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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

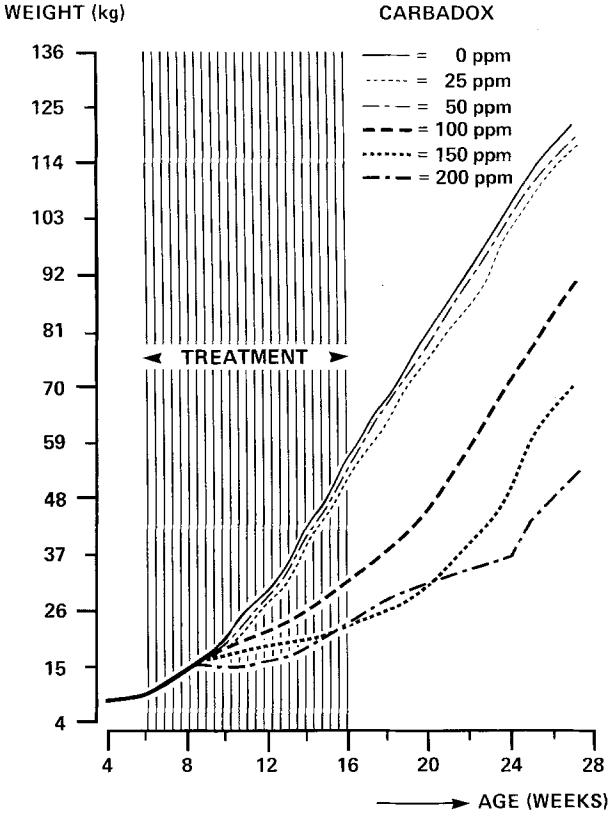

Figure 1. Effects of different dosages of carbadox, administered in feed for 10 weeks, on the growth of pigs. The values presented are average weights of the pigs in a given dosage group (n = 6). Throughout the experiment the range of individual weights was less than ± 10% of the average given. Differences 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.

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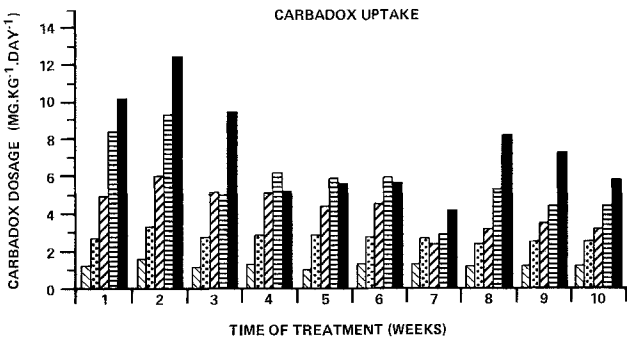

Figure 2. Uptake of carbadox by the pigs in different dosage groups. The bars are in sets of increasing dosage per week, from left to right: 25, 50, 100, 150 and 200 ppm respectively. Note that after the 2nd week of treatment the reduced feed consumption by the higher dosage groups can result in a 'non-toxic' dose of carbadox.

parts of the feed charge prepared. It was verified that the feed given to the animals in different groups was identical in all other aspects.

At weekly intervals the animals were weighed and heparinized blood samples were taken. Sodium and potassium levels in plasma were measured with standard atomic absorption (AAS) assays. Throughout this and other experiments high levels of potassium were measured in plasma of young pigs compared to values reported for other mammalian species. This may be due to the stress experienced by the animals during blood-sampling,

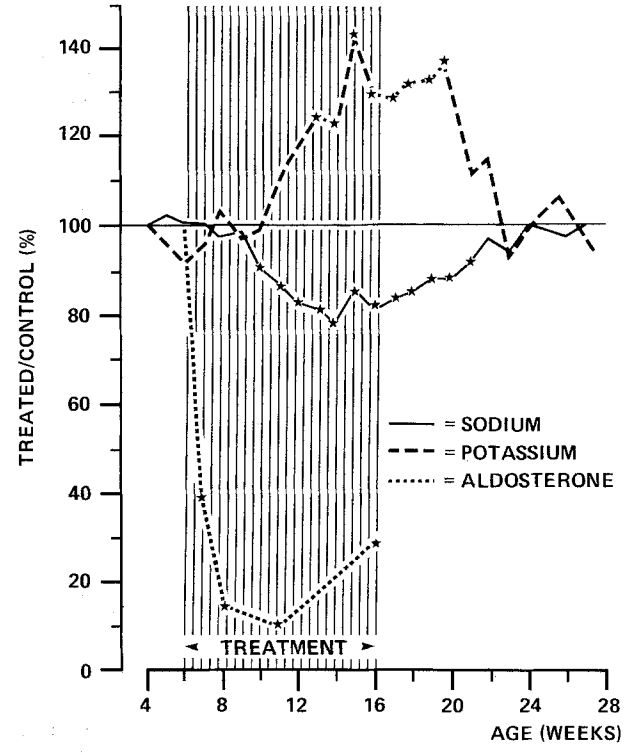

Figure 3. Effect of the administration of 150 mg carbadox per kg feed on the plasma levels of aldosterone, sodium and potassium of pigs. Blood samples were taken at weekly intervals but only at some points in time were the samples obtained measured for aldosterone. The values given are the averages (n = 6) relative to the average values measured simultaneously in untreated animals. Significant differences between the measurements from the 150 ppm group and from the 0 ppm group are indicated with an asterisk (Wilcoxon's test, $p < 0.05$). 100% = 136.2 ± 4.1 mM Na^+; 6.14 ± 0.76 mM K^+ and 1.25 ± 0.23 nM aldosterone (m ± SEM). The increase of the relative aldosterone levels at 16 weeks is due to the age-dependent decrease in aldosterone levels of the 0 ppm animals.

as no correlation was found with occasionally hemolyzed blood samples. Aldosterone levels in plasma were estimated with a RIA-method by Dr W. Schopman at the Bergweg Hospital, Rotterdam. Tritiated aldosterone was used to correct for the extraction, and spiked samples were used to determine the recovery.

Values given are mean and standard error of the mean. Differences were considered to be real when tests gave probability levels smaller than 0.05. Carbadox was given as a commercial product as a 10% feed-premix (Mecadox) by Pfizer.

Results. After in-feed administration of carbadox in dosages of 100 ppm and higher in young pigs, growth retardation became apparent after 4 weeks (fig. 1). Also the feed consumption in the higher disage groups was reduced after the 2nd week of treatment (fig. 2). The average feed consumption of the control and lower dosage groups throughout the treatment period was about 50 $g \cdot kg^{-1}$ b.wt $\cdot day^{-1}$, whereas that of the high dosage groups decreased to values of 20 $g \cdot kg^{-1}$ b.wt $\cdot day^{-1}$ and less. After withdrawal of carbadox the daily weight gain of the pigs recovered after delays, the duration of which increased with the dosage of carbadox given. The backlog in weight, however, remained during the observation period. In this dose-response study the effects of carbadox at the dosage of 150 ppm, the dosage often used as treatment of weaned pigs against diarrhoea, on the plasma levels of aldosterone, sodium and potassium were also studied and compared with the 0 ppm group.

One week after the first administration of carbadox-containing feed at the level of 150 ppm, a significant decrease in the aldosterone levels in plasma was observed. Subsequently the sodium and potassium levels in plasma changed. From the observations presented in figure 3 it is clear that changes in aldosterone plasma levels precede those in sodium and potassium levels. Sodium and potassium plasma levels returned to normal after carbadox administration had been discontinued.

The hypoaldosteronism observed was corroborated at the autopsies of pigs that had been given carbadox. As shown in figure 4, carbadox given for 5 weeks in dosages of 150 ppm caused an almost complete disappearance of the zona glomerulosa as a distinct structure in the adrenals. Other parts of the adrenals did not exhibit significant differences, on microscopic examination, from those of control animals. In other organs no differences, or only minor ones, between carbadox-treated and control animals were observed microscopically after 5 weeks; the minor differences seemed to be related to a disturbance of the electrolyte homeostasis, e.g. necrosis in the collecting tubules in the renal medulla.

Discussion. The present observations seem to imply that carbadox induces hypoaldosteronism via a direct action on the zona glomerulosa. In vitro experiments with adrenal tissue are needed for a more definitive assessment of this assumption. The indirect evidence for this view is, however, rather strong: a) In the case of high, toxic dosages of carbadox changes in the structure of the cells in the glomerular zone are observed relatively early. b) The elevated plasma potassium levels are of a magnitude reputed to triple the rate of the aldosterone production[3]; the decrease in sodium concentration should reinforce this effect. These feedback mechanisms appear not to counteract the carbadox-induced disturbance of electrolyte homeostasis, underlining the dysfunction of the zona glomerulosa due to carbadox. The time sequence and the direction of the observed changes, primarily of aldosterone levels, then of sodium and potassium levels, also implicate the adrenal glomerular zone as the primary site of action of carbadox. c) The absence of visible changes in the zona fasciculata and zona reticularis of the adrenal cortex indicates that ACTH does not play a role in the mediation of the carbadox-induced effects[4]. d) Another pathway that might be involved in the decrease of the aldosterone levels evoked by carbadox is the renin-angiotensin-aldosterone system (RAAS). Drugs like captopril which inhibit the conversion of angiotensin I into angiotensin II are known to lower aldosterone levels. The often

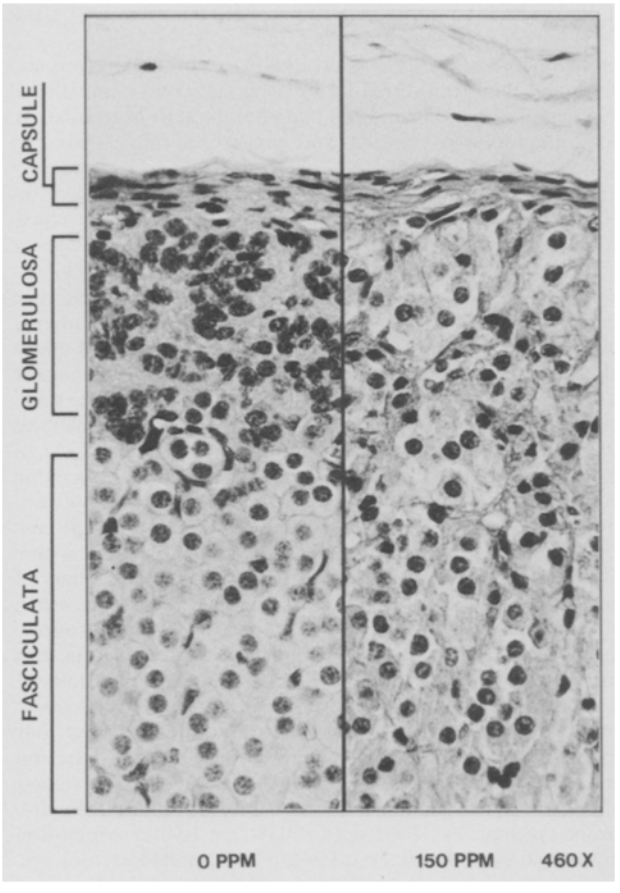

Figure 4. Effect of feeding piglets 150 mg carbadox per kg feed for 5 weeks on the structure of the glomerular zone of the adrenal cortex. The left-hand micrograph represents the control situation, the right-hand micrograph that of the carbadox treatment. Note the complete hydropic appearance of the zona glomerulosa, while the underlying zona fasciculata does not show significant changes.

large decreases in aldosterone levels observed in man with captopril are, however, qualified as a return to normal from elevated levels[5]. Furthermore, extirpation of the kidneys, the major source of renin, does not decrease the aldosterone levels to the extent observed with carbadox[6].

Hence it appears that carbadox interferes directly with the aldosterone-releasing functions of the zona glomerulosa. Giurgea et al.[7] also reported effects of in-feed administration of carbadox on the adrenals of chickens. Although the data given with regard to the amount of carbadox per kg b.wt per day and the in-feed concentration of carbadox seem to be inconsistent, evidence is presented that in chickens carbadox alters the ascorbic acid content of the adrenals. These alterations might reflect effects on the glucosteroid-producing zona fasciculata and zona reticularis, sites of action for which the present results did not give indications. This difference might be attributable to the species difference, but it is possible that also in pigs other parts of the adrenals would be affected with much higher dosages; we interpreted the dosages used in chickens[7] as being at least 10 times higher (mg carbadox $\cdot$ kg^{-1} b.wt $\cdot$ day^{-1}) than in the present study with pigs.

The lack of growth promoting activity of carbadox in dosages up to 50 ppm in this study can be attributed to the 'good health', 'good feed' and 'good housing' conditions of the experimental animals. The toxic activity of carbadox observed, however, indicates a safety-factor smaller than two for the advised dosage of carbadox as a feed additive. With regard to its preventive efficacy against anaerobic pathogens for swine the in vitro MIC-values[8] seem to indicate that this efficacy can be achieved with lower dosages.

An intriguing question is how the majority of the animals treated with 150 ppm carbadox survived and apparently recovered after a 10-week treatment. Probably the residual mineralocorticoidal activity of glucocorticoids, together with that of the renin-angiotensin part of the RAAS provide a mechanism for a minimal survival. Furthermore, the reduced feed consumption by the high dosage-groups seemed to have limited the intoxication. Urine-drinking invariably accompanies dosages of 100 ppm carbadox or higher. This behavior is suggestive of salt-craving, as is known from patients with Addison's disease. In cases of Addison's disease ultimately the whole adrenal cortex becomes dysfuncitonal, whereas carbadox, in the dosages used, selectively affects only the zona glomerulosa of the adrenal cortex. The toxic activity of carbadox as reported above might, however, provide an experimental model for testing aldosterone substitution therapies.

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Inhibition of aldosterone synthesis induced by flow-stop in the Mongolian gerbil adrenal gland superfused in vitro

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Summary. Stopping of superfusion flow for short periods resulted in a significant accumulation of aldosterone within the Mongolian gerbil adrenal gland superfused in vitro. Aldosterone amounts in the first 2-min samples after the re-starting of superfusion were positively correlated with the length of flow-stop; however, they were significantly lower than calculated amounts: 5-min stop: 37 ± 1% inhibition, 10-min stop: 51 ± 1% inhibition. In addition, aldosterone secretion was significantly suppressed during prolonged incubation. The results suggest that aldosterone and glucocorticosteroid amounts in adrenal tissue may modulate basal corticosteroidogenesis and that self-suppression forms an important part of the control mechanisms involved in corticosteroidogenesis.

Key words. Adrenals; in vitro superfusion; aldosterone; Mongolian gerbils.