

MATERIALS AND METHODS

Mature female and male Wistar rats were purchased from National Laboratory Animal Center, Salaya Campus, Mahidol University. All animals were housed in a controlled room with temperature of 25 ± 1 °C and the light on from 06.00 to 18.00 h. Rat chow and tap water were available ad libitum.

The animals were left undisturbed for 2 weeks and then, in female, daily vaginal cycle was examined by vaginal smear. After appearance of 2 consecutive normal estrous cycles and during either late proestrus or estrous stage, they were mated with male animals. The day that the sperms were found in vaginal smear was defined as day 1 of pregnancy. The pregnant rats gave pups on approximately day 21 of pregnancy. The day of birth was designated as day 1 of the neonate. Only neonatal female rats were used for the experiments. Subcutaneous injections of 40% solution of MSG were administered at a dose of 1, 2, 4 mg/gm body weight to pups on day 1, 3, 5, 7 and 9 postnatally. Control rats received injections of physiological saline, the volume injected being adjusted to that in which 4 mg/gm body weight MSG-treated group was administered. Animals were weaned on day 21 of age. After that, they were separated from the mothers, grouped according to treatment and maintained 2 rats/cage.

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Growth Parameters

After weanling, animals were placed two per cage according to treatment condition. Food intake was measured by placing a weighed amount of food into individual cage food-holders, provided with a trap to catch spilled food particles. Every 4 days, the remaining food was removed and weighed together with trapped particles. The difference between the original amount of food and that left was taken as the amount ingested. At the same time, tapeline was used to determine nasoanal length under ether anesthesia and body weights were also recorded using the balance. The indication of stunting was assessed as the Lee index, calculated by dividing the cube root of the body weight in grams with nasoanal length in centimeters and then multiplying by 10.

$$\text{Lee index} = \frac{\sqrt[3]{\text{body weight (gm)}}}{\text{nasoanal length (cm)}} \times 10$$

This index is frequently used as a measure of body fat.

Reproductive Capacity

After weanling, all rats were monitored daily for vaginal opening by careful inspection. Estrous cycle was examined by vaginal smear at 9.00 h and carried out for 30 days starting from the 60 day of age. To examine the estrous cycle, briefly, a

drop of normal saline was administered into vaginal canal. Then, the vaginal fluid was taken and smeared on a slide, stained with methylene blue and these slides were inspected under light microscope. Estrous cycle was simply divided into four stages : proestrus (nucleated epithelial cells), estrus (completely cornified cells), metestrus (cornified and nucleated cells with some leucocytes), diestrus (preponderance of leucocytes).

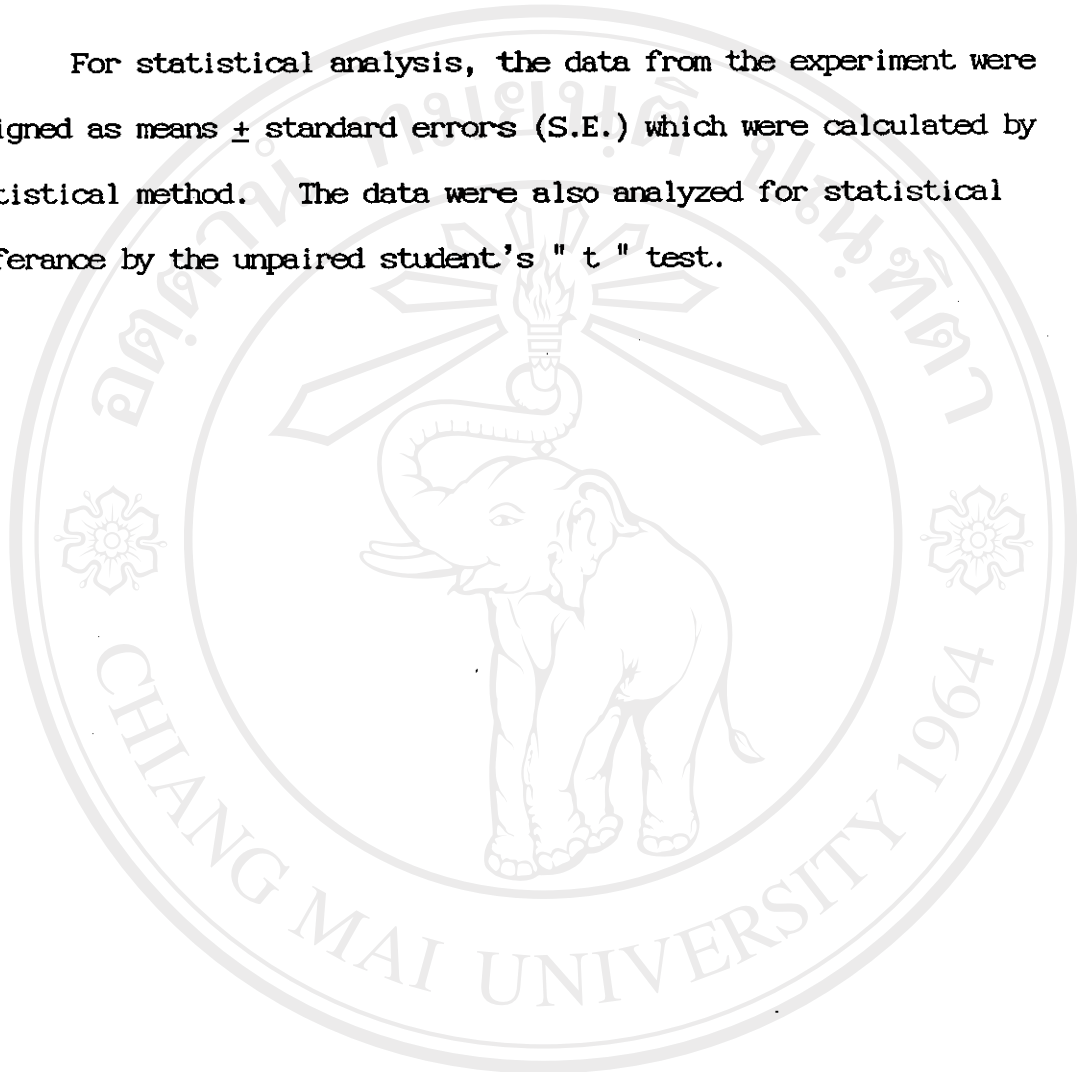
At 90 day of age, all groups were divided into two subgroups, one for studying the reproductive capacity, another one for organ weight determination. In the first subgroup, animals were mated with normal adult male. The female rats were mated with the normal adult male in 3:1 ratio. Copulation was daily verified by detection of sperms in the vaginal smear. Percentage of reception and pregnancy, duration of pregnancy, number and mean birth weight of litters were carefully recorded. In the case that reception was not indicated, to assure the animals rejection, the animals were left together for as long as 60 days.

Determination of Organ Weight

Another subgroup of animals was killed by decapitation at around 120 day of age. The pituitary gland, uterus and ovary were carefully removed, cleaned and weighed on electronic balance (R300s Satorius Research). All weights were recorded as wet weights as well as relative weights (organ weight per 100 gm of body weight).

Statistical Analysis

For statistical analysis, the data from the experiment were assigned as means \pm standard errors (S.E.) which were calculated by statistical method. The data were also analyzed for statistical difference by the unpaired student's "t" test.



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