonium (TMA)-sulfate (0.1 M), Tris, and the chelators in order to remove extravesicular K^+ or Na^+. Vesicle concentration was established by phosphorous analysis[12].

The efflux of K^+ at 25 °C from the liposomes was followed with a K^+ electrode (Beckman 39047) connected by a choline chloride salt bridge to a reference electrode as previously described[4]. Efflux of Na^+ was recorded in the same way using a Beckman Na^+-selective electrode.

Results and discussion. When liposomes containing K_2SO_4 (0.1 M, pH 8.7) are added to a medium of TMA-sulfate (0.1 M, pH 8.7), there is an initial electrode response due to the small amount of extravesicular K^+ which is present (fig. 1). After this deflection, the electrode shows that there is only a very slow leak of K^+ from these liposomes at 25 °C and that the K^+ can be released by disrupting the vesicles with Triton X-100 (fig. 1). Addition of the monovalent cation/H^+ exchanger nigericin[13] results in a rapid and nearly complete release of the sequestered K^+ (fig. 1). The rate of K^+ release is proportional to the concentration of nigericin added in the concentration range from 5×10^{-11} M to 2×10^{-10} M and a plot of K^+ released $\cdot min^{-1}$ vs nmole nigericin is linear with the slope of this plot corresponding to 460 ngion $K^+ \cdot sec^{-1} \cdot nmole^{-1}$ of nigericin. Addition of valinomycin to provide an exogenous uniport pathway for K^+ efflux[12] results in only a small increase in the rate of K^+ loss from the vesicles (fig. 1). Increasing the H^+ conductivity of the liposomes by addition of high concentrations of an uncoupler (m-chlorocarbonylcyanidephenylhydrazone, CCP, in figure 1) also does not affect the release of K^+ from these liposomes, either in the absence of a pH gradient (as shown in figure 1) or when the vesicles are sus-