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STUDIES OF THE EFFECTS OF DEXAMETHASONE SODIUM PHOSPHATE ON LENGTH OF LONG BONES (TIBIA) OF MICE

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ABSTRACT

Seventy two immature male and female mice were taken and divided into three groups, 24 mice remained untreated and 24 male and female mice were treated upto six weeks with daily dose of Dexamethasone phosphate. Twenty four similar mice were given injections of vehicle in which the drug dexamethasone was suspended and this acted as placebo group.

Length of the tibia in treated group was less but the difference was not statistically significant in two weeks experimental animals. It was significant in 4 and 6 weeks.

Introduction

The physiological significance of adrenal began when a description was given by Addison. Brown-Sequard (cited by Goodman and Gilman 1975) performed experiments on the subjects in whome the adrenalectomy was done and he proved the adrenals to be essential for life, later it was recognised that the cortex of the gland was the life maintaining portion.

Glucose and glycogen formed under the influence of adrenal cortex during fasting appeared to be derived from tissue proteins (Long et al., 1940). From these studies emerged the concept of two types of adrenal cortical hormones, the miner-alocoticoid relating with the salt and electrolyte balance and glucocorticoids concerning with carbohydrate metabolism. The concept of glucocorticoids and mineralocorticoids still continue.

The concentration between glucocorticoids and glycogen deposition was first emphasised by Long et al. (1940) and it represents a cardinal manifestation of the effects of glucocorticoids on metabolism. In vitro studies of muscles from glucocorticoid treated animals show deficiencies in ribosomal functions and the mitichondria (Bullock 1970).

Since glucocorticoids induce osteoporosis in long bones so their use needs precautionary measures. The long use also reduces skeletal growth in children. Tissue metabolism occurs in the bone matrix which interfere with ossification by decreasing the surface on which bone mineral can be deposited (Bernick and Ershoff, 1963).

Systemic corticosteroid therapy produces injurious side effects on bones (Heimann and Frebergar, 1960). There is decrease bone formation and sometimes excessive bone break down with increased urinary excretion of bone forming minerals (Eisenberg and Gorden 1961). Gorden (1961) emphasised that all the available corticoids rapidly produce calcium wasting and cause clinical wasting. In many skeletal diseases bone formation and bone resorption tend to change in the same direction though unequally (Ivey 1981). The tendency of corticosteroids to produce a state of negative calcium balance aggravated by the low rate of bone formation will limit the subjects ability to replace resorption of bone (Drake 1983). Long term steroid treatment is not advisable because it induces osteoporosis (Cope, 1972).

Anatomy of the Tibia of Mice:

The skeleton of the leg is formed by the stout tibia and a short extremely slender fibula. Proximally the tibia possess two condyles articulating with the femoral condyles. On the anterior surface of the tibia is a narrow ridge or crest. Distally the tibia expands and forms a median projection. The median projections the medial malleolus. The thin fibula is lateral to the tibia.

Development and growth of the bone starts with the process by osteogenesis. Osteoclasts secrete phosphate enzymes causing precipitation of calcium salts and form calcified bone matrix. As long as each bone is growing each osteoblast divide into two daughter cells one migrate to the periphery while the other secretes one matrix and surrounds itself by a lacuna and becomes an adult bone called osteocyte (Arey, 1974).

According to embryological origin there are two types of bone development intramembranous and intracartillaginous or endochondrial. In intramembranous bone formation it develops directly on or within membrane. In endochondral bone formation cartilage is first removed, later to be replaced by bone (Copenhaver et al, 1978).

Materials and Methods

Seventy two immature mice (36 females and 36 males) of J.P.M.C. breed weighing between 15 to 17 Gm and aged between 4 and 4-U2 weeks were taken from the animal house of Jinnah Post Graduate Medical Centre. The mice were maintained on normal standard diet.

Two days before experiment the animals were marked by cutting their toes.

Grouping* of *Animals

The animals were divided into three main groups.

Group A (normal control)

This main group of 24 mice (12 females and 12 males) served as control in order to see the various changes in bone growth at intervals of 2, 4 and 6 weeks, these animals were further divided into 3 sub groups.

Sub group A_1F

This group consisted of 4 female mice they were sacrificed 2 weeks after the commencement of the experiment and served as control for animals of treated and placebo sub groups.

Sub group A_2f

This group consisted 4 female mice and were killed 4 weeks after the commencement of the experiment.

Sub group A_3F

This consisted of 4 female mice were killed 6 weeks after the commencement of the experiment.

Sub group A_1M

The consisted of 4 male mice. They were sacrificed 2 weeks after the commencement of the experiment and served as control for animals treated and placebo groups.

Sub group A_2M

This group consisted of 4 female mice. These animals were killed 4 weeks after the commencement of experiment.

Sub group A3A1

This group consisted of 4 mice and were killed 6 weeks after commencement of experiment.

Group B Treated

This group consisted of 24 mice (12 females and 12 males). The animals were given dexamethasone sodium phosphate by daily I/M injection. The dose was calculated at 0.I6ug/G of the body weight of the hormone in a volume of 0.12 ml. These animals were further divided into three sub groups similar to those of control.

Sub group B_1F
Four female mice *were* sacrificed after two weeks of experiment

Sub group B_2F
Four female animals were sacrificed after four weeks of experiment

Sub group B_3F
Four female mice were sacrificed after six weeks of experiment

Sub group B_1M
Four male mice were sacrificed after two weeks of experiment

Sub group B_2M
Four male mice were sacrificed after four weeks of experiment

Sub group B_3M
Four male mice were sacrificed after six weeks of experiment

Group C (Placebo)
This group of mice consisted of 24 mice (12 female and 12 males) and was given placebo treatment by I/m injection of 0.32 ml of the solvent used for the corticosteroids.

Like the other groups this group was further divided into three sub groups.

Sub *group C_1F*
Four female mice were sacrificed after two weeks of experiment

Sub group C_2F
Four female mice were sacrificed after four weeks of experiment

Sub group C_3F
Four female mice were sacrificed after six weeks of experiment

Sub group C_1M
Four male mice were sacrificed after two weeks of experiment

Sub group C_2M
Four male mice were sacrificed after four weeks of experiment

Sub group C_3M
Four male mice *were* sacrificed after six weeks of experiment

Administration of Drug

Injection dexamethasone sodium phosphate MSD (Decadrone) was used each ml containing 4 mg of the drug in 1 nil for mouse 0.16 ug/g of body weight was administrated i.e. about the twice of the human corresponding dose. Amount of drug for 15 gm of the mouse was = 15 x 0.16 = 2.4 ug.

Placebo contained

Creatinine	8 mg
Sodium citrate	10 mg
Sodium hydroxide	10 mg
Methyl paraben	0.2 mg

Dissolved in 1 ml of solution

0.1 ml of the solution was used.

Removal of Tibia*:*

The tibias were removed and weighed by torsion balance and was processed.

Results and Discussion

The length of the tibia was measured and recorded as shown in the following.

The *length of the Tibia*

The mean length of group A_1F was 1.70 ± 0.01 cm, it was compared with B_1F where the mean length was 1.68 ± 0.01 cm the difference was statistically not significant (P>0.05 table 1).

Mean length of C_1F was 1.65 ± 0.10, when it was compared with A_1F group. It was not significant.

The mean length of group A_1M was 1.6 ± 0.04 cm, it was compared with B_1M it was 1.64 ± 0.01 cm. The difference was statistically not significant (P0.05 table 1).

The length of group A_1M was compared with C_1M the mean length of C_1M was 1.72 ± 0.01 cm. The difference was statistically significant (P<0.05 table 2).

The mean length of A_2F was 1.81 ± 0.01 when it was compared with the mean length of B_2F. The mean length was 1.77 ± 0.01 cm. The difference was statistically significant (P <0.05 table 1).

The mean length of group A_2M was 1.80 ± 0.01 cm when it was compared with B_2M the mean length was 1.65 ± 0.01 cm. The difference was statistically significant ($P<0.05$ table 2).

The mean length of group A_2F was compared with C_2F. The length of C_2F was 1.85 ± 0.004 cm the difference was statistically significant ($P < 0.05$ table 1).

Similarly the mean length of group A2M was compared with C2M it was 1.78 ± 0.04 cm. It was statistically not significant ($P> 0.05$ table 2).

The mean length of A_3F was 1.86 ± 0.01 cm, whereas the mean length of B_3F was 1.81 ± 0.01 cm, it was statistically significant ($P<0.05$ table 1).

The mean length of A_3M was 1.87 ± 0.01 cm and the mean length of B_3M was 1.77 ± 0.01 cm. The difference was statistically significant ($P< 0.05$ table 2).

The mean length of A_3F was compared with C_3F. The mean length of this group was 1.85 ± 0.01 cm, the difference was not significant ($P > 0.05$ table 3).

The mean length of A_3M was compared with C_3M which was 1.88 ± 0.01 statistically was not significant ($P > 0.05$ table 2).

Conclusion

The length of the tibia in treated group was less but the difference was not statistically significant in two weeks experimental animals. It was significant in 4 weeks and 6 weeks of experimental animals.

Table 1
Mean length of Tibia in female mice

Group No.	Sub group	No. of animals	No. of tibia	*Mean length of tibia (cms)	P value of difference as compared to control group (A)
	A_1F	4	8	1.70 ± 0.01	
A	A_2F	4	8	1.81 ± 0.01	
	A_3F	4	8	1.86 ± 0.01	
	B_1F	4	8	1.68 ± 0.02	Not significant (P > 0.05)
B	B_2F	4	8	1.77 ± 0.01	Significant (P < 0.05)
	B_3F	4	8	1.81 ± 0.01	Significant (P < 0.05)
	C_1F	4	8	1.65 ± 0.01	Not significant (P > 0.05)
C	C_2F	4	8	1.85 ± 0.004	Significant (P < 0.05)
	C_3F	4	8	1.85 ± 0.01	Not significant (P > 0.05)

*S.E. Mean standard error

Table 2
Mean length of Tibia in male mice

Group No.	Sub groups	No. of animals	No. of tibia	*Mean length of tibia (cms)	P value of difference as compared to control group (A)
	A_1M	4	8	1.60 ± 0.04	
A	A_2M	4	8	1.80 ± 0.01	
	A_3M	4	8	1.87 ± 0.01	
	B_1M	4	8	1.64 ± 0.02	Not significant (P > 0.05)
B	B_2M	4	8	1.65 ± 0.01	Significant (P < 0.05)
	B_3M	4	8	1.77 ± 0.01	Significant (P < 0.05)
	C_1M	4	8	1.72 ± 0.01	Not significant (P > 0.05)
C	C_2M	4	8	1.78 ± 0.04	Significant (P < 0.05)
	C_3M	4	8	1.88 ± 0.01	Not significant (P > 0.05)

*S.E. Mean standard error

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