

dark yellow and those of the male white. The ovary was isolated and cut into 2 pieces. One piece (about 1 g) was fixed with Bouin's fluid for the histological examination. The other piece (about 4 g) was homogenized in 40 ml of 70% ethanol with a glass-fitted homogenizer and then centrifuged at  $10000 \times g$  for 30 min at  $4^\circ\text{C}$ . The precipitate was lyophilized and weighed. The weight of the precipitate was considered as the dry weight of the ovary. The supernatant was dried under reduced pressure below  $50^\circ\text{C}$  and the residue was dissolved in 2 ml of distilled water. An equal volume of chloroform was then added to remove the lipids. The aqueous part was withdrawn and lyophilized. The residue was kept in a freezer at  $-80^\circ\text{C}$  until the activity was examined. The respiration-stimulating activity of the sample for the *H. pulcherrimus* spermatozoa was assayed by polarographical measurement of oxygen consumption in a sperm suspension as reported elsewhere<sup>5</sup>. The activity of each sample was expressed by dividing the rate at half-

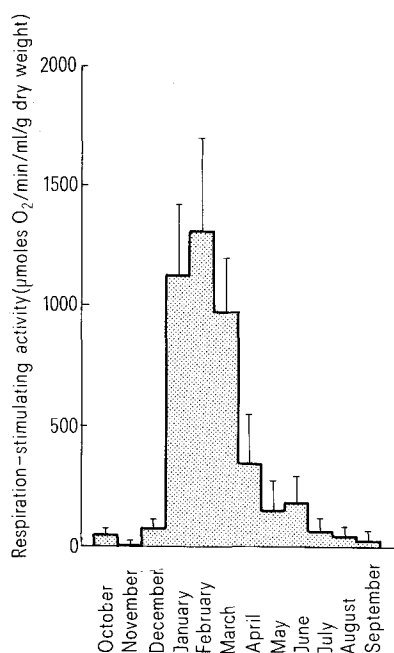


Figure 2. Annual cycle of respiration-stimulating activity in extracts from ovaries of *H. pulcherrimus*. 5 specimens were collected on October 10, November 21, December 11, January 8, February 7, March 10, April 12, May 10, June 9, July 11, August 8 and September 9, respectively. Thin sections of the specimens from October to February were examined under a microscope.

maximal stimulation by the dry weight of the ovary. For histological examination, a fixed piece of an ovary was dehydrated with an ethanol series and embedded in paraffin. Sections  $8 \mu\text{m}$  thick were cut and stained with Mayer's hematoxylin and eosine.

**Results and discussion.** Figure 1 shows cross sections of *H. pulcherrimus* ovaries. From October to December, the ovaries were filled with accessory cells containing globules (fig. 1, A–C). The reproductive season began at the end of January in 1981. In January and February, mature eggs occupied all the space within the ovary (fig. 1, D and E). These morphological changes were essentially the same as those reported by Masuda and Dan<sup>10</sup>. They indicated that ovaries from April to November remained at the same morphological stage. Our interest was in whether these ovaries at different developmental stages had respiration-stimulating activity towards spermatozoa and if not, when this activity appears in the ovary. As shown in figure 2, extracts prepared from ovaries from January to March showed high respiration-stimulating activity, but extracts from ovaries between April and December did not show as much activity. Furthermore, extracts prepared from testes showed no detectable respiration-stimulating activity. These results indicate that the appearance of the respiration-stimulating activity parallels the maturation of the ovary and the peptide is present before the egg is spawned, since no ovaries spawned naturally or artificially in January. Therefore, it is concluded that the peptide is not synthesized by the egg after spawning. At present time, we do not know which cells are responsible for the peptide synthesis, nor how the peptide is transported into the egg jelly coat, but we think that our results will be useful for the study of the biosynthesis of the peptide.

- 1 We thank the Misaki Marine Biological Station for kindly supplying us with specimens. This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.
- 2 Department of Biology, College of General Education, University of Chiba, 1-33 Yayoi-cho, Chiba 280 (Japan).
- 3 Noto Marine Laboratory, Kanazawa University, Ogi, Uchiura, Ishikawa 927-05 (Japan).
- 4 A. Tyler, Exp. Cell Res. 10, 377 (1956).
- 5 H. Ohtake, J. exp. Zool. 198, 303 (1976).
- 6 E. Vasseur and M. Hagström, Ark. Zool. 37A, 1 (1946).
- 7 J.R. Hansbrough and D.L. Garbers, J. biol. Chem. 256, 1447 (1981).
- 8 N. Suzuki, K. Nomura, H. Ohtake and S. Isaka, Biochem. biophys. Res. Commun. 99, 1238 (1981).
- 9 J.R. Hansbrough and D.L. Garbers, J. biol. Chem. 256, 2235 (1981).
- 10 R. Masuda and J.C. Dan, Biol. Bull. 153, 577 (1977).

### Effects of monosodium glutamate on circulating concentrations of luteinizing hormone and growth hormone in young growing domestic fowl (*Gallus domesticus*)<sup>1</sup>

C. G. Scanes and A. Camaratto

Department of Animal Sciences, Cook College, Rutgers, The State University, New Brunswick (New Jersey 08903, USA), 30 June 1981

**Summary.** The administration of monosodium glutamate (MSG) at a dose of 4 g/kg in 5-day-old fowl or 5 daily injections of MSG (total dose 20 g/kg) in 1- to 5-day-old fowl did not affect body growth in either male or female domestic fowl. Neither MSG treatment schedule affected either testis weight or the circulating concentration of luteinizing hormone (LH). A small, but significant decrease in the plasma concentration of growth hormone (GH) was observed in female chicks which had received daily MSG injections.

Postnatal administration of high doses (4 g/kg) of monosodium glutamate (MSG) to laboratory rodents induces lesions in the hypothalamus<sup>2</sup>. In particular, there appears to

be destruction of neurons in the arcuate nucleus<sup>3</sup>. This is accompanied by decreased concentrations of dopamine and an enzyme of cholinergic synapses (choline acetyl trans-

Effect of monosodium glutamate (MSG) on body and gonadal growth and circulating concentrations of growth hormone (GH) and luteinizing hormone (LH)  $\pm$  SEM in young domestic fowl (N = 10 throughout)

Treatment <sup>a</sup>	Sex	Plasma GH concentration (ng/ml) % <sup>b</sup>				Plasma LH concentration <sup>c</sup> (ng/ml)	Body weight at 6 weeks old (kg)	Gonad weight (g)
		at 2 weeks	4 weeks	6 weeks				
Single injection of MSG	M	178 $\pm$ 49	54 $\pm$ 4	34 $\pm$ 5	103 $\pm$ 10.0 (30)	7.7 $\pm$ 0.64 (30)	1.28 $\pm$ 0.045	368 $\pm$ 28
Single injection of vehicle	M	220 $\pm$ 42	52 $\pm$ 14	26 $\pm$ 4	100 $\pm$ 11.9 (30)	7.3 $\pm$ 0.63 (30)	1.36 $\pm$ 0.028	326 $\pm$ 23
Single injection of MSG	F	100 $\pm$ 23	31 $\pm$ 4	17 $\pm$ 2	81 $\pm$ 7.3 (30)	4.8 $\pm$ 0.43 (30)	1.04 $\pm$ 0.020	-
Single injection of vehicle	F	135 $\pm$ 33	39 $\pm$ 6	19 $\pm$ 2	100 $\pm$ 8.6 (30)	5.9 $\pm$ 0.50 (30)	0.99 $\pm$ 0.038	-
Multiple injection of MSG	M	110 $\pm$ 18	79 $\pm$ 9	23 $\pm$ 2	88 $\pm$ 6.5 (30)	6.4 $\pm$ 0.64 (30)	1.25 $\pm$ 0.039	325 $\pm$ 22
Multiple injection of vehicle	M	150 $\pm$ 37	77 $\pm$ 19	26 $\pm$ 2	100 $\pm$ 11.7 (30)	7.6 $\pm$ 0.61 (30)	1.32 $\pm$ 0.032	308 $\pm$ 19
Multiple injection of MSG	F	75 $\pm$ 16,*	27 $\pm$ 12	15 $\pm$ 2	60 $\pm$ 6.3 (30)**	5.2 $\pm$ 0.51 (30)	1.03 $\pm$ 0.027	-
Multiple injection of vehicle	F	140 $\pm$ 26*	46 $\pm$ 5	21 $\pm$ 2	100 $\pm$ 10.8 (30)	5.8 $\pm$ 0.37 (30)	1.01 $\pm$ 0.017	-

<sup>a</sup> Chicks received either a single s.c. injection of MSG (4 g/kg) or its saline vehicle at 5 days post hatching or multiple injections (at days 1, 2, 3, 4 and 5 post hatching) of MSG (total dose 20 g/kg) or its vehicle control. <sup>b</sup> Cumulative GH data expressed as a percentage of the mean GH concentration of the vehicle treated group at each age. <sup>c</sup> Mean LH concentration  $\pm$  SEM (N) from terminal blood samples taken at 2- or 4- or 6-week-old chicks. \* Different  $p < 0.05$ ; \*\*  $p < 0.005$ , by Student's t-test.

ferase) in both the arcuate nucleus and the median eminence. No changes in hypothalamic norepinephrine concentrations are observed. Postnatal MSG injections are followed in rats by elevated circulating concentrations of prolactin in females, no changes in plasma luteinizing hormone (LH) levels and decreased plasma concentrations of growth hormone (GH)<sup>3,4</sup>, the latter due to suppression of episodic secretory pulses<sup>5</sup>. In the domestic fowl, perinatal MSG administration is followed by extensive loss of hypothalamic neurons<sup>6,7</sup>. There is however, no data on the effects of MSG on endocrine parameters in birds. The present communication examines the effect of MSG on growth and the circulating concentrations of LG and GH in growing chicks.

**Materials and methods.** Domestic fowl of a broiler strain were supplied by Cobb Inc. (Concord, MA) at 1 day old. Males and females were reared separately. Chicks were placed into 8 groups of 10. These were maintained in separate ground cages on deep litter and exposed to a 16L:8D photoperiod. Food and water was available ad libitum. The 1st male and female groups of chicks received a single s.c. injection of MSG (4 g/kg) at 5 days old<sup>6,7</sup>. The 2nd groups received injections of the saline vehicle. The 3rd male and female groups had daily s.c. injection of MSG from 1 to 5 days old (total dose 20 g/kg), while the 4th groups received vehicle control.

At 2, 4, and 6 weeks old, the birds were weighed and terminal blood samples were taken from 10 chicks from each group following decapitation. Plasma concentrations of GH and LH were determined by homologous radioimmunoassay<sup>8,9</sup>. At the 6-week sampling time, testicular weights were also determined.

**Results and discussion.** The table summarizes data on the effect of perinatal MSG administration of endocrine parameters in growing chicks. Neither a single nor 5 daily injections of MSG had any observable effects on growth as indicated by body weight at 6 weeks old (table) or at 2 or 4 weeks old (data not shown). Similarly MSG had no effects on reproduction, affecting neither testis weight nor the plasma concentration of LH. There was a consistent trend for the circulating concentration of GH to be depressed following daily injection of MSG or a single injection of MSG to female chicks. Indeed in female chicks, multiple MSG administration significantly depressed the plasma concentration of GH (table). It may be noted that circulating concentrations of GH decreased with age. This result is similar to a number of earlier studies.

Robinson et al. established that a single s.c. injection of 4 g/4 kg MSG at 5 days old was followed by extensive

damage in the chicken hypothalamus. In particular, there appears to be a 87% loss of neurons in the rotundas nuclei and a 63% in an area dorsolateral to the ventromedial hypothalamus (VMH)<sup>6,7</sup>. They observed, however, little effect in ventromedial hypothalamus or elsewhere in the hypothalamus. The lack of a measurable effect of an identical single injection of MSG on any parameter measured suggests that neither the rotundas nuclei or the area dorsolaterally to the VMH are of major importance to the control of GH and LH secretion (and hence of growth and gonadal development). Daily MSG administration may be expected to cause greater damage within the hypothalamus, particularly as the blood-brain barrier for glutamate is not well developed prior to 5 days of age in the fowl<sup>10</sup>. This method of MSG administration did not affect growth, testis weight, or the plasma concentration of LH and had only a small effect on the circulating GH concentration. The latter contrasts with the suppression of GH secretion in MSG-treated rats<sup>3-5</sup>. The absence of a major effect of MSG on GH secretion in the young fowl may be related to the importance of dopaminergic neurons in the hypothalamic control of GH. In MSG-treated rats, the low plasma concentration of GH correlates with decreased dopamine levels in the arcuate nucleus and median eminence. It may be noted that birds have relatively few dopaminergic neurons in their hypothalami<sup>11</sup>.

- 1 Acknowledgments. Paper of Journal Series, New Jersey State Experiment Station, project No. 18141 and 18443, supported by State and Hatch Act funds and grants from the National Science Foundation (PCM 80227227) and the Upjohn Company.
- 2 Burde, R.M., Schainker, B., and Kayes, J., *Nature* 233 (1971) 58.
- 3 Nemeroff, C.B., Konkol, R.J., Bissette, G., Youngblood, W., Martin, J.B., Blazneau, P., Rone, M.S., Prange, A.J., Brees, G.R., and Kizer, J.S., *Endocrinology* 101 (1977) 613.
- 4 Bakke, J.L., Lawrence, N., Bennet, J., Robinson, S., and Bowers, C.Y., *Neuroendocrinology* 26 (1978) 220.
- 5 Millard, W.J., Gordon, K., Martin, J.B. Jr., Audet, J., Sagar, S., and Martin, J.B., *Fedn Proc.* 39 (1980) 980.
- 6 Snapir, N., Robinson, B., and Perek, M., *Path. Eur.* 8 (1974) 265.
- 7 Robinson, B., Snapir, N., and Perek, M., *Poult. Sci.* 53 (1974) 1539.
- 8 Follett, B.K., Scanes, C.G., and Cunningham, F.J., *J. Endocr.* 52 (1972) 359.
- 9 Harvey, S., and Scanes, C.G., *J. Endocr.* 73 (1977) 321.
- 10 Fifkova E., and van Harreveld, A., *Exp. Neurol.* 28 (1970) 286.
- 11 Calas, A., *Adv. biochem. Psychopharmac.* 16 (1977) 79.