DISCUSSION

Many effects of MSG on growth and reproductive capacity of female rats were shown in this study. Relating to growth parameters, the body weight, nasoanal length, Lee index and amount of food intake were recorded. MSG-treated female rats showed a decrease in rate of growth and turned to obese. The mean body weights of all MSG-treated groups were significantly less than those of the littermate control throughout the period of examination, except that at day 90 of age the body weight of MSG_4 group was not significantly different when compared with that of control group. The nasoanal lengths of all MSG-treated groups were significantly affected, mostly marked in the MSG_4 group. The Lee indexes, the measurement of obesity, in all MSG-treated groups were higher than those in control group and the highest values were found in MSG_4 group. From the physical appearance, all of the MSG-treated rats looked obese, and inspection of the body cavity by autopsy (at around 120 day of age) indicated a striking accumulation of fat in all the body depots, especially in the abdominal cavity (data not shown). This appears to agree with the previous work of Rodriquez-Sierra, et al. (1980) that body weights of MSG-treated rats were less than those of control during development (1-60 day of age). Though the nasoanal length and Lee index were not determined in their study, their data showed that at about the same chronological age (~ 6 months), MSG-treated rat showed clearly obesity as this observation. In addition, Redding, et al. (1971) reported that body weights and nasoanal lengths at 40 day of age were significantly

reduced in MSG-treated female rats, while, at 110 day of age, the body weights had no significant difference from those of control. This was consistent with our data demonstrating that at 90 day of age for MSG_4 group of animals. However, in the present study, the nasoanal length was significantly shorter and Lee index was elevated in the MSG-treated rat when compared with the control rat. Throughout the experimental period, food consumption was determined to find whether the obesity in animals treated with MSG, if any, was due to overeating. As shown in Table 4 and Figure 4, all MSG-treated groups of animals ate no more than the control group. This finding is supported by other investigators (Olney, 1967; Redding, et al., 1971; Araujo and Mayer, 1973) that hyperphagia was not observed in MSG-treated animals (mice and rat).

Many effects produced by MSG in this experiment are similar to a syndrome seen in weanling rats with electrolytic lesions in the ventromedial nucleus (VMN). VMN is known as a satiety center. Stimulation of this nucleus causes complete satiety. Conversely, destructive lesions of it cause results exactly opposite to those caused by stimulation, that is voracious and continued eating until the animal become extremely obese, sometime as large as four times of the normal size (Guyton, 1991). However, experimentally, Han, et al. (1965) demonstrated that hypothalamic lesion in weanling rats induced by bilateral electrolytic lesion technique caused obesity without overeating. In addition, in 1966 and 1967, Bernardis and Skelton studied about growth and obesity in rats after placement of VMN lesions at four different ages. They found that, in terms of Lee's index, all

lesioned rats were obese when compared with their respective control, but only some showed significant hyperphagia. This disturbance in growth was conclusively and mostly marked in youngest brain. Furthermore, they also observed that the number of the pituitary acidophils known to elaborate GH was significantly reduced in some lesioned rats. These results led them to suggest that hypothalamic obesity of rats are associated with growth impairment and may be metabolically linked to an insufficiency of growth hormone secretion.

The defect in GH secretion associated with obesity was found in the genetically obese Zucker rats, a model of spontaneous obesity due to autosomal recessive gene (Finkelstein, et al., 1986). The mean plasma GH levels in these obese rats were significantly depressed when compared with those in their lean (nonobese) littermate. During the 6-h sampling period, all lean animals displayed a pulsatile pattern of GH release, with at least one peak exceeding 800 ng/ml, the time between the first and second peaks ranged from 2-3 h, whereas only 2 of 6 obese rats displayed a single peak greater than 800 ng/ml. The length of femur and tibia were, as an independent mean of assessing the ability of GH to affect growth of these bones, significantly shorter in the obese individuals. Many groups of investigators formerly reported that neonatal MSG administration resulted in a significant reduction of the pituitary contents of growth hormone, causing a marked disturbance in episodic GH secretion and plasma GH concentration (Redding, et al., 1971; Nemeroff, et al., 1981).

These data, taken together, suggest that stunting of growth and the obesity observed in all MSG-treated groups does not correspond

with eating behaviors of the animals, but may be associated with hormonal imbalance, particularly, insufficiency of growth hormone secretion. Consequently, defective GH release in MSG-treated rats could possibly be related to some abnormalities at three possible sites. There could be due to, frist, a circulating inhibitor of GH release in the systemic blood, secondly, abnormally functioning pituitary somatotrops, or thirdly, a dysfunction in central regulation of GH secretion.

The rate of growth hormone secretion increases and decreases within minutes, sometimes for a reason that are not at all understood, but at other times definitely in relation to individual state of nutrition and stress. For examples, the extremely high levels of growth hormone frequently occur during starvation, the condition of hypoglycemia stimulates growth hormone secretion while excitement and trauma diminish it. However, in this experiment, all animals were controlled under the same condition with water and food available ad libitum, so the defect of GH release in MSG-treated rats is unlikely to be due to these factors.

By autopsy, the pituitary weight and size in MSG-treated rats were strikingly reduced from control rats (Table 8.). Both absolute and relative weights of pituitary gland in MSG_2 and MSG_4 groups were significantly reduced from control while only relative weight of MSG_1 group was significantly reduced. This observation is supported by many studies showing that neonatal treatment of MSG caused atrophy of pituitary gland (Nemeroff, et al., 1987; Terry, et al., 1981; Dada, et al., 1984). Terry, et al. (1981) reported that

there was a marked decrease in total GH content and concentration in pituitary from MSG-treated rats. However, Dada, et al. (1981) found that although serum GH concentration in the female MSG-treated rats was depressed when compared with those in their saline-treated littermate. However, basal GH release and anterior pituitary (AP) GH concentration were no difference between the two groups. For this point of view, the present study does not directly answer the question of whether the expectable depressed GH secretion in MSG-treated rats was the result of abnormally functioning pituitary gland. Up to the present, there have been no reports of any gross pathological change in the AP after MSG treatment. Millard, et al. (1982) found that the AP, despite being smaller in size and weight, contained normal amount of both GH and PRL while the amount of TSH, LH and FSH were slightly elevated. Moreover, Nemeroff, et al. (1978) concluded that MSG is not toxic to the AP, since their MSG-treated animals displayed either a normal or an elevated AP release of LH and TSH in response to exogenous LHRH and TRH. Katakami, et al. (1984) showed that the intravenous administration of PGE_1, possessing the capacity to stimulate the AP, produced an abrupt GH release in MSG-treated rats as in control animals. This indicates that the reduced GH secretion in MSG-treated rat is not due to an impaired function of pituitary somatotrophs.

However, it is known that growth hormone secretion is controlled almost entirely by two humoral factors secreted in the hypothalamus and then transported to the AP through the hypothalamic-hypophysial portal system. These are growth hormone-releasing hormone

(GHRH) and growth hormone inhibitory hormone (GHIH), also called somatostatin (SS or SRIF) (Guyton, 1991). The reduced GH release in MSG-treated rats may result from decreased levels of stimulatory GHRH or from increased secretion of SRIF.

Neonatal treatment with MSG is known to cause destruction of the hypothalamic arcuate nucleus (ARC), effective doses produced 80-90% loss of neuron perikarya in the ARC leaving axons in passage and glia cells intact. Adjacent nuclear areas, including the VMN are spared (Olney, 1969; Nemeroff, et al., 1978; Terry, et al., 1981; Millard, et al., 1982; Antoni, et al., 1983). From these findings, the selective effects of MSG have been suggested as a useful tool for studying ARC function in neuroendocrine regulation.

The decreased GH secretion in MSG-treated rats was not due to increased SRIF from the hypothalamus, since the anterior hypothalamic periventricular regions containing SRIF cells still appeared to be intact (Millard, et al., 1982), and despite the destruction of 80-90% of cell bodies within the ARC by neonatal MSG treatment, levels of SRIF within the medial basal hypothalamus were unchanged (Nemeroff, et al., 1977). Millard, et al. (1982) suspected that if a persistent increase in SRIF secretion was the primary cause of the reduced GH levels in MSG-treated rats, it would be expectable that inactivation of circulating SRIF by administration of effective anti-SRIF would produce a marked elevation in GH peak amplitude to levels comparable to those in control animals. However, this was not found in the MSG-treated animal in their study. Thus, alternately, it was suggested that the MSG-treated rat might be dificiency in

hypothalamic GHRH. Consistent with this hypothesis is the fact that the ARC is thought to be a major neural center for GHRH activity. A study of Bloch, et al.(1984) by immunofluorescence technique supported this idea. They presented that GHRH-producing neurons were located mainly in the ARC and MSG treatment resulted in the complete loss of GHRH-immunoreactive cell bodies within this nucleus and prevoked a selective disappearance of GHRH-immunoreactive fiber in the median eminence. Millard, et al. (1982) studied GH secretory mechanism in response to compounds such as morphine sulfate (MS), a potent stimulant to GH release in the rat. Morphine, administered peripherally, was reported to act within the hypothalamus and not on the AP to cause a rapid dose dependent increase in plasma GH levels. Their control animals showed a significant elevation of plasma GH levels at all doses of MS used. On the other hand, MSG-treated animals showed a significant elevation of plasma GH in response to only the high doses of MS (1.0 and 3.0 mg/kg). They suggested that MS might act to facilitate GH release by affecting GHRH but not SRIF release since MS is known to stimulate GH secretion with no effect on hypophysial portal blood levels of SRIF. Therefore, the blunted response to MS in MSG-treated animals reflected the loss of GHRH as the result of ARC damage.

As shown in Table 5 and Figure 5, large does of neonatal MSG treatment resulted in a delayed vaginal opening. This seems to agree with the observations of the other investigators (Pizzi and Barnhart, 1977; Rodriquez-Sierra, et al., 1980). It is believed that during the period from birth to puberty, a neural mechanism is operating to

prevent the normal pulsatile release of LHRH. However, up to the present, the nature of this neural mechanism inhibiting the LHRH pulse is still unknown (Ganong, 1989). Wheaton, et al. (1975) reported that large amounts of LHRH was found in the arcuate-median eminence region, the region that is believed to control most female sexual activity. Since MSG-induced lesion was repeatedly reported to be specific in the ARC in experimental animals when administered during neonatal period, thus delayed vaginal opening may probably result from decreased LHRH production in the ARC. Nevertheless, although serum gonadotrophic hormones were not determined in the present study, it is expectable that the delayed vaginal opening was resulted from a decreased estrogen output, since vaginal opening is known to be triggered by an initial release of large amounts of ovarian estrogen (Pizzi, et al., 1977). It is worthy to realize that a decrease in gonadotropin or gonadal steroid at the time of puberty may result from disturbances of their organ of origin itself. The panhypopituitary dwarf does not pass through puberty and never secrete a sufficient quantity of gonadotropic hormone to develop adult sexual functions (Guyton, 1991). Thus, the delayed puberty in MSG-treated rat might result from an abnormal function of ovary in producing estrogen output and/or abnormal function of pituitary gland in releasing gonadotropin and/or damaging part of the ARC of hypothalamus, a center that produces LHRH.

In the present study, several indicators of MSG-induced reproductive dysfunction were observed in adult rats as well. The MSG-treated female showed an irregular estrous cycle (% regular cycle in

MSG_1 group = 80%, in MSG_2 group = 57% and in MSG_4 group = 0%) whereas all of control animals showed a regular estrous cycle. In MSG_2 and MSG_4 groups, there were found significantly prolonged estrous period and shortened proestrous period. These findings are supported by other investigators who observed some irregularities in the estrous cycle such as prolonged estrus (Matsuzawa, et al., 1979) and decreased incidence of proestrus (Pizzi, et al., 1977) in MSG-treated rat. Bakke, et al. (1978) also reported that the MSG- treated rats had more days of cornification in each cycle than the control.

The cycle alterations of the reproductive system are normally regulated by hormones from the anterior pituitary-gonadal axis. The irregularity in the estrous cycle observed, indicates that gonadotropin release was also impaired after puberty. It is difficult to explain why MSG-treated rats increase the incidence of estrus, but, at least, it implies that the amount of estrogen secreted by ovary was sufficient to initiate vaginal change. However, these animals were not shown any rises a display of lordosis behavior. Rodriquez-Sierra, et al. (1980) reported that ovariectomized MSG-treated rats injected with estradiol benzoate followed by a progesterone injection 2 day later did not exhibit sexual receptivity to male rats while all the control rats displayed lordosis. These support this present findings that, in mating experiments, peroentages of reception were reduced with increases in dosage of MSG treatment. There were only 83.3% and 54% reception in MSG_2 and MSG_4 animals, respectively, while in control and MSG_1, animals were shown 100% reception. It is interesting that MSG-treated rats were observed with decreased incidence of proestrus.

Elias and Blake (1981) studied the characterization of FSH and LH hormone secretion during estrous cycle in rat. The results showed that serum LH concentrations were relatively low throughout the estrous cycle, except for the afternoon and evening of proestrus. In the afternoon of proestrus, serum FSH concentration rose in association with a rise in serum LH levels. It is well accepted that LH is necessary for final follicular growth and ovulation. Without this hormone, even though large quantities of FSH are available, the follicle will not progress to the stage of ovulation.

In MSG-treated rats, serum LH levels were significantly reduced (Clemens, et al., 1978). In addition, Sridaran, et al. (1981) also found that in 4 mg/gm body weight MSG-treated rats, plasma LH levels after ovariectomy were lower than in control animals both in amplitude and frequency of LH pulses. Thus, the decreasing incidence of pro-estrus may relate to decrease in preovulatory surge of LH. This idea is supported by the report of Rodriquez-Sierra, et al. (1980) that percentage of female rat ovulating was significantly reduced if treated neonatally with MSG. Additionally, the histological study of gonads by Paisarn (1987) was reported that the MSG-treated group of animals, forced fed with 4 mg of MSG solution per gm body weight on day 7 of age, showed reduction of number of graafian follicle and corpora lutea which was significantly different from those of the control animals.

In mating of MSG-treated females with normal males, only 33% of pregnancy were shown in MSG_4 group of animals. Consequently, when pregnancy terminated, the MSG-treated female gave significantly smaller litter size. The reduced fertility in females observed here

might be due to a decreased incidence of ovulation and/or number of evagination of ovum when ovulated.

In this present study, the durations of pregnancy were also recorded, nevertheless, no differences between MSG-treated and control groups were found. The observation of the offspring of MSG-treated female rats mated with normal male in terms of the average birth weight of pups MSG_1, MSG_2 and MSG_4 groups of animals showed no significant difference when compared with those in control group. This finding is similar to the report of Bakke, et al. (1978) in which they suggeted that large litter size may be associated with smaller pups.

In this study, both absolute and relative weights of uteruses of all MSG-treated groups were not significantly different from control group. This finding is similar to the studies of Trentini,et al. (1974) and Bakke, et al. (1978) who reported that no significant difference was noted between uterine weights of MSG-treated rats and control rats. In addition, Paisarn (1988) also reported that dry uterine weight of MSG-treated rat showed no significant difference when compared with those of control rat.

As shown in Table 8 and Figure 15, in MSG_4 group were significantly reduced, either in absolute or relative ovarian weights. It is possible that the reproductive dysfuntion found in this study was associated with impaired ovarian function. However, some of MSG_4 rats which had 100% abnormal estrous cycle were fertile. It is interesting that in MSG-treated rats, the ovarian weight was reduced in association with reduction of pituitary gland weight. In MSG_2 and MSG_4 groups were

found significantly reduced pituitary gland in both absolute and relative weight. As discussed above, the reproductive abnormality may result from disturbance of gonadotropin secretion from pituitary gland. Greeley, et al. (1978) found basal FSH level in adult female rats treated with MSG as neonate to be only 60% of that observed in controls, but the difference was not statistically significant. Clemens, et al. (1978) also reported the reduction of pituitary weight and either normal or low basal serum LH levels. However, there have been no evidence that the disturbances in gonadotropin secretion in MSG-treated rat is in the pituitary gland itself. The rise in serum LH concentration in response to LHRH was, in supporting the above conclusion, reported with no diminishment, both in intact and ovariectomized treated with MSG as neonate (Clemen, et al., 1978; Sridaran, et al., 1981).

On the other hand, cyclic regulation of gonadotropin release in rats with marked arcuate neuron damage was seemingly disturbed, as indicated by the disrupted estrous cycle. It is clear that MSG produces lesions in the arcuate nucleus of the hypothalamus (Olney, 1969; Holzwath-Mc Bride, et al., 1967; Tanaka, et al., 1978; Lamperti and Baldwin, 1978; Rodriquez-Sierra, et al., 1983; Katakami, et al., 1984). Therefore, it is possible that the lack of ovarian cyclicity and infertility in most of the MSG-treated rats was due to a disruption in the neural control of tonic secretion of gonadotropins resulting in a poorly developed ovary.