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Differential effects of nitrofurans on the production/release of steroid hormones by porcine adrenocortical cells in vitro

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Abstract

Changes in the biogenesis of corticosteroids caused by nitrofurans were studied. The three nitrofurans used: furazolidone, furaltadone and nitrofurantoin, altered the steroid production/release by porcine adrenocortical cells in vitro during 1 h incubations. With pregnenolone as a substrate the nitrofurans inhibited aldosterone production/release. Although the nitrofurans differed in potency (nitrofurantoin > furazolidone > furaltadone) maximum inhibition occurred at 100 μM. In this concentration the nitrofurans changed also the release/production of other corticosteroids. The output of corticosterone and cortisol decreased by 50%. The production/release of deoxycortisol stayed the same. In contrast the output of progesterone and 17α-hydroxyprogesterone increased to more than 200% of control. The nitrofurans slightly reduced the output of androstenedione. No significant increases of the production/release of other steroids (testosterone, dehydroepiandrosterone, estradiol-17β and estrone) by the cell suspension could be observed. The profile of the nitrofuran-induced changes lead to the conclusion that nitrofurans interfere with mitochondrial enzymes. These enzymes, presumably cytochrome $P450_{11,18}$ mediate the hydroxylation and the oxidation at C11 and C18, the final steps in the biogenesis of aldosterone, corticosterone and cortisol. The rapid and reversible fall in the output of these steroids occurs in vitro at concentrations which are below therapeutic blood concentrations seen in vivo. At higher concentrations the nitrofurans hinder the biogenesis of androgens. Thus nitrofurans can also affect steps in the steroid biogenesis located in the endoplasmatic reticulum. © 1997 Elsevier Science B.V.

Keywords: Steroid hormones; Aldosterone; Corticosterone; Cortisol; Deoxycortisol; Progesterone; 17α-hydroxyprogesterone; Androstenedione; Steroid biogenesis; Pregnenolone; Adrenocortical cells, in vitro, porcine; Nitrofurans; Furazolidone; Furaltadone; Nitrofurantoin

1. Introduction

Nitrofurans are synthetic antibacterial drugs that in vitro are active against a wide range of pathogens (Dodd and Stillman, 1944). Although developed as disinfecting wound dressing (Snyder et al., 1945), they are currently used primarily to treat infections by Gram-negative pathogens. Furthermore, they are also active against some Gram-positive bacteria, fungi and protozoa. Notwithstanding their widespread use for half a century, bacterial or protozoal resistance is rare.

The safety margin of these compounds, however, is narrow. Nitrofurans are assumed to be carcinogenic and mutagenic (Anon, 1991; Van Koten-Vermeulen et al., 1993). Therefore, in human medicine now only nitrofurantoin is licensed for the treatment of urinary tract infections. In some countries nitrofurans are still authorized for the treatment of eye infections and of some protozoal contagious diseases.

Nitrofurans are widely used in animal husbandry as antiprotozoal and antibacterial agents (Paul, 1956). In pig farming, furazolidone is added to feed as a growth-promoter and prophylactic medicine against bacterial enteritis. In poultry and pig farming, furaltadone dissolved in drinking water is used to treat various infections. In some small farm animals, such as rabbits and mink, nitrofurans are also given as prophylactic treatment. The use of nitrofurans in veterinary praxis to treat food producing animals, however, is being curbed by licensing authorities because of the alleged carcinogenic and mutagenic nature of the nitrofurans (Anon, 1991, 1993).

In animal husbandry symptoms of acute intoxication by furazolidone and other nitrofurans can mostly be attributed to inhibition of oxidative enzymes such as monoaminooxy-

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dase-inhibition (Van Miert et al., 1984). Symptoms of intoxication by carbadox and other quinoxalines are different and include hypoaldosteronism (Van der Molen et al., 1986, 1989). Van der Kerk (1985) reported a toxic interaction between furazolidone and carbadox in pigs, the symptoms of which indicated an acute quinoxaline intoxication, without furazolidone symptoms.

In a study using a suspension of porcine adrenocortical cells, we found that quinoxalines reduced the output of aldosterone. This inhibition developed rather slowly and was virtually irreversible (Jager et al., 1994). In the same study we found that nitrofurans rapidly and reversibly reduced the output of aldosterone in vitro. This effect of the nitrofurans occurs in concentrations well below blood concentration found in vivo during treatment with nitrofurans. The present study aims at characterizing this in vitro effect. This mode of action might relate to the toxic interaction between nitrofurans and quinoxalines and to the carcinogenic activity of the nitrofurans in vivo. Furazolidone, furaltadone, and nitrofurantoin were selected for study because they are the most widely used nitrofurans in veterinary and human medicine.

2. Materials and methods

Adrenals of pigs (life-weight 90–100 kg) were removed at a slaughterhouse. Immediately after the abdomens were opened, the adrenals were removed and stored in ice-cold saline solution (0.9% NaCl). Within 2 h the surrounding tissue, capsule and medulla of the adrenals were removed and the remaining tissue was minced. Minced cortical tissue suspended in Krebs solution (0.2 g/ml) was treated with collagenase: 10 mg collagenase/g tissue at 37°C for 45 min in a shaking water-bath. Depending on the size of the experiment, cells from up to 50 adrenals were pooled. After being passed through a 100 μm sieve, the cells were washed twice with Krebs solution, as follows. The cell suspension was centrifuged ($100 \times g$) for 10 min, the supernatant was decanted and the cells in the pellet were resuspended in fresh medium. Cells were kept suspended overnight in RPMI-solution (about 10^6 cells/ml) at 4°C. The next morning the cells were transferred to fresh RPMI-solution. The cell suspension was usually prepared on one day and the experiment was started the next morning. The release of aldosterone by the cell suspension and the number of viable cells decreased only slightly during overnight storage (about 10%). Longer storage resulted in unacceptable losses of cells and activity.

Just before each experiment cell viability was determined by trypan blue exclusion. The final suspension of the cell pellet was made on the basis of the viability results. Throughout the experiments cell suspensions of 1×10^6 cells/ml were used. All incubations were done at 37 ± 0.5°C in a shaking water-bath. RPMI-solution with 1 μM pregnenolone was used to preincubate the cell suspension for 30 min. Usually incubations of 1 h were used with fresh RPMI-solution and substrate. Dose–response studies were carried out with 2 ml samples of the adrenocortical cell suspension with substrate and vehicle or with substrate, vehicle and test compound. Incubation was stopped by immersing the vials in boiling water for 10 min. The vials were then centrifuged, and the supernatant was stored at −20°C until the analysis by radioimmunoassay. All steroids measured were stable during boiling and storage at −20°C up to 1 year.

The survival of the cells was checked by measuring the lactate-dehydrogenase activity (Bergmeyer et al., 1965) of cells and supernatant before and after incubation with test compounds (Legrand et al., 1992). The survival rate was determined as the lactate-dehydrogenase activity of the cells after incubation relative to that obtained before incubation; this rate was generally more than 95%. Lactate-dehydrogenase activity in the medium after incubation was below detection level, and therefore less than 0.1% of the lactate-dehydrogenase activity in the cells.

Steroid hormones released by the cell suspension into the medium were measured by specific radioimmunoassay. Results were expressed as ng steroid produced/released by 10^6 cells per h. Aldosterone, cortisol, corticosterone, testosterone, estrone, estradiol (estradiol-17β), progesterone, 17α-hydroxyprogesterone, androstenedione and dehydroepiandrosterone were measured with radioimmunoassay-kits from D.P.C., deoxycortisol with radioimmunoassay-kits from ICN Biomedicals. See for the details of the radioimmunoassay Table 1 in Jager et al. (1996).

The Krebs-solution used contained (mM): NaCl (118); KCl (5.4); $NaHPO_4$ (1.6); $NaHCO_3$ (21.9); $CaCl_2$ (2.5); $MgSO_4$ (1.2); glucose (11.1). The solution was equilibrated with carbogen (5% CO_2 and 95% O_2) and the pH was 7.4 at 37 ± 0.5°C. The RPMI-solution was prepared according to the instructions from the supplier (#041-02409, GIBCO). The pH was 7.4 at 37 ± 0.5°C. Visible color changes of the pH-indicator occurred at pH 8.0 (purple) and at pH 6.5 (yellow). The buffer capacity in these experimental circumstances was 8.3 for OH^--ions and 5.6 for H^+-ions.

All data are given as means ± standard error of the mean (m ± S.E.M.). In figures the S.E.M. is only presented if it exceeds the dimensions of the symbol. Statistical tests were made according to procedures given by Diem (1980). Differences between the production/release of steroids by control and drug-treated cell suspensions were evaluated with the *t*-test. Differences were assumed to be real when tests gave probability levels less than 5%; *n* indicates the number of independent observations; *N* indicates the number of different sets of experiments of which the control responses were lumped. The effect of drugs on steroid production/release by cell suspensions are expressed as ratio (percentage) of the steroid production/release measured after treatment and that after concurrent vehicle treatment. The term 'release' is used in this paper to indicate production and/or release.

The drugs used were: collagenase (type 1A; Sigma C-9891), DMSO (Merck), furaltadone-HCl (Orphahell), furazolidone (Orphahell), nitrofurantoin (Orphahell), pregnenolone (Sigma P-9129). All drugs were routinely added dissolved in dimethyl sulfoxide (DMSO). DMSO in the concentration used (176 mM) did not affect the release of the steroids measured. After the drugs were added, the pH of the solution was adjusted to 7.4, when necessary.

3. Results

The earlier account that nitrofurans inhibit the aldosterone release was reproduced using this time pregnenolone as substrate. The dose–response curves shown in Fig. 1 agree with those reported using deoxycorticosterone as substrate (Jager et al., 1994). This in vitro system reveals a small potency difference between the three nitrofurans: nitrofurantoin > furazolidone > furaltadone. Nitrofurantoin (10 μM), furazolidone (25 μM) and furaltadone (50 μM) each induced a 50% reduction in the aldosterone release. Notwithstanding this potency difference maximal inhibition occurred by all nitrofurans at 100 μM.

Using this maximal concentration and a submaximal concentration (25 μM) we looked for possible effects on the release of other steroids (Fig. 2). In the low concentration, nitrofurans hardly changed the release of cortisol and corticosterone but 100 μM clearly reduced the release. Another dose dependent action of nitrofurans was the increase of the output of progesterone and its 17-hydroxy derivative. Some changes were observed in the release of androstenedione.

A subsequent, separate and more detailed study of the influence of nitrofurans on the release of steroid hormones confirmed the previous observation (Table 1). The nitrofurans did not alter the release of deoxycortisol. Androstenedione release however, was reduced significantly by furazolidone and the high and low concentration furaltadone, but not the middle concentration, and unchanged in the presence of nitrofurantoin. Furthermore, a dose dependent increase in the release of progesterone and 17α-hydroxyprogesterone was observed. The release of aldosterone, corticosterone and cortisol was not equally susceptible to the inhibiting actions of nitrofurans. The aldosterone output was clearly the most sensitive, followed by that of cortisol, whereas corticosterone, which is also a substrate in the aldosterone synthesis, was the least susceptible. The potency order of the nitrofurans in reducing the output of cortisol or corticosterone is not different from that observed with aldosterone. The adrenocortical cell suspension can release testosterone in quantities well above detection level in the presence of drugs like spironolactone (Jager et al., 1996). Nevertheless the treatments summarized in Table 1 were unable to increase the testosterone output above detection level. The same holds for dehydroepiandrosterone, estradiol and estrone.

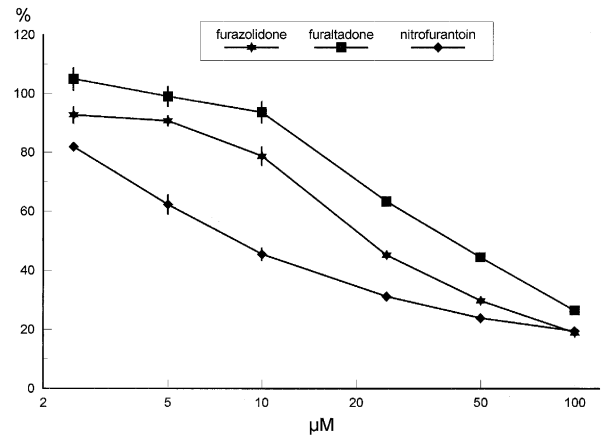

Fig. 1. Dose–inhibition curves of the nitrofurans furazolidone, nitrofurantoin, and furaltadone on the aldosterone release by the adrenocortical cell suspension with pregnenolone (1 μM) as substrate. 100% = 2.27 ± 0.08; 2.06 ± 0.05 and 2.32 ± 0.10 ng aldosterone/10^6 cells per h ($n = 4$) for furazolidone, nitrofurantoin and furaltadone, respectively.

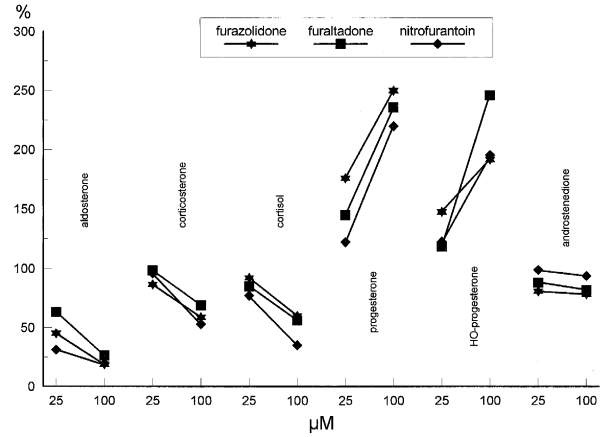

Fig. 2. Effect of furazolidone, furaltadone and nitrofurantoin (25 and 100 μM) on the steroid hormones released by porcine adrenocortical cells in vitro. Pregnenolone (1 μM) was used as substrate during the 1 h incubation. 100% = 2.22 ± 0.05 ng aldosterone/10^6 cells per h; 109 ± 2 ng corticosterone/10^6 cells per h; 27 ± 1 ng cortisol/10^6 cells per h; 36 ± 1 ng progesterone/10^6 cells per h; 18.4 ± 0.4 ng 17α-hydroxyprogesterone/10^6 cells per h; 2.3 ± 0.1 ng androstenedione/10^6 cells per h ($n = 4$).

4. Discussion

The in vitro inhibition by ten different nitrofurans of the aldosterone release (Jager et al., 1994) was characterized using furazolidone, furaltadone, and nitrofurantoin. The results clearly indicate that nitrofurans inhibit the mitochondrial enzymes involved in the final phase of the biogenesis of aldosterone, cortisol and corticosterone (Ohnishi et al., 1988). In a previous study we found that aldosterone synthesis is also inhibited when corticosterone, deoxycorticosterone or progesterone are used as substrates

Table 1
Nitrofuran-induced alterations in the release of steroids [a] by porcine adrenocortical cell suspensions [b]

Treatment	μM	Aldosterone (%)	Corticosterone (%)	Cortisol (%)	Deoxycortisol (%)	Progesterone (%)	HO-progesterone (%)	Androstenedione (%)
Control (ng/10^6 cells per h)		2.16±0.11	113±7	26±1	9.4±0.3	41±4	17.0±0.4	2.0±0.1
Furazolidone	25	45±1 [d]	87±5	92±3	88±9	176±14 [d]	148±16	81±4 [d]
	100	19±1 [d]	59±4 [d]	60±5 [d]	94±8	250±21 [d]	192±17 [d]	78±5 [d]
Furaltadone	25	63±1 [d]	99±1	85±5	94±7	145±12 [d]	119±5 [d]	88±2 [d]
	50	44±1 [d]	91±3	78±5 [d]	117±10	156±13 [d]	178±8 [d]	91±6
	100	26±1 [d]	69±1 [d]	56±6 [d]	99±4	236±9 [d]	246±11 [d]	82±6 [d]
Nitrofurantoin	10	46±1 [d]	106±2	87±2 [d]	87±8	106±2	105±6	98±2
	25	31±1 [d]	96±3	77±7 [d]	111±6	122±8 [d]	123±10	99±7
	100	20±1 [d]	53±2 [d]	35±3 [d]	119±8	220±6 [d]	196±9 [d]	94±3

[a] The release of dehydroepiandrosterone, testosterone, estrone and estradiol remained below detection limits.
[b] Release during treatment is expressed as a percentage (mean±S.E.M.; $n=48$; $N=12$) of the control release (1 h incubation with 1 μM pregnenolone and vehiculum).
[c] 17α-hydroxyprogesterone.
[d] Significant difference from 100% ($P<0.05$; t-test).

instead of the presently used pregnenolone (Jager et al., 1994). As the activity of the nitrofurans with these four substrates was similar, the nitrofurans probably inhibited the enzymes involved with the transformations at C-18; these reactions use corticosterone as substrate. Also the parallel pathway, which bypasses corticosterone seemed to be blocked by nitrofurans. From the present results it has to be concluded that also the hydroxylation at C-11 is affected. Thus the nitrofurans rapidly and reversibly inhibit all mitochondrial transformations from deoxycorticosterone to corticosterone and aldosterone and from deoxycortisol to cortisol.

It is now generally (Orth et al., 1992) assumed that one cytochrome enzyme, P-$450_{11,18}$, mediates these mitochondrial transformations (Yanagibashi et al., 1986). Different isomers of this enzyme seem to exist in the zona glomerulosa and in the zona fasciculata (Lauber et al., 1987; Müller et al., 1991). An inhibition of this enzyme by nitrofurans seems to explain our findings. The higher susceptibility of the aldosterone release to the inhibitory action of nitrofurans reflects the fact that corticosterone is also an intermediate in the biogenesis of aldosterone (Yanagibashi et al., 1988). A substrate reduction might thus add to the enzyme inhibition.

Another explanation of our observations might be that the nitrofurans inhibit the transport of the deoxysteroids across the mitochondrial membranes. At present we have no data to suggest in favor of either inhibition of the biotransformation or inhibition of the substrate supply in the mitochondria. Both explanations however, do not exclude each other.

Surprisingly the release of deoxycortisol was not enhanced in the presence of nitrofurans. Assuming that the same holds for deoxycorticosterone this might indicate a product regulated feedback in the biogenesis of these intermediate steroids. In an earlier study (Jager et al., 1996) we found that in the 0.3–10 μM range with pregnenolone as substrate increasing concentrations increased the release of corticosteroids, except for deoxycortisol and cortisol. The release of these steroids reached a maximum with 1–3 μM pregnenolone and higher concentrations of pregnenolone decreased the output. This bell-shaped dose–response curve of deoxycortisol with pregnenolone and the constant release of deoxycortisol with different concentrations K^+ also imply that the biogenesis of deoxycortisol is cellularly regulated (Jager et al., 1996).

17α-Hydroxyprogesterone is not only substrate in the biogenesis of deoxycortisol but also of androstenedione (see Jager et al., 1996, Fig. 6). Again changes in the release of androstenedione in the presence of nitrofurans did not reflect those of 17α-hydroxyprogesterone. If significant changes are observed then the release of androstenedione is lower while 17α-hydroxyprogesterone is higher. Changes in the release of androstenedione induced by altered K^+ or substrate concentrations or with ACTH do seem to follow changes in the release of 17α-hydroxyprogesterone (Jager et al., 1996). Therefore it is likely that nitrofurans also inhibit the C17,C20-lyase side chain cleavage. This effect of nitrofurans is however, marginal at concentrations which induce an blockade of the mitochondrial biogenesis of aldosterone, corticosterone and cortisol. Our observation that the middle concentration furaltadone did not induce a significant change can be attributed to variability in marginal effects. It is remarkable that the potency order of the three nitrofurans with regard to this effect (furazolidone > furaltadone > nitrofurantoin) differs from that of the inhibition of the mitochondrial steroids. This might indicate that this effect is mediated via a different mode of action and/or that it is not an attribute of all nitrofurans. Another extrapolation is that the relatively small or insignificant changes in androstenedione release, while concurrently a substrate is released at twice the control levels, hides a 50% inhibition of the C17,C20-lyase.

Quinoxalines such as carbadox and their major metabolites induced a slowly developing but virtually irreversible inhibition of the C18 transformations from corticosterone to aldosterone. But these compounds hardly affected the alternative pathway from deoxycortisol (Jager et al., 1994). As these drugs are given continuously, the biogenesis of aldosterone in vivo is likely to be seriously impaired, even when used in the advised dosages (Van der Molen et al., 1989). The animals might compensate with enhanced production via the alternative pathway and/or by enhanced release of other corticosteroids with mineralocorticosteroid activity. The rapid shutdown of the mitochondrial biogenesis of corticosteroids by nitrofurans will then cause hypoaldosteronism, which is typical for a quinoxaline intoxication.

The assumption that nitrofurans are carcinogenic drugs is solely based on long term feeding experiments with relatively high doses of nitrofurans to rodents. In these experiments the incidence of mammary tumors and neoplasia in the ovaries and testes was significantly enhanced. Also the onset of spontaneous mammary tumors in some strains was months earlier during treatment with nitrofurans (see Van Koten-Vermeulen et al., 1993). As most of the enzymes involved in the biogenesis of adrenal steroids are also present in other steroid hormone producing organs, a comparable differential effect on the biogenesis and release of steroid hormones is to be expected. A decrease of the circulating concentration of corticosteroids with an increase in the circulation of progestagens comparable to the extent observed in vitro might by itself bring about the carcinogenic activity observed (Zumoff et al., 1981; Labrie et al., 1987; Gomes et al., 1988; Najid and Habrioux, 1990). It seems that nitrofurans might not be carcinogenic drugs, and that the carcinogenic activity found must be attributed to experimental artifacts.

The presently reported effects of nitrofurans on the steroid biogenesis in vitro were observed at concentrations below or within the range of concentrations found in vivo

during treatment (see Jager et al., 1994). Antibacterial treatments are short and the side effects of nitrofurans concerning steroid biogenesis are not only fast in onset, but also reversible, as observed with aldosterone (Jager et al., 1994). Prolonged administration of nitrofurans causes a prolonged disturbance of the steroid balance, which is likely to cause numerous side effects. It seems therefore moot to weigh the benefits of relatively short antibacterial or antiprotozoal treatments with nitrofurans against carcinogenic risks. Nevertheless, treatment with nitrofurans during periods in which the steroid hormones have a critical role, such as during pregnancies, rapid growth and weaning, does have the potency to cause serious bodily and behavioral malformations and malfunctions (Roberts et al., 1987; Vahl and Stappers, 1985; Zimmermann et al., 1993).

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References

Anon, 1991. Nitrofurans; Withdrawal of Approval of New Animal Drug Applications. USDHHS, FDA, Rockville, MD, pp. 1–52.

Anon, 1993. Commission Regulation (EEG) No 2901/93 of 18 October 1993 amending Annexes I, II, III and IV to Council Regulation (EEG) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medical products in foodstuffs of animal origin. Official Journal of the European Communities, L 264.

Bergmeyer, H.U., Bent, E., Heff, B., 1965. Lactic dehydrogenase. In: Bergmeyer, H.U. (Ed.), Method of Enzymatic Analysis. Verlag Chemie, Weinheim, pp. 736–743.

Diem, K., 1980. Wissenschaftliche Tabellen/Geigy, Teilband Statistik, 8th ed. Ciba-Geigy, Basel.

Dodd, M.C., Stillman, W.B., 1944. The in vitro bacteriostatic action of some simple furan derivatives. J. Pharmacol. Exp. Chemother. 82, 11–18.

Gomes, P., Cassanas, G., Halberg, F., Hermida, R., Robel, P., Baulieu, E.-E., Lakatua, D., Haus, E., 1988. Taux sanguin du sulfate de la déhydroépiandrostérone (DHEA-S) et risque de cancer du sein [Blood concentration of dehydroepiandrosterone sulfate (DHEA-S) classifies for the risk of developing breast cancer]. C.R. Acad. Sci. Paris 306, 261–264.

Jager, L.P., de Graaf, G.J., Widjaja-Greefkes, H.C.A., Accord-Burleson, C.C.F., Van den Dungen, H.M., Baars, A.J., 1994. Effects of feed additives and veterinary drugs on aldosterone production and release by porcine adrenal cells in vitro. J. Vet. Pharmacol. Ther. 17, 175–185.

Jager, L.P., de Graaf, G.J., Widjaja-Greefkes, H.C.A., 1996. Screening for drug-induced alterations in the production and release of steroid hormones by porcine adrenocortical cells in vitro. Toxicol. in Vitro 10, 595–608.

Van der Kerk, P., 1985. Geneesmiddel intoxicaties bij biggen na langdurig gebruik van gemedicineerd voer [Toxic effects in piglets following long-term feeding of medicated feed]. Tijdschr. Diergeneesk. 110, 181–184.

Van Koten-Vermeulen, J.E.M., Wouters, M.F.A., Van Leeuwen, F.X.R., 1993. Furazolidone. Report 40th JECFA meeting. WHO-Food Additives Series 31, 85–123.

Labrie, F., Poulin, R., Simard, J., Hubert, J.-F., Spinola, P., Marchetti, B., 1987. Adrenal steroids exert potent estrogenic action in both normal and cancer tissue. In: Klijn, J.G.M., Paridaens, R., Foekens, J.A. (Eds.), Hormonal Manipulation of Cancer: Peptides, Growth Factors, and New (Anti)Steroidal Agents. Raven Press, New York, NY, pp. 7–16.

Lauber, M., Sugano, S., Ohnishi, T., Okamoto, M., Müller, J., 1987. Aldosterone biosynthesis and cytochrome P-450$_{11\beta}$: Evidence for two different forms of enzyme in rats. J. Steroid Biochem. 26, 693–698.

Legrand, C., Bour, J.M., Jacob, C., Capiaumont, J., Martial, A., Marc, A., Wudtke, M., Kretzmer, G., Demangel, C., Duval, D., Hache, J., 1992. Lactate dehydrogenase activity of the number of dead cells in the medium of cultured eukaryotic cells as marker. J. Biotechnol. 25, 231–243.

Müller, J., Schmid, C., Böni-Schnetzler, M., Lauber, M., 1991. Two forms of cytochrome P-450$_{11\beta}$ in rat zona glomerulosa cells: A short review. Endocr. Res. 17, 165–184.

Najid, A., Habrioux, G., 1990. Biological effects of adrenal androgens on MCF-7 and BT-20 human breast cancer cells. Oncology 47, 269–274.

Ohnishi, T., Wada, A., Lauber, M., Yamano, T., Okamoto, M., 1988. Aldosterone biosynthesis in mitochondria of isolated zones of adrenal cortex. J. Steroid Biochem. 31, 73–81.

Orth, D.N., Kovacs, W.J., DeBold, C.W., 1992. The adrenal cortex. In: Wilson, J.D., Foster D.W. (Eds.), Williams Textbook of Endocrinology. Saunders, Philadelphia, pp. 489–619.

Paul, H.E., 1956. Research background on the nitrofurans. In: Reid, W.M. (Ed.), Proc. 1st Natl. Symp. on Nitrofurans in Agriculture. Michigan State University, East Lansing, pp. 6–11.

Roberts, E., Bologa, L., Flood, J.F., Smith, G.E., 1987. Effects of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice. Brain Res. 406, 357–362.

Snyder, M.L., Kiehn, C.L., Christopherson, J.W., 1945. Effectiveness of a nitrofuran in the treatment of infected wounds. I. Preliminary report. Milit. Surg., pp. 380–384.

Vahl, H.A., Stappers, H.P., 1985. Effecten van groeibevorderaars en furazolidon bij slachtkuikens [Effects of feeding growth-stimulating agents and furazolidone to broilers]. Tijdschr. Diergeneeskd. 110, 185–188.

Van Miert, A.S.J.P.A.M., Vroomen, L.H.M., Jager, L.P., 1984. Farmacologie en toxicologie van furazolidon en carbadox; een literatuuronderzoek [Pharmacology and toxicology of furazolidone and carbadox, a study of the literature]. Tijdschr. Diergeneesk. 109, 928–933.

Van der Molen, E.J., De Graaf, G.J., Spierenburg, T.J., Nabuurs, M.J.A., Baars, A.J., Jager, L.P., 1986. Hypoaldosteronism in piglets induced by carbadox. Experientia 42, 1247–1249.

Van der Molen, E.J., Baars, A.J., De Graaf, G.J., Jager, L.P., 1989. Comparative study of the effect of carbadox, olaquindox and cyadox on aldosterone, sodium and potassium plasma levels in weaned pigs. Res. Vet. Sci. 47, 11–16.

Yanagibashi, K., Haniu, M., Shively, J.E., Shen, W.H., Hall, P., 1986. The synthesis of aldosterone by the adrenal cortex. Two zones (fasciculata and glomerulosa) possess one enzyme for 11β-, 18-hydroxylation, and aldehyde synthesis. J. Biol. Chem. 261, 3556–3562.

Yanagibashi, K., Shackleton, C.H.L., Hall, P.F., 1988. Conversion of 11-deoxycorticosterone and corticosterone to aldosterone by cy-

tochrome P-450 11β-/18-hydroxylase from porcine adrenal. J. Steroid Biochem. 29, 665–675.

Zimmermann, H., Gutte, G., Kern, M., Golbs, S., Ludewig, T., 1993. Reproduktionstoxikologische Grundlagenuntersuchungen zur Wirkung von Furazolidon auf die Achse Hypothalamus-Hypophyse-Gonaden der männlichen Ratte [Reproductive-toxicologic investigations concerning the effect of furazolidone on the axis hypothalamus–hypophysis–gonads of male rats]. Dtsch. Tierärztl. Wochenschr. 100, 91–94.

Zumoff, B., Levin, J., Rosenfeld, R.S., Markham, M., Strain, G.W., Fukushima, D.K., 1981. Abnormal 24-hr mean plasma concentrations of dehydroisoandrosterone and dehydroisoandrosterone sulfate in women with primary operable breast cancer. Cancer Res. 41, 3360–3363.