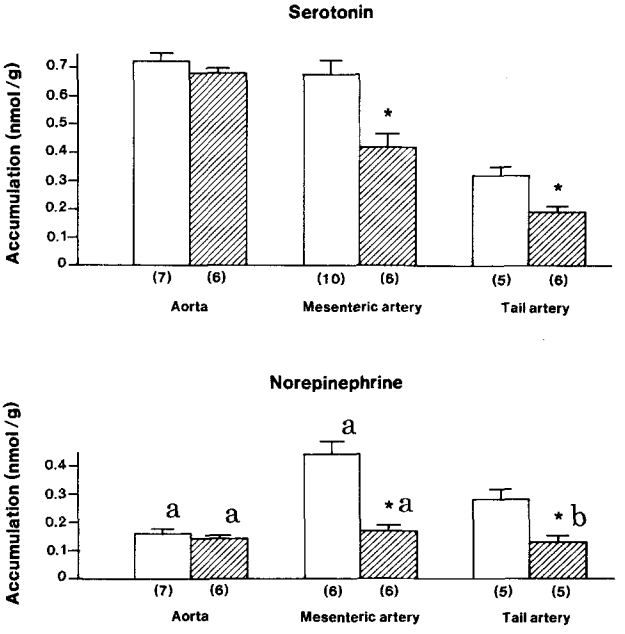

Effects of adrenergic denervation with 6-OHDA on accumulations of 5-HT and NE (0.1 μM, respectively) in rat aortae, mesenteric and tail arteries. Distribution of these amines in the extracellular space was corrected by substracting [^{3}H]-sorbitol uptake. Open column: control, hatched column: 6-OHDA pretreated. *indicates statistically significant from control tissues ($p < 0.01$). [a] ($p < 0.01$); [b] ($p < 0.05$) significantly smaller than the respective values for 5-HT. Parentheses indicate number of experiments.

The extracellular space measured by [^{3}H]-sorbitol in intact and 6-OHDA-treated arteries were 0.50 ± 0.02 ($n = 6$) and 0.47 ± 0.01 ml/g ($n = 5$) in aorta, 0.50 ± 0.01 ($n = 8$) and 0.49 ± 0.01 ml/g ($n = 8$) in mesenteric arteries and 0.48 ± 0.02 ($n = 4$) and 0.47 ± 0.01 ml/g ($n = 5$) in tail arteries, respectively.

Discussion. The exact sites of 5-HT accumulation in vascular walls still remain controversial. It has been reported that 5-HT is taken up by nerve tissues in dog cerebral arteries and saphenous vein[3]. Iwasawa and Gillis[4] reported that the uptake sites of 5-HT and NE are pharmacologically different in rabbit perfused lung tissues. In contrast, Paiva et al.[7] reported that the distribution and uptake sites of 5-HT and NE are essentially the same in the dog saphenous vein. Results of the present study show that in the rat aorta, mesenteric and tail arteries 5-HT accumulation occurs in both neuronal and extraneuronal tissue, but to a greater extent in the latter. This is based on the observation that more than 50% of 5-HT accumulation remains in the adrenergically denervated aorta, mesenteric and tail arteries. Furthermore, adrenergic denervation using 6-OHDA did not significantly affect the accumulation of both 5-HT and NE in the aorta which is known to receive sparse or no sympathetic innervation[8], indicating that accumulation of both amines in the aorta is primarily extraneuronal. In contrast, adrenergic denervation results in less than 50% of NE content in the mesenteric and tail arteries, indicating that NE mainly accumulates in neuronal tissues in these arteries.

The primary finding of the present study is that a greater accumulation of 5-HT occurs in extraneuronal tissue than neuronal tissues in the aorta (sparse adrenergic innervation), mesenteric (moderate adrenergic innervation)[9] and tail arteries (dense adrenergic innervation)[10] of the rat. Preliminary results indicate that removal of endothelial cells in the aorta only slightly inhibits 5-HT accumulation, suggesting that extraneuronal accumulation of this amine probably occurs in medial muscle layers. This is supported by the finding from autoradiographic studies at light microscopic level that 5-HT accumulation is largely associated with smooth muscle cells in the rat aorta (Fukuda et al., unpublished observation). The greater extraneuronal uptake of 5-HT therefore emphasizes the significant role of the vascular smooth muscle in regulating the hemostatic and hemodynamic effects of 5-HT in the rat.

The exact mechanism of extraneuronal accumulation of 5-HT remains unclear. Since 5-HT-accumulation in the denervated aorta was not affected by pretreatment with NE, and the accumulation of 5-HT is much higher (4.7 times) than that of NE in the aorta, the mechanism of extraneuronal accumulation of 5-HT is probably different from that of NE.

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Serotonin metabolism in the CNS in cerebellar ataxic mice

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Summary. The metabolism of 5-hydroxytryptamine (5-HT) in the CNS was investigated in four kinds of morphologically different ataxic mice; reeler, staggerer, weaver and Purkinje cell degeneration mutants, and in hypocerebellar mice experimentally produced by injection of cytosine arabinoside. 5-HT and 5-hydroxyidoleacetic acid concentrations and tryptophan hydroxylase (TrpOH) activity were measured in the cerebrum, cerebellum and brain stem, respectively. TrpOH activity was significantly reduced only in the reeler mouse. The enhancements of the cerebellar 5-HT metabolism observed in the ataxic mice other than the reeler were supposed to be pseudo-enhancements subsequent to the cerebellar hypoplasia.
Key words. Serotonin metabolism; tryptophan hydroxylase; cerebellar ataxic mice.

Ataxic mice have extensively been studied morphologically[1,2] and physiologically[3] as animal model for cerebellar ataxia. These mice are known to show cerebellar hypoplasia and many different types of cytoarchitectural changes. Though many reports have indicated that the levels of free amino acids[4], acetylcholine[5] and noradrenaline metabolism[6,7] are altered in the ataxic mice there are, so far as we know, no reports on 5-hydroxytryptamine (5-HT) metabolism in the ataxic mice, except for the report on cytosine arabinoside (ara-C) treated mice by Tsuji et al.[8]. Some histochemical[9] studies showed that 5-HT neurons from raphe nuclei innervated the cerebellar cortex and intracerebellar nuclei. Furthermore, 5-HT neurons were electrophysiologically shown to have some influences on cerebellar activity. Therefore, the present study was undertaken to investigate central 5-HT metabolism in the ataxic mice, by measuring the tryptophan hydroxylase (TrpOH) activity in the brain stem, and 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) contents in the cerebellum and cerebrum, respectively.

Materials and methods. The ataxic mice used were the reeler, staggerer, weaver and Purkinje cell degeneration (PCD) mutants. They were originally obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and raised in our institution. Mice homozygous for the mutation were obtained by intercrossing pairs heterozygous for autosomal recessive gene. In all cases, the homozygous mutants were easily recognized by their ataxia. Behaviorally normal littermates (genetically non-carrier or heterozygous carrier) were used as controls. The ara-C treated hypocerebellar mice were produced by injection of ara-C into ICR mice according to the method of Shimada et al.[10] and non-treated ICR mice were used as controls. The number of animals used are given in parentheses in table 1. The mice were sacrificed at the age of 6 or 7 weeks by decapitation.

5-HT and 5-HIAA quantitation in the cerebellum and cerebrum was carried out by the method of Mefford[11], using high-performance liquid chromatography. TrpOH activity in the brain stem was assayed by the method of Boadle-Biber et al.[12].

Results. The wet weights of the organs removed from each mouse are shown in table 1. All of the affected mice showed a significantly reduced cerebellar wet weight. The ataxic mice had also a significantly smaller cerebrum and brain stem in comparison with each control. Nevertheless, the reductions of wet weight in the cerebrum were not so obvious as in the cerebellum.

Both 5-HT and 5-HIAA concentrations per wet weight were siginficantly increased in the cerebellum of all affected mice compared with controls (table 2). 5-HT and 5-HIAA concentrations in the cerebrum had no significant changes in any affected mice. The TrpOH specific activity in the reeler showed a significant change.

Calculated from tables 1 and 2 only the reeler mouse revealed a significant increase in 5-HT and 5-HIAA content per whole cerebellum (19.8 ± 1.6 ng and 4.8 ± 0.5 ng for reeler; 12.8 ± 0.5 ng and 3.1 ± 0.5 ng for control, respectively). The reeler mouse also had a significantly lower level of TrpOH activity per brain stem (6.0 ± 0.6 ng/min as 5-hydroxytryptophan (5-HTP) for reeler; 9.2 ± 0.5 ng/min as 5-HTP for control). In contrast, none of the other affected mice showed any significant changes of total 5-HT and 5-HIAA contents and total TrpOH activity compared with controls.

Discussion. The concentrations of 5-HT and 5-HIAA per wet weight in the cerebellum were significantly increased in all affected mice. However, calculating 5-HT and 5-HIAA concentrations as the total content in the whole cerebellum, no significant increases were confirmed except for the reeler. The degree and extent of increase in 5-HT and 5-HIAA concentrations were well correlated with the degree of cerebellar weight loss.

To assay the activity of TrpOH, the rate limiting enzyme in 5-HT synthesis, is very important in a detailed analysis of 5-HT metabolism. It has been reported that most of the TrpOH is synthesized in the cell bodies of 5-HT neurons in the nucleus raphe. We assayed TrpOH activity in the brain stems of the ataxic mice because the brain stems of these ataxic mice, including the reeler, has been shown to be morphologically normal. No significant changes of TrpOH activity were observed except for the reeler. Its activity in the reeler was significantly decreased.

These results suggest that the increase in 5-HT metabolism observed in the cerebellum of staggerer, weaver, PCD and ara-C treated mice is a pseudo-increase resulting from cerebellar hypoplasia, and 5-HT metabolism in these ataxic mice is not basically altered. In addition, we suppose that 5-HT metabolism in the reeler may be significantly decreased.

Table 1. Wet weights in mg of organs from ataxic and control mice

			Cerebellum	Cerebrum	Brain stem
Reeler	Affected	(13)	16.4 ± 0.5**	278.4 ± 4.6**	43.9 ± 1.1**
	Control	(22)	47.5 ± 1.0	310.5 ± 4.0	50.1 ± 0.7
Staggerer	Affected	(8)	7.1 ± 0.4**	260.7 ± 6.1**	39.4 ± 0.7
	Control	(12)	42.1 ± 0.8	298.7 ± 2.7	46.3 ± 1.6
Weaver	Affected	(7)	14.8 ± 0.6**	270.5 ± 6.7**	42.5 ± 1.9
	Control	(18)	42.5 ± 1.,5	314.1 ± 3.3	48.3 ± 1.2
PCD	Affected	(7)	31.6 ± 0.7**	279.1 ± 7.9	43.6 ± 1.6
	Control	(14)	46.9 ± 1.1	297.5 ± 2.4	47.3 ± 0.8
Ara-C treated mouse	Affected	(10)	20.3 ± 1.0**	296.5 ± 6.5**	45.8 ± 1.4
	Control	(14)	58.8 ± 1.8	334.0 ± 6.8	54.7 ± 1.9

Mean ± SE, ** $p < 0.001$.

Table 2. 5-HT and 5-HIAA concentrations and TrpOH activity

		Cerebellum 5-HT (ng/g w.wt)	Cerebellum 5-HIAA (ng/g w.wt)	Cerebrum 5-HT (ng/g w.wt)	Cerebrum 5-HIAA (ng/g w.wt)	Brain stem TrpOH 5-HTP (ng/min/g w.wt)
Reeler	Affected	1216 ± 36**	298 ± 31**	1702 ± 26	312 ± 18	138 ± 12*
	Control	270 ± 12	66 ± 4	1573 ± 26	285 ± 12	182 ± 9
Staggerer	Affected	1526 ± 75**	576 ± 39**	1799 ± 84	384 ± 29	120 ± 15
	Control	296 ± 10	87 ± 4	1785 ± 53	314 ± 11	178 ± 14
Weaver	Affected	1055 ± 68**	245 ± 12**	1720 ± 72	336 ± 25	143 ± 14
	Control	283 ± 19	105 ± 8	1539 ± 37	314 ± 18	134 ± 17
PCD	Affected	522 ± 45**	128 ± 13	1826 ± 70	289 ± 11	92 ± 7
	Control	288 ± 10	112 ± 10	1742 ± 52	347 ± 24	67 ± 6
Ara-C treated mouse	Affected	705 ± 18**	198 ± 20**	1538 ± 31	346 ± 18	120 ± 15
	Control	242 ± 29	65 ± 3	1728 ± 97	330 ± 13	178 ± 14

Mean ± SE, * $p < 0.005$; ** $p < 0.001$.

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Hypoaldosteronism in piglets induced by carbadox

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Summary. An exploratory study was made of the mechanisms underlying the toxic action of carbadox in young pigs: dehydration, loss of appetite and at autopsy seemingly specific and selective structural alterations of the glomerular zone of the adrenal cortex. Administration of carbadox in the feed, in dosages of 150 ppm (approximately 6 $mg \cdot kg^{-1}$ $b.wt \cdot day^{-1}$) caused a rapid decline in the plasma aldosterone levels (to 10% of control) followed by significant changes in the sodium and potassium levels in blood. Characteristic for the toxic action of carbadox are the rapid and seemingly selective and specific alterations in the aldosterone-releasing zona glomerulosa of the adrenals. Our results indicate that with carbadox a functional and possibly reversible extirpation of the adrenal zona glomerulosa can be achieved in pigs.
Key words. Carbadox; aldosterone; adrenal damage; zona glomerulosa; electrolyte homeostasis; pig.

Quinoxaline-di-N-oxide derivatives like carbadox (3-(2 quinoxalinyl-methylene)carbazic acid methyl ester N^1, N^4-dioxide) are widely used as growth-promoting feed additives in pig husbandry. Carbadox also has a prophylactic efficacy against anaerobic spirochetes such as *Treponema hyodysenteriae*, a pathogen implicated in swine dysentery. The mechanism(s) underlying the growth-promoting activity of carbadox, administered to pigs from weaning up to the age of 4 months, is unclear. Pathological examinations of pigs from herds apparently suffering from feed-poisoning in which an overdose of cabadox was suspected indicated adrenal damage and dehydration as characteristic changes[2].

In a preliminary study, mimicking a case of feed-poisoning, pathological changes were observed in the zona glomuerulosa of the adrenals after feeding 7-month-old pigs a toxic dose of 500 mg carbadox per kg feed (15 $mg \cdot kg^{-1}$ $b.wt \cdot day^{-1}$). These changes increased with time. Already after two days the pathological changes were well outside the range of normal variation, although the animals had not yet developed any observable sign of intoxication, such as reduced appetite. Also the side-effects, such as dehydration, observed in veterinary practice with carbadox in dosages of 100 ppm (100 $mg \cdot kg^{-1}$ feed) and higher in young pigs (1–4 months old), suggested an interaction of carbadox with the mineralocorticoid activity[2].

Thus an interaction of carbadox with mineralocorticoid hormonal activity might underly the effects of toxic dosages of carbadox. The present study was aimed at the determination of the safety margin of carbadox, as reflected in the growth of young pigs, and at testing the hypothesis that carbadox in high dosages interferes with the electrolyte homeostasis via aldosterone.

Materials and methods. Groups of 6 weaned piglets (commercial hybrids: Dunel, of both sexes), 4 weeks old, were allowed to equilibrate for 2 weeks. The pens had a day-and-night regimen of 12 h and the temperature and humidity were kept at 20 ± 2 °C and 55 ± 5% respectively. Water and feed were given ad libitum throughout the experiment. Up to the age of 18 weeks all animals received the same piglet feed and thereafter the same grower-finisher feed. Carbadox was added in the feed-mill to

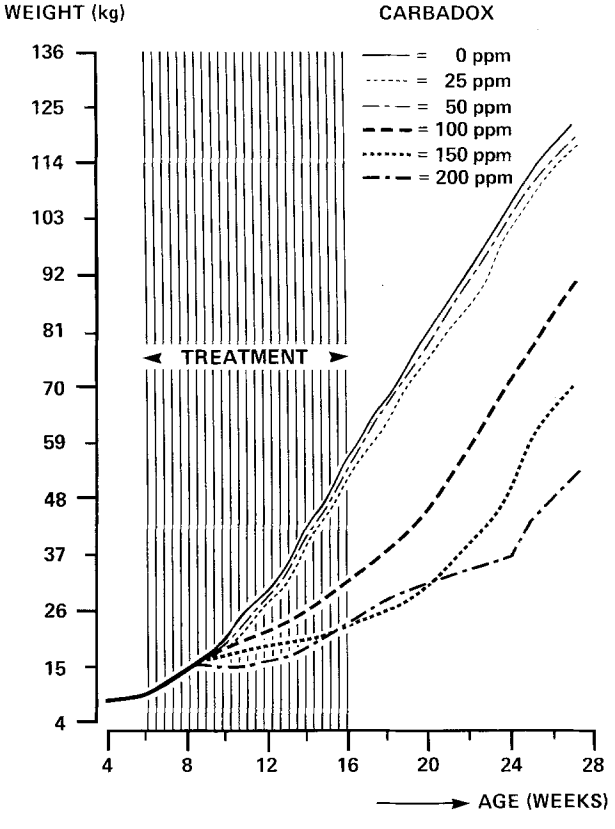

Figure 1. Effects of different dosages of carbadox, administered in feed for 10 weeks, on the growth of pigs. The values presented are average weights of the pigs in a given dosage group (n = 6). Throughout the experiment the range of individual weights was less than ± 10% of the average given. Differences from the 0 ppm group are statistically significant ($p < 0.05$, Student's t-test) for the dosage groups of 100, 150 and 200 ppm, starting at the age of 11, 11 and 10 weeks, respectively.