Modeling the Effects of Prenatal Exposure to Aspirin on the Postnatal Development of Rat Brain

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Abstract: Three growth models were used to examine the effects of prenatal exposure to aspirin on the postnatal development of brain parts. A total of 60 pregnant rats which were divided into three experimental groups and a control group were exposed to aspirin doses of 12.5, 25, 37.5 mg/kg, and distilled water, respectively. The brain parts of 200 rat pups starting from the first week after birth until the fifth week were weighted and the length and width of the cerebrum and cerebellum were measured to determine the parameters of the growth models. The results indicated that the three models successfully predicted the growth of the different brain parts and that aspirin decreased the total brain weight, cerebrum length and width, and decreased the cerebellum length and width at aspirin dose of 37.5 mg/kg. Further analysis is needed to investigate if aspirin effects were carried out through its role in inhibiting prostaglandin production and consequently affecting the activity of the hypothalamus-pituitary axis.

Key words: Brain Growth, Growth Models, NSAID, Acetylsalicylic acid, Cerebrum, Cerebellum, Brain development

INTRODUCTION

It is well known that aspirin (acetylsalicylic acid) inhibits the production of cyclooxygenase (COX), which is necessary for the production of prostaglandins (Vane and Botting, 1998). As a result, it is widely used for the treatment of pain, fever, inflammation, and the reduction of platelet aggregation (Craig and Stitzel, 1997). Although aspirin has been known to cross the placenta, and circulate in fetal blood (Wilson *et al.*, 1997), it is prescribed to pregnant women for a variety of reasons (Fenster *et al.*, 1999). A number of studies have examined the positive or negative effects of aspirin on the brain. Hwang *et al.* (2004) studies the role of aspirin in suppressing the growth of C6 glioma cells

through its effect on prostaglandin E2 synthesis. De Cristobal *et al.* (2002) examined the protective role of aspirin against brain cell oxidative damage. Montine *et al.* (2002) has suggested that at least some non-steroidal anti-inflammatory drugs may influence the initiation of Alzheimer's disease by reducing cerebral prostaglandin elevation that may occur in middle age. Furthermore, a number of studies have indicated that aspirin restricts brain growth (Bonthius and West, 1989); significantly accentuate the alcohol induced crainofacial anomalies and growth retardation (Padmanabhan *et al.*, 1994), and increase brain tumor volume, cell proliferation and vascular density when administered in high doses (Arrieta *et al.*, 2001).

Modeling and mathematical calculations have been used to investigate the relative growth of different body parts. For example, Boire and Baron (1994) used allometry for the comparison of brain, and main brain subdivisions in birds, while Cabana *et al.* (1990) investigated brain and body growth of Mongolian gerbils, and Wanderley *et al.* (1990) examined the relative growth of brain in the human fetuses. Finally, Burri *et al.* (2003) to investigate postnatal marsupial lung development.

According to Garman *et al.* (2001), every single study that was used to assess neurologic development in the laboratory animals needs a careful qualitative, and quantitative investigation. These quantitative methods include modeling the growth of different brain, and body parts based on measurements, such as length, width, and weight of brain parts. Therefore, the aim of this study is to examine the effects of prenatal exposure to aspirin on the postnatal brain development, using growth models in order to fulfill the objectives of the present research.

MATERIALS AND METHOD

Chemicals

Aspirin (acetylsalicyclic acid) powder was purchased from Chemie S. A. (Spain), dissolved in distilled water, and given at doses of 12.5, 25 and 37.5 mg/Kg.

Treatments, brain removal and brain measurements

A total of 60 pregnant Sprague Dawley (SD) rats housed in (60 X 40 X 20 cm) cages in the animal house of the Hashemite University were divided equally into four groups and were fed standard commercial rat diet and water. A control group, which received distilled water and three experimental groups, received aspirin doses of 12.5, 25 and 37.5 mg/Kg respectively. All administrations were oral and were given daily from the fifth day of gestation until birth. The rats were subjected to routine examination, and testing for the presence of pathogens, or, diseases by the staff of the animal house. At day 20 of gestation, the rats were housed singly in hard bottom cages (40 X 20 X 15 cm), and were checked, regularly, for the presence of pups to determine the day of birth.

Two hyndred pups were used starting from the first week after birth until the fifth week. At each week, 10 pups from each group were deeply anesthetized with ether, and weighted using analytical balance. Pups were perfused intracardially through the left ventricle with 0.1 M phosphate buffer (pH 7.4) for brain fixation. This was followed by perfusing an equal volume of fixative, containing 20% buffered formalin fixative (Kiernan, 1999). Each brain was carefully removed and rapidly immersed for 3 days in 20% buffered formalin for further fixation, to prevent any alteration in the size of the brain (Kiernan, 1999). The brains then were weighed; the cerebellum was separated from cerebrum; and the length and width of each brain part were measured, using a digital caliper (E-Base, MC 02050282-1, China).

Modeling and data analysis

Cerebrum length and width, cerebellum length and width and brain weight were analyzed using three growth models (Elkarmi and Abu Eideh, 2006, Elkarmi and Ismail, 2006 and Aiba and Kohyama, 1996).

Richard's growth model: $X = X\infty\ [1\text{-}A*e^{-kt}]$,
Von Bertalanffy's growth model:
$X = X\infty\ [1\text{-}\ e^{-k(t-t0)}]$
Gompertz growth model: $X = X\infty\ [e^{(-A*e(-k*t))}]$

Where X is the measured parameter, X∞ is the measure parameter at time ∞ (long period of time); A is a constant; k is the growth coefficient; and t0 is the age at which the measure parameter is theoretically nil. The motivation for using three models instead of one was intended to give further support, reliability and accuracy to the findings of one particular model. Although the three models may seem mathematically close, they differ in the way they determine the growth parameters. Thus, confirmation and validation of the calculations may well be achieved. The parameters X•, k, A and t0 were calculated by the quasi-Newton method (Ismail and Elkarmi, 2006) by a computer-based statistical software (STATISTICA software, StatSoft, Inc., OK, USA). This method uses the means of the results of each week for each group (n=50 for each group) as to calculate the growth parameters. In the quasi-Newton method, the second order (partial) derivatives of the loss functions are asymptotically estimated, and used to determine the movement of the model parameters from iteration to iteration. Therefore, the model can successfully predict the growth pattern of the brain part, and any individual variation in the dimensions of the brain part; or, alteration due to shrinkage will be eliminated. Furthermore, an Analysis of Variance is carried out to determine if the differences between the results of each group are statistically significant.

RESULTS

The three growth models successfully predicted the brain growth of rat pups with a very high correlation coefficient (r) as shown in tables (1 to 3). There are differences in the growth of the cerebrum, and cerebellum between the control group, and the three groups subjected to aspirin. The growth coefficient (k) determined the rate of growth of each brain parameter; and thus, the higher it is, the higher the growth of the brain part. The growth coefficient for cerebrum length, as predicted by the Richard's growth model, decreased in the three treatment groups when compared with the control group (k=1.3, 0.75, 0.6, and 0.7 for the control group, group 1, group 2 and group 3 respectively). The other growth models showed the same pattern of growth. As a result, the growth of the cerebrum length was lower in the aspirin treated groups than in the control group. This growth pattern applies also, but to a lesser extent, to cerebrum width (k=1.6, 1.5, 1.3 and 1.1 for the control group, group 1, group 2, and group 3, respectively). The main difference for cerebellum width was evident in group 3, which is the one that was treated with the highest aspirin dose (k=1.05, 1.1, 1.0 and 0.65 for the control group, group 1, group 2, and group 3, respectively). Similarly, the main difference for cerebellum length was evident in group 3 (k=0.95, 0.9, 0.8 and 0.7 for the control group, group 1, group 2, and group 3, respectively). The Von Bertalanfi's and Gompertz growth models indicated the same growth patterns for the cerebrum and cerebellum length and width. It is interesting to note that group 3 showed the lowest growth coefficient for most brain parameters, with the exception of cerebrum length, where group 2 was the lowest. The means used for the calculations of the model parameters for each week of each group are presented in table (4). The ANOVA test indicated that there are statistically significant differences between the groups ($p<0.000$). The means of the measured values for the cerebrum, and cerebellum length and width displayed in figures (1 to 4) indicate that the growth of the measured brain part increases rapidly in the first two weeks; then, it either stops to increase; or, increases at a slow rate. They all show the exponential growth pattern during the five weeks of growth predicted by the three models. Besides, the growth coefficient of the total brain weight decreased in the three treatment groups, when compared with the control group (k=1.1, 0.85, 0.75 and 0.8 for the control group, group 1, group 2, and group 3, respectively).

TABLE 1
Results of Richard's growth model listing the growth parameters for the control and experimental groups

	Control Group	Group 1 (12.5mg/Kg)	Group 2 (25mg/Kg)	Group 3 (37.5mg/Kg)
Brain Weight (mg)				
∞	1371.073	1457.490	1459.192	1354.060
A	1.105	0.952	0.810	0.978
K	1.1	0.85	0.75	0.8
r	0.988	0.971	0.998	0.999
Cerebrum length (mm)				
∞	11.152	11.457	11.807	11.574
A	0.887	0.412	0.504	0.642
K	1.3	0.75	0.6	0.7
r	0.996	0.943	0.997	0.999
Cerebrum width (mm)				
∞	14.706	14.987	15.007	14.759
A	0.526	0.596	0.391	0.385
k	1.6	1.5	1.3	1.1
r	0.971	0.966	0.997	0.990
Cerebellum width (mm)				
∞	11.630	11.754	11.643	11.692
A	0.795	0.887	0.690	0.552
k	1.05	1.1	1.0	0.65
r	0.999	0.997	0.999	0.982
Cerebellum length (mm)				
∞	7.507	7.663	7.769	7.534
A	0.985	0.816	0.793	0.827
k	0.95	0.9	0.8	0.7
r	0.992	0.991	0.982	0.982

∞: Parameter at age ∞ (maximum weight, length or width)

k: growth coefficient

r: correlation coefficient

A: growth model constant

TABLE 2
Results of Von Bertalanfi's growth model listing the growth parameters for the control and experimental groups

	Control Group	Group 1 (12.5mg/Kg)	Group 2 (25mg/Kg)	Group 3 (37.5mg/Kg)
Brain weight (mg)				
∞	1391.313	1727.878	1682.442	1452.778
to	0.099	0.481	0.504	0.281
K	0.9	0.3	0.32	0.5
r	0.976	0.907	0.977	0.988
Cerebrum length (mm)				
∞	11.137	11.450	11.717	11.579
to	0.072	0.931	0.650	0.443
K	1.35	0.7	0.65	0.7
r	0.997	0.939	0.997	0.999
Cerebrum width (mm)				
∞	14.716	15.002	15.007	14.837
to	0.742	0.619	0.939	1.139
k	1.5	1.4	1.3	0.9
r	0.967	0.960	0.997	0.983
Cerebellum width (mm)				
∞	11.630	11.754	11.714	11.692
to	0.229	0.120	0.462	0.594
k	1.05	1.1	0.9	0.65
r	0.999	0.997	0.999	0.982
Cerebellum length (mm)				
∞	7.507	7.698	7.816	7.375
to	0.015	0.25	0.279	0.049
k	0.95	0.85	0.75	0.85
r	0.992	0.989	0.978	0.990

∞: Parameter at age ∞ (maximum weight, length or width)

k: growth coefficient

r: correlation coefficient

A: growth model constant

TABLE 3
Results of Gompertz growth model listing the growth parameters for the control and experimental groups

	Control Group	Group 1 (12.5mg/Kg)	Group 2 (25mg/Kg)	Group 3 (37.5mg/Kg)
Brain weight (mg)				
∞	1370.331	1584.35	1523.498	1489.107
A	1.497	0.8717	0.9013	0.995
K	1.2	0.5	0.6	0.5
r	0.985	0.918	0.989	0.974
Cerebrum length (mm)				
∞	11.209	11.439	11.646	11.524
A	0.916	0.474	0.662	0.841
K	1.2	0.8	0.75	0.8
r	0.991	0.941	0.997	0.998
Cerebrum width (mm)				
∞	14.703	14.977	15.013	14.768
A	0.584	0.704	0.414	0.412
k	1.65	1.6	1.3	1.1
r	0.971	0.968	0.997	0.988
Cerebellum width (mm)				
∞	11.643	11.875	11.761	11.680
A	0.975	0.954	0.728	0.671
k	1.1	1.0	0.9	0.7
r	0.998	0.994	0.998	0.978
Cerebellum length (mm)				
∞	7.472	7.747	7.823	7.557
A	1.415	0.933	0.957	1.086
k	1.1	0.85	0.8	0.75
r	0.992	0.982	0.971	0.975

∞: Parameter at age ∞ (maximum weight, length or width)

k: growth coefficient

r: correlation coefficient

A: growth model constant

TABLE 4
Mean values of the measured parameters
for the control and experimental groups (mean ± standard error)

	Brain weight (mg)	Cerebrum length (mm)	Cerebrum width (mm)	Cerebellum length (mm)	Cerebellum width (mm)
Control					
Week 1	852.39 ± 31.320	8.43 ± 0.109	13.10 ± 0.169	4.57 ± 0.102	8.38 ± 0.178
Week 2	1241.04 ± 20.351	10.49 ± 0.091	14.58 ± 0.086	6.57 ± 0.155	10.53 ± 0.086
Week 3	1345.54 ± 36.076	11.08 ± 0.085	14.79 ± 0.144	7.22 ± 0.093	11.3 ± 0.087
Week 4	1312.12 ± 31.148	11.01 ± 0.128	14.5 ± 0.161	7.20 ± 0.071	11.37 ± 0.077
Week 5	1351.33 ± 22.084	11.05 ± 0.096	14.60 ± 0.058	7.35 ± 0.090	11.62 ± .053
Group 1					
Week 1	830.75 ± 41.641	9.04 ± 0.214	12.91 ± 0.294	5.05 ± 0.204	8.27 ± 0.213
Week 2	1302.17 ± 31.312	10.91 ± 0.126	14.94 ± 0.14	6.83 ± 0.127	10.68 ± 0.096
Week 3	1323.75 ± 33.95	10.84 ± 0.148	14.84 ± 0.132	7.24 ± 0.09	11.26 ± 0.105
Week 4	1364.84 ± 43.281	10.97 ± 0.171	14.79 ± 0.139	7.32 ± 0.087	11.53 ± 0.113
Week 5	1445.34 ± 34.263	11.40 ± 0.142	14.89 ± 0.123	7.64 ± 0.129	11.85 ± 0.111
Group 2					
Week 1	897.42 ± 24.144	8.56 ± .1038	13.39 ± 0.128	4.87 ± .0895	8.70 ± 0.145
Week 2	1206.65 ± 25.798	9.95 ± 0.147	14.66 ± 0.110	6.77 ± 0.09	10.53 ± 0.087
Week 3	1329.64 ± 27.943	10.93 ± 0.127	14.83 ± 0.141	7.42 ± 0.125	11.23 ± 0.112
Week 4	1383.23 ± 38.083	11.16 ± 0.093	14.98 ± 0.135	7.34 ± 0.074	11.45 ± 0.067
Week 5	1445.18 ± 53.746	11.56 ± 0.1	14.99 ± 0.2	7.51 ± 0.102	11.66 ± 0.097
Group 3					
Week 1	754.32 ± 28.233	7.86 ± 0.1317	12.82 ± 0.160	4.31 ± 0.10	8.13 ± 0.1515
Week 2	1101.14 ± 15.909	9.83 ± 0.107	14.31 ± 0.072	6.18 ± 0.104	10.26 ± 0.059
Week 3	1231.63 ± 21.541	10.63 ± 0.130	14.43 ± 0.103	7.07 ± 0.069	10.95 ± 0.096
Week 4	1283.36 ± 23.873	11.03 ± 0.107	14.66 ± 0.073	6.95 ± 0.061	11.13 ± 0.095
Week 5	1339.35 ± 19.709	11.43 ± 0.08	14.75 ± 0.075	7.20 ± 0.081	11.21 ± 0.107

DISCUSSION

There is a strong support for the use of modeling to determine the growth of different body parts. Growth models and allometry have been used to determine the growth of brain, and body parts by a number of researchers (Dawood *et al.*, 1988, Pesce and Carli, 1988, Shea *et al.*, 1987, and Boire and Baron, 1994). Therefore, these can be a useful tool, if not the best method, in studies similar in aim to the current study.

The theoretical maximum of each parameter is the expected value of either the maximum length, or, width of the cerebrum or the cerebellum of rats in each experimental group as predicted by each model. The age at which the brain parameter is theoretically nil, indicated by (to) helps in determining the growth pattern of each brain parameter.

It is interesting to note that there was an effect of aspirin on brain growth, evident in the decrease in the total brain weight, cerebrum length and cerebrum width. A decrease in cerebellum width and

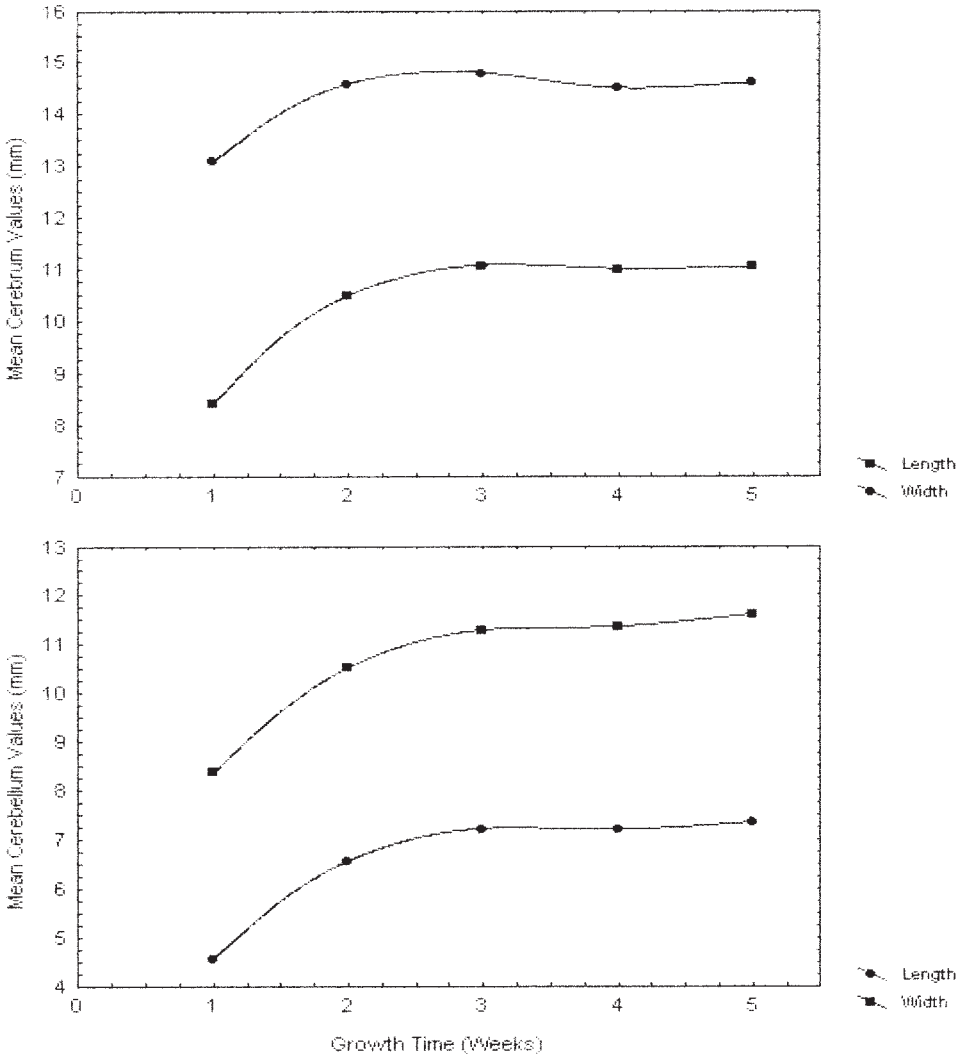

Figure 1: Cerebrum and Cerebellum mean values (mm) listed according to the week of growth for the control group

length was observed at the highest dose of aspirin (37.5 mf/Kg). In addition, the results indicate that the higher the dose of aspirin, the more the decrease in the growth of the brain part. The pattern of growth of each brain part for each group, as shown in the figures (1–4) indicates that there was an expected pattern of growth of the brain parts as far as the weight and size is concerned. These growth patterns, moreover, indicated that there were differences between each group in the growth of each brain part. Such differences can be determined by the differences in the growth parameters (k, r, A and to), calculated for each group. Unfortunately, few studies have been carried out to examine the effects of prenatal exposure to aspirin on the postnatal development of brain parts. Bonthius and West (1989), for instance, reported that aspirin augments alcohol in restricting brain growth in the neonatal rat. Padmanabhan *et al.* (1994) reported that pre-treatment with aspirin accentuate the prenatal mortality in the TO mouse fetuses. Al-Qaisi *et al.* (2006) used allometry to study postnatal brain

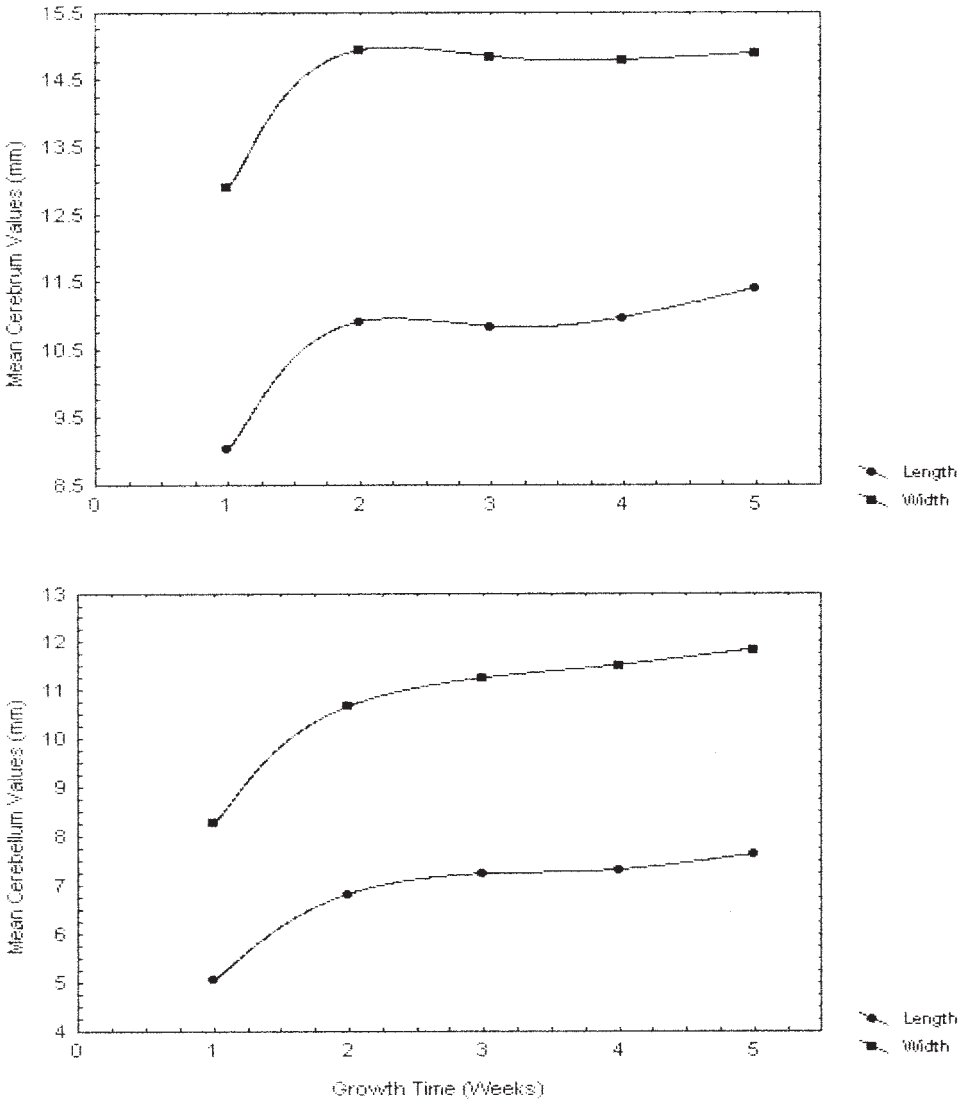

Figure 2: Cerebrum and Cerebellum mean values (mm) listed according to the week of growth for group 1 (12.5 mg/Kg)

development after being exposed to aspirin prenatally, and reported that aspirin has an effect on the development of brain parts.

Although further studies are needed to provide an explanation of the effect of aspirin on the development of brain parts, a possible mechanism that might explain this effect can be related to the role of aspirin in inhibiting prostaglandin production, which modulate the activity of the hypothalamus-pituitary axis. A number of researchers have reported various results due to this role of aspirin inhibition of prostaglandin production. Amateau and McCarthy (2004) has, for example, reported that aspirin inhibition of prostaglandin E2 (PGE2) production can interfere with the sexual development of rats through affecting the masculinization carried out by steroid hormones of some aspects of the rat brain. Hwang *et al.* (2004) has also reported that aspirin and indomethacin inhibit PGE2 synthesis in C6 glioma cells, and that low dose aspirin

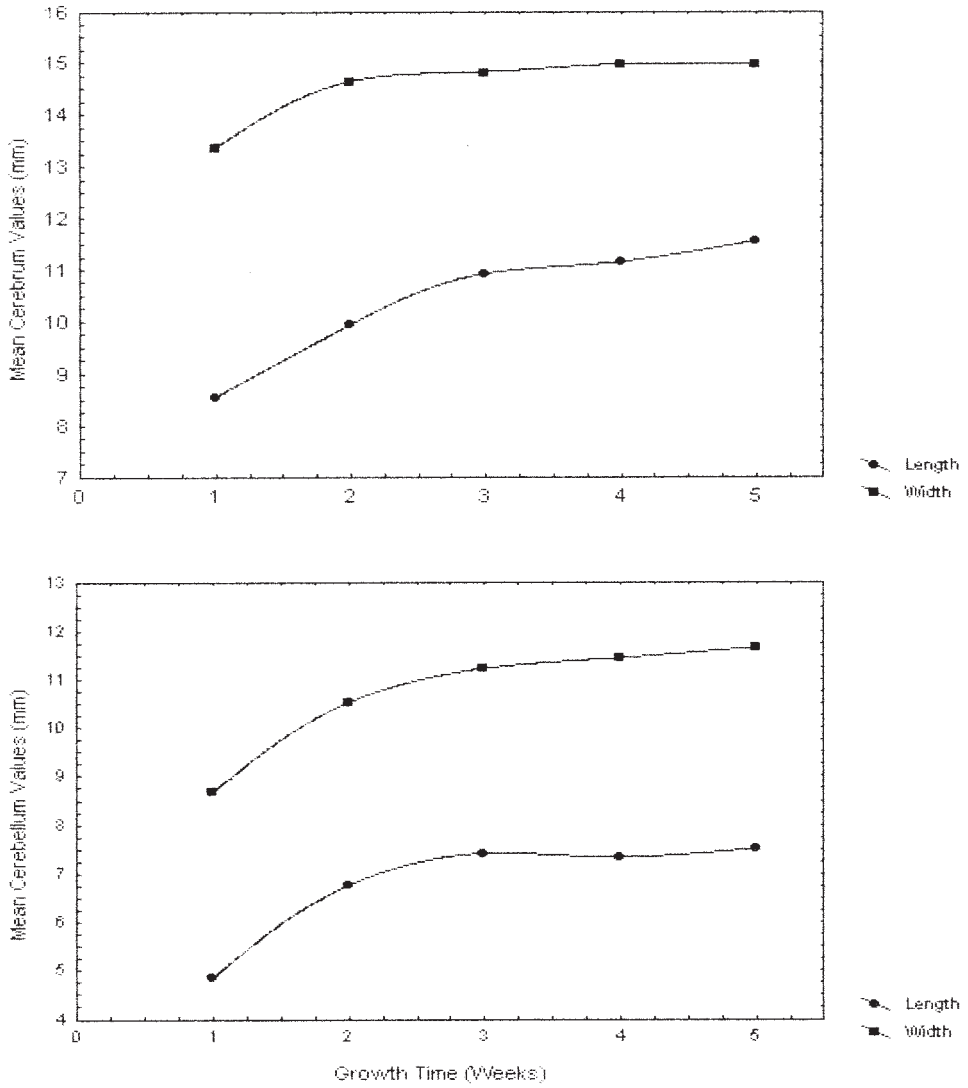

Figure 3: Cerebrum and Cerebellum mean values listed according the week of growth for group 2 (25 mg/Kg).

is as effective as high dose aspirin. Montine *et al.* (2002) has shown that non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin suppressed cerebral levels of PGE2. Di Luigi *et al.* (2001) has pointed out that prostaglandins modulate the activity of the hypothalamus-pituitary axis, and that aspirin ingestion significantly blunts the serum ACTH, beta-endorphin, cortisol and growth hormone. Michel *et al.* (2003) has stressed that acetylsalicylic acid affects the body weight, and the corticotropin-releasing hormone in rats. Accordingly, our assumption is that the inhibition of prostaglandin production, which reportedly has an affect on the activity of the hypothalamus-pituitary axis, may be a possible explanation of the effect of aspirin on the growth of the brain parts. Further studies are recommended to be made as to provide further support to this assumption.

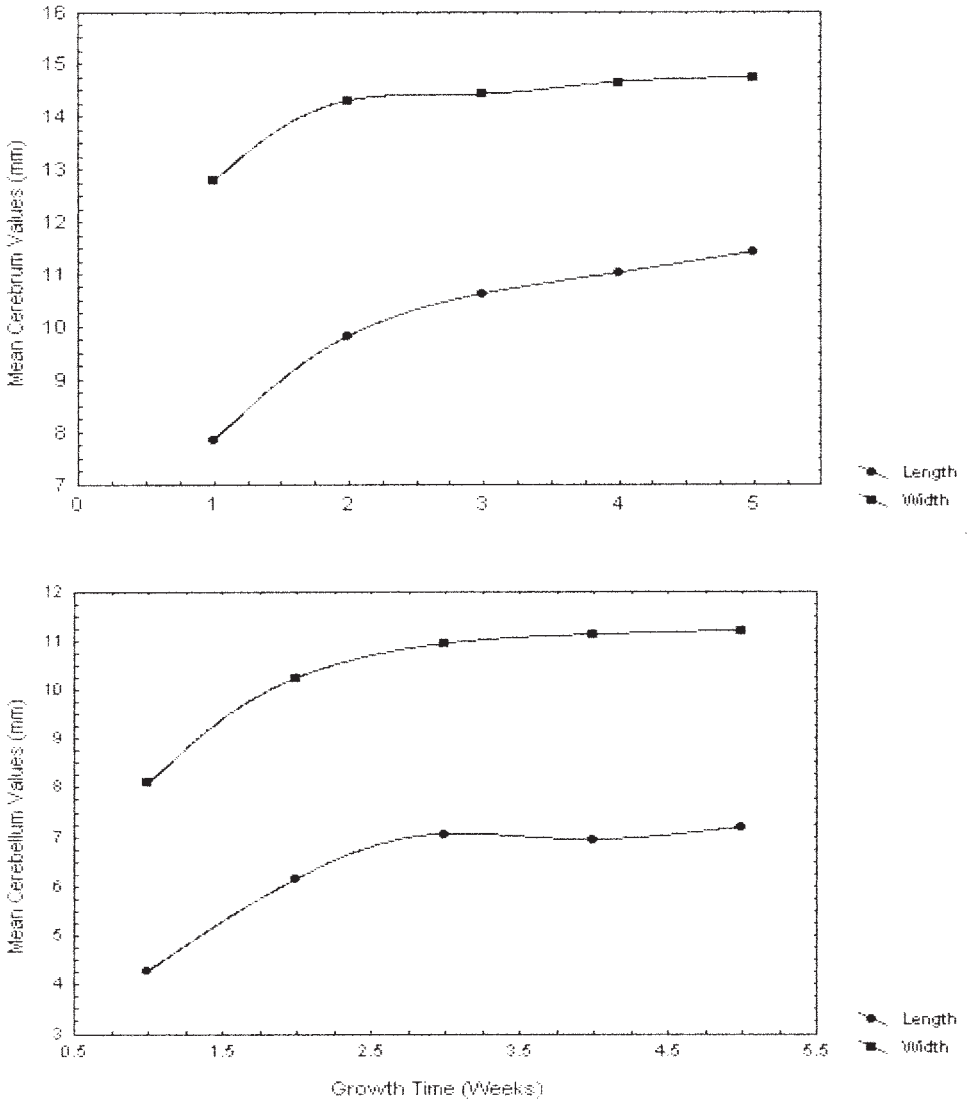

Figure 4: Cerebrum and Cerebellum mean values listed according to the week of growth for group 3 (37.5 mg/Kg)

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