FULL PAPER *Pathology*

Neurotoxicity Induced by a Single Oral Dose of Aniline in Rats

Yoshimasa OKAZAKI[1)*], Kotaro YAMASHITA[1)], Masato SUDO[1)], Minoru TSUCHITANI[1)], Isao NARAMA[2)], Ryoji YAMAGUCHI[3)] and Susumu TATEYAMA[3)]

[1)]Mitsubishi Chemical Safety Institute Ltd., 14 Sunayama, Hasaki, Kashima, Ibaraki 314–0255, [2)]Research Institute of Drug Safety, Setsunan University, 45–1 Nagaotohge-cho, Hirakata, Osaka 573–0101, and [3)]Department of Veterinary Pathology, Faculty of Agriculture, Miyazaki University, 1–1 Gakuenkibana-dai Nishi, Miyazaki 889–2155, Japan

(Received 7 November 2000/Accepted 29 January 2001)

ABSTRACT. The neurotoxicity of aniline and its age-dependent responses were investigated in male rats. Groups of 6 rats, 4-week-old, were treated once with aniline (500, 750 or 1,000 mg/kg) or olive oil by gavage. Additional groups of 6 rats, 7- or 10-week-old, were treated once with 800 mg/kg of aniline or olive oil. Paralytic gait or hindlimb paralysis was observed between post-treatment days 8 and 15 in two out of six rats receiving 1,000 mg/kg of aniline at 4 weeks of age. On post-treatment day 15, spongy change in the white matter of the spinal cord was observed in all rats receiving 750 or 1,000 mg/kg of aniline at 4 weeks of age. The lateral and ventral columns of the thoracic spinal cord were the most severely affected. Spongy change in the facial nerve and spinal trigeminal tracts of pons and medulla oblongata, and mild degeneration of the peripheral nerves was found in 3 out of 6 rats receiving 1,000 mg/kg of aniline. At the ultrastructural level, the spongy change was due to distention of the myelin sheath and splitting of the intraperiod line. Axons were well preserved in the affected nerve fibers. No abnormalities were seen in the neuronal cell bodies. Although transient cyanosis was observed in all rats receiving 800 mg/kg of aniline at 7- or 10-week-old, as well as in rats receiving 750 or 1,000 mg/kg of aniline at 4-week-old, no treatment-related neurobehavioral or morphologic abnormalities were found in the former. These findings demonstrate the neurotoxicity of orally administered aniline for rats, depending upon the age of the animal at the time of administration.

KEY WORDS: aniline, hindlimb paralysis, neurotoxicity, rat, spongy change with myelin vacuolation.

J. Vet. Med. Sci. 63(5): 539–546, 2001

Aniline is one of the oldest and most widely used industrial chemicals, and is used primarily as an intermediate in the synthesis of other organic compounds, such as rubber processing chemicals, antioxidants, dyestuffs, photographic chemicals, pharmaceuticals, and agricultural chemicals [12, 15].

Several toxic effects of aniline have been reported in humans and laboratory animals [8, 12, 29]. Symptoms of aniline toxicity in humans following acute exposure include cyanosis due to methemoglobinemia, headache, nausea, vomiting, confusion, vertigo, tinnitus, ataxia, weakness, numbness in dactyls, lethargy, drowsiness or coma [8, 29]. Symptoms following chronic exposure include loss of appetite, decreased body weight, headaches, visual disturbances, and skin lesions [29]. Additionally, toxic effects of aromatic amines including aniline to the nervous system was reported and called Excitement-Hypotony Syndrome [19]. Although there are some evidences for central nervous symptoms in aniline intoxication as described above, the details are unclear.

In toxicologic studies using experimental animals, the major acute toxic effects of aniline have been reported as methemoglobinemia and hemolysis [12, 13]. In chronic toxicity and carcinogenicity studies of rats, the following toxic effects were also observed: suppression of body weight gain [7]; decrease in steroidogenesis and reduced amount of lipid accumulation in the ovaries and adrenal glands [10, 16]; hemosiderosis in the liver and kidneys; splenic fibrosis; increased incidence of endometrial stromal polyps, splenic fibrosarcomas, and hemangiosarcomas [6, 7, 12].

It has been reported that nitrobenzene, the raw material for aniline production, induced encephalopathy in rats [5, 21]. The anilides, aniline-derivatives, also induced paralysis and vacuolation of the white matter of the cerebellum and spinal cord in rabbits [24]. However, the influence of aniline itself to the nervous system has not been fully examined in animals.

The purpose of this study was to identify possible neurotoxicities in rats treated once with aniline by gavage. Since susceptibility to the toxic effects of several neurotoxicants is often age-dependent [18], three different age groups of rats, 4, 7 and 10 weeks, were used in this study.

MATERIALS AND METHODS

Chemicals: Aniline ($C_6H_5NH_2$, 99% pure, Lot No. 10521BR, CAS no. 62–53–3) was obtained from Aldrich Chemical Company Inc. (Milwaukee, U.S.A.). The purity was determined to be 99.9% by gas liquid chromatography, and the contamination of nitrobenzene was less than the limit of detection (≤ 0.001%; Certificate published by Aldrich Chemical Company Inc.). Olive oil was purchased from Maruishi Seiyaku Co., Ltd., (Osaka, Japan) and used as the vehicle solution. The dosing solutions were prepared immediately prior to application, mixing aniline with olive oil.

* CORRESPONDENCE TO: OKAZAKI, Y., Mitsubishi Chemical Safety Institute Ltd., 14 Sunayama, Hasaki, Kashima, Ibaraki 314–0255, Japan.

Table 1. Treatment groups

Group	Dose level (mg/kg)	Age of dosing (week)	Group size
I	0	4	6
II	500	4	6
III	750	4	5(1)[a]
IV	1000	4	6
V	0	7	6
VI	800	7	6
VII	0	10	6
VIII	800	10	6

a) One out of 6 rats was omitted from statistical analysis of this study, because of the accidental death.

Animals: Three-week-old, male Crj:CD(SD)IGS rats were obtained from Japan Charles River Inc. (Kanagawa, Japan). The animals were housed in polycarbonate cages (2 rats/cage), and the animal room was maintained at a temperature of 20°C to 24°C, with a relative humidity of 40% to 70%, and a 12-hr light/dark cycle. The rats were fed a standard laboratory diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and sterilized water *ad libitum*. Healthy rats were used for the study after an acclimation period of at least 5 days.

The animals were cared for according to the principles outlined in the guide for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our institution.

Treatment of aniline, clinical observation, and body weight: Forty-eight rats, 4 weeks of age, were stratified by body weight, and were randomly assigned to 8 experimental groups (six rats per group) to create similar initial body weights. Treatment groups and experimental design are shown in Table 1. Four of these groups (Groups I to IV) were orally treated once with olive oil, 500, 750, 1,000 mg/kg of aniline, respectively. The dosage level of 1,000 mg/kg was estimated as the maximum tolerate dose at which acute death did not occur in rats receiving aniline (personal data). Two groups (Groups V and VI) given a single oral dose of 800 mg/kg of aniline or olive oil at 7 weeks of age. The remaining groups (groups VII and VIII) were orally treated once with 800 mg/kg of aniline or olive oil at 10 weeks of age. The dosage level of 800 mg/kg in Groups VI and VIII was estimated as the maximum dose at which the majority of animals escaped from acute death in each age group, although rats showed cyanosis, prone position, and decrease in spontaneous activity (personal data).

All rats were carefully observed for mortality and clinical signs at 30 min, 1 hr, and 3 hr after dosing and once a day thereafter for the following 14 days. Body weights were recorded before the dosing and on post-treatment days (days) 3, 7, 10, 13, and 15. In Group III, one rat died on day 1, due to the technical error of dosage. Therefore, this animal was omitted from evaluation in this study.

Pathologic examination: All surviving rats were killed on day 15, under deep anesthesia by an intraperitoneal injection of sodium pentobarbital. The rats were killed by exsanguination from the right atrium and perfused via the left ventricle with phosphate buffer solution (PBS) containing heparin (1,000 IU/*l*), followed by reperfusion with PBS containing paraformaldehyde (4%) and glutaraldehyde (1%). PBS (50 m*l*/rat) and fixative (100 m*l*/100 g body weight) were fed from containers held at 150 cm above the animal. After perfusion, central and peripheral nervous tissues were post-fixed in the same fixative over 24 hr. Cross or sagittal sections of the following tissues were collected and examined: cerebral cortex, basal ganglia, hippocampus, thalamus, hypothalamus, midbrain, cerebellum, pons, medulla oblongata, trigeminal nerve including Gasserian ganglion, spinal cord with dorsal roots, ventral roots and dorsal root ganglia. In addition, the transverse sections of the proximal portion of both sciatic and tibial nerves, and the longitudinal sections of the consecutive portion of each nerve were examined. The tissue samples of the spinal cord were collected from cervical (C3-C7), thoracic (T5-T10) and lumbar (L1-L3) vertebrae. Skeletal muscle tissue obtained from the gastrocnemius was additionally examined in Groups I and IV. The samples were processed for paraffin embedding, sectioned at 5 μm in thickness, and stained with hematoxylin and eosin (HE) or double-stained with luxol fast blue and HE (LFB-HE). For electron microscopy, the tissue fragments from the spinal cord of thoracic (T8) vertebrae were fixed in a paraformaldehyde (4%) and glutaraldehyde (1%) solution for approximate 24 hr, and postfixed in 1% osmium tetroxide for 2 hr. These tissue samples were embedded in epoxy resin. Semithin sections were stained with toluidine blue. Ultrathin sections stained with uranyl acetate and lead citrate were observed using a transmission electron microscope at 80 kV (JEM-100CX II, JEOL Ltd., Japan).

Statistical methods: Body weight data in Groups I to IV was analyzed by multiple comparison tests. They were first analyzed by Bartlett's test. If the group variance was determined to be homogenous, all groups were compared by a one-way analysis of variance. If Bartlett's test indicated heterogeneous variance, the Kruskal-Wallis test was employed, and Dunnett's test was used when there was a significant difference between the groups. Student's t test was employed for the analysis of body weight data between Groups V and VI, or between Groups VII and VIII. Results of body weights were expressed as mean ± standard deviation (S.D.). The histopathological data was analyzed by a x b Chi-square test, and when there was a significant statistical difference, Armitage's Chi-square test was used to compare the difference between each treatment group and the age-matched control group. Values of $p<0.05$ were considered significant for these statistical tests.

RESULTS

Clinical signs and body weight: All rats that received 750 mg/kg (Group III) or 1,000 mg/kg (Group IV) of aniline showed cyanosis between days 2 and 4 or days 2 and 5, respectively. Paralytic gait was observed in two rats of Group IV between days 8 and 15, and especially, one rat out

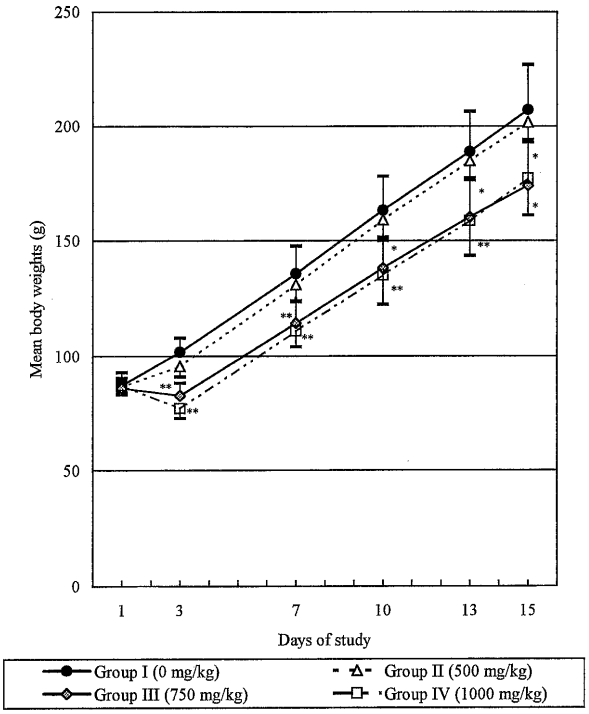

Fig. 1. Mean body weights of Groups of rats that received 0 (I), 500 (II), 750 (III), or 1,000 (IV) mg/kg of aniline at 4 weeks of age. Error bars reveal the standard deviation. * $p<0.05$, ** $p<0.01$ compared with that of control (Group I), by multiple comparison test.

of them showed hindlimb paralysis between days 9 and 13. Forelimbs were not clinically affected in any aniline-treated rats. No apparent neurobehavioral abnormalities were found in Groups I, II, or III.

All rats in Groups VI and VIII showed cyanosis between days 2 and 5. This sign had completely disappeared by day 6. One animal of Group VIII died on day 3, without recovery from acute toxic symptoms such as cyanosis, prone position, and decrease in spontaneous activity. No apparent neurobehavioral abnormalities were found in Groups V, VI, VII, or VIII.

Mean body weights of Groups III and IV between days 3 and 15 were significantly lower than those of Group I (Fig. 1). There were no significant differences between Groups I and II. Mean body weights of Groups VI and VIII between days 3 and 13 were significantly lower than those of each control group, but the significant differences disappeared on day 15 (Fig. 2).

Light and electron microscopic changes: Spongy change in the white matter of the spinal cords was observed in all rats that received 750 (Group III) or 1,000 (Group IV) mg/kg of aniline (Table 2). The lesions were present throughout the whole length of the spinal cord and distributed symmetrically in all levels of the spinal cord. The thoracic level was more severely affected than the cervical and lumbar levels. The severity of the spongy change in the spinal cord was classified into 3 grades as follows: severe, lateral and ventral columns extensively presented spongy state (Fig. 3a), and the lesions in the dorsal column were milder than those of other columns; moderate, spongy change was located mainly in the outer ascending tracts, such as lateral and ven-

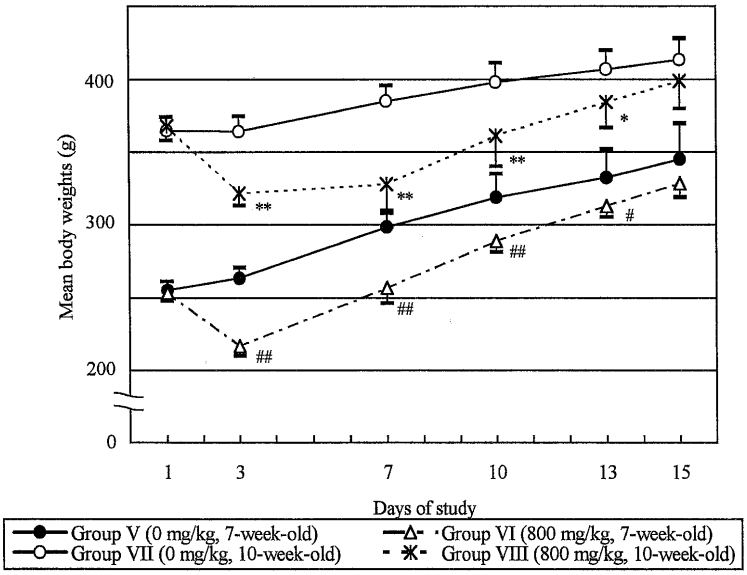

Fig. 2. Mean body weights of Groups of rats that received 0 (V) or 800 (VI) mg/kg of aniline at 7 weeks of age, and 0 (VII) or 800 (VIII) mg/kg of aniline at 10 weeks of age. Error bars reveal the standard deviation. # $p < 0.05$, ## $p < 0.01$ compared with that of age-matched control group (Group V), by Student's *t* test; * $p<0.05$, ** $p<0.01$ compared with that of age-matched control group (Group VII), by Student's *t* test.

Table 2. Distribution, severity and incidence of central and peripheral nervous lesions in rats receiving aniline or olive oil at 4-week-old

Tissues and findings	Group / Dosage level (mg/kg) / Number of animals examined		I 0 6	II 500 6	III 750 5	IV 1000 6
Pons and Medulla oblongata	Spongy change	+[a)]	0[b)]	0	0	2
Spinal cord Cervical	Spongy change	+	0	0	2	3(**)
		++	0	0	0	2
		+++	0	0	0	1
Thoracic	Spongy change	+	0	0	2(**)	0(**)
		++	0	0	3	4
		+++	0	0	0	2
Lumbar	Spongy change	+	0	0	2	2(*)
		++	0	0	0	3
Trigeminal nerves	Nerve fiber degeneration	+	0	0	0	3(*)
Sciatic nerves	Nerve fiber degeneration	+	0	0	0	3(*)
Tibial nerves	Nerve fiber degeneration	+	0	0	0	3(*)

a) Histologic grades: +; slight or sporadic, ++; moderate or multifocal, +++; severe or diffuse.
b) Numerals represent the number of animals bearing the lesion.
(*) $p<0.05$, (**) $p<0.01$ compared with that of age-matched control group (Group I), by Armitage's Chi-square test.

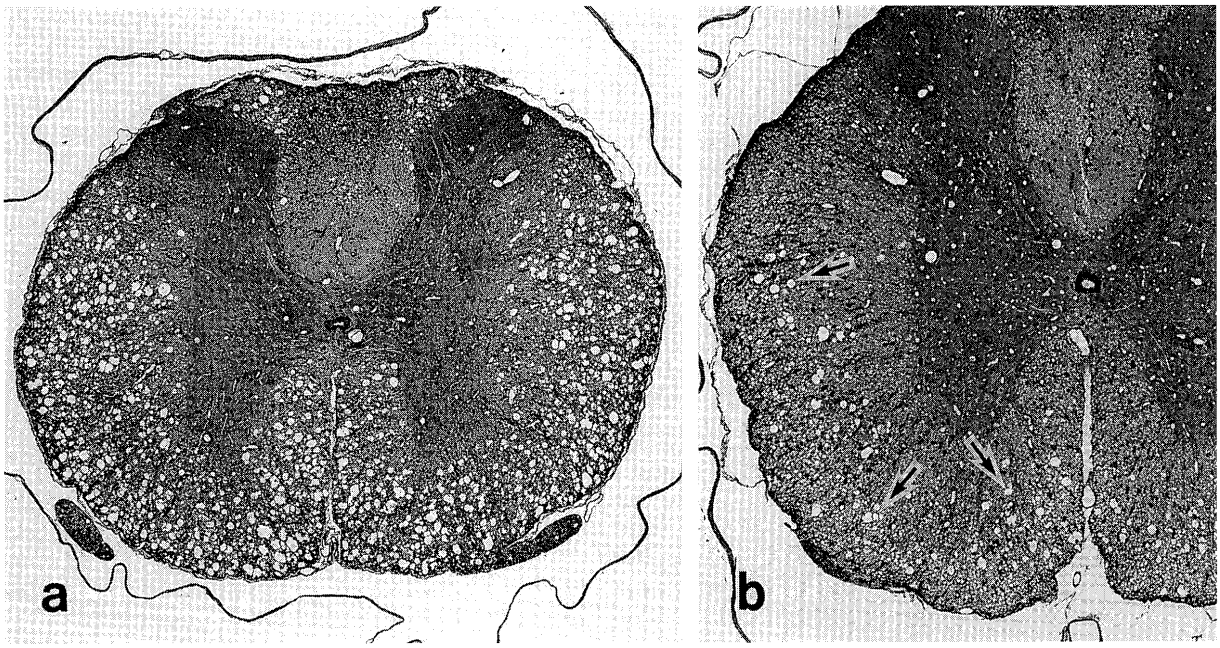

Fig. 3. Photomicrographs of the thoracic spinal cord of rats that were treated once with aniline at 4 weeks of age; post-treatment day 15.
(a) Severe spongy change extended to all columns of the white matter in a rat receiving 1,000 mg/kg of aniline (Group IV). HE. × 50.
(b) Vacuoles were seen in the outer, ascending tracts in both lateral and ventral columns (arrows) of the white matter in a rat receiving 750 mg/kg of aniline at 4 weeks of age (Group III). HE. × 70.

tral spinothalamic tracts, spinotectal tracts or ventral spinocerebellar tracts (Fig. 3b); slight, only a few vacuoles were found in the same tracts described in moderate lesion. In the severe lesions, there were various shape and size of the vacuoles, and some vacuoles fused and formed large loculi traversed by thin tissue strands (Fig. 4a). Macrophages were occasionally found in the vacuolated spaces, and some macrophages engulfed the myelin debris in their cytoplasm (Fig. 4b). Electron microscopically, the vacuoles corresponded to the distension of the myelin sheath, essentially due to the splitting of the intraperiod line of the myelin sheath (Figs. 5a and 5b). The axons of affected fibers were well preserved. A small amount of membranous debris were found sporadically in the cytoplasm of some oligodendrocytes in severe lesions. However, this was not a prominent feature and it was difficult to clearly distinguish the pathognomonic changes from the artifactual changes.

In 3 out of 6 rats that received 1,000 mg/kg of aniline at

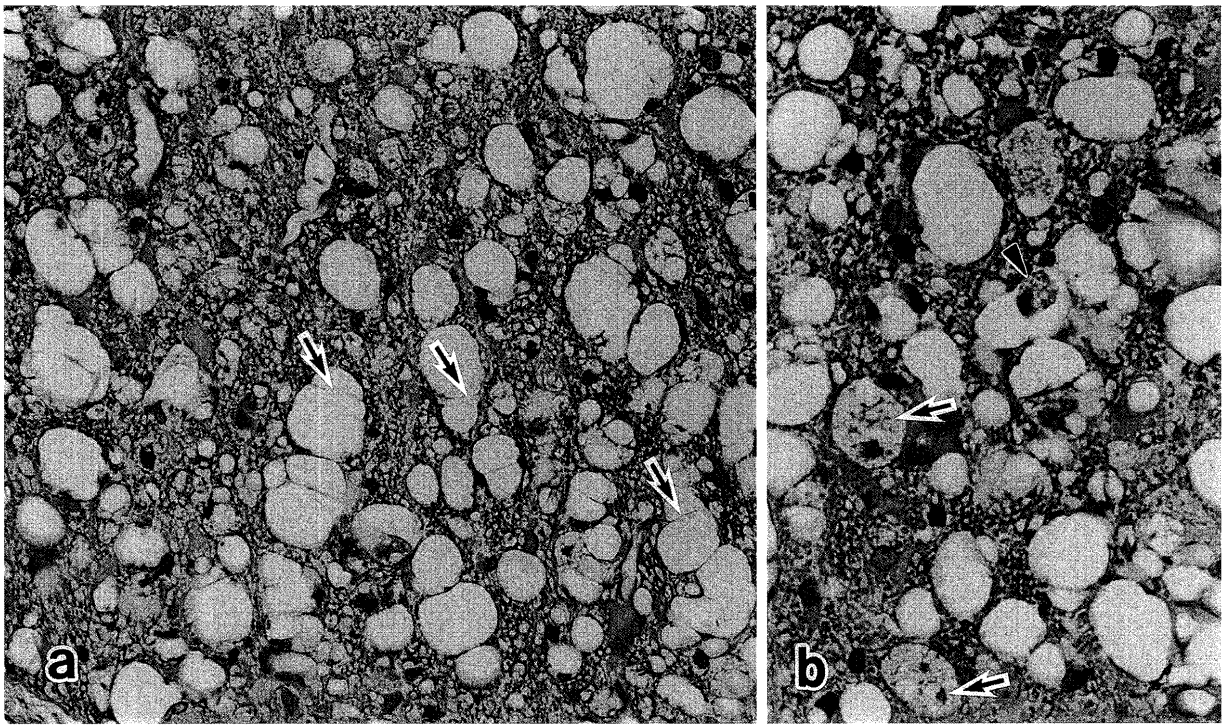

Fig. 4. Photomicrographs of the thoracic spinal cords of rats that were treated once with 1,000 mg/kg of aniline at 4 weeks of age; post-treatment day 15. (a) Loculate vacuoles traversed by thin tissue strands (arrows). LFB-HE. × 400. (b) Macrophage in the vacuolated space (arrowhead). Arrows show the macrophage engulfing myelin debris. LFB-HE. × 530.

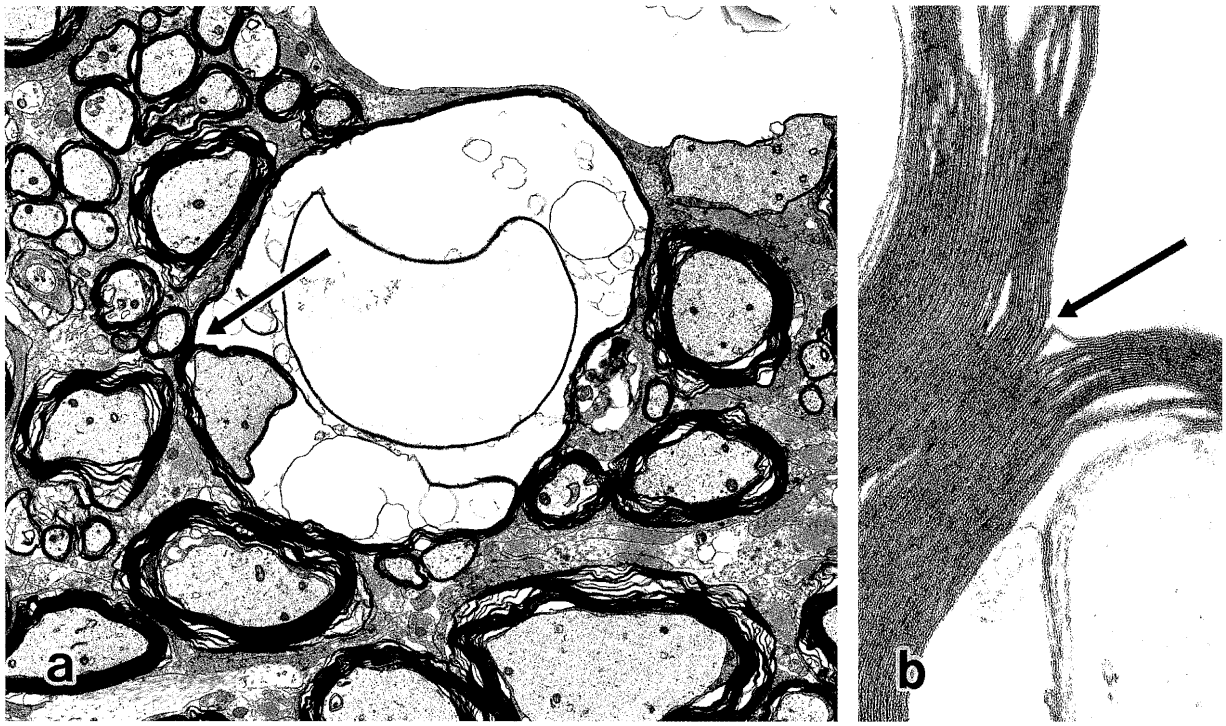

Fig. 5. Electron micrographs of the thoracic spinal cords of rats that were treated once with 1,000 mg/kg of aniline at 4 weeks of age; post-treatment day 15. (a) Splitting of the intraperiod line in the myelin sheath (long arrow). Axons are well preserved, × 5,000. (b) Higher magnification of the splitting point is indicated by the long arrow, × 73,000.

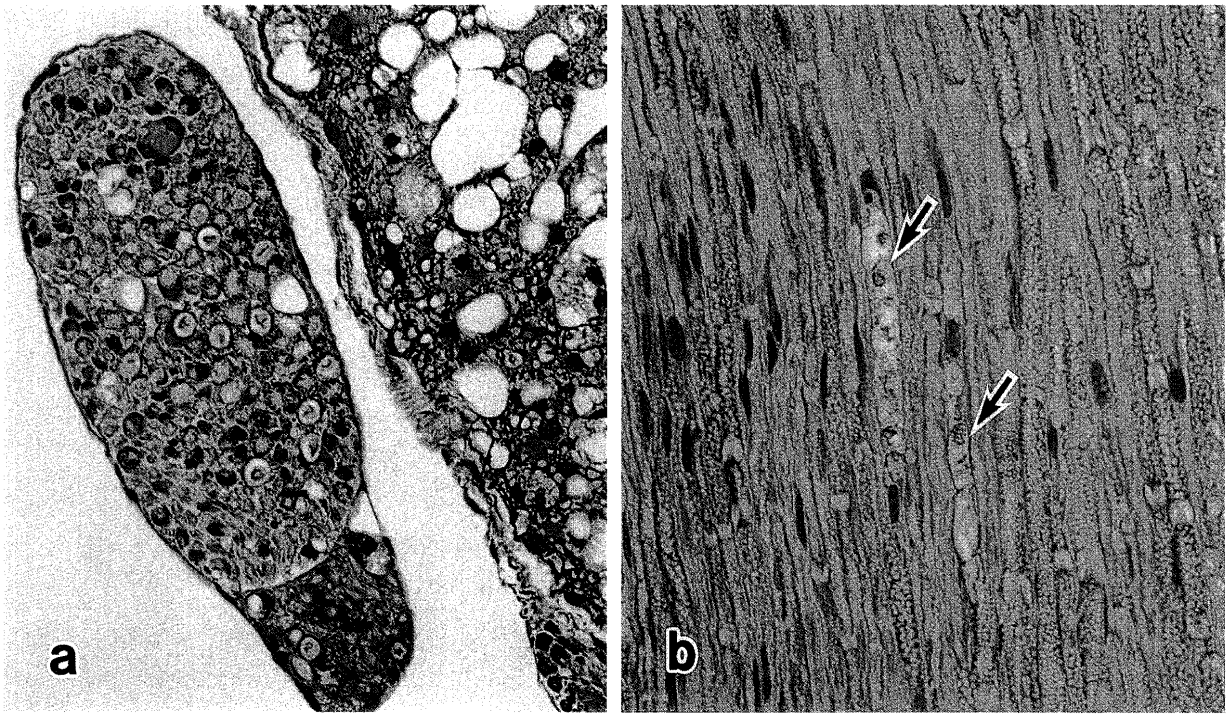

Fig. 6. Photomicrographs of the peripheral nerves of rats that were treated once with 1,000 mg/kg of aniline at 4 weeks of age; post-treatment day 15. (a) Demyelination in the ventral root of the spinal nerves. LFB-HE. × 430. (b) Nerve fiber degeneration in the sciatic nerves. The digestion chambers include myelin-debris that stain blue with luxol fast blue (arrows). LFB-HE. × 430.

4-week-old, the following lesions were also observed: spongy state in the facial nerve tracts and the spinal trigeminal tracts in the pons and medulla oblongata (Table 2); nerve fiber degeneration including demyelination were sporadically seen in the dorsal and ventral spinal roots (Fig. 6a), but the lesions were sporadic and restricted to the thoracic level; slight and sporadic nerve fiber degeneration that was characterized by the formation of the digestion chamber was also found in the trigeminal, sciatic and tibial nerves (Fig. 6b). The same rats presented moderate to severe lesions over a length of the spinal cord, and two rats out of them showed paralytic gait or hindlimb paralysis. No remarkable changes were found in any neuronal cells, ganglion cells, or skeletal muscles.

No treatment-related neurohistopathologic abnormalities were seen in any rats of Groups I, II, V, VI, VII, or VIII.

DISCUSSION

From this study, it is evident that a single oral dose of aniline induces neurotoxicity characterized by paralytic gait or hindlimb paralysis, and spongy change of the white matter of the central nervous system (CNS). The degeneration of fibers was observed in the peripheral nerves, although the lesions were much less pronounced than that in CNS. The induction of the neurotoxic changes in this study was restricted to 4-week-old rats that received 750 or 1,000 mg/kg of aniline. No treatment-related paralytic gait, paralysis, or neurohistopathological changes were found in any 7- or 10-week-old rats receiving 800 mg/kg of aniline.

Similar vacuolation of the myelin sheath is produced by several other compounds, including triethyl tin (TET) [2, 28], hexachlorophene [28], isonicotinic acid hydrazide (INH) [2, 4, 28], and cuprizone [2, 20]. Nitrobenzene (NB) is the raw material in aniline-production [9]. NB produces intramyelinic vacuolation in the white matter of the brain stem and cerebellum in rats [5, 21]. Morgan *et al.* suggested that NB-induced intramyelinic vacuolation was the result of uncoupling of the mitochondrial oxidative phosphorylation in the oligodendrocytes [21]. The inhibition of mitochondrial oxidative phosphorylation interferes with ATP production, and consequently, the transmembranous energy-bound electrolyte transport is disturbed [28]. Normal production and maintenance of myelin sheath in CNS are dependent on the oligodendrocyte. Therefore, when the function of oligodendrocyte is impaired, the loosening in the intraperiod region of the myelin sheath develops easily [17, 28]. The inhibition of oxidative phosphorylation is induced by nitrosobenzene, which is one of the common metabolites of NB and aniline [1]. The structural and metabolic similarities between NB and aniline strongly suggest the common process that lead to intramyelinic vacuolation.

The degenerative changes of oligodendrocytes have been reported in INH intoxication in dogs [4] and cuprizone intoxication in rodents [20]. In this study, only a few membranous debris were found in some oligodendrocytes. Since

the debris was not a prominent feature, it was difficult to clearly distinguish the pathognomonic changes from the artifactual changes. Further investigations will be needed concerning the relationships between functions and morphology of oligodendrocytes under aniline intoxication.

The most important toxic event in the aniline intoxication is the production of reactive oxygen species (ROS) due to the damage to erythrocytes [14]. Recently, Smith *et al.* reported that oligodendrocytes were most sensitive to oxidative stress *in vitro* among the glial cells [25]. Petito *et al.* also described that oligodendrocytes were more sensitive than neurons in certain brain regions of rats after brief cerebral global ischemia [22]. Therefore, the effect of ROS may be the another point to be considered in the aniline neurotoxicity.

Fatty acid anilides, one of the aniline-derivatives, is the causative agent of toxic oil syndrome [28]. Rodrigo *et al.* reported that repeated doses of anilides to rabbits induced ataxia and paralysis, and the vacuolation of the white matter of the cerebellum and spinal cords, necrosis of Purkinje's cells in the cerebellum, as well as muscular degeneration and eosinophilic interstitial pneumonitis [24]. Autoimmune toxicity or eosinophil related allergic response has been suggested as the mechanisms of the neurotoxicity [24]. However, no inflammatory responses were found in either nervous tissues nor skeletal muscles in this study, and the number of white blood cells was within the normal range in rats treated with aniline (data not shown).

Aniline-induced neurotoxicity in rats was dependent on the age of the animal at the time of administration. There are differences in proportion of lipids between immature myelin and mature one. Galactolipid gives stiffness to myelin sheath [18]. Immature myelin contains a higher proportion of phospholipid and lower amounts of galactolipid. With age, the proportion of galactolipid increases and the myelin sheath matures biochemically [18]. It has been reported that the synthetic activity of lipids in the myelin sheath from the spinal cord remained high for up to 30 days of age [26]. Therefore, the biochemic immaturity of myelin sheath might be relevant to the age-dependent neurotoxicity of aniline in rats.

The age-related difference in the levels of hepatic enzymes is another point of view with respect to the age-dependent neurotoxicity of aniline. In experimental animals and humans, aniline is acetylated or hydroxylated by the drug-metabolizing enzyme system in the liver [23]. In general, the activities of hepatic drug-metabolizing enzymes of rats increase with age, and their levels reach a plateau between 14 and 52 weeks of age in male rats [11]. Thus the activities of hepatic drug-metabolizing enzymes in 4-week-old rats are lower than those in 7- or 10-week-old rats. The possibility exists that the lower metabolic activity in liver of 4-week-old rats is relevant to the age-dependent neurotoxicity of aniline.

In this study, the spongy state was most prominent at the thoracic level among the spinal cord, and the lesion located mainly in the outer ascending tracts of the white matter. The similar distribution of the lesions, involving the surface or subpial fibers in the white matter at the thoracic level of the spinal cord, is found in several other diseases affecting the spinal cord [28]. This area appears to be the most vulnerable among the spinal cord.

The lesions in the peripheral nerves were much less pronounced than that in CNS. The different susceptibilities between central and peripheral nervous tissues are found in several other compounds, including TET, INH, and cuprizone [28]. The following factors had been described in the literature: species or age of animals used in the studies, dosage level of chemicals, or duration of treatment [4, 28]. It also remains to be investigated whether there are different vulnerabilities between oligodendrocyte and schwann cell under aniline intoxication.

Aniline is widely used as an intermediate and raw material in several industrial products, including agricultural chemicals. Based on the increase in methemoglobin in blood in exposed workers, a threshold limit value - time weighted average (TLV-TWA) of 2 ppm is recommended for aniline [3, 29]. Focused on the blood methemoglobin level after single oral dose of aniline, the no-effect dose in adult rats was 20 mg/kg body weight. The corresponding no-effect dose in healthy adult humans was 15 mg/person [13]. Those results reported by Jenkins *et al.* mean that aniline is more toxic in humans than in rats. Although there is no available data on the toxicity of aniline in human infant, our results reveals that aniline-induced neurotoxicity is age-dependent in rats and premature generation is more susceptible than the older one. Aniline and its derivatives also occur in various products in the home, such as paints, varnishes, marking inks and some kinds of polishes [8]. Therefore, toxicologic investigations of aniline are very important for not only concerning the industrial hazards but also environmental health, especially in young humans and animals. Further morphologic, biochemic and metabolic investigations are required to clarify the mechanisms of aniline-induced neurotoxicity.

ACKNOWLEDGMENTS. The authors wish to thank Ms. M. Sekine and Ms. H. Shigematsu for assistance in the neurobehavioral investigation, Ms. E. Okabe and Ms. C. Asano for assistance in necropsy, and Mr. T. Ito, Ms. K. Okazaki, Ms. M. Morino, Ms. M. Yamamoto, Ms. M. Onozawa and Ms. T. Hayashi for excellent technical assistance in tissue preparation.

REFERENCES

1. Abou-Khalil, S., Abou-Khalil, W. H. and Yunis, A. A. 1980. Differential effects of chloramphenicol and its nitrosoanalogue on protein synthesis and oxidative phosphorylation in rat liver mitochondria. *Biochem. Pharm.* 29: 2605–2609.
2. Adornato, B. and Lampert, P. 1971. Status spongiosus of nervous tissue, electron microscopic studies. *Acta Neuropathol. (Berl.).* 19: 271–289.
3. American Conference of Governmental Industrial Hygienists. 1991. Aniline and homologues. pp. 68–70. *In*: Documentation

of the Threshold Limit Values and Biological Exposure Indicies, 6th ed., Cincinnati, ACGIH.
4. Blakemore, W. F., Palmer, A. C. and Noel, P. R. B. 1972. Ultrastructural changes in isoniazid-induced brain oedema in the dog. *J. Neurocytol.* 1: 263–278.
5. Bond, J. A., Chism, J. P., Rickert, D. E. and Popp, J. A. 1981. Induction of hepatic and testicular lesions in Fischer-344 rats by single oral doses of nitrobenzene. *Fundam. Appl. Toxicol.* 1: 389–394.
6. Bus, J. S. and Popp, J. A. 1987. Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally-related compounds. *Fd. Chem. Toxic.* 25: 619–626.
7. Goodman, D. G., Ward, J. M. and Reichardt, W. D. 1984. Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, p-chloroaniline, azobenzene, o-toluidine hydrochloride, 4,4'-sulfonyldianiline, or D & C Red No. 9. *J. Natn. Cancer Inst.* 73: 265–273.
8. Gosselin, R. E., Hodge, H. C., Smith, R. P. and Gleason, M. N. 1976. Aniline. pp. 29–35. *In*: Clinical Toxicology of Commercial Products, Acute Poisoning, 4th ed. (Gosselin, R. E., Hodge, H. C., Smith, R. P. and Gleason, M. N. eds.), The Williams & Wilkins, Baltimore.
9. Guy, R. 1998. Nitrobenzene. pp. 421–423. *In*: Encyclopedia of Toxicology, vol. 2 (Wexler, P. ed.), Academic Press, San Diego.
10. Hatakeyama, S., Kovacs, K., Yeghiayan, E. and Blascheck, J. A. 1971. Aniline-induced changes in the corpora lutea of rats. *Am. J. Obstet. Gynecol.* 109: 469–476.
11. Imaoka, S., Fujita, S. and Funae, Y. 1991. Age-dependent expression of cytochrome P-450s in rat liver. *Biochim. Biophys. Acta.* 1097: 187–192.
12. International Agency for Research on Cancer. 1982. Aniline and aniline hydrochloride. pp. 39–61. *In*: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 27. IARC, Lyon.
13. Jenkins, F. P., Robinson, J. A., Gellatly, J. B. M. and Salmond, G. W. A. 1972. The no-effect dose of aniline in human subjects and a comparison of aniline toxicity in man and the rat. *Fd. Cosmet. Toxicol.* 10: 671–679.
14. Khan, M. F., Boor, P. J., Gu, Y., Alcock, N. W. and Ansari, G. A. S. 1997. Oxidative stress in the splenotoxicity of aniline. *Fundam. Appl. Toxicol.* 35: 22–30.
15. Kouris, C. S. and Northcott, J. 1963. Aniline and its derivatives. pp. 411–419. *In*: Encyclopedia of Chemical Technology, 2nd ed., vol. 2 (Kirk, R. E. and Othmer, D. F. eds.), John Wiley & Sons, New York.
16. Kovacs, K., Blascheck, J. A., Yeghiayan, E., Hatakeyama, S. and Gardell, C. 1971. Adrenocortical lipid hyperplasia induced in rats by aniline: a histologic and electron microscopic study. *Am. J. Pathol.* 62: 17–34.
17. Kreutzberg, G. W., Blakemore, W. F. and Graeber, M. B. 1997. Cellular pathology of the central nervous system. pp. 85–156. *In*: Greenfield's Neuropathology, 6th ed., vol. 1 (Graham, D. I. and Lantos, P. L. eds.), Arnold, London.
18. Krinke, G. J. and Eisenbrandt, D. L. 1994. Nonneoplastic changes in the brain. pp. 6–19. *In*: Pathobiology of the Aging Rat, vol. 2 (Mohr, U., Dungworth, D. L. and Capen, C. C. eds.), ILSI Press, Washington, D. C.
19. Laborit, H., Jouany, J. M. and Gerard, J. 1960. Introduction into the study of the antioxydants and the structures of single electrons in biology—1st part—General study. *Agressologie* 1: 63–79 (in French).
20. Love, S. 1988. Cuprizone neurotoxicity in the rat: morphologic observations. *J. Neurol. Sci.* 84: 223–237.
21. Morgan, K. T., Gross, E. A., Lyght, O. and Bond, J. A. 1985. Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. *NeuroToxicology* 6: 105–116.
22. Petito, C. K., Olarte, J-P., Roberts, B., Nowak T. S. Jr. and Pulsinelli, W. A. 1998. Selective glial vulnerability following transient global ischemia in rat brain. *J. Neuropath. Exp. Neurol.* 57: 231–238.
23. Radomski, J. L. 1979. The primary aromatic amines: their biological properties and structure-activity relationships. *Annu. Rev. Pharmacol. Toxicol.* 19: 129–157.
24. Rodrigo, J., Robles, M., Mayo, I., Pestana, A., Marquet, A., Larraga, V. and Munoz, E. 1983. Neurotoxicity of fatty acid anilides in rabbits. *Lancet* 1: 414–416.
25. Smith, K. J., Kapoor, R. and Felts, P. A. 1999. Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol.* 9: 69–92.
26. Smith, M. E. 1973. A regional survey of myelin development: some compositional and metabolic aspects. *J. Lipid Res.* 14: 541–551.
27. Summers, B. A., Cummings, J. F. and de Lahunta, A. 1995. Degenerative diseases of the central nervous system. pp. 208–350. *In*: Veterinary Neuropathology (Summers, B. A., Cummings, J. F. and de Lahunta, A. eds.), Mosby, St. Louis.
28. Verity, M. A. 1997. Toxic disorders. pp. 755–811. *In*: Greenfield's Neuropathology, 6th ed., vol. 1 (Graham, D. I. and Lantos, P. L. eds.), Arnold, London.
29. Weiss, G. 1986. Aniline. pp. 123. *In*: Hazardous Chemicals Data Book, 2nd ed. (Weiss, G. ed.), Noyes Data, New Jersey.

た．血中フレカイニド濃度は，QRS 間隔（r＝0.935，P＜0.001）および QT 間隔（r＝0.753，P＜0.001）は有意な相関を示した．以上のことから，4および6 mg/kg のフレカイニド経口投与により除細動に必要な血中濃度が得られ，また，それは速やかに減少することが明らかとなった．

2例の2カ月齢の猫で大きな心雑音が聴取され，うち1例では鬱血性心不全を呈していた．心エコー検査では，左室の求心性肥大と左室流出路障害が認められた．2例は，それぞれ5カ月齢と1歳齢で死亡し，病理検査により肥大型心筋症と確定診断された．

病　理　学：

アニリンの神経毒性誘発能と投与時週齢の違いによる神経毒性発現の感受性の違いを雄の SD 系ラットを用いて検討した．アニリンの500，750または1,000 mg/kg を4週齢のラットに，800 mg/kg を7または10週齢のラットにそれぞれ単回経口投与し，一般状態を15日間観察した．7および10週齢ラットの投与量は，過半数の動物が投与直後の急性影響で死亡しない最大の用量とした．各々の週齢の動物にオリーブ油を投与する溶媒対照群を設けた．動物数は各群6匹とした．観察期間終了後に中枢および末梢神経組織の病理組織学的検査と脊髄の電子顕微鏡学的検査を行った．4週齢時にアニリンの1,000 mg/kg を投与した6例中2例が投与後8～15日目に麻痺性歩行または後肢麻痺を呈した．病理組織学的には脊髄白質の海綿状変化が750および1,000 mg/kg 群の全例にみられた．病変の程度は胸部脊髄の側索および腹索で強かった．1,000 mg/kg 群の6例中3例では橋および延髄に軽度の海綿状変化および軽度の末梢神経線維の変性が認められた．脊髄の海綿状変化は髄鞘の intraperiod line の解離によるものであった．7または10週齢時にアニリンの800 mg/kg を投与したラットには神経症状，神経病変ともにみられなかった．大量のアニリンをラットに単回経口投与することで神経毒性が誘発されることが確認された．また，この神経毒性の発現は投与時の週齢に依存し，若齢ラットに投与した場合に限られることが示された．

伊東細胞（脂肪摂取細胞）は肝臓のディッセ腔に存在し様々な働きを持っている．今回，ブロイラー鶏の健常な肝臓および胆汁をうっ帯させた肝臓における伊東細胞について免疫組織化学および電子顕微鏡学的に検索した．免疫組織化学的に伊東細胞は，HHF35抗筋系アクチン抗体，ビメンチン，デスミン，グリア線維性酸性蛋白（GFAP），神経特異性エノラーゼ（NSE），クロモグラニン A，サイトケラチンに対し陽性を示した．これらの陽性細胞は肝小葉にび漫性に認められた．肝外胆管結紮を行った肝臓では，胆汁うっ滞，線維化，胆管および伊東細胞の増殖が認められた．伊東細胞はしばしば線維化領域で観察され，正常な肝臓の伊東細胞よりも細胞の大きさを増し強い免疫陽性反応を示した．電子顕微鏡学的検索では，肝外胆管を結紮した肝臓では伊東細胞は膠原線維の産生と密接に関係していた．これらの所見により，胆汁がうっ滞した鶏の肝臓では伊東細胞が肝細胞の障害に対して活発に反応し，肝臓の線維形成機序に重要な役割をもつことが示唆された．