

result of the reserve of flexibility which the red cells have in these larger vessels rather than of functional physiological significance.

R. SKALAK

Department of Civil Engineering and Engineering Mechanics,
Columbia University, New York 10027

P-I BRÄNEMARK

Laboratory of Experimental Biology,
Department of Anatomy, University of Gothenburg, Gothenburg, Sweden

References and Notes

1. M. I. Gregersen, C. A. Bryant, W. E. Hamnerle, S. Usami, S. Chien, *Science* **157**, 825 (1967).
2. P-I. Bränemark, "Intravascular Anatomy of Blood Cells in Man," in preparation.

3. E. H. Bloch, *Amer. J. Anat.* **110**, 125 (1962).
4. P-I. Bränemark and J. Lindström, *Biorheology* **1**, 139 (1963).
5. P-I. Bränemark, in *Proceedings of the Fourth International Congress on Rheology* (Interscience, New York, 1965), p. 459.
6. M. M. Guest, T. P. Bond, R. G. Cooper, J. R. Derrick, *Science* **142**, 1319 (1963).
7. A. C. L. Barnard, L. Lopez, J. D. Hellums, *Microvascular Res.* **1**, 21 (1968).
8. R. M. Hochmuth and S. P. Suter, *Bibl. Anat.*, in press.
9. H. Wang and R. Skalak, *Office of Naval Research Rep. NR 062-393-1*, Columbia University, New York (1967).
10. P. B. Canham and A. C. Burton, *Circ. Res.* **22**, 405 (1968).
11. Y. C. Fung, *ibid.* **25**, 1761 (1966).
12. ——— and P. Tong, *J. Biophys.* **8**, 175 (1968).
13. P. A. G. Monro, *Bibl. Anat.*, in press.
14. Supported by grants from NIH (HE 5724-08), NSF, and Swedish Medical Research Council. We thank Miss Y. Winsnes for research assistance.

30 January 1969

Brain Lesions, Obesity, and Other Disturbances in Mice Treated with Monosodium Glutamate

Abstract. *In newborn mice subcutaneous injections of monosodium glutamate induced acute neuronal necrosis in several regions of developing brain including the hypothalamus. As adults, treated animals showed stunted skeletal development, marked obesity, and female sterility. Pathological changes were also found in several organs associated with endocrine function. Studies of food consumption failed to demonstrate hyperphagia to explain the obesity. It is postulated that the adult syndrome represents a multifaceted neuroendocrine disturbance arising from the disruption of developing neural centers concerned in the mediation of endocrine function.*

Parenterally administered monosodium glutamate (MSG) produces an acute degenerative lesion in the inner retina of normal neonatal mice (1). Although the acute lesion has been described both light and electron microscopically (2) and several biochemical parameters have been studied (3), the specific mechanisms underlying the effect of MSG on retinal neurons have not been definitively clarified. That MSG treatment might have a similar deleterious effect on neurons in other regions of the central nervous system (CNS) has apparently not been considered. A suspicion that hypothalamic lesions might be associated with glutamate treatment was aroused by the observation that several months after neonatal mice were treated with glutamate, for purposes of inducing retinal pathology (4), they became quite obese. Data establishing that glutamate treatment does induce brain lesions are now presented, and a preliminary characterization is given of a syndrome resulting from glutamate treatment which features obesity as its most striking characteristic.

Ten litters of Swiss albino mice, 2

to 9 days old, were killed from 1 to 48 hours after a single subcutaneous injection of MSG (dosages varied from 0.5 to 4 mg/g), and brains were examined by light microscopy for acute pathology. Brain lesions characterized by intracellular edema and neuronal necrosis developed within a few hours

of treatment at every dose tested, including 0.5 mg/g (Fig. 1a). Certain structures located in a paramedian plane and bordering on the roof and floor of the third ventricle were preferentially affected. At the base of the brain, preoptic and arcuate nuclei of the hypothalamus were selectively destroyed along with scattered neurons within the median eminence (nuclei tuberales). No acute changes were found in other hypothalamic areas or in the pituitary. Dorsally, the subcommissural and subfornical organs and neuronal groups contiguous with them were involved, including the medial habenular nuclei and neurons of the rostral hippocampus (dentate gyrus). Acute lesions were also found in brains of adult mice given high doses (5 to 7 mg/g) of MSG subcutaneously (Fig. 1b). Whether lower dosages than those tested might induce neuronal pathology in either the immature or mature CNS requires further systematic investigation. Brain lesions were also found in the C57BL/6 strain of mice and in albino rats after MSG treatment in the neonatal period.

To study the possibility of long-range effects accruing from glutamate treatment of the neonate I followed five litters of Swiss albino mice, consisting of 38 healthy animals, from birth to 9 months of age. Twenty animals received subcutaneous injections of MSG daily from 1 to 10 days after birth, according to a dose schedule described by Cohen (4); 18 controls received no treatment. All animals were weighed individually on a weekly

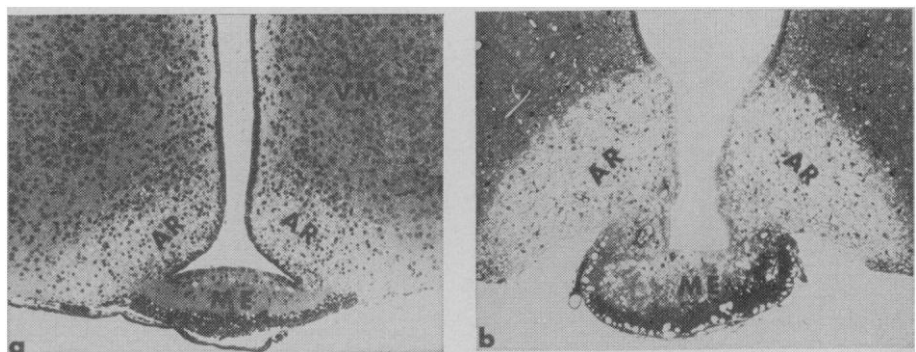


Fig. 1. (a) Section through hypothalamus of 5-day-old Swiss albino mouse showing lesion formation 3 hours after a subcutaneous dose of MSG (1 mg/g). Scattered neurons in the median eminence (ME) are necrotic with bloated cytoplasm and pyknotic nuclei. The majority of neurons in the arcuate nuclei (AR) are necrotic, but those of the ventromedial nuclei (VM) are unaffected ($\times 100$). (b) Section through hypothalamus of adult C57BL/6 mouse 3 hours after a subcutaneous dose of MSG (6 mg/g). The arcuate nuclei (AR) are completely destroyed along with neuronal constituents in the median eminence (ME). Capillary lumina are empty and widely dilated because this animal was killed by perfusion of glutaraldehyde through the ascending aorta ($\times 115$).

basis from the 1st to 5th months, and data on food consumption were gathered weekly on all males for the same period. Animals were given free access to Purina mouse breeder chow in containers designed to avoid food loss by spillage or soiling of food by urine and feces.

Treated animals appeared stunted at termination of treatment on the 10th day after birth and remained smaller than controls on the 30th day. Data on growth presented separately for females (Fig. 2a) and males (Fig. 2b) illustrate that the rate of weight gain for experimental animals was greater than

for controls throughout the period from 30 to 150 days. Treated animals surpassed controls in weight at a mean age of approximately 45 days, and at 150 days the range of weights for treated animals showed no overlap with those of controls. Experimental females in this series gained more weight by comparison to controls of their own sex than did treated males.

Data on food consumption compiled for all males of the five litters studied are included in Fig. 2b for direct comparison with their growth data for comparable periods. Contrary to expectation, treated animals consumed slightly less food than controls in every period for which data were collected. Food consumption was also measured on all animals, both male and female, from the same five litters for 4 hours of unrestricted eating after the 24 hours during which food was withheld. Mean per capita consumption over the 4 hours was 2.5 g for controls and 1.7 g for experimental animals.

Treated animals continued to gain weight on unrestricted diets beyond the age of 5 months (Fig. 3). Despite weight excesses, however, treated animals were approximately 10 percent shorter in mean body length than controls. These differences were reflected in measurements of the long bones and spines of animals x-rayed at 9 months. Treated animals were quite lethargic as adults, and they lacked the sleekness of body coat seen in controls (Fig. 3). The reproductive capacity of treated females was also affected in that they consistently failed to conceive in spite of adequate exposure, from 5 to 9 months of age, to both treated and normal males. Mating of treated males with untreated females resulted in pregnancies and normal offspring.

Autopsies performed at 9 months of age revealed massive accumulations of adipose tissue in experimental animals compared with small to moderate amounts in controls. The livers of treated animals showed fatty changes, and the ovaries contained approximately twice as many atretic follicles as were found in controls. The uteri of treated animals were easily distinguished from controls by their slender, attenuated appearance. The endometrium was thinner and contained only small secretion-poor glands. The testes of treated males were indistinguishable from controls. Findings suggestive of a mild adrenocortical hypertrophy remain under study at this time. The pars nervosa and pars intermedia of pitui-

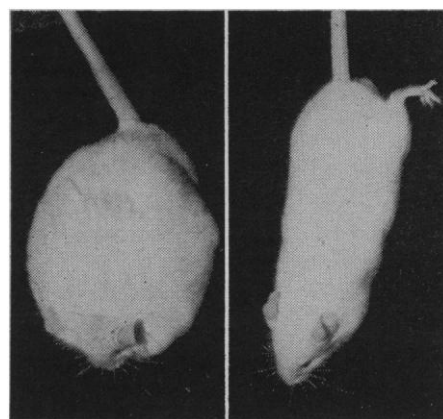


Fig. 3. A 9-month-old Swiss albino mouse (left) which was treated, as a newborn, with MSG is shown beside the heaviest untreated male (right) from the same litter. The experimental animal weighed 84 g compared to 44 g for the control. In addition, the treated animal is shorter than the control, and his body coat is not as sleek as that of the control.

tary glands from experimental animals appeared normal but an overall reduction in mass and in the number of cells was evident in the pars distalis (adenohypophysis).

Three additional litters of Swiss albino mice (10 experimental and 13 control animals) were used to test the obesity-inducing potential of a single subcutaneous injection of MSG (3 mg/g) 2 days after birth. Treated animals in this series were on the average 16.9 g heavier than littermate controls at 9 months of age. However, this represented a more slowly developing and less severe syndrome than was created by daily treatments for the first 10 days of life.

These observations, linking MSG treatment of the neonatal mouse with a syndrome of manifestations, including skeletal stunting, marked adiposity, and sterility of the female, coupled with histopathological findings in several organs associated with endocrine function, suggest a complex endocrine disturbance. In view of the additional finding of lesions in regions of the brain thought to function as neuroendocrine regulatory centers (5), a unitary hypothesis might be constructed relating all or most of the findings to the neonatal disruption of neuronal development in these centers. Since acute degenerative changes were not found in neonatal pituitary glands, the small size of adult pituitary glands from treated animals suggests an interference in some extrapituitary influence (perhaps hypothalamic) on the development of this gland.

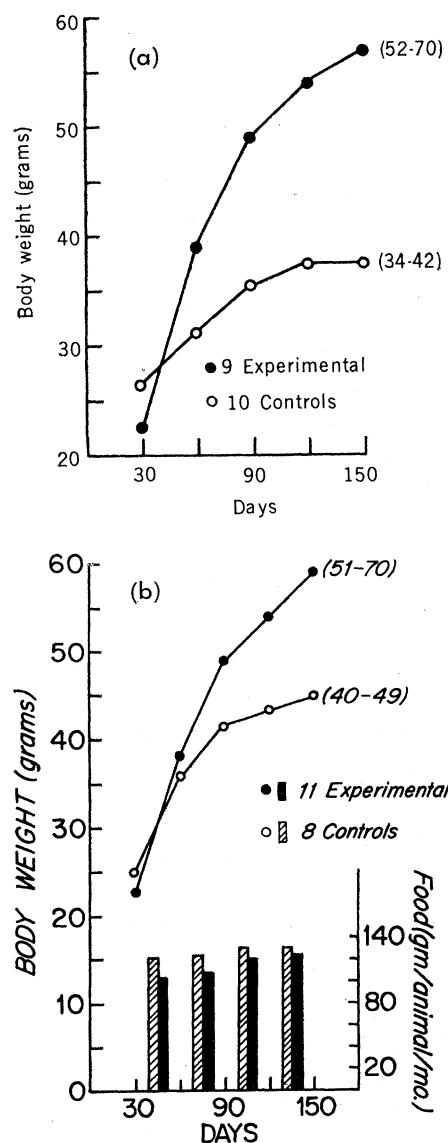


Fig. 2. (a) Composite growth records for experimental and control females from five litters of mice covering the 1st to 5th months of life. Weight ranges on the 150th day are in parentheses. (b) Composite growth records and data on food consumption for experimental and control males from five litters of mice covering the 1st to 5th months of life. Ranges of weight on the 150th day are in parentheses.

Obesity, the most striking clinical manifestation of MSG treatment, has been produced experimentally in mice treated with two other chemical compounds, gold thioglucose (GTG) (6) and bipiperidyl mustard (7). In each case, however, animals were treated in adulthood, lesions were reported in the ventromedial nucleus ("satiety center") of the hypothalamus, and treated animals were considered hyperphagic. In that hypothalamic lesions in MSG-treated animals routinely spared ventromedial nuclei and these animals were consistently hypophagic by comparison with littermate controls, a mechanism other than appetite disturbance must be considered. Whether a regulatory mechanism affecting fat metabolism in the mouse can be localized to the arcuate nucleus, or other brain areas selectively destroyed by MSG treatment, requires further study.

The assumption that MSG is an entirely innocuous substance for human consumption has been questioned recently in view of its role in the Chinese restaurant syndrome (8). The finding that neuronal necrosis can be induced in the immature mouse brain by 0.5 mg/g of MSG raises the more specific question whether there is any risk to the developing human nervous system by maternal use of MSG during pregnancy. The primate placenta maintains amino acids in consistently higher concentrations in the fetal circulation than are found in the maternal circulation, the ratio for glutamic acid being greater than 2:1 (9). In fact, when high

doses of phenylalanine were given to a pregnant rhesus monkey, the ratio of mother to fetus for this amino acid remained unchanged so that exceedingly high fetal blood levels resulted (9). The possibility that brain lesions could occur in the developing primate embryo in response to increased glutamic acid concentrations in the maternal circulation, therefore, warrants investigation.

JOHN W. OLNEY

Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri

References and Notes

1. D. R. Lucas and J. P. Newhouse, *Amer. Med. Ass. Arch. Ophthalmol.* **58**, 193 (1957).
2. J. W. Olney, in press.
3. J. K. Freedman and A. M. Potts, *Invest. Ophthalmol.* **1**, 118 (1962); *ibid.* **2**, 252 (1965).
4. A. I. Cohen, *Amer. J. Anat.* **120**, 319 (1967).
5. E. Scharrer and B. Scharrer, *Neuroendocrinology* (Columbia Univ. Press, New York, 1963).
6. G. Brecher and S. Waxler, *Proc. Soc. Exp. Biol. Med.* **70**, 498 (1949); J. Mayer, *Physiol. Rev.* **33**, 472 (1953); A. H. Perry and R. A. Liebelt, *Proc. Soc. Exp. Biol. Med.* **106**, 55 (1961); R. L. Deter and R. A. Liebelt, *Tex. Rep. Biol. Med.* **22**, 229 (1964).
7. R. J. Rutman, F. S. Lewis, W. D. Bloomer, *Science* **153**, 1000 (1966).
8. H. H. Schaumburg and R. Byck, *N. Engl. J. Med.* **279**, 105 (1968); M. Ambros, N. Leavitt, L. Mormotek, S. Wolsilina, *ibid.*, p. 105; H. H. Schaumburg, R. Byck, R. Gerstl, J. H. Mashman, *Science* **163**, 826 (1969).
9. G. R. Kerr and H. A. Waisman, in *Amino Acid Metabolism and Genetic Variation*, W. L. Nyan, Ed. (McGraw-Hill, New York, 1967), p. 429.
10. Supported in part by PHS grants NB-04816, MH-07081, MH-13002, and MH-38894. I thank Drs. E. Robins, A. I. Cohen, M. Constant, and D. Kipnis for advice, and Miss S. Freeman for the original observation that glutamate-treated mice appeared abnormally fat.

11 March 1969

Deoxyribonucleic Acid Methylase Activity in Pea Seedlings

Abstract. *Deoxyribonucleic acid methylase activity has been detected in a preparation of disrupted nuclei prepared from pea seedlings. S-Adenosyl-L-methionine acted as a donor of methyl groups, and the product of the reaction was identified as 5-methylcytosine. The reaction had a sharp temperature optimum at about 30°C and was unusual in that the DNA methylase was able to methylate DNA in the crude extract.*

5-Methylcytosine is a minor component of the DNA of many organisms (1), but it is a major component of the DNA's of higher plants (2). In bacterial DNA's only about 1 percent and in animal DNA's only about 5 percent of the cytosines are methylated (1), but in plant DNA's between 20 and 30 percent of the cytosine is methylated. In bacteria and in animal tissues, 5-methylcytosine is the product of highly specific enzymes, called DNA methylases, which transfer methyl groups

from S-adenosyl-L-methionine to specific sites in DNA of high molecular weight (3). Despite the superabundance of 5-methylcytosine in plant DNA's, nothing has been known about its biosynthesis. To our knowledge this is the first report of DNA-methylase activity in a higher plant, pea seedlings.

The assay for DNA-methylase activity of pea seedlings was identical with that developed for detection of DNA-methylase activity in extracts of mammalian tissues (4). This assay measures

incorporation of radioactive methyl groups from ^3H -methyl-S-adenosyl-L-methionine into material which survives deproteinization by the Marmur procedure (5), is precipitable in cold 5 percent perchloric acid, and is not degraded by 0.5M NaOH at 60°C. This assay removes protein and RNA that is methylated by other enzymes present in the crude nuclear extracts. In plants deproteinization has the additional virtue of removing some green material that interferes with scintillation counting of the product (Table 1). Most of the radioactivity remaining in acid-precipitable material after this procedure appeared to be in DNA, as it was rendered acid-soluble by treatment with pancreatic deoxyribonuclease (Table 1). The product of the reaction was further characterized by hydrolysis in 90 percent formic acid at 180°C for 30 minutes to generate the free bases,

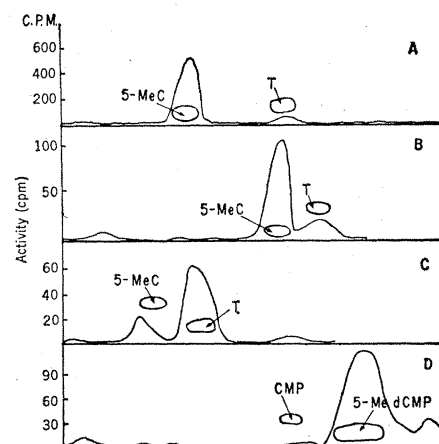


Fig. 1. Chromatography of the methylated product. The reaction mixture and conditions of incubation were the same as those described under Table 1. Product measured by the standard assay procedure described in the text. (A) The product was hydrolyzed at 180°C for 45 minutes with 0.3 ml of 90 percent formic acid and chromatographed in butanol:H₂O:NH₃ for 16 hours. (B) A second sample of the formic acid hydrolyzate was chromatographed in isopropanol:HCl for 20 hours. (C) A third sample of the formic acid hydrolyzate was treated with HNO₃ for 4 hours at 20°C and chromatographed in isopropanol:HCl for 20 hours. (D) The final alkali stable sediment was washed with ether, dried, and then hydrolyzed with 20 µg of pancreatic deoxyribonuclease (Worthington) in 2.5 ml of tris (pH 7.5) 0.01M containing 0.005M MgCl₂ for 3 hours at 37°C. Then 0.3 ml of 0.5M tris (pH 8.5) was added with 210 µg of venom phosphodiesterase (*Crotalus adamanteus*) (Worthington), and the incubation was continued for 3 more hours. The material was dried, resuspended in H₂O, applied to Whatman No. 3 paper, and chromatographed for 16 hours in borate buffer.