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Somatic, Behavioral, and Reproductive Disturbances in Mice Following Neonatal Administration of Sodium L-Aspartate

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PIZZI, W. J., J. M. TABOR AND J. E. BARNHART. *Somatic, behavioral, and reproductive disturbances in mice following neonatal administration of sodium L-aspartate.* PHARMAC. BIOCHEM. BEHAV. 9(4) 481–485, 1978.—Sodium L-aspartate (ASP) was administered to neonatal mice according to an increasing dose schedule from Days 2–11 after birth. Adult ASP-treated animals showed large increases in body weight over controls along with stunted body length. The ASP group also showed decreases in locomotor and exploratory behavior. Reproductive dysfunction occurred in both female and male ASP-treated animals. Among treated animals, females had fewer pregnancies and smaller litters while males showed reduced fertility. Evidence of multiple endocrine dysfunction in ASP-treated animals was reflected by decreased pituitary, thyroid, ovaries and testes weights, along with delayed onset of puberty in females. These results demonstrate that sodium L-aspartate produces a syndrome similar to that seen following the administration of monosodium L-glutamate.

Aspartate Obesity Activity Reproductive dysfunction

NEONATAL administration of the dicarboxylic amino acids, glutamate and aspartate, has been shown to produce destruction of neurons in the central nervous system (CNS), particularly those neurons in the arcuate nucleus of the hypothalamus [3, 6, 7, 8]. Early exposure to both monosodium L-glutamate (MSG) and sodium L-aspartate (ASP) results in CNS lesions which seem to parallel the distribution of the circumventricular organs [9]. Furthermore, the lesions produced by ASP are reported to be indistinguishable from those produced by MSG [8].

Obesity [1,10], stunted body length [1, 4, 6, 10] and reproductive dysfunction [5, 6, 10] have all been found to follow early administration of MSG. Schainker and Olney [13] reported physiological changes in adult mice following neonatal administration of ASP that were identical to the syndrome which occurs following MSG. Their findings included obesity, skeletal stunting, reduced weights of the adenohypophysis and gonads, while histological examination revealed an almost total absence of neurons in the arcuate nucleus. At this time no additional reports of the concomitant developmental, physiological and behavioral abnormalities resulting from administration of ASP have appeared.

This report presents data which support the somatic and endocrine findings reported by Schainker and Olney [13]. In addition to these findings this study shows that ASP-treated adult mice are hypoactive and manifest reproductive dysfunction. These results, along with delayed vaginal canalization in ASP-treated females, indicate an impairment in the hypothalamic-hypophysial regulation of endocrine function and reproductive capacity.

METHOD

Animals

Animals were 123 albino mouse pups from 10 litters ([Ha](ICR) strain, Blue Spruce Farms, Altamont, N.Y.). Animals were born in the laboratory, randomly assigned to either treatment or control groups and were housed in standard polycarbonate cages with their dams. At 30 days of age the animals were sexed and weaned. All animals were fed standard laboratory chow and given water ad lib. The colony was maintained on a 12 hr on/off lighting schedule throughout the experiment.

Drug Treatment

Sodium L-aspartate was administered to all treated animals from days 2–11 of life following the original schedule of Potts, Modrell and Kingsbury [12], which was also used by several other investigators [6, 10, 11, 14] in studying the toxicological properties of monosodium L-glutamate. Essentially this schedule calls for the subcutaneous administration of a gradually increasing dose of sodium L-aspartate from 2.2 mg/g body weight to 4.4 mg/g body weight. Control animals received equal volumes of bacteriostatic saline. The sodium L-aspartate was purchased from K & K Labs Inc., Plainview, New York.

Somatic Evaluation

Following drug treatment animals were weighed every 10 days on an electronic balance. Animals were placed in a paper cup to reduce movements, and weighed to a hundredth

of a gram. Growth curves were followed for 150 days. At the conclusion of all experiments the animals were anesthetized and body length was calculated as the distance from the tip of the snout to the anal orifice.

Reproductive Testing

Female reproductive function was assessed by creating mating environments each composed of 2 control females, 2 ASP-treated females, and 1 control male. Male reproductive function was tested by pairing 1 ASP-treated male with 1 control female. All matings started when the animals reached 150 days of age and ended at 180 days of age. The females were weighed every 7 days and a rapid increase in weight of 5.0 g predicted pregnancy with 100% accuracy. Pregnant females were placed in individual cages.

Behavioral Testing

Male animals were tested on 2 measures of locomotor and exploratory activity starting between 190 and 195 days of age. The first measure utilized the open field test. This measure consisted of placing each mouse in the center of a 76 cm by 76 cm box which was lined off to make up 36 squares. The measure of activity was the total number of squares entered in a 3-min period. The criterion for entrance into a square consisted of the animal putting its 2 front paws into a particular square. Animals were tested once each day for 2 consecutive days and assigned a mean score. The apparatus for the poke test was a modification of that described by Boissier and Simon [2]. It consisted of a black plywood board of 37.5 cm by 37.5 cm by 1.3 cm in which were located 16 equidistant holes with a dia. of 4.5 cm. The board was surrounded by walls which suspended it 35.0 cm above the floor. The walls extended 20.5 cm above the poke board surface. Each animal was placed in the center of the board and the number of pokes recorded over a 5-min period. A poke was defined as the insertion of the animal's head, past the level of its eyes, into any hole. Animals were tested once each day for 3 consecutive days and assigned a mean score. Autopsies were performed when males were between 200 and 280 days old and females were between 220 and 300 days of age. Body weights along with the weights of pituitary, thyroid, testes, ovaries and adrenals were recorded.

RESULTS

Female mice treated neonatally with sodium L-aspartate showed a significant delay in vaginal canalization (39.21 days for ASP-treated females as opposed to 31.11 days for control females, $p<0.001$). At 150 days of age, the last recorded body weight prior to mating (Fig. 1), both male and female ASP-treated animals weighed more than controls (males, Cont.=38.27 vs. ASP=48.12, $p<0.001$; females, Cont.=31.10 vs. ASP=42.35, $p<0.001$). ASP-treated males were less active than control males on both measures of activity (open field: mean scores, Cont.=160, ASP=108, $p<0.05$; poke test: mean scores, Cont.=61, ASP=36, $p<0.02$). All data reported above were statistically analyzed using two-tailed *t*-tests.

Table 1 summarizes the findings on reproductive testing from both ASP-treated female and male mice mated with control animals of the opposite sex. ASP-treated females became pregnant less often than control females; however, neither litter size nor birth weight were significantly different. The lack of a significant effect on litter size is most likely due to the small number of ASP-treated females who became pregnant. Experimental males did not impregnate control females as often as did control males. Offspring were weighed every 5 days until day 30. The offspring of ASP-treated males had lower body weights at birth and at 30 days

TABLE 1

SODIUM L-ASPARTATE AND REPRODUCTION

Number of Pregnancies		Litter Size (Mean)		Mean Birth Weight (g)		Mean Weight at 30 days of age (g)	
ASP & Control Females Mated with Control Males							
Controls	ASP	Controls	ASP	Controls	ASP	Controls	ASP
16/16	2/16	12.18 (±0.96)	6.00 (±2.00)	1.62 (±0.01) N=194	1.62 (±0.03) N=6	20.09 (±0.84) N=127	NA*
$p<0.005$ Chi Square		NS Mann-Whitney		NS *t*-test			
ASP & Control Males Mated with Control Females							
Controls	ASP	Controls	ASP	Controls	ASP	Controls	ASP
8/8	3/14	11.50 (±1.47)	11.00 (±1.52)	1.65 (±0.01) N=91	1.47 (±0.02) N=33	19.21 (±0.40) N=69	16.87 (±1.07) N=19
$p<0.0005$ Fisher's		NS Mann-Whitney		$p<0.01$ *t*-test two-tailed		$p<0.02$ *t*-test two-tailed	

SEM in parentheses

*All offspring were killed or abandoned by the mothers

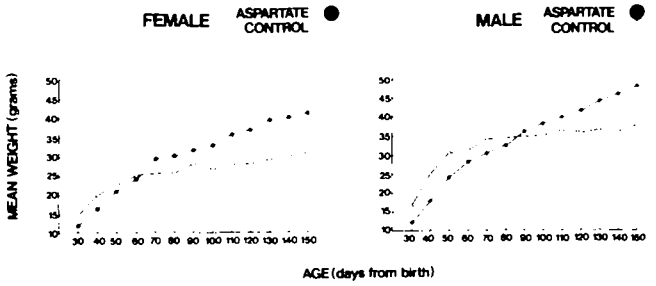

FIG. 1. Mean weights of ASP-treated and control groups plotted in 10-day intervals.

of age. No data were obtained for offspring of ASP-treated females following the initial birthweight, since these pups were either killed by their dams or left to die.

Table 2 shows the mean body weights, body lengths and mean weights of the endocrine glands at autopsy. ASP-treated males had significantly heavier body weights, shorter body lengths, along with significantly lower weights of pituitary, thyroid and testes when compared with control males (all findings significant at $p<0.001$). ASP-treated females showed the same pattern with smaller and lighter ovaries ($p<0.01$). Neither group showed differences in adrenal gland weights when compared with controls. All ASP-treated groups showed large accumulations of adipose tissue throughout the body (Fig. 2).

DISCUSSION

This report demonstrates that sodium L-aspartate administered to neonatal mice leads to a number of somatic, behavioral, and reproductive disturbances in the adult animal. The syndrome includes obesity and stunted body length, along with significantly reduced weights of pituitary, thyroid, ovaries and testes. The ASP-treated females had a delayed onset of puberty as measured by the occurrence of vaginal canalization and displayed reproductive deficits with significantly fewer pregnancies, and a reduced number of offspring following a successful pregnancy. The fact that the 2 ASP-treated females either killed off or abandoned their pups is of potential interest but may not be specific to the drug treatment, since a sample of only 2 ASP-treated females is too small to draw any definite conclusions. We have not seen this pattern of offspring neglect in MSG-treated animals, and it may represent a normal process of species preservation. ASP-treated males also showed reproductive deficits by impregnating significantly fewer females under controlled mating conditions. At this time no apparent explanation is offered for the reduced body weights at birth and 30 days of age in the offspring of ASP-treated males. Testing of male animals on 2 tasks of locomotor and exploratory behavior showed reduced levels of activity in ASP-treated males on both tasks. Furthermore, the increased lethargy in ASP-treated males shows a marked similarity to our previous findings with MSG-treated animals [10].

The occurrence of reproductive deficits in both the female and male mouse along with the reduced endocrine gland weights strongly suggest that sodium L-aspartate administered during the neonatal period results in hypothalamic damage leading to multiple endocrine dysfunction. The multiple endocrine dysfunction hypothesis can account for many of the other findings in this report including obesity, stunted body length, and hypoactivity. Further, the striking similarity between these data and our previous findings with monosodium L-glutamate [10,11] suggest that the various dysfunctions which follow the administration of these amino acids most probably result from the same mechanism. This conclusion is further supported by the detailed anatomical studies of Olney and his colleagues [8,13] showing that both MSG and ASP produce indistinguishable hypothalamic lesions.

While the dose schedule used in this study was designed to maximize the occurrence of toxic effects, there is evidence that lower doses can also have toxic consequences.

TABLE 2

SODIUM L-ASPARTATE AND REPRODUCTION

	Control (±SEM)	ASP-Treated (±SEM)	
Mean Autopsy Weights of Endocrine Glands ASP-Treated and Control Males			
Body weight (g)	35.66 (±1.30)	48.97 (±1.83)	$p<0.001$
Body length (mm)	107.16 (±1.27)	100.09 (±0.74)	$p<0.001$
Pituitary (mg)	2.03 (±0.14)	0.74 (±0.08)	$p<0.001$
Thyroid (mg)	3.48 (±0.14)	2.16 (±0.19)	$p<0.001$
Testes (mg)	293.16 (±9.92)	198.47 (±8.10)	$p<0.001$
Adrenals (mg)	5.63 (±0.23)	5.78 (±0.31)	NS
Mean Autopsy Weights of Endocrine Glands ASP-Treated and Control Females			
Body weight (g)	31.72 (±0.70)	48.37 (±1.26)	$p<0.001$
Body length (mm)	109.10 (±0.48)	99.40 (±0.66)	$p<0.001$
Pituitary (mg)	2.40 (±0.09)	0.97 (±0.08)	$p<0.001$
Thyroid (mg)	3.75 (±0.07)	2.06 (±0.11)	$p<0.001$
Ovaries (mg)	14.55 (±1.39)	9.31 (±0.93)	$p<0.01$
Adrenals (mg)	7.87 (±0.31)	7.39 (±0.33)	NS

All p values are two-tailed (t-test)

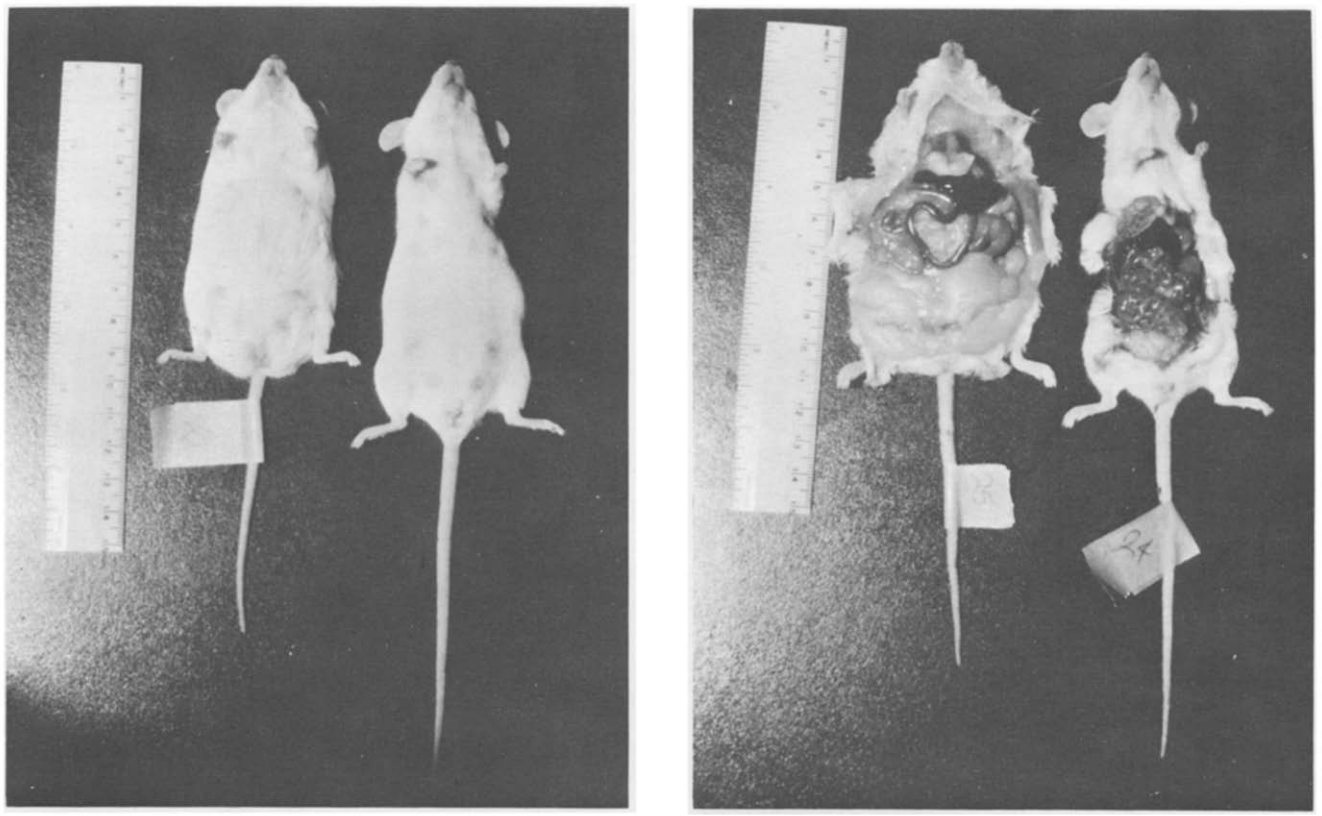

FIG. 2. (Left) Randomly selected female mice. The control female mouse (right) was treated with the control vehicle from Days 2–11 of life, while the obese mouse (left) was treated with ASP from Days 2–11. (Right) Randomly selected female mice treated according to the above description. The ASP-treated animal (left) shows an extreme accumulation of adipose tissue and reduced body length.

Olney and Ho [8] have shown that a single oral administration (by feeding tube) of MSG at a dose of 0.5 mg/g body weight produces damage to the arcuate nucleus of the hypothalamus in the neonatal mouse. Having found that a 0.5 mg/g dose of MSG combined with a 0.5 mg/g dose of ASP administered orally to neonatal mice results in the same degree of hypothalamic damage as that seen after a 1 mg/g dose of either substance administered alone, Olney and Ho [8] concluded that ASP and MSG are additive in their neurotoxic effects. Since both of these amino acids are used or are proposed for regular use in frequently consumed foods and beverages (ASP is 1 of 2 components of a new sweetening agent which has been proposed as a replacement for saccharin), well designed parameter studies should be conducted in several independent laboratories, aimed at determining the safety factor for these substances in both immature and mature organisms, along with any homergistic effects which may result from their combined intake.

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REFERENCES

1. Araujo, P. E. and J. Mayer. Activity increase associated with obesity induced by monosodium glutamate in mice. *Am. J. Physiol.* **225**: 764–765, 1973.
2. Boissier, J. R. and P. Simon. Dissociation a deux composantes le comportment d'investigation de la souris. *Arch. int. Pharmacodyn. Ther.* **147**: 372-387, 1964.
3. Lemkey-Johnston, N. and W. A. Reynolds. Nature and extent of brain lesions in mice related to ingestion of monosodium glutamate: A light and electron microscope study. *J. Neuropath. exp. Neurol.* **33**: 74–97, 1974.
4. Matsuyan, S. Studies on experimental obesity in mice treated with monosodium glutamate. *Jap. J. vet. Sci.* **32**: 206, 1970.
5. Nagasawa, H., R. Yanai and S. Kikuyama. Irreversible inhibition of pituitary prolactin and growth hormone secretion and of mammary gland development in mice by monosodium glutamate administration neonatally. *Acta endocr.* **75**: 249–259, 1974.
6. Olney, J. W. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science* **164**: 719–721, 1969.
7. Olney, J. W. Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. *J. Neuropath. exp. Neurol.* **28**: 455–474, 1969.
8. Olney, J. W. and O. L. Ho. Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature, Lond.*, **227**: 609–610, 1970.

9. Olney, J. W., V. Rhee and T. DeGubareff. Neurotoxic effects of glutamate on mouse area postrema. *Brain Res.* **120:** 151–157, 1977.
10. Pizzi, W. J. and J. E. Barnhart. Effects of monosodium glutamate on somatic development, obesity, and activity in the mouse. *Pharm. Biochem. Behav.* **5:** 551–557, 1976.
11. Pizzi, W. J., J. E. Barnhart and D. J. Fanslow. Monosodium glutamate administration to the newborn reduces reproductive ability in female and male mice. *Science* **196:** 452–454, 1977.
12. Potts, A. M., K. W. Modrell and C. Kingsbury. Permanent fractionation of the ERG by sodium glutamate. *Amer. J. Ophthal.* **50:** 900–907, 1960.
13. Schainker, B. and J. W. Olney. Glutamate-type hypothalamic-pituitary syndrome in mice treated with aspartate or cysteate in infancy. *J. Neural Trans.* **35:** 207–215, 1974.
14. Trentini, G. P., A. Botticelli and C. S. Botticelli. Effect of monosodium glutamate on the endocrine glands and on the reproductive function of the rat. *Fert. Steril.* **25:** 478–483, 1974.