Growth of suckling rats after treatment with dexamethasone or cortisol

Implications for steroid therapy in human infants

S. W. DE SOUZA and B. P. F. ADLARD

From the Department of Child Health, University of Manchester, Manchester

De Souza, S. W., and Adlard, B. P. F. (1973). *Archives of Disease in Childhood,*
48, 519. **Growth of suckling rats after treatment with dexamethasone or
cortisol: implications for steroid therapy in human infants.** Immature rats
were treated with a single dose of dexamethasone (1 mg/kg or 20 mg/kg body weight).
At both dose levels the subsequent growth of whole body, brain, and thymus was
markedly impaired. Cortisol had smaller but similar effects. These results are
discussed in the context of possible glucocorticoid therapy in newborn infants.

There is experimental evidence suggesting that
glucocorticoids may be useful in the prevention of
hyaline membrane disease (DeLemos *et al.*, 1970;
Kotas *et al.*, 1971). Large doses of glucocorticoids
are, however, well known to cause atrophy of
lymphoid tissue and impair brain growth (Howard,
1965; Schapiro, 1965) in immature animals. Thus,
before glucocorticoids are used in clinical practice,
some consideration must be given to the possible
harmful effects of these drugs on the developing
organism (Reynolds, 1971). In addition, it has
been suggested (Howard, 1968) that repeated stress
may interfere with brain growth and cell division
through increased adrenocortical secretion and
raised plasma corticosteroid levels.

In the work reported here, *dexamethasone* was
administered to developing rats in a single dose
(1 mg/kg) comparable with that used clinically in
infants, and in a higher dose (20 mg/kg) which has
been found necessary to prevent the occurrence of
cerebral oedema in asphyxiated immature rats (De
Souza and Dobbing, 1973). Immature rats were also
treated daily with *cortisol* either in a high dose (5 or
50 mg/kg) or in an amount (1 mg/kg) within the
range which might be secreted in a stressed infant
(Bertrand *et al.*, 1963; Aarskog, 1965; Alleyne and
Young, 1967).

Rats aged 4 or 5 days were used, since at this age
rat brain is at a stage of development comparable
with that of the human baby brain towards the latter
part of the last trimester of pregnancy (Dobbing,
1970).

Methods

Animals. Rats of a black- and white-hooded strain
were used. At birth, litters were reduced to either 6
(dexamethasone experiment, all males where possible) or
10 (cortisol experiment, equal numbers of each sex)
animals.

Steroids. Dexamethasone phosphate (sodium salt)
was a gift from Merke, Sharp and Dohme Ltd.,
Hoddesdon, Herts. Cortisol hemisuccinate (sodium
salt) was purchased from Sigma Chemical Co., Kingston
upon Thames, Surrey.

Brain dissection. Brain was separated from spinal
cord at the foramen magnum. Cerebellum was
routinely removed and weighed separately. The
remainder of the brain is referred to as 'forebrain and
brainstem'. The term 'whole brain' includes
cerebellum.

Dexamethasone experiment. Rats in each litter
were paired according to body weight and sex. Each
member of a pair received, at 5 days of age, one dose of
either dexamethasone phosphate dissolved in water, or
an equal volume of isotonic glucose. (The quantity of
glucose given was negligible compared with circulating
amounts.) The dose of steroid was 1 mg/kg in 7 litters
and 20 mg/kg in 5 litters. Two animals (one
dexamethasone-treated rat together with its matched
glucose-injected control) were killed from each litter at
ages 7, 10, and 21 days.

Cortisol experiment. 6 litters of 10 animals each
were used. At 4 days of age 1 animal of each sex was
killed from each litter. The remaining 8 animals were
randomly assigned to one of 4 treatment groups, each
consisting of 1 member of each sex. Three groups
received cortisol hemisuccinate (1 mg/kg, 5 mg/kg, 50

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TABLE I

Body, brain, and thymus weight in 7-day-old and 21-day-old rats after one dose of dexamethasone at 5 days of age

	Age (dy)	Dexamethasone (1 mg/kg)			Dexamethasone (20 mg/kg)		
		Control	Experimental	Deficit (%)	Control	Experimental	Deficit (%)
Body weight (g)	7	16±2	13±1‡	19	14±3	11±1*	21
	21	49±5	44±7†	10	57±12	40±16†	30
Forebrain + brainstem weight (mg)	7	616±46	562±48†	9	595±71	526±66‡	12
	21	1147±50	1047±92*	9	1137±98	1032±170§	9
Cerebellum weight (mg)	7	46±7	37±5†	18	42±8	35±6§	16
	21	174±10	154±21*	12	167±35	128±35§	23
Thymus weight (mg)	7	49±10	14±5‡	72	37±16	13±6*	64
	21	195±28	142±44†	27	224±54	103±56†	54

Note: Results are given as mean ±SD. Each experimental (dexamethasone-treated) animal was paired with a littermate control of the same sex and similar body weight at 5 days of age. There were 4 to 7 pairs (each from a different litter) for each age and dose. Results were compared using the paired comparisons 't' test.
*$P<0.05$, †$P<0.01$, ‡$P<0.001$, § not significant.

mg/kg body wt, intraperitoneally) dissolved in isotonic saline daily between 4 to 8 days of age. One group (controls) received the same volume of isotonic saline for the same period. All animals were killed at 9 days of age, 24 hours after the last injection.

DNA determination. Total brain DNA, a measure of total numbers of cells, was determined according to Zamenhof *et al.* (1964).

Results

Dexamethasone at both doses used resulted in an acute reduction in growth of body, brain, and thymus to 7 days of age (Table I). Thymus was markedly more affected than whole body, while deficits in cerebellar weight were greater than those of the remainder of the brain. The extent of growth retardation in 10-day-old dexamethasone-treated rats, previously reported in preliminary form (Adlard and De Souza, 1973), was similar to that of 7-day-old animals.

Ultimately, some recovery of growth appeared to occur in animals given the lower dose of dexamethasone (1 mg/kg) since weight deficits at 21 days of age were generally less than those at 7 days (Table I). However, such recovery was not evident for the high dose (20 mg/kg) of dexamethasone.

Deficits in brain weight in dexamethasone-treated animals at 10 and 21 days of age were associated with greater reductions in total brain

TABLE II

Whole brain DNA in 7-day-old, 10-day-old, and 21-day-old rats after one dose of dexamethasone at 5 days of age

Dose (mg/kg)	Age (dy)	μmol DNA-P per brain		Deficit in total DNA (%)	Deficit in brain weight (%)
		Control	Experimental		
1	7	1·62±0·26	1·55±0·11§	—	9
	10	3·02±0·51	2·63±0·42‡	13	8
	21	4·23±0·74	3·74±0·47§	—	9
20	10	2·86±0·29	2·18±0·47*	24	14
	21	4·33±0·63	3·86±0·63†	11	11

Note: Cerebellum was pooled with forebrain and brainstem for these analyses. Results are given as mean ±SD. Each experimental (dexamethasone-treated) animal was paired with a littermate control of the same sex and similar body weight at 5 days of age. There were 3 to 7 pairs (each from a different litter) for each age and dose. Results were compared using the paired comparisons 't' test. All experimental groups showed significant (P <0.05) deficits in brain weight.
*P <0.05, †P <0.01, ‡P <0.001, § not significant. DNA-P, DNA-phosphorus.

TABLE III

Effect of daily injections of cortisol on the growth of 4- to 9-day-old rats

Age (dy)	Treatment (no.)	Body weight (g)	Forebrain + brainstem weight (mg)	Cerebellum weight (mg)	Thymus weight (mg)
4	Control (12)	10·5 ±1·1	419 ±26	23·8 ±3·4	28·5 ±3·1
9	Control (11)	18·8 ±2·1	769 ±38	72·8 ±5·9	64·6 ±14·2
9	Cortisol 5 × 1 mg/kg (11)	18·5 ±1·7§	763 ±30§	71·3 ±6·0§	56·3 ±10·0§
9	Cortisol 5 × 5 mg/kg (8)	17·3 ±2·0§	749 ±37§	66·8 ±8·1§	43·7 ±6·6† (58%)
9	Cortisol 5 × 50 mg/kg (11)	15·6 ±2·5† (39%)	725 ±42* (13%)	59·6 ±5·8‡ (27%)	24·3 ±6·4‡ (111%)

Note: Results are given as mean ± SD. Percentages in parentheses represent apparent inhibition of growth between 4 and 9 days of age, calculated for each body or organ weight as

$$100 - \frac{(\text{Mean experimental wt at 9 dy} - \text{mean control wt at 4 dy}) \times 100}{\text{Mean control wt at 9 dy} - \text{mean control wt at 4 dy}}$$

Differences between experimental (cortisol-treated) and control animals at 9 days of age were compared using Student's 't' test.
*P <0·05, †P <0·01, ‡P <0·001, § not significant.

DNA (Table II), which were statistically significant except at 21 days in the lower dosed animals.

Repeated large daily doses of cortisol impaired growth of body, brain, and thymus (Table III), though this effect was clearly smaller than with comparable amounts of dexamethasone.

Discussion

The 5-day-old rat brain and the human brain in the perinatal period are in a similar phase of development (Dobbing, 1968, 1970). The adult number of neurons (with few exceptions) have already formed, glial cells have begun to appear, and the establishment of neuronal interconnexions has begun. In the human brain there is still appreciable glial cell formation during the first 18 postnatal months (Dobbing, 1970). Therefore, any adverse effects of steroid treatment in 4- and 5-day-old rats could, by implication, occur perinatally in human babies.

Deficits in brain weight in rats treated with dexamethasone and those treated with cortisol could be due partly to a reduction in cell numbers, though other developmental processes, including myelination (Howard, 1965), may also be altered. Other studies suggest that shortly after treatment of animals with cortisol cell mitosis is inhibited in brain (Baláses and Cotterrell, 1972) and thymus and lymph nodes (Stevens, Colessides, and Dougherty, 1965). In the present study cerebellar growth was more retarded than that of the remainder of the brain after glucocorticoid treatment, a type of selectivity also observed after undernutrition (Culley and Lineberger, 1968; Chase, Lindsley, and O'Brien, 1969) and hyperphenylalaninaemia (Chase and O'Brien, 1970) in developing rats. A possible

explanation may be found in the very rapid rate of cell formation in the cerebellum during early postnatal life (Dickerson and Dobbing, 1966; Altman, 1970).

The rate of cortisol secretion is reported to be in the range of 1·0 to 1·5 mg/kg per 24 hr in newborn infants (Aarskog, 1965) and somewhat lower in older children (Bertrand *et al.*, 1963). Neither body nor brain growth in the suckling rat were significantly affected by cortisol in a dose of 5 mg/kg per 24 hr; thymus growth was reduced at this dose, but not significantly so at 1 mg/kg per 24 hr (Table III). If it is reasonable to extrapolate between rat and man it seems likely that increased adrenocortical secretion induced by stress is unlikely to affect brain growth and that any effects on thymus growth would be relatively small.

However, the possible long-term effects of treating pregnant women or newborn babies with glucocorticoids must be considered. Cortisol-treated animals are not only growth retarded, but also show certain behavioural abnormalities (Howard and Granoff, 1968; Schapiro, Salas, and Vukovitch, 1970). In addition, retarded development of lymphoid tissue could have adverse effects on the body's immune mechanisms.

It can be argued, however, that a direct extrapolation between rat and man is difficult, since human development takes place over a much longer period so that possible adverse effects of steroids are grossly exaggerated when studied in a rapidly growing species such as the rat. There is evidence also that the rat may be more sensitive than man to the thymolytic effects of glucocorticoids (Claman, 1972). Nevertheless, women who received glucocorticoids in pregnancy are known to have had

growth-retarded babies (Warrell and Taylor, 1968). It is clear that administered steroids have a profound and deleterious effect on growth, including that of the brain. This must be carefully borne in mind when their use is being considered in the newborn baby.

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Correspondence to Dr. S. W. De Souza, Department of Child Health, University of Manchester, Clinical Sciences Building, York Place, Manchester M13 0JJ.