

## Chapter 4

### Results

#### 1. Effect of DMPA on milk secretion

Results were presented in Table 1 and Figure 3. The results obtained in Figure 1 showed that after day 11 in DMPA treated group and day 13 of lactation in control group were greater than the earlier days. Milk yield on day 12, 13, 16 and 17 of lactation in DMPA treated group was greater significant different than the control (Table 1). In DMPA treated group milk yield on day 12, 13 and 17 of lactation when compared to day 8, the day before DMPA administration was significant difference. According to the second nursing the weight gain of the pups after day 13 was higher when compared to the appropriate day of the control, indicating the more refilling rate of the DMPA group.

#### Pituitary weight and Cell types population

Results from table 2 showed that the average pituitary weight per 100 gm. body weight at autopsy on day 19 post-delivery mean values were not differ in statistics in all groups ranging from  $3.23 \pm 0.1$  and  $3.26 \pm 0.2$  mg.

Using the Periodic acid Schiff's reagent-Iron Hematoxylin-Orange-G technique, the cytoplasm of

pituitary acidophils, basophils and chromophobes were stained orange, pink and light grey, respectively. As shown in Table 2 and Figure 4, pituitary acidophils, basophils and chromophobes of DMPA treated lactating rats with suckled pups were 51.4, 5.0 and 43.3 percent respectively, whereas those of control lactating rats with suckled pups were 41.8, 4.9 and 53.3 percent respectively. The number of acidophils of DMPA rats with suckled pups were increased significantly when compared with the controls, but the chromophobes population significantly decrease. However, gonadotroph populations were not changed in these two groups of lactating mothers.

Pituitary acidophils, basophils and chromophobes of the DMPA lactating rats with no suckling were 39.4, 5.2 and 55.3 percent respectively whereas the control lactating rats with no suckling were 34.5, 4.6 and 60.9 percent respectively. Although acidophil populations of DMPA treated group were slightly higher than the control but none of the cell types of DMPA treated group differed in statistics from the vehicle injected group.

Table 1 Effect of DMFA on milk secretion in lactating rats \*

Day 9 Post-delivery Treatment	Body Weight <sup>2</sup> of mother (gm.) (M ± S.E.)	Based on	Milk yield (gm.) on different days of lactation <sup>1</sup>											
			7	8	9	10	11	12	13	14	15	16	17	18
DMFA 5 µg/gm.	306.7±9.1	1 <sup>st</sup> nursing (6 hrs.)	3.4±0.4	3.7±0.3	4.2±0.5	3.4±0.4	4.8±0.4	4.5±0.5	5.6±0.4	5.3±0.4	5.7±0.3	5.7±0.5	6.4±0.5	5.7±0.4
		2 <sup>nd</sup> nursing (4 hrs.)	2.7±0.3	2.7±0.3	2.7±0.8	3.8±0.6	2.3±0.2	3.1±0.4	3.2±0.5	3.2±0.4	2.4±0.3	3.0±0.3	2.6±0.3	2.3±0.3
		Total (10 hrs.)	6.1±0.2	6.4±0.3	6.8±0.3	7.2±0.5	7.0±0.3	8.6±0.8	8.8±0.7	8.5±0.3	8.2±0.3	8.7±0.5	9.0±0.3	8.0±0.4
Control	302.6±11.5	1 <sup>st</sup> nursing (6 hrs.)	3.2±0.6	4.2±0.6	4.4±0.5	3.6±0.9	4.2±0.8	4.5±0.6	4.2±0.6	5.6±0.7	4.2±0.3	5.4±0.6	5.6±0.6	4.9±0.6
		2 <sup>nd</sup> nursing (4 hrs.)	2.3±0.2	2.2±0.2	1.9±0.2	3.3±0.4	2.0±0.3	2.4±0.6	2.0±0.3	2.0±0.2	2.8±0.5	1.6±0.1	1.8±0.2	2.0±0.3
		Total (10 hrs.)	5.6±0.4	6.5±0.4	6.3±0.5	6.9±1.0	6.2±0.5	6.9±1.0	6.2±0.7	7.6±0.9	7.0±0.5	7.0±0.6	7.4±0.6	6.9±0.6
Unpaired t-test between DMFA and Control		1 <sup>st</sup> nursing	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
		2 <sup>nd</sup> nursing	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.	<0.05	<0.05	N.S.	<0.05	<0.05	N.S.
		Total (10 hrs.)	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	<0.05	N.S.	N.S.	<0.05	<0.05	N.S.

\* = DMFA 5 µg/gm body weight was injected intramuscularly on day 9 of lactation

1 = Estimated by averaging from body weight gain of 5 pups after suckling on different day

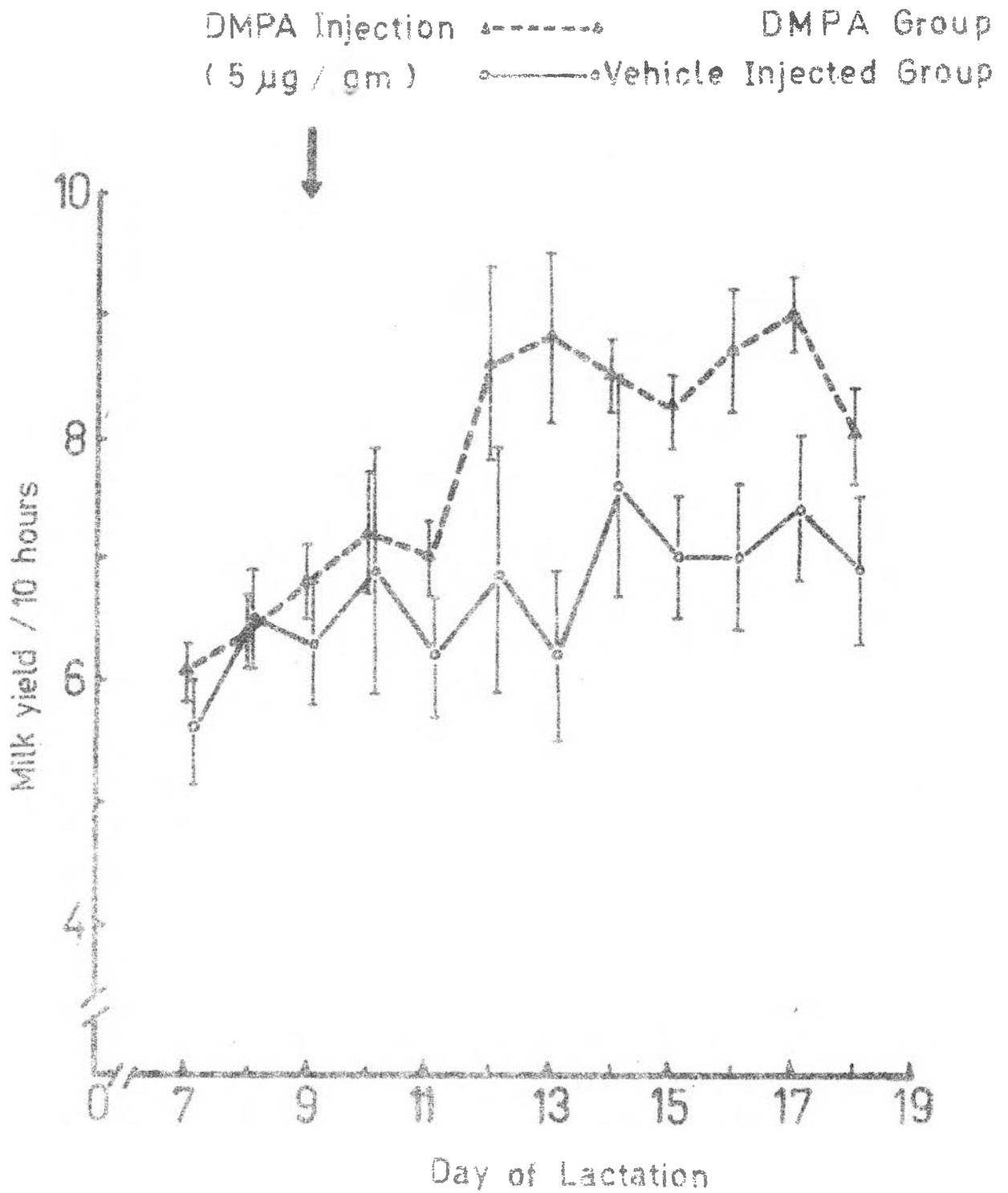
2 = Obtained on post-partum day

Abbreviation :

M = Mean

S.E. = Standard error of mean

Figure 3 Effect of a single injection of DMPA on milk secretion of lactating rats nursing 5 pups.



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Table 2 Effect of a single injection of DMPA (5 µg/gm. body weight) on population of pituitary cell types and mammary gland histology in lactating rats \*

Group of Animals	Mother's ** body weight (gm.) M ± S.E.	Pituitary/ weight/100gm. body weight µgm. M ± S.E.	Day 19 Post-delivery observations						
			Pituitary Cell Types Population			Histology of Mammary Glands			
			Acidophils(%) M ± S.E.	Basophils (%) M ± S.E.	Chromophobes(%) M ± S.E.	Highly active animal/total	Moderately active animal/total	Inactive animal/total	Involution animal/total
a) Control rats with suckled pups.	302.6 ± 11.5	3.26 ± 0.2	41.8 ± 2.2 <sup>b</sup>	4.9 ± 0.3	53.3 ± 2.0 <sup>b</sup>	1/10	3/10	4/10	2/10
b) DMPA rats with suckled pups.	302.7 ± 9.1	3.23 ± 0.1	51.4 ± 1.2 <sup>a,c,d</sup>	5.0 ± 0.2	43.3 ± 1.4 <sup>a,b,d</sup>	3/10	5/10	2/10	-
c) Control rats with no suckling	296.9 ± 10.0	3.5 ± 0.2	34.5 ± 1.9 <sup>b</sup>	4.6 ± 0.8	60.9 ± 2.0 <sup>b</sup>	-	-	-	10/10
d) DMPA rats with no suckling	306.2 ± 14.1	3.3 ± 0.1	39.4 ± 1.9 <sup>b</sup>	5.2 ± 0.5	55.3 ± 2.3 <sup>b</sup>	-	-	-	10/10

\* = DMPA or Vehicle was injected on day 9 of Lactation

\*\* = Weighed on day of delivery

a, b,... = Significantly difference from group a, b,..... respectively at  $p < 0.01$

Abbreviations:

M = Mean

S.E. = Standard error of mean

Figure 4.

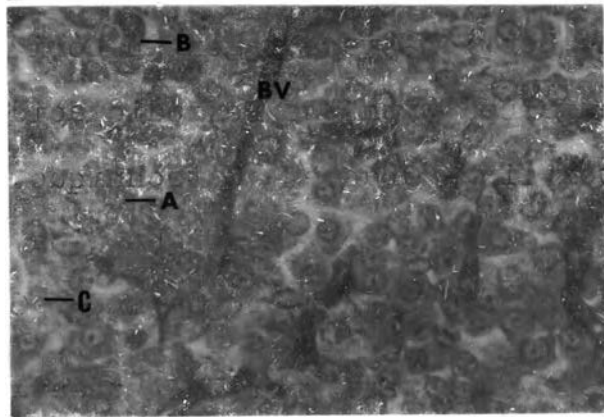
Histology of anterior pituitary in rats on day 19 of lactation. Sections were taken in horizontal plane at 4 micra and stained with the Periodic acid Schiff's reagent Iron - Hematoxylin - Orange - G technique. Each photograph was taken at the region of the right wing of the gland. The magnification of the photomicrograph was 600 times.

Fig 4.1 Anterior pituitary of DMPA treated lactating rats on day 9 of lactation. Notice that number of acidophils are much greater than the control in Fig 4.2, but only a few chromophobes were present.

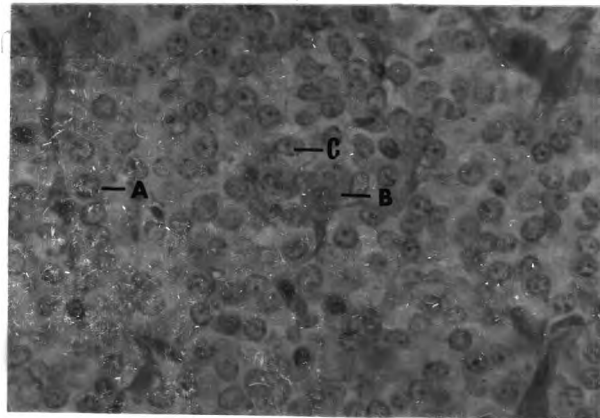
Fig 4.2 Anterior pituitary of control lactating rats. Number of acidophils are much lesser than DMPA treated in Fig 4.1 while tremendous number of chromophobes occupied most of the area in the photograph.

Abbreviation :

- A = acidophils
- B = basophils
- C = chromophobes



4.1



4.2

### Histology of mammary glands

The results obtained from the histological technique by using Hematoxylin-Eosin staining procedure, the sections of mammary gland in the lactating rats were classified into four categories.

1. Highly active All of the mammary alveoli are still distended with milk secretion.
2. Moderately active More than half of mammary alveoli generally present with secretion while the others were not definitely active.
3. Inactive Epithelial cells of the mammary alveoli showed sign of collapse but occasionally traces of secretion still remained in some alveoli.
4. Involution The alveoli had collapsed and revealed a shrunken appearance and definite signs of regression, the secretion in alveoli was absent or rare.

As shown in table 2, according to this classification 3/10, 5/10 and 2/10 of lactating rats administered DMPA were highly active, moderately active and inactive respectively, whereas 1/10, 3/10, 4/10 and 2/10 of the controls were highly active, moderately active, inactive and involution respectively. Histological finding of the highly active mammary alveoli of DMPA group contained more secretory product than the controls, as shown in



Figure 5.1 and 5.2

In non suckling group, the mammary gland's tissue of rats eighteen days after litters removal were involuted rapidly. A large amount of adipose tissue had invaded the gland parenchyma and the number of alveoli was diminished. The alveoli owing to a decrease in size and the degeneration of glandular cells diminished greatly and loss their lumen. But some secretion still remained in the mammary ducts and alveoli as shown in Figure 6.3. No mark difference between DMPA administration and the control rats, as shown in Figure 6.1 Figure 6.2.

Results obtained from the whole mount technique showed that the degree of mammary gland stimulation in lactating rats administered DMPA had no mark difference from the control rats as shown in Figure 7.1 and Figure 7.2. Duct systems were covered with dense clusters of alveoli. In litter removal rats, the degree of mammary gland stimulation was also shown no mark difference between DMPA rats and the control rats as shown in Figure 7.3 and Figure 7.4.

Figure 5

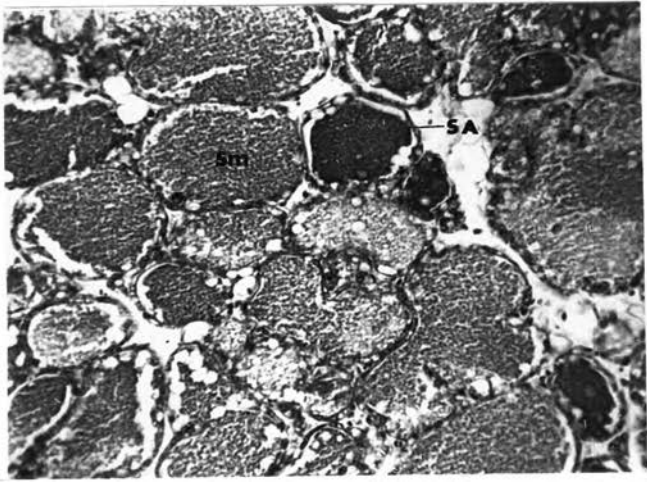
Effect of DMPA administered to lactating rats on day 9 of lactation on histology of mammary gland at autopsy on day 19 post-delivery comparing to the control. Sections were taken in horizontal plane at 6 micra and stained with Hematoxylin and Eosin. The magnification of each photomicrograph was 150 times.

Fig 5.1 Typical example of highly active mammary alveoli of DMPA injected lactating rats. Mammary alveoli were highly distended with secretory material. This animal was among 3 out of 10 as classified highly active mammary alveoli.

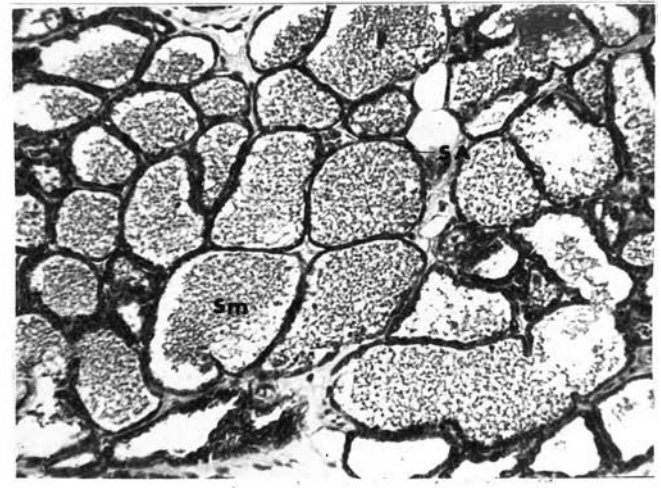
Fig 5.2 An Example of highly active mammary alveoli of control lactating rats. Secretory material in mammary alveoli was looser when compared to Fig 5.1. This animal was among 1 out of 10 as classified highly active mammary alveoli.

Fig 5.3 Typical example of moderately active mammary alveoli of DMPA injected lactating rats. More than half of mammary alveoli generally present with secretion. This animal was among 5 out of 10 as classified moderately active mammary gland.

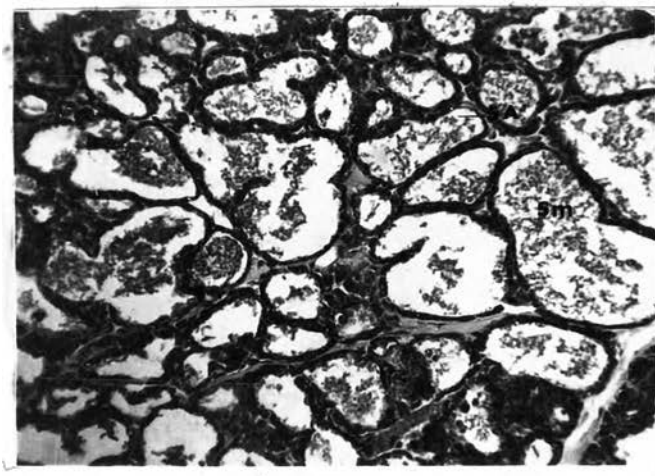
Fig 5.4 Typical example of moderately active mammary alveoli of control lactating rats. More than half of mammary alveoli generlly present with secretion. This animal was among 3 out of 10 as classified moderately active mammary alveoli.



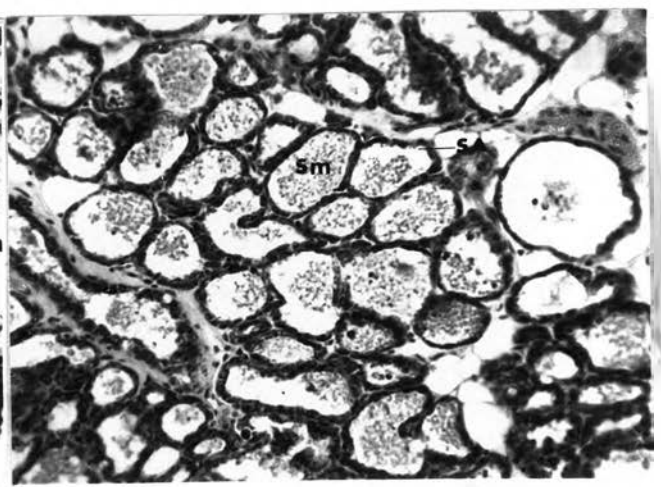
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5.2



5.3

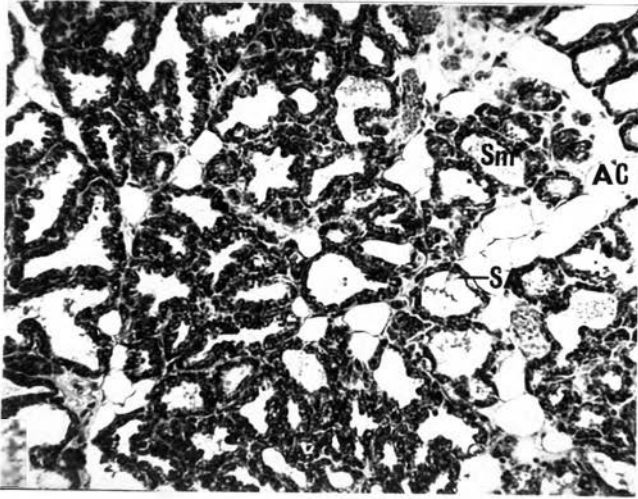


5.4

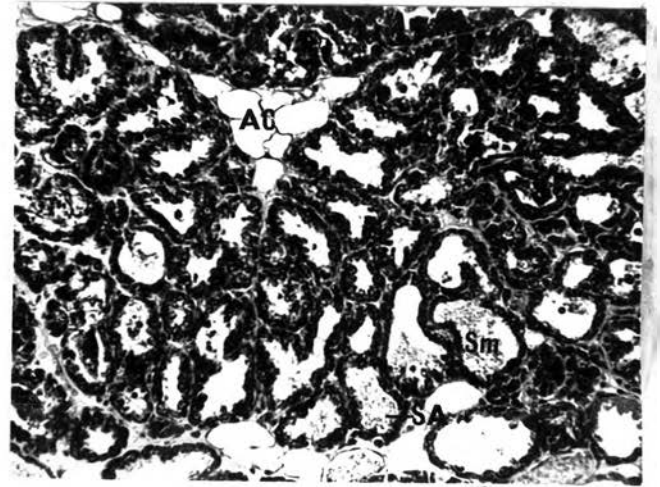
- Fig 5.5 Example of inactive mammary alveoli of DMPA injected lactating rats, which showed sign of collapse and occasionally traces of secretion still remained in some alveoli. This animal was among 2 out of 10 as classified inactive mammary alveoli.
- Fig 5.6 Typical example of inactive mammary alveoli of control lactating rats, which showed sign of collapse and occasionally traces of secretion still remained in some alveoli. This animal was among 4 out of 10 as classified inactive mammary alveoli.
- Fig 5.7 Example of involution mammary alveoli, the alveoli collapsed and revealed a shrunken appearance and definite signs of regression, the secretion in alveoli was absent or rare. This was found only in control lactating rats, and was among 2 out of 10 as classified involution mammary alveoli.

Abbreviations :

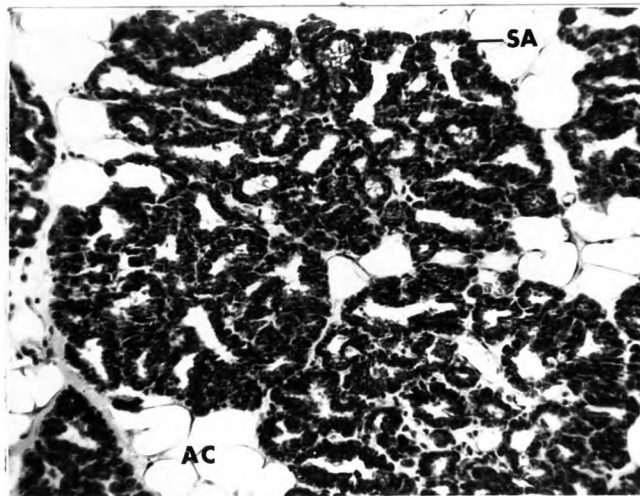
- AC = adipose cell  
IC = interlobular connective tissue  
SA = secretory alveoli  
Sm = secretory material



5.5



5.6



5.7

Figure 6

Histology of mammary gland of rats 18 days after litters were removed. DMPA or its vehicle was injected on day 9 post-delivery. Sections were taken at 6 micra in horizontal plane and stained with Hematoxylin and Eosin. The magnification of each photomicrograph was 150 times.

Fig 6.1 Typical example of mammary alveoli of rats treated with DMPA on day 9 post-delivery.

A large amounts of adipose tissue had invaded the gland parenchyma and the number of alveoli was diminished. Some secretion still remained in the alveoli.

Fig 6.2 Typical example of mammary alveoli of control rats. Some secretion still remained in the alveoli. No mark difference when compared to DMPA treated group in Fig 6.1.

Fig 6.3 Some secretion still remained in the mammary ducts and alveoli which were found both in DMPA treated and control rats.

Abbreviation :

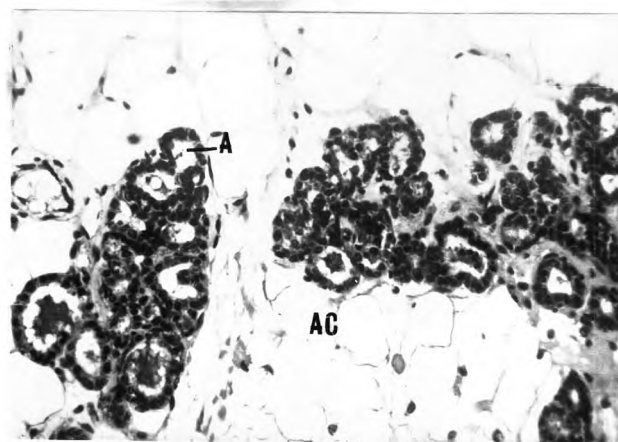
A = alveoli

AC = adipose cell

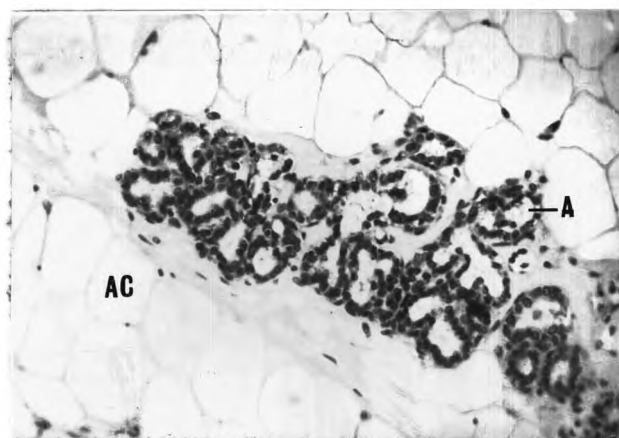
D = duct

IC = interlobular connective tissue

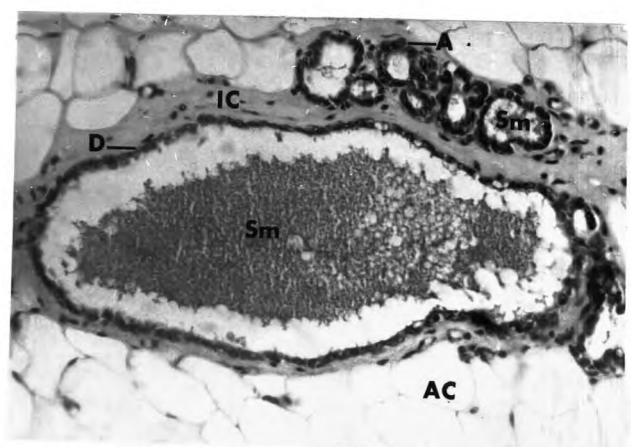
Sm = secretory material



6.1



6.2



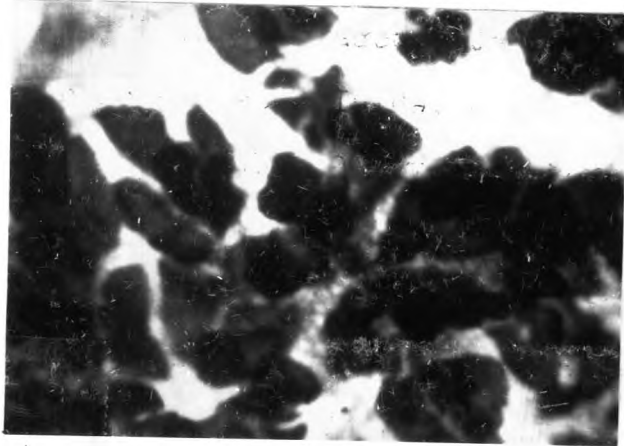
6.3

Figure 7

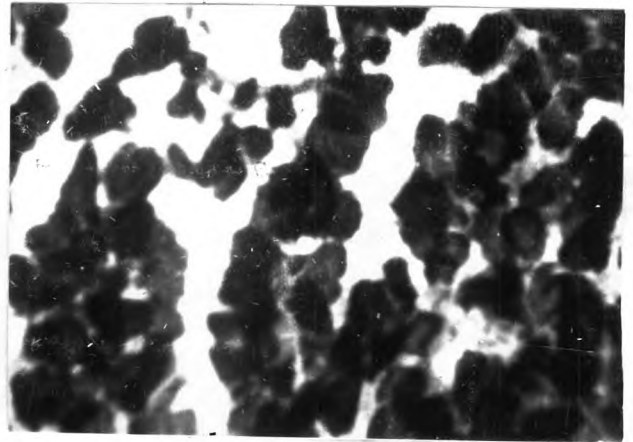
Whole mount technique showing the lobulo-alveolar systems of mammary gland in lactating rats and rats 18 days after litters were removed at autopsy on day 19 post-delivery, in which DMPA or its vehicle was injected intramuscularly on day 9 post-delivery. Glandular tissues were stained with Hematoxylin. The magnification of each photomicrograph was 13 times.

- Fig 7.1 Typical example of whole mount of mammary lobulo-alveolar systems of lactating rats treated with 5  $\mu$ g/gm. DMPA on day 9 post-delivery. Duct systems were covered with dense cluster of alveoli.
- Fig 7.2 Typical example of whole mount of mammary lobulo-alveolar systems of control lactating rats. Duct systems were also covered with dense cluster of alveoli. No mark difference when compared to DMPA treated lactating rats in Fig 7.1.
- Fig 7.3 Typical example of whole mount of mammary lobulo-alveolar systems of rats 18 days after litters were removed and injected with DMPA on day 9 post-delivery. There was much regress in duct systems when compared to lactating rats in Fig 7.1 and 7.2.
- Fig 7.4 Typical example of whole mount of mammary lobulo-alveolar systems of control rats 18 days after litters were removed. There was also much regress in duct system but no mark difference when compared to DMPA treated rats in Fig. 7.3.

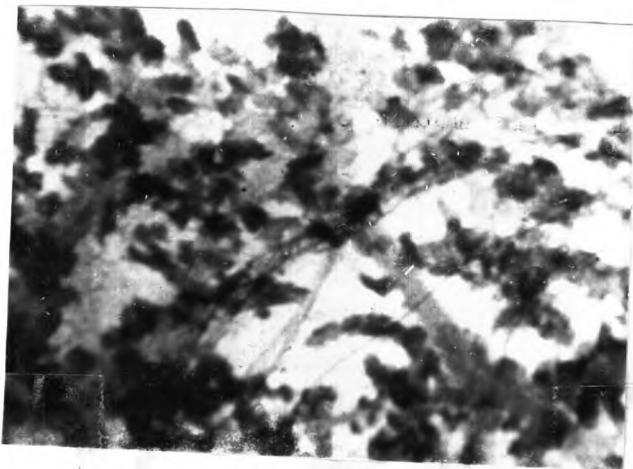




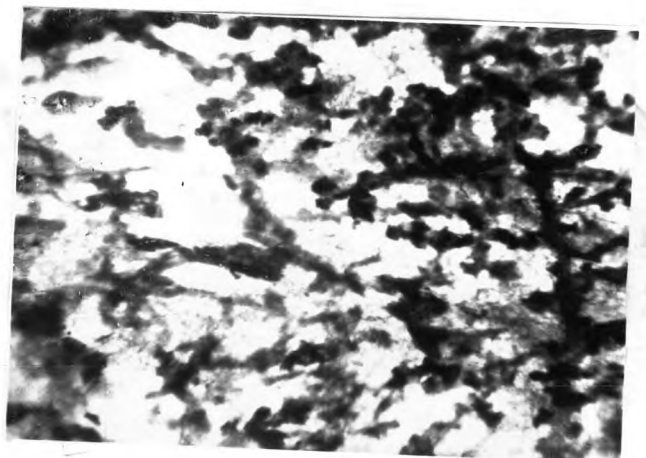
7.1



7.2



7.3



7.4

## 2. Effect on growth rates of the litters

As shown in Table 4 and Figure 8, there were no differences between the daily body weight of the twenty-eight control and thirty-two DMPA treated female rats as observed till 60 days of age. Similarly, there were also no differences between the daily body weight of nineteen controls and nineteen DMPA treated males.

## 3. Effect of sexual maturation of female litters

Results of Table 3 showed that DMPA administered to lactating rats at dose of 5  $\mu\text{g}/\text{gm}$ . body weight on day three post-partum significantly delayed by a few days of vaginal opening from  $36.2 \pm 0.3$  days to  $37.6 \pm 0.5$  days and delayed the onset of the first estrous cycle from  $39.3 \pm 0.9$  days to  $44.3 \pm 1.4$  days.

Effect on reproductive capacity of these litters showed that mean duration of pregnancy and number of litters borned were not differ between both groups. There were no apparent abnormalities in their litters. However, lactating capacity to be a mother assessed by growth rates of the  $F_2$  litters as compared with normal rats showed significantly higher than the control during some intervals both in male and female litters (Table 5 and Figure 9).

Table 4 Mean body weight of control and experimented (DMPA injected to mother on day 3 of lactation) male and female rats.

Age (days)	Control Females (M ± S.E.)	DMPA females (M ± S.E.)	Control males (M ± S.E.)	DMPA males (M ± S.E.)
1	6.3 ± 0.2	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.9
2	6.8 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
3	7.9 ± 0.3	7.6 ± 0.3	7.9 ± 0.3	7.6 ± 0.3
4	9.6 ± 0.2	9.2 ± 0.2	10.1 ± 0.2	9.5 ± 0.3
5	11.2 ± 0.3	10.8 ± 0.2	11.6 ± 0.4	11.0 ± 0.3
6	12.9 ± 0.4	12.6 ± 0.3	13.4 ± 0.5	12.3 ± 0.4
7	14.6 ± 0.5	14.4 ± 0.4	15.3 ± 0.6	14.7 ± 0.5
8	16.6 ± 0.6	16.6 ± 0.4	17.4 ± 0.7	17.0 ± 0.6
9	18.7 ± 0.7	19.1 ± 0.5	19.3 ± 0.8	19.3 ± 0.8
10	21.0 ± 0.8	21.4 ± 0.6	22.2 ± 0.9	21.5 ± 1.0
11	23.4 ± 0.8	23.9 ± 0.7	24.5 ± 0.9	24.1 ± 1.0
12	25.8 ± 1.0	26.1 ± 0.7	26.8 ± 1.0	26.1 ± 1.0
13	28.5 ± 1.0	28.8 ± 0.7	29.5 ± 1.1	28.7 ± 0.9
14	30.8 ± 1.0	31.3 ± 0.8	31.8 ± 1.2	31.1 ± 1.0
15	33.5 ± 1.1	33.6 ± 1.0	34.4 ± 1.2	33.3 ± 1.1
16	36.0 ± 1.1	36.3 ± 1.0	36.7 ± 1.3	35.6 ± 1.2
17	38.2 ± 1.2	38.6 ± 0.9	39.1 ± 1.3	38.0 ± 1.0
18	40.6 ± 1.3	41.3 ± 0.9	41.3 ± 1.4	40.6 ± 1.0
19	43.5 ± 1.4	41.2 ± 0.8	44.2 ± 1.6	43.3 ± 1.0
20	46.7 ± 1.4	47.5 ± 0.8	47.6 ± 1.7	46.6 ± 1.1
21	50.4 ± 1.4	51.6 ± 0.9	50.5 ± 1.7	49.9 ± 1.1
22	55.1 ± 1.5	56.7 ± 0.9	55.8 ± 2.1	55.9 ± 1.3
23	60.2 ± 1.5	61.8 ± 1.0	62.4 ± 1.8	60.3 ± 1.3
24	65.4 ± 1.6	66.5 ± 1.1	68.1 ± 2.2	66.3 ± 1.4
25	68.2 ± 1.6	71.5 ± 1.2	71.8 ± 2.1	72.0 ± 1.7
26	73.4 ± 1.7	76.6 ± 1.4	78.1 ± 2.3	77.7 ± 1.7
27	79.0 ± 1.9	81.6 ± 1.2	83.2 ± 2.4	82.3 ± 1.8
28	83.9 ± 1.8	86.5 ± 1.3	89.9 ± 2.3	87.9 ± 2.2
29	86.6 ± 1.4	90.0 ± 1.3	91.8 ± 2.1	93.2 ± 1.9
30	94.4 ± 1.9	95.3 ± 1.4	98.5 ± 2.2	98.6 ± 1.9
31	98.5 ± 2.1	100.2 ± 1.7	105.9 ± 2.3	103.4 ± 2.2
32	103.5 ± 2.2	104.5 ± 1.6	112.8 ± 2.6	109.4 ± 2.0
33	109.0 ± 2.2	109.1 ± 1.6	120.3 ± 2.6	115.9 ± 2.3
34	113.8 ± 2.2	114.0 ± 1.6	126.8 ± 2.6	122.3 ± 2.6
35	119.8 ± 2.3	118.3 ± 1.6	134.0 ± 3.0	128.6 ± 2.5
36	123.9 ± 2.3	123.5 ± 1.4	140.7 ± 2.7	133.1 ± 3.3
37	128.9 ± 2.2	128.7 ± 1.4	146.7 ± 2.8	139.4 ± 3.5
38	133.8 ± 2.5	133.9 ± 1.6	149.8 ± 2.8	145.6 ± 4.4
39	137.1 ± 2.5	136.9 ± 1.5	156.9 ± 3.3	153.0 ± 3.8
40	141.2 ± 2.3	141.8 ± 1.4	165.6 ± 4.0	161.4 ± 4.0
41	146.3 ± 2.1	146.1 ± 1.7	172.1 ± 3.5	171.6 ± 5.1
42	149.0 ± 2.4	150.7 ± 1.7	175.3 ± 4.4	173.8 ± 4.1
43	153.2 ± 2.8	156.2 ± 1.7	179.2 ± 5.7	181.5 ± 5.2
44	159.0 ± 2.4	160.5 ± 1.8	191.2 ± 6.9	195.6 ± 6.7
45	158.5 ± 2.5	164.1 ± 2.0	195.0 ± 6.2	194.2 ± 5.0
46	157.8 ± 5.6	165.9 ± 5.8	200.5 ± 10.9	196.9 ± 6.2
47	171.6 ± 4.9	168.8 ± 2.8	205.7 ± 8.1	203.3 ± 5.3
48	169.4 ± 2.3	170.0 ± 3.4	219.5 ± 5.6	196.9 ± 8.9
49	170.7 ± 3.3	174.3 ± 3.8	222.8 ± 8.7	214.9 ± 4.7
50	172.4 ± 5.7	176.6 ± 4.6	230.5 ± 8.0	232.7 ± 7.9
51	175.6 ± 3.9	180.9 ± 3.8	238.8 ± 6.2	239.4 ± 8.4
52	179.9 ± 2.2	190.9 ± 3.3	237.6 ± 6.5	246.9 ± 5.5
53	184.4 ± 4.6	190.1 ± 4.5	245.6 ± 8.5	242.1 ± 6.8
54	188.2 ± 4.6	195.5 ± 5.4	256.1 ± 10.1	250.2 ± 7.0
55	191.8 ± 2.9	198.2 ± 4.6	256.9 ± 9.4	258.0 ± 11.0
56	195.2 ± 4.0	200.0 ± 5.1	262.0 ± 9.3	258.5 ± 12.7
57	196.4 ± 4.7	201.0 ± 4.3	263.0 ± 6.0	263.8 ± 6.5
58	200.0 ± 4.1	201.6 ± 4.0	263.8 ± 5.9	271.6 ± 7.0
59	201.9 ± 2.5	202.9 ± 3.2	269.3 ± 7.0	282.9 ± 7.6
60	199.2 ± 4.6	209.1 ± 7.7	270.0 ± 7.1	284.0 ± 6.5

Figure 8 Mean body weight of control and experimented (DMPA injected to mothers) male and female rats.

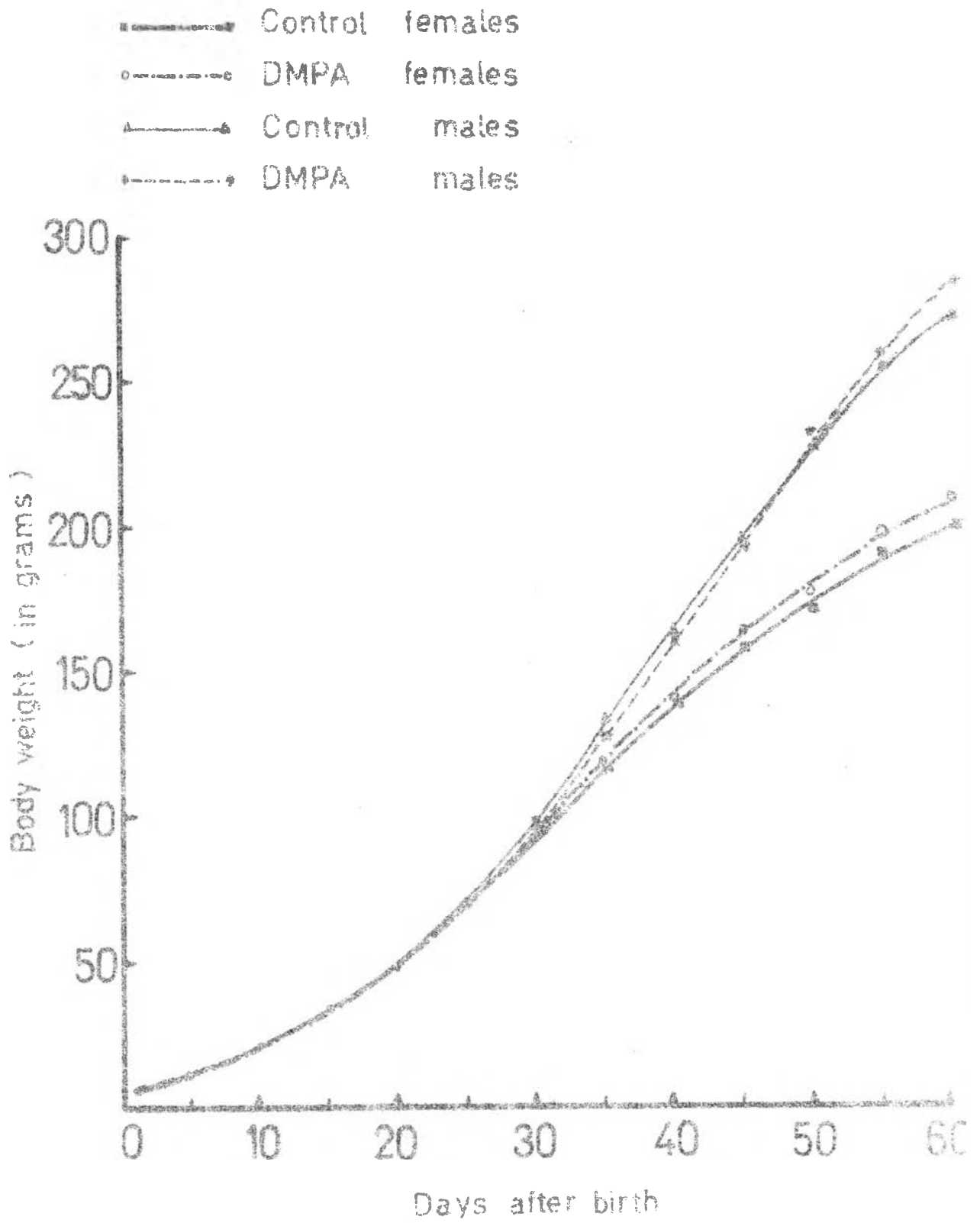


Table 3 Effect of a single injection of DMPA(5  $\mu$ g/gm) on development of sexual characteristics of female litters in lactating rats \*

Measurements	Normal Mothers M $\pm$ S.E.	DMPA Mothers M $\pm$ S.E.	Student t test
Age of Vaginal canalization	36.2 $\pm$ 0.3 <sup>(1)</sup>	37.6 $\pm$ 0.5 <sup>(2)</sup>	p < 0.05
Age of the first oestrous cycle	39.3 $\pm$ 0.9	44.3 $\pm$ 1.4	p < 0.01
Duration of pregnancy	22.3 $\pm$ 0.1	22.4 $\pm$ 0.1	N.S.
Number of litters	10.7 $\pm$ 0.4	10.4 $\pm$ 0.5	N.S.

\* = DMPA was injected on day 3 of lactation

(1) = mean value of 26 female litters of control mothers

(2) = mean value of 32 female litters of DMPA mothers

Abbreviation :

M = mean

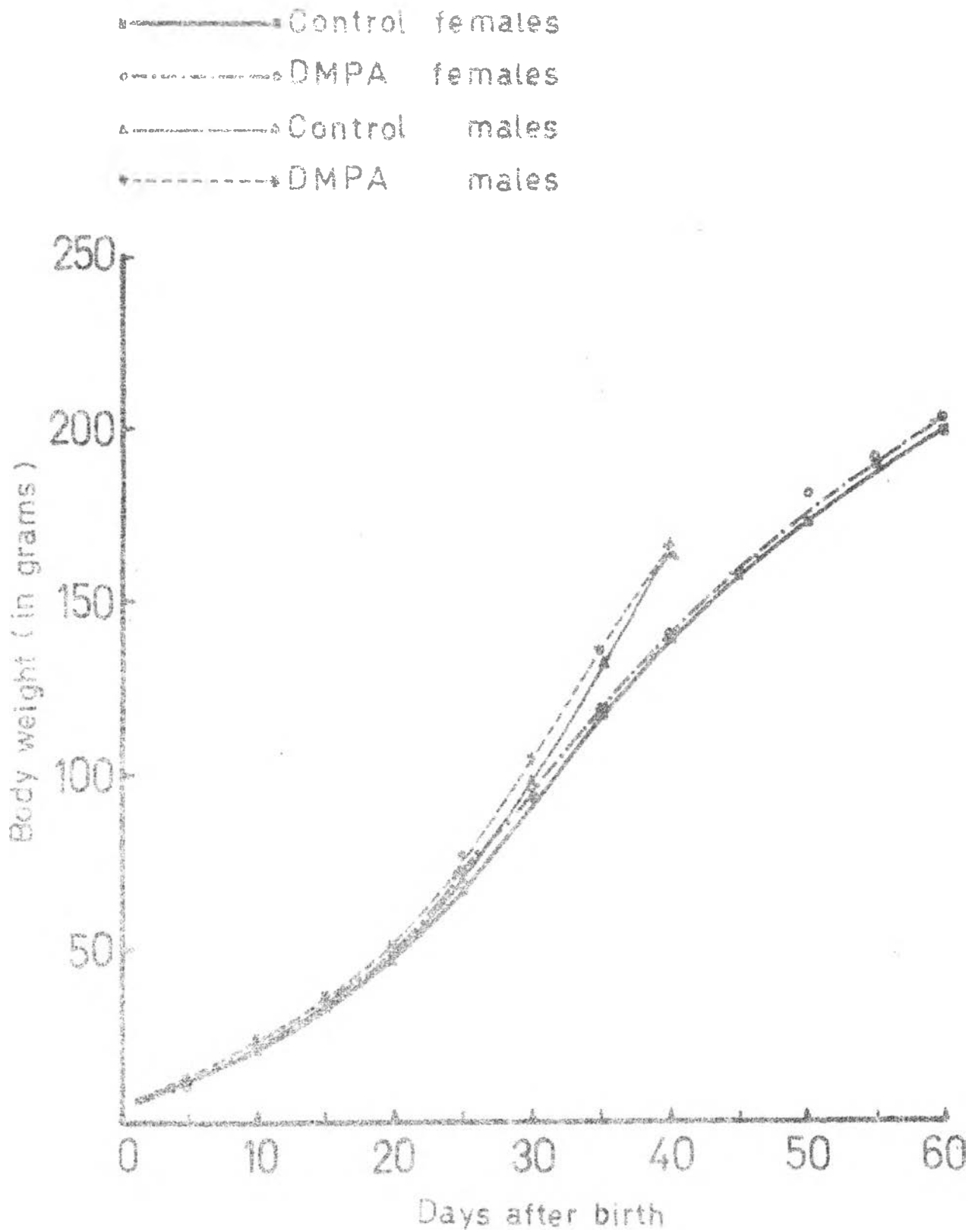
S.E. = standard error of mean

Table 5 Mean body weight of male and female litters of female rats (DMPA injected to mother) as compared with the controls

Age(days)	Control Females M ± S.E.	DMPA Females M ± S.E.	Control males M ± S.E.	DMPA males M ± S.E.
1	6.3 ± 0.2	6.6 ± 0.1	6.2 ± 0.1	6.6 ± 0.1
2	6.8 ± 0.2	7.4 ± 0.1	5.8 ± 0.2	7.4 ± 0.1
3	7.9 ± 0.3	8.5 ± 0.1	7.9 ± 0.3	8.5 ± 0.1
4	9.6 ± 0.2	10.0 ± 0.1	10.1 ± 0.2	10.7 ± 0.2
5	11.2 ± 0.3	11.8 ± 0.1	11.6 ± 0.4	12.5 ± 0.2
6	12.9 ± 0.4	13.7 ± 0.2	13.4 ± 0.5	14.5 ± 0.3
7	14.6 ± 0.5	17.8 ± 0.2	15.3 ± 0.6	16.7 ± 0.3
8	16.6 ± 0.6	18.1 ± 0.3	17.4 ± 0.7	19.3 ± 2.4
9	18.7 ± 0.7	*21.0 ± 0.3	19.8 ± 0.8	22.1 ± 0.5
10	21.0 ± 0.8	*23.6 ± 0.4	22.2 ± 0.9	*24.6 ± 0.5
11	23.4 ± 0.8	26.1 ± 0.4	24.5 ± 0.9	27.0 ± 0.6
12	25.8 ± 1.0	28.6 ± 0.5	26.8 ± 1.0	29.1 ± 0.6
13	28.5 ± 1.0	*31.3 ± 0.5	29.5 ± 1.1	32.3 ± 0.7
14	30.8 ± 1.0	34.1 ± 0.5	31.8 ± 1.2	35.0 ± 0.7
15	33.5 ± 1.1	*36.9 ± 0.6	34.4 ± 1.2	*37.7 ± 0.8
16	36.0 ± 1.1	39.0 ± 0.6	36.7 ± 1.3	40.2 ± 0.8
17	38.2 ± 1.1	*41.3 ± 0.6	39.1 ± 1.3	42.5 ± 0.8
18	40.6 ± 1.2	43.6 ± 0.8	41.3 ± 1.4	44.8 ± 1.0
19	43.5 ± 1.3	45.9 ± 0.7	44.2 ± 1.6	47.5 ± 0.9
20	46.7 ± 1.4	49.4 ± 0.8	47.6 ± 1.7	51.1 ± 1.2
21	50.4 ± 1.4	53.8 ± 0.9	50.5 ± 1.7	56.2 ± 1.2
22	55.1 ± 1.5	58.9 ± 0.9	55.8 ± 2.1	61.6 ± 1.2
23	60.2 ± 1.5	63.4 ± 1.0	62.4 ± 1.8	67.4 ± 1.2
24	65.4 ± 1.6	68.5 ± 1.0	68.1 ± 2.2	73.0 ± 1.2
25	68.2 ± 1.6	*73.4 ± 1.0	71.8 ± 2.1	*78.4 ± 1.3
26	73.4 ± 1.7	77.8 ± 1.0	78.1 ± 2.3	82.4 ± 1.1
27	79.0 ± 1.9	82.5 ± 1.0	83.2 ± 2.4	89.4 ± 1.2
28	83.0 ± 1.8	85.1 ± 1.1	89.9 ± 2.3	94.3 ± 1.2
29	86.0 ± 1.4	*91.5 ± 1.1	91.8 ± 2.1	100.6 ± 1.2
30	94.4 ± 1.9	96.2 ± 1.1	98.5 ± 2.2	105.7 ± 1.3
31	98.5 ± 2.1	100.6 ± 1.2	105.9 ± 2.3	110.8 ± 1.3
32	103.5 ± 2.2	104.9 ± 1.3	112.8 ± 2.6	116.0 ± 1.4
33	109.0 ± 2.2	108.9 ± 1.2	120.3 ± 2.6	123.4 ± 1.5
34	113.8 ± 2.2	113.0 ± 1.2	126.8 ± 2.6	129.0 ± 1.7
35	119.8 ± 2.3	119.1 ± 1.1	134.0 ± 3.0	136.5 ± 1.9
36	123.9 ± 2.3	123.2 ± 1.2	140.7 ± 2.7	143.3 ± 2.0
37	128.9 ± 2.2	127.7 ± 1.2	146.7 ± 2.8	148.9 ± 1.8
38	133.8 ± 2.5	132.2 ± 1.3	149.8 ± 2.8	153.2 ± 2.1
39	137.1 ± 2.5	136.8 ± 1.3	156.9 ± 3.3	159.6 ± 2.3
40	141.2 ± 2.3	141.3 ± 1.3	165.6 ± 4.0	166.7 ± 2.6
41	146.3 ± 2.1	146.9 ± 1.2	172.1 ± 3.5	173.6 ± 2.8
42	149.0 ± 2.4	149.2 ± 1.3	175.3 ± 4.4	*180.2 ± 3.1
43	153.2 ± 2.8	152.9 ± 1.5	-	-
44	159.0 ± 2.4	158.3 ± 1.6	-	-
45	158.5 ± 2.5	159.8 ± 1.8	-	-
46	157.8 ± 5.6	163.3 ± 1.8	-	-
47	171.6 ± 4.9	164.7 ± 2.0	-	-
48	169.4 ± 2.3	*177.8 ± 3.6	-	-
49	170.7 ± 3.5	*180.5 ± 4.9	-	-
50	172.4 ± 5.7	*181.2 ± 5.0	-	-
51	175.6 ± 3.9	*183.8 ± 5.3	-	-
52	179.9 ± 2.2	*185.6 ± 5.5	-	-
53	184.4 ± 4.6	184.5 ± 5.7	-	-
54	188.2 ± 4.6	185.9 ± 2.2	-	-
55	191.8 ± 2.9	190.5 ± 2.8	-	-
56	195.2 ± 4.0	196.6 ± 5.0	-	-
57	196.4 ± 4.7	200.0 ± 4.8	-	-
58	200.0 ± 4.1	201.1 ± 5.0	-	-
59	201.9 ± 2.5	203.8 ± 7.8	-	-
60	199.2 ± 4.6	204.0 ± 2.3	-	-

\* Significant different at P < 0.05

Figure 9 Lactational performance of female litters of female rats 47  
whose mother received DMPA on day 3 of lactation.



#### 4. Effect on growth and sexual maturation of male litters

When they reached a suitable weight (about 3 months of age) and expected to have sexual maturity were mated with normal female rats. Result showed that mean duration of pregnancy and the number of litters borned were consistently normal and showed no apparent difference from the control rats.

Similar to the  $F_1$  female rats whose their mother received DMPA during lactation, the  $F_1$  male rats sired normal healthy pups and these pups showed significantly higher of the body weight than the control pups in some interval of age (Figure 10, Table 6).



**Table 6** Mean body weight of male and female litters of male rats (DMPA injected to mother) as compared with the controls

Age(days)	Control Females (M ± S.E.)	DMPA Females (M ± S.E.)	Control males (M ± S.E.)	DMPA males (M ± S.E.)
1	6.3 ± 0.2	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
2	6.8 ± 0.2	7.0 ± 0.1	6.8 ± 0.2	7.0 ± 0.1
3	7.9 ± 0.3	7.9 ± 0.1	7.9 ± 0.3	7.8 ± 0.1
4	9.6 ± 0.2	9.7 ± 0.1	10.1 ± 0.2	10.1 ± 0.2
5	11.2 ± 0.3	11.4 ± 0.2	11.6 ± 0.4	11.9 ± 0.2
6	12.9 ± 0.4	13.2 ± 0.2	13.4 ± 0.5	13.8 ± 0.3
7	14.6 ± 0.5	15.0 ± 0.2	15.3 ± 0.6	15.4 ± 0.3
8	16.6 ± 0.6	17.1 ± 0.3	17.4 ± 0.7	18.2 ± 0.3
9	18.7 ± 0.7	19.4 ± 0.3	19.8 ± 0.8	20.5 ± 0.4
10	21.0 ± 0.8	21.8 ± 0.3	22.2 ± 0.9	22.9 ± 0.4
11	23.4 ± 0.8	24.2 ± 0.4	24.5 ± 0.9	25.3 ± 0.4
12	25.1 ± 1.0	26.7 ± 0.4	26.8 ± 1.0	28.1 ± 0.5
13	28.5 ± 1.0	29.1 ± 0.5	29.5 ± 1.1	30.7 ± 0.5
14	30.8 ± 1.0	32.7 ± 0.5	31.8 ± 1.2	33.4 ± 0.5
15	33.5 ± 1.1	34.4 ± 0.6	34.4 ± 1.2	36.4 ± 0.5
16	36.0 ± 1.1	36.8 ± 0.6	36.7 ± 1.3	39.0 ± 0.5
17	38.2 ± 1.2	39.2 ± 0.6	39.1 ± 1.3	41.6 ± 0.6
18	40.6 ± 1.3	40.4 ± 0.7	41.3 ± 1.4	43.3 ± 0.5
19	43.5 ± 1.4	44.1 ± 0.9	44.2 ± 1.6	44.6 ± 0.7
20	46.7 ± 1.4	47.5 ± 1.0	47.6 ± 1.7	50.2 ± 0.8
21	50.4 ± 1.4	52.3 ± 1.0	50.5 ± 1.7	*55.9 ± 0.9
22	51.1 ± 1.5	56.8 ± 1.5	55.8 ± 2.1	*59.8 ± 1.0
23	60.2 ± 1.5	61.8 ± 1.7	62.4 ± 1.8	64.8 ± 1.0
24	65.4 ± 1.6	66.7 ± 1.6	68.1 ± 2.2	70.4 ± 1.1
25	68.2 ± 1.6	71.4 ± 1.5	71.8 ± 2.1	75.6 ± 1.1
26	73.4 ± 1.7	75.7 ± 2.0	78.1 ± 2.3	81.3 ± 1.4
27	79.0 ± 1.9	80.7 ± 1.4	83.2 ± 2.4	86.0 ± 1.6
28	83.9 ± 1.8	84.8 ± 1.8	89.9 ± 2.3	91.8 ± 1.5
29	86.6 ± 1.4	88.4 ± 1.5	91.8 ± 2.1	*96.0 ± 2.3
30	94.4 ± 1.9	91.8 ± 1.6	98.5 ± 2.2	101.8 ± 2.5
31	98.5 ± 2.1	101.8 ± 1.4	105.9 ± 2.3	107.1 ± 1.9
32	103.5 ± 2.2	106.4 ± 1.7	112.8 ± 2.6	114.2 ± 2.0
33	109.0 ± 2.2	108.7 ± 1.4	120.3 ± 2.6	*127.3 ± 2.8
34	113.8 ± 2.2	112.5 ± 1.5	126.8 ± 2.6	128.3 ± 2.4
35	119.8 ± 2.3	118.3 ± 1.7	134.0 ± 3.0	133.3 ± 2.3
36	123.9 ± 2.3	123.4 ± 1.6	140.7 ± 2.7	140.0 ± 2.5
37	128.9 ± 2.2	128.7 ± 1.5	146.7 ± 2.8	146.1 ± 2.6
38	133.8 ± 2.5	132.9 ± 1.6	149.8 ± 2.8	*153.5 ± 2.6
39	137.1 ± 2.5	137.2 ± 1.4	156.9 ± 3.3	159.2 ± 3.4
40	141.2 ± 2.3	142.3 ± 1.5	165.6 ± 4.0	167.4 ± 3.0
41	146.3 ± 2.1	148.5 ± 1.5	172.1 ± 3.5	175.5 ± 2.8
42	149.0 ± 2.4	149.7 ± 1.6	175.3 ± 4.4	*182.4 ± 2.9
43	153.2 ± 2.8	155.1 ± 1.6	-	-
44	159.0 ± 2.4	158.6 ± 1.8	-	-
45	158.5 ± 2.5	162.1 ± 1.9	-	-
46	157.8 ± 5.6	165.9 ± 1.6	-	-
47	171.6 ± 4.9	168.4 ± 1.9	-	-
48	169.4 ± 2.3	170.8 ± 0.3	-	-
49	170.7 ± 3.5	172.4 ± 2.7	-	-
50	172.4 ± 5.7	175.2 ± 2.5	-	-
51	175.6 ± 3.9	180.1 ± 2.7	-	-
52	179.9 ± 2.2	185.2 ± 2.0	-	-
53	184.4 ± 4.6	192.2 ± 2.7	-	-
54	188.2 ± 4.6	190.5 ± 8.0	-	-
55	191.8 ± 2.9	189.1 ± 7.3	-	-
56	195.2 ± 4.0	190.0 ± 2.5	-	-
57	196.4 ± 4.7	195.0 ± 6.5	-	-
58	200.0 ± 4.1	-	-	-
59	201.9 ± 2.5	-	-	-
60	199.2 ± 4.6	208.6 ± 3.0	-	-

\* Significant different at P < 0.05

Figure 10 Ability to sire normal healthy pups of male litters whose mother received DMPA on day 3 of lactation

- Control females
- - -●- - - DMPA females
- ▲— Control males
- - -●- - - DMPA males

