with a rubber band. Similarly, at the same temperature, 1st instar nymphs were kept in 6 glass jars (30 × 10 cm) in batches of 40. The crickets were supplied with fresh food daily. The 6th instar nymphs were taken out of the jars for testing.

6 insecticides, namely chlordane, malathion, fenitrothion, pyrethrum, rotenone and sevin were tested (using technical grades). For each insecticide a solution at 1 concentration (0.005%) was prepared with acetone as solvent. Using one ml. of solution, a film of each insecticide was prepared on a round filter paper placed on the bottom of a 500 ml. glass beaker.

After the filter papers had been dried under a ceiling fan for 15 min, 10 nymphs were released in each beaker for 24 h at 28 ± 2 °C.

The beakers were covered with muslin cloth fastened by rubber bands to prevent the escape of the insects. It was ascertained that the insects were in contact with the treated surface all the time and did not crawl on the walls of the beaker. Each concentration was replicated 6 times for insects reared under crowded conditions as well as for nymphs reared in isolation. A separate check was run for each insecticide using acetone alone. For the assessment of toxic effect, mortality counts were taken 24 h after the release of insects in the beaker. The nymphs were examined individually with the naked eye; moribund insects were taken as dead. Mortality in the controls ranged from 0 to 2%. Nymphs which survived were transferred to their respective glass jars and observations were recorded on adult emergence.

Results and discussion. It is evident from the table that insects reared under crowded conditions were more susceptible to all the insecticides than those reared under isolated conditions. Among the insecticides chlordane was found to be the most toxic because the mortality rate was 100% with 'crowded' as well as with 'isolated-reared' insects. Sevin was found least toxic because the mortality rate was nil in insects reared in isolation, while 20% mortality was observed in insects reared under crowded conditions. The decreasing order of the toxicity of the insecticides was: chlordane > malathion > fenitrothion > pyrethrum > rotenone > sevin.

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Diabetic syndrome in the Chinese hamster induced with monosodium glutamate[1]

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Summary. Neuronal necrosis in the arcuate and ventromedial hypothalamus regions is easily induced in 1-day-old Chinese hamsters by the administration of monosodium glutamate (MSG). New-born Chinese hamsters injected with MSG showed no sign of obesity, even when grown up, but apparently developed a diabetic syndrome.

Since the first report by Olney[3] on the neuropathogenic action of monosodium glutamate (MSG) in mice, this amino acid has fascinated many workers in the fields of neurology and endocrinology. There seems to be almost universal agreement that neuronal necrosis is provoked in the preoptic and arcuate nuclei and median eminence regions of MSG-treated mice with subsequent development of obesity[4,5].

During our metabolic and pathological studies of the Chinese hamster treated with MSG, we confirmed occurrence of neuronal necrosis in the brain of the treated animals essentially similar to those described by Olney[3] in mice. In contradiction to the general agreement, however, new-born Chinese hamster injected with MSG showed no sign of obesity, even when grown up, but apparently developed a diabetic syndrome. We describe here the brain lesions and diabetic syndrome found in our experiment. To examine the acute effects of MSG on the brain, 11 litters of 1-day-old Chinese hamsters (random bred in our laboratory) were s.c. injected with the amino acid at a dose of 4 mg/g b. wt or a corresponding volume of 0.85% saline (control). 1–48 hours after the treatment, they were anesthetized and sacrificed for the study. Paraffin sections of the brain were stained with Mayer's hematoxylin and eosin and examined by light microscope.

Histological changes in the brain were observed 1 h after injection of MSG. The majority of neurons around the 3rd ventricle showed enlargement of nuclei. After 6 h, necrotic neurons with pyknotic nuclei were frequently observed. Loss of neurons was noticeable. Almost the whole area of the arcuate nucleus was involved, and the lesion extended to the ventromedial nucleic region (figure 1). Also, many neurons in the cerebral cortex underwent necrotic change. No histological change was found in the brains of control hamsters.

For the study of chronic effects, MSG was s.c. injected to 82 new-born Chinese hamsters at a dose of 4 mg/g b. wt daily during the first 3 days of the neonatal stage, and 73 hamsters were treated with a corresponding volume of saline in a similar way. The MSG-treated (n = 70) and control (n = 68) hamsters were weaned at 3 weeks of age. They were housed in plastic cages, given free access to food and water, and maintained on a 16-h light schedule. Thus, we closely followed up these 138 animals from 3 weeks through 30 weeks of age. All animals were weighed weekly. Urinary glucose was examined by Tes-tape and urinary ketones by Ketostix weekly during the period of experiment.

The first occurrence of glycosuria (3–4 plus) was noticed in 6 out of 70 MSG-treated hamsters at 4 weeks of age. Then, the number of animals with glycosuria increased with age in the treated group. During the period from 13 weeks through 30 weeks of age, heavy glycosuria was demonstrated in 94.3–95.7% of the treated hamsters. However, no

ketonuria was detected in the treated animals during the period of the experiment. No glycosuria nor ketonuria was found in the control hamsters.

Chinese hamsters with glycosuria (n=39) apparently showed hyperglycemia, at overnight fasting levels of 249.5±16.7 (mean±SEM), in contrast to a level of 77.5±2.4 in the controls (n=40). Glucose tolerance curves for the hamsters with persistent glycosuria demonstrated a high attainment with a maximum level of 832 mg/100 ml, and tardy descent, a typical pattern seen in diabetics (figure 2).

Metabolic data were recorded for 7 days during the period from 24 to 26 weeks of age with the aid of metabolic cages for mice. Treated animals, both male and female, consumed an appreciably larger amount of food than controls (1.5–2.0 times). More significant was the case with water consumption (5–7 times). The urine of the treated hamsters was 9–13 times as much as that of controls. However, the weight gain of the treated animals was noticeably smaller than that of controls from 14 weeks through 30 weeks of age. The same held good for body length. Pancreatic islets from the treated hamsters with hyperglycemia were found to undergo pathological changes. The number of islets decreased slightly. Complete or partial degranulation of B cells was one of the most remarkable findings. In most cases, B cells tended to show nuclear hypertrophy, frequently with obscure cell contour and loss of uniformity in nuclear size. Vacuolization of B cells was not uncommon finding. A fairly large amount of glycogen was clearly demonstrated in the corresponding loci by the PAS method (figure 3).

Many workers have described acute damge induced in the hypothalamus of new-born mice[3,4], Syrian hamsters[6] and monkeys[7] after injection of MSG, and the lesions involved a definite area around the arcuate nucleus of the hypothalamus. In mice with the lesion, a marked obesity developed, paradoxically with no hyperphagia[3]. In our study, the brain damge acutely induced with MSG in new-born Chinese hamsters was essentially similar to that described for other animals in its pathological nature. However, the induced areas in the brain of Chinese hamsters extended to the region of ventromedial hypothalamus (VMH), generally accepted as the 'satiety center' and the animals with the lesion conspicuously showed hyperphagia. It is of great interest that no obesity developed in these hyperphagic hamsters. Recent investigations have thrown much light on the mechanism of development of hyperphagia and obesity following VMH injury, and it seems to be a recent tendency

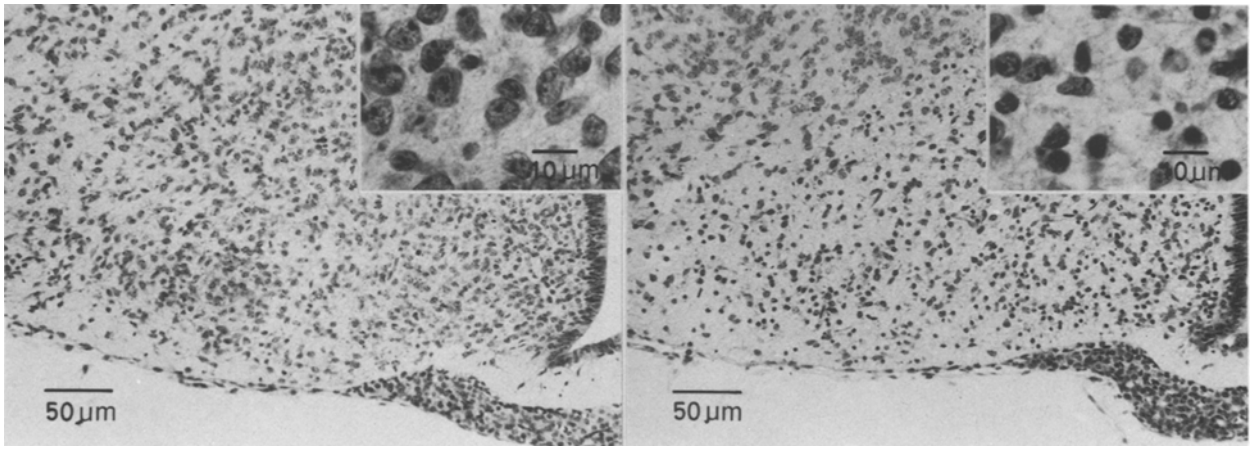

Fig. 1. Hypothalamus of a 1-day-old Chinese hamster sacrificed 6 h after s.c. injection of MSG (right) at a dose of 4 mg/g or a corresponding volume of saline (left). In the hypothalamus from a MSG-treated hamster, the majority of neurons around the 3rd ventricle are necrotic with pyknotic nuclei, and loss of neurons is noticeable. The arcuate nucleus is involved, and the lesion extends to the ventromedial nucleic region. Perfusion fixation through the ascending aorta with saturated picric acid containing 2.4% glutaraldehyde and 4% acetic acid. Hematoxylin and eosin stain. Insets show high power views of neurons in the ventromedial nucleic region.

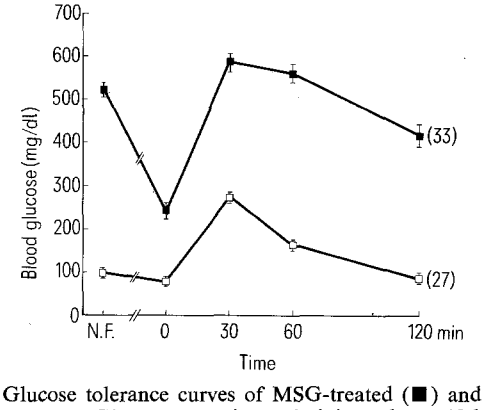

Fig. 2. Glucose tolerance curves of MSG-treated (■) and control (□) hamsters. Glucose was i.p. administered to 18-h fasted hamsters (26–27 weeks of age) at a dose of 1 mg/g b.wt. Blood was taken from the orbital sinus. Arabic figures in the parentheses mean the number of hamsters used. Vertical lines show mean±SEM. N.F. denotes nonfasting level.

to support a view that hyperphagia and obesity are both secondary to the rise in insulin levels following VMH injury[8–10]. Very recently, Inoue et al.[11] have shown that freeing the pancreas from its usual neural influences markedly reduced the effect of VMH lesions on the body weight and food intake of rats.

Cameron et al.[12] described that a diabetic syndrome was experimentally induced in KK mice by the administration of MSG, and glycosuria was detected in approximately 40% of the treated mice together with a marked obesity. They stated that MSG-induced arcuate nucleic lesions may unmask the genetic tendency to diabetes in this strain. In the present study, we have no evidence on which to affirm or deny the effect of unidentified genetic factors on the development of diabetes.

Like et al.[13] noticed B cell degranulation, intracytoplasmic vacuoles with dissolution of cytoplasmic glycogen in spontaneously diabetic, non-ketotic Chinese hamsters. Islet pathology in the MSG-treated hamsters is very similar to their findings.

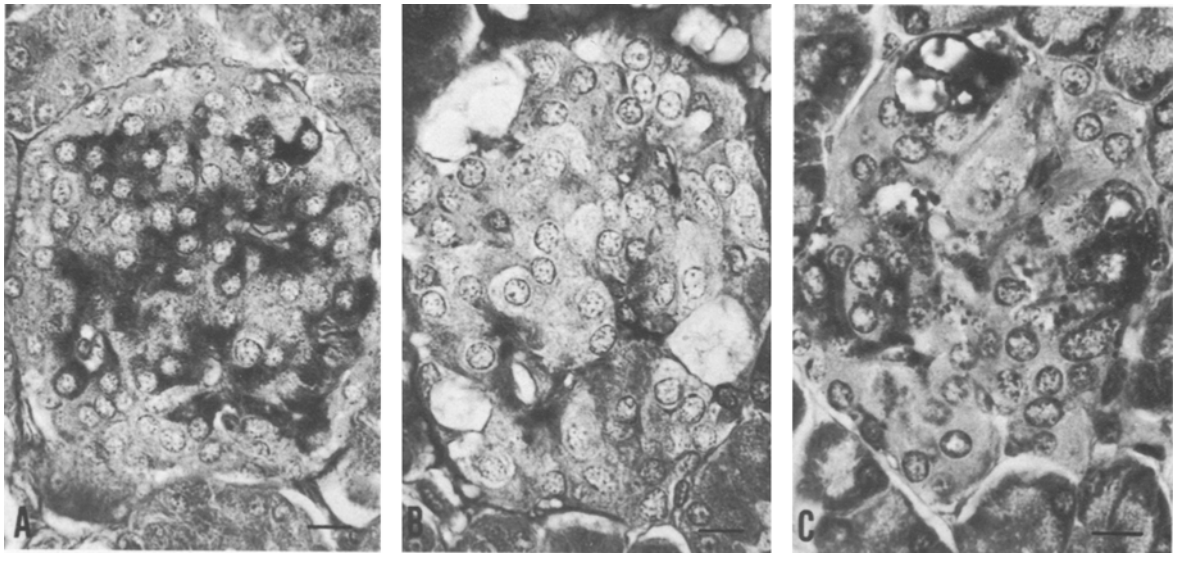

Fig. 3. Pancreatic islets from a normal Chinese hamster (A), and from a MSG-treated hamster (B and C) 30 weeks after the treatment. In the normal pancreatic islet, B cells are well granulated. Islets from the treated hamster show degranulation and vacuolation of B cells, and accumulation of glycogen. Immersion fixation with saturated picric acid containing 2.4% glutaraldehyde and 4% acetic acid. Sections were stained with aldehyde-fuchsin combined with kernechtrot and one-step trichrome (A and B) and the PAS method (C). The horizontal bar represents 10 μm.

The results of the present study show that the VMH lesion is easily induced in new-born Chinese hamsters by the administration of MSG. However, no hypothalamic obesity develops in the VMH-lesioned animals irrespective of their marked increase in food intake. A fact that diabetic syndrome with no obesity is induced with MSG in the Chinese hamster should be emphasized and this new experimental procedure may serve for the study of a possible role of the hypothalamus in the development of diabetes.

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Renal effects of 8-substituted derivatives of adenosine 3′,5′-cyclic monophosphate in dogs[1]

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Summary. The renal effects of nine, 8-substituted derivatives of 3′,5′-cyclic monophosphate were studied in anesthetized mongrel dogs. Infusion of the compounds into a renal artery resulted in an increase of renal blood flow without any effect on blood pressure. Diuretic and natriuretic effects are evident with 6 of the 9 derivatives. As these 8-substituted analogues exert renal effects in a manner similar to that seen with the parent nucleotide, cyclic adenosine 3′,5′-monophosphate, they may serve as useful pharmacological agents in vivo.

Using isolated and purified enzyme systems, several 8-substituted derivatives of adenosine 3′,5′-cyclic monophosphate (cAMP) have been shown to possess activating ability for cAMP-dependent protein kinases of bovine brain[3,4] and rat liver[4], and this activating ability is comparable to, or even surpasses, that of the parent nucleotide cAMP. These derivatives also stimulate glycogenolysis in rat liver slices[4], steroidgenesis in adrenal cells and lipolysis in the isolated rat epididymal lipocyte[5]. Matsubara and Imai[6] reported that these 8-substituted compounds produce definite positive inotropic and chronotropic effects in isolated guinea-pig atria. These in vitro studies suggested that the 8-substituted cAMP derivatives may be capable of producing cAMP-like biological effects in vivo. As cAMP has been shown to be involved in water permeability and electrolyte transport in the kidney[7-11], we carried out renal clearance studies in an attempt to screen the renal pharmacological effects of 9 different 8-substituted derivatives of cAMP.