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Comparative Study In The Growth Pattern Of Prepubertal, Pubertal And Adult Visceral Organs Of Male And Female Mice.

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ABSTRACT

This study investigated the growth of various organs in 72 male and female mice using increase in weight as the index of growth. The mice were randomly selected from the offspring of the same breeding stock. They were given food and drinking water ad libitum until they were sacrificed at 3, 6 and 12 weeks of age. At each age, 12 males and 12 females were sacrificed by severing the spinal cord at the atlanto-occipital joint. The live body weight of each mouse was determined. Following death, skeletal muscles (triceps brachii and gastrocnemius muscles) and internal organs (lungs, heart, kidneys, liver and spleen) were dissected and their weights were determined. Humerus and femur were also dissected out from each mouse and their lengths were determined. The muscle mass index (milligram muscle weight per gram body weight) was determined for each muscle. The percentage of body weight contributed by each internal organs was calculated.

The study demonstrated that although sex differences were not evident in the weights of these organs at 3 weeks of age, there were sex differences in the weights and growth patterns of muscles, bones and internal organs of male and female mice at 6 weeks and 12 weeks of age. It was suggested that these differences may have arisen from the possible significant influence of sex hormones on the growth and development of these organs in both male and female mice.

KEYWORDS: Mice, Organs, Growth, Sex differences

Several factors are thought to influence the process of growth at different stages in the course of development. Balinsky (1970) suggested that the process of growth may be controlled by the genotype in two different ways. Firstly, the genetic constitution may act directly on the ability of cells to grow and proliferate. Alternatively, the gene action may be indirect through its primary effect to modify in a certain way, the differentiation of the anterior lobe of the hypophysis responsible for secretion of growth hormones. This accounts for the hereditary differences in the sizes of animals.

In addition to growth hormone, the anterior lobe of the hypophysis is also responsible for the release of certain gonadotrophic hormones. The stimulatory action of these hormones on the gonads results in the production of sex hormones namely, testosterone and oestrogen in males and females respectively (Warren *et al.*, 1975; Armstrong and Papkoff, 1976).

It has been suggested that hormones particularly, androgens may have a direct or at least, an indirect stimulatory influence on the development of muscles (Goldspink, 1972). Thus, the muscles of adult male mice were found to be larger than those of adult female mice (Goldspink and Rowe, 1968). A similar observation was made in rats by Cheek *et al.* (1968). The sex difference was found to be due to greater increase in the sizes of myofibres in males than females. There was no differences in the number of myofibres in the muscles of both sexes of mice (Rowe and Goldspink, 1969; Aberle and Doolittle, 1976). Studies by Venable (1966a), as well as, Ihemelandu and Ibebunjo (1992) revealed that retardation of muscle growth resulted from the castration of male mice. Venable (1966a; 1966b) demonstrated that replacement therapy using testosterone, following castration of males, caused considerable hypertrophy of the fibers in rat levator ani muscles. It was noted that the hypertrophy of the muscles was a result of increase in myofibrilar material of the fibres. There was no change in the number of fibres either during the replacement therapy or during the atrophy of the muscles following castration. At the cellular level, an increased incorporation of

labeled amino acids into muscle proteins following administration of androgens to both intact and castrated animals was reported by Buresova *et al.* (1969). Androgens have therefore been shown to increase the rate of protein synthesis in most muscles and so, influence the rate and extent of development of muscle fibres.

Large doses of oestrogen administered within the first 5 days of postnatal life have been found to produce a depressant effect on the development of various muscles in sexually immature female rabbits (Ihemelandu, 1980), as well as, male and female mice (Ihemelandu, 1984). Ihemelandu (1987) observed lack of differences in he muscle weights of prepubertal male and female mice at 4 weeks of age. The same author reported greater muscle weights in the males than the females at 5 and 6 weeks of age. According to him, this period coincided with the period of significant increase in the secretion of testosterone and oestrogen in the males and females respectively hence, the differences in their muscle weights. Furthermore, in the [presence of oestrogen, there were no differences between the muscle mass of developing male and female mice (Ihemelandu, 1981). These observations suggest that the smaller muscle mass of adult females when compared to adult males may be attributed to the inhibitory effect of oestrogen on muscle development in the females. It has been demonstrated that oestrogen brought about this inhibition of muscle mass development by limiting the size of individual muscle fibre (Ihemelandu, 1984).

Although sex-differences and the influence of sex hormones on muscle and bone growth and development have been extensively studied as indicated by the review above, there is paucity of information on the possible sex-differences in the pattern of growth and development on visceral organs. The present study was therefore designed to investigate the probable influence of sex on the growth of visceral organs. It seeks to study the growth patterns of these visceral organs and bones in male and female mice at prepubertal, pubertal and adult ages, using increase in weight as the index of growth as describes by Balinsky (1970).

MATERIALS AND METHODS

Male and female mice used as the breeding stock in this study were randomly selected from a group of inbred albino mice maintained for research in the Department of Veterinary Anatomy, University of Nigeria Nsukka. They were fed a commercially prepared diet and drinking water *ad libitum*. Following acclimatization for a period of two weeks, the female mice of the breeding stock were mated with the males at a ratio of one male to three females. After parturition, the neonates were left with their dams until weaning at 21 days of age. 72 mice (36 males and 36 females) were randomly selected from the offspring of the breeding stock and were used as the experimental animals in this study. They were housed in cages with aluminum bottom and screen top according to sex. These mice were fed the commercially prepared diet and drinking water *ad libitum* until they were sacrificed at 3, 6 and 12 weeks of age. These ages were chosen to represent prepubertal, pubertal and adult ages respectively (Bennet and Vickery, 1970). At each age, 12 males and 12 females were sacrificed by severing the spinal cord at the atlanto-occipital joint.

Prior to sacrificing each mouse, the live body weight was determined. Following death, triceps brachii and gastrocnemius muscles were dissected and weighted. The mean weight of the right and left muscles was determined. Visceral organs including the heart, lungs, liver, spleen and kidneys were dissected from each mouse and the weight of each organ was determined. The muscle mass index (milligram muscle weight per gram body weight) was determined for each muscle. Similarly, the percentage of body

weight contributed by each internal organ (gram organ weight per gram body weight multiplied by 100) was determined. These allometric parameters yielded size-independent dimensional constants and thus, allowed comparison of organ weights in individuals of varying body weights over a wide range of body sizes (Stahl, 1965). In addition, the relative growth rate of each visceral organ was determined by dividing the mean weight of the organ at 6 and 12 weeks of age by its mean weight at 3 weeks of age. Two bones were immersed in water over-night to facilitate separation of soft tissues from them. The length of each bone from the articular head to the trochlear was measured using a venier caliper. The mean length of left and right bones was determined.

Statistical Analysis

Means and Standard errors were calculated for each group of observations. The data obtained were subjected to statistical analysis using student 't' test to determine whether significant differences exist between observed means (Fisher and Yates, 1967). The data on relative growth rate of internal organs are presented with bar charts.

RESULTS

Comparison of the body weights of male and female mice (Table 1) indicated that although there was no significant difference ($p > 0.05$) between the sexes at 3 weeks of age, the male mice weighed significantly heavier ($p < 0.01$) than the females at 6 and 12 weeks of age. Furthermore, the weights of muscles, as well as, the length of bones, which did not differ significantly ($p > 0.05$) between male and female mice at 3 weeks of age, differed significantly ($p < 0.01$) between sexes at 6 and 12 weeks of age. The males possessed heavier muscles and longer bones than the females. However, the length of femur was similar ($p > 0.05$) in male and female mice at 12 weeks of age. Comparison of the muscle mass indices of the muscles of male and female mice (Table 2) showed that there were no significant differences ($p > 0.05$) between sexes at 3 weeks of age. The muscle mass indices to triceps bracii ($p < 0.01$) and gastrocnemius ($p < 0.05$) muscles were significantly greater in the male mice than the females at 6 and 12 weeks of age.

While the weights of heart ($p < 0.01$) and spleen ($p < 0.05$) were significantly different between male and female mice at 3 weeks of age, the weights of lungs, liver and kidneys were not significantly different ($p > 0.05$) in the two sexes of mice at this age (Table 3). The weights of heart ($p < 0.01$), lungs ($p < 0.05$), liver ($p < 0.01$), spleen ($p < 0.01$) and kidneys ($p < 0.01$) were significantly greater in male mice than females at 6 weeks of age (Table 3). At an adult age of 12 weeks, the weights of heart ($p < 0.01$), liver ($p < 0.01$) and kidneys ($p < 0.01$) were still significantly greater in the males. There were no significant differences ($p > 0.05$) in the weights of spleen and lungs of male and female mice at this age (Table 3). When the percentage of body weight contributed by each organ in male and female mice were compared (Table 4), the results revealed that no significant differences ($p > 0.05$) existed between sexes at 3 weeks of age. The only exception was the percentage of body weight contributed by the heart, which was significantly greater ($p < 0.05$) in males than female mice. The percentage of the body weight contributed by the heart ($p < 0.05$), liver ($p < 0.01$), spleen ($p < 0.05$) and kidneys ($p < 0.01$) were significantly greater in the males at 6 weeks of age (Table 4). At this age however, the percentage of body weight contributed by the lungs did not differ significantly ($p < 0.05$) between sexes. The percentage of body weight contributed by the liver did not differ significantly ($p > 0.05$) between sexes at 12 weeks of age, but that contributed by the heart ($p < 0.05$) and kidneys ($p < 0.01$) remained significantly greater in the male mice.

However, the lungs ($p < 0.05$) and spleen ($p < 0.01$) contributed significantly greater percentages to the body weight of female mice than that of male mice at 12 weeks of age (Table 4).

The results of the comparison of the relative growth rate of visceral organs of male and female mice revealed that at 6 weeks of age (Figure 1), all the organs studied had higher relative growth rates in male than female mice. This higher relative growth rate of the organs of male mice continued at 12 weeks of age (Figure 2) in the liver, kidneys and heart. The lungs and spleen of male mice had lower relative growth rate than those of female mice at 12 weeks of age.

Table 1. Comparison of Body weights (g), Muscle weight (mg) and length of bones (cm) using student 't' test

Age (weeks)	Parameter	Males	Females	T-value
	Body weight	9.3±0.04	9.2±0.4	0.167
	Muscles			
	Triceps brachii	26.9±1.3	27.9±1.7	0.447
3 weeks	Gastrocnemius	39.4±2.9	38.2±2.8	0.069
	Bones			
	Humerus	0.44±0.01	0.45±0.01	0.524
	Femur	0.53±0.01	0.56±0.02	1.049
	Body weight	27.3±0.8	18.3±0.4	9.368**
	Muscles			
	Triceps brachii	83.8±3.0	50.6±1.6	9.477**
6 weeks	Gastrocnemius	144.2±4.6	88.3±3.7	9.036**
	Bones			
	Humerus	0.76±0.02	0.61±0.01	7.035**
	Femur	0.99±0.02	0.83±0.01	8.390**
	Body weight	34.0±1.2	27.3±1.0	4.202**
	Muscles			
	Triceps brachii	134.2±4.8	84.2±3.5	8.068**
12 weeks	Gastrocnemius	199.1±9.1	149.7±6.4	4.249**
	Bones			
	Humerus	0.89±0.01	0.82±0.02	3.283**
	Femur	1.19±0.02	1.16±0.02	1.049**

Values represent mean ± standard error for each measurement.

df = **p < 0.01

Table 2. Comparison of muscle mass indices (mg/g) of muscles using student 't' test

Age	Muscles	Males	Females	T-value
	Triceps brachii	2.89+0.07	3.02+0.09	1.083
3 weeks	Gastrocnemius	4.20+0.17	4.11+0.15	0.421
	Triceps brachii	3.07+0.07	2.78+0.08	2.508**
6 weeks	Gastrocnemius	5.28+0.07	4.84+0.19	2.093*
	Triceps brachii	3.95+0.08	3.08+0.06	8.699**
12 weeks	Gastrocnemius	5.85+0.16	5.48+0.11	1.809*

Values represent mean ± standard error for each measurement.

df = 22 *p<0.05 **p<0.01

Table 3. Comparison of weights of internal organs (g) using student 't' test.

Age	Organs	Male	Female	T-value
3 weeks	Heart	0.07+0.003	0.06+0.002	2.909**
	Lungs	0.12+0.01	0.12+0.1	0
	Liver	0.45+0.03	0.44+0.03	0.247
	Spleen	0.05+0.003	0.04+0.004	1.915*
	Kidneys	0.13+0.005	0.13+0.01	0
6 weeks	Heart	0.15+0.01	0.09+0.003	9.277**
	Lungs	0.25+0.02	0.18+0.01	2.357*
	Liver	1.66+0.05	0.89+0.04	12.314**
	Spleen	0.16+0.01	0.08+0.01	6.850**
	Kidneys	0.40+0.01	0.20+0.01	15.216**
12 weeks	Heart	0.18+0.01	0.13+0.004	5.270**
	Lungs	0.27+0.02	0.30+0.04	0.671
	Liver	1.56+0.08	1.24+0.05	3.185**
	Spleen	0.12+0.01	0.12+0.01	0
	Kidneys	0.54+0.02	0.32+0.02	7.690**

Values represent mean ± standard error for each measurement.
df = 22 *p<0.05 **p<0.01

Table 4. Comparison of the percentage of body weight contributed by each internal organ (%) using student 't' test.

Age	Organs	Males	Females	T-value
3 weeks	Heart	0.71+0.03	0.64+0.02	1.780*
	Lungs	1.26+0.08	1.31+0.12	0.332
	Liver	4.80+0.11	4.76+0.11	0.246
	Spleen	0.49+0.03	0.47+0.04	0.383
	Kidneys	1.36+0.03	1.41+0.03	1.172
6 weeks	Heart	0.54+0.02	0.50+0.02	1.713*
	Lungs	0.91+0.08	0.99+0.06	0.766
	Liver	6.07+0.10	4.87+0.13	7.125**
	Spleen	0.57+0.03	0.43+0.05	2.451*
	Kidneys	1.47+0.05	1.11+0.02	5.896**
12 weeks	Heart	0.51+0.01	0.48+0.01	2.171*
	Lungs	0.80+0.04	1.07+0.13	1.949*
	Liver	4.55+0.12	4.55+0.06	0
	Spleen	0.35+0.02	0.44+0.02	3.567**
	Kidneys	1.60+0.04	1.16+0.03	8.569**

Values represent mean ± standard error for each measurement
df = 22 *p<0.05 **p<0.01

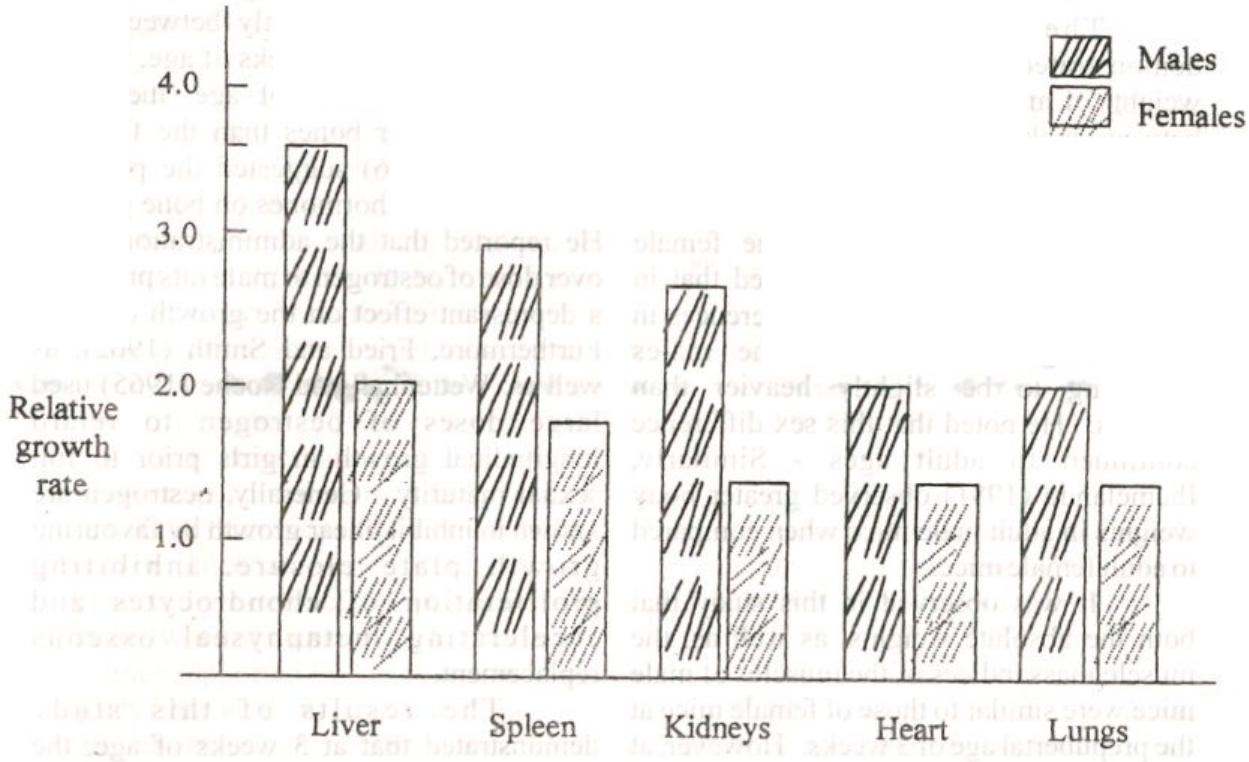

Figure 1: The relative growth rates of internal organs of male and female mice at 6 weeks of age.

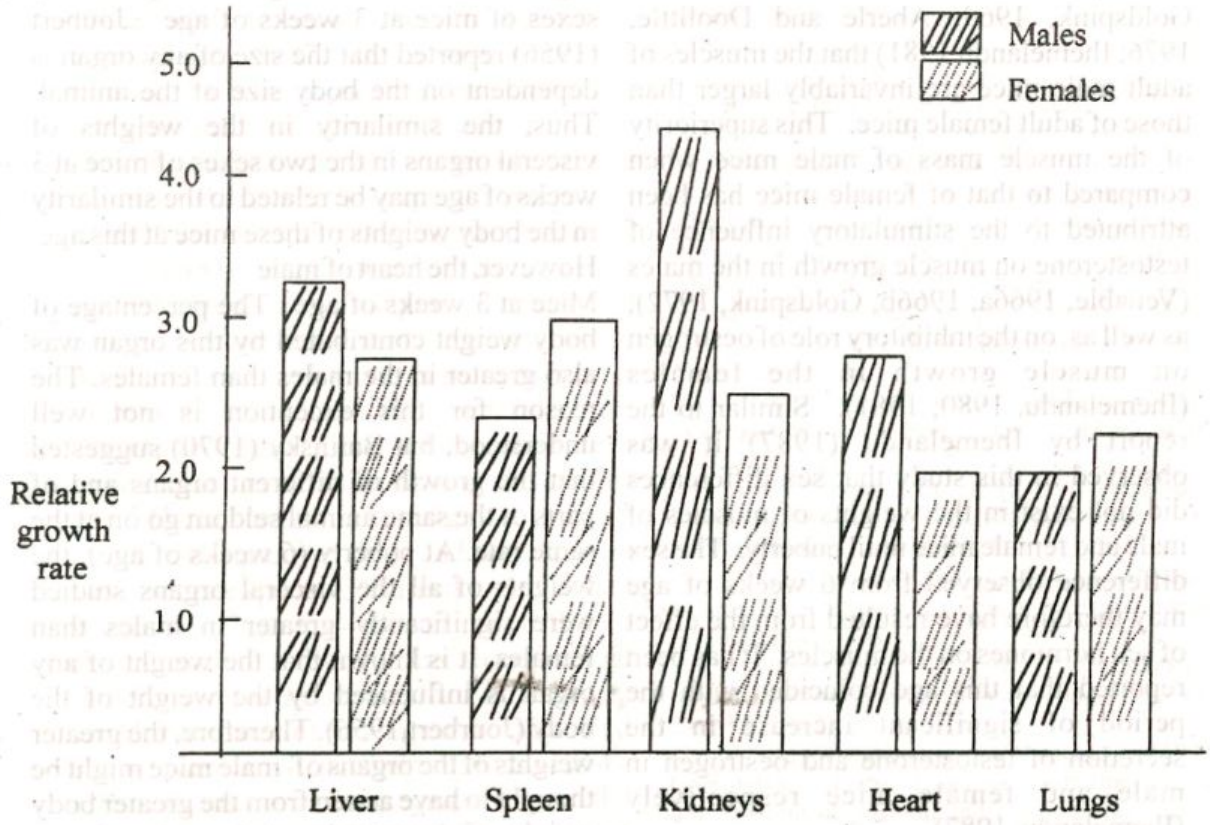

Figure 2: The relative growth rates of internal organs of male and female mice at 12 weeks of age.

DISCUSSION

The results of this study demonstrated that although the body weights of mice did not differ significantly between males and females at 3 weeks of age, sex differences were apparent at 6 weeks and 12 weeks of age, with the male mice weighing heavier than the female mice. Meara (1947) had reported that in most species, there exist sex differences in body weights of animals, the males appearing to be slightly heavier than females. He noted that this sex difference continued to adult ages. Similarly, Ihemelandu (1981) observed greater body weights in adult male mice when compared to adult female mice.

It was observed in this study that both the absolute weights, as well as, the muscle mass indices of the muscles of male mice were similar to those of female mice at the prepubertal age of 3 weeks. However, at 6 weeks and 12 weeks of age, these parameters were consistently greater in male mice than female mice. This agrees with reports by previous workers (Goldspink and Rowe, 1968; Rowe and Goldspink, 1969; Aberle and Doolittle, 1976; Ihemelandu 1981) that the muscles of adult male mice are invariably larger than those of adult female mice. This superiority of the muscle mass of male mice when compared to that of female mice has been attributed to the stimulatory influence of testosterone on muscle growth in the males (Venable, 1966a; 1966b; Goldspink, 1972), as well as, on the inhibitory role of oestrogen on muscle growth in the females (Ihemelandu, 1980; 1981). Similar to the report by Ihemelandu (1987) it was observed in this study that sex differences did not exist in the weights of muscles of male and female mice until puberty. The sex difference observed from 6 weeks of age may therefore have resulted from the effect of sex hormones on the muscles. It has been reported that this age coincided with the period of significant increase in the secretion of testosterone and oestrogen in male and female mice respectively (Ihemelandu, 1987).

The observations of the present study indicated that the length of long bones did not differ significantly between male and female mice at 3 weeks of age, but at 6 weeks and 12 weeks of age, the males possessed longer bones than the females. Riesenfeld (1976) suggested the probable influence of sex hormones on bone growth. He reported that the administration of an over dose of oestrogen to male rats produced a depressant effect on the growth of tibia. Furthermore, Fried and Smith (1962), as well as, Wettenhall and Roche (1965) used large doses of oestrogen to retard longitudinal growth in girls prior to full sexual maturity. Generally, oestrogen are known to inhibit linear growth by favouring growth plate closure, inhibiting proliferation of chondrocytes and accelerating metaphyseal osseous replacement.

The results of this study demonstrated that at 3 weeks of age, the weights of liver, kidneys, lungs and spleen did not differ significantly between male and female mice. Similarly, the percentage of body weight contributed by each of these organs did not differ significantly in both sexes of mice at 3 weeks of age. Joubert (1956) reported that the size of any organ is dependent on the body size of the animal. Thus, the similarity in the weights of visceral organs in the two sexes of mice at 3 weeks of age may be related to the similarity in the body weights of these mice at this age. However, the heart of male
Mice at 3 weeks of age . The percentage of body weight contributed by this organ was also greater in the males than females. The reason for this exception is not well understood, but Balinsky (1970) suggested that the growth of different organs and of parts of the same animal seldom go on at the same rate. At puberty (6 weeks of age), the weights of all the visceral organs studied were significantly greater in males than females. It is known that the weight of any organ is influenced by the weight of the body (Jourbert,1956). Therefore, the greater weights of the organs of male mice might be thought to have arisen from the greater body weight of these males at 6 weeks of age. However, organ weights in males remained

greater than those of females when expressed as percentages of the body weight. Since percentages contribution to body weight is an allometric index that yields a size independent dimensional constant, it allows comparison of organ weights in individuals of varying body weight (Stahl,1965). It follows therefore that the organ weights of male mice were indeed greater than those of female mice at 6 weeks of age. The sex difference can not be attributed to the influence of body weight. The only exception was the lungs whose percentage contribution to body weight did not differ significantly between male and female mice at 6 weeks of age. This observed greater weights of organs of male mice when compare to female mice is further supported by the observation in this study that at 6 weeks of age, the relative growth rate of all the internal organs studied were higher in males than females. Mice attain puberty at 6 weeks of age (Bennet and Vickery, 1970), thus, this age marks the period of significant increase in the secretion of testosterone and oestrogen in male and female mice respectively (Ihemelandu, 1987). It is possible therefore that these sex hormones may be responsible for the sex differences observed in the growth of organs in male and female mice at this age. At 12 weeks of age, the weights of heart, liver and kidneys remained greater in males than females, but sex differences were not observed in the weights of lungs and spleen. Similarly, the relative growth rate of he heart, liver and kidneys were higher in male than female mice at 12 weeks of age. The relative growth rate of lungs and spleen were higher in females than males at this age. These variations in the growth pattern of internal organs of male and female mice were not unexpected. Balinsky (1970) had suggested that the growth of different organs and of parts of the same animal seldom go on at the same rate. Furthermore, the ultimate size of any organ is influenced by he function it performs (Goss, 1964). These factors may have given rise to the variations observed in the growth pattern of visceral organs in both male and female mice.

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