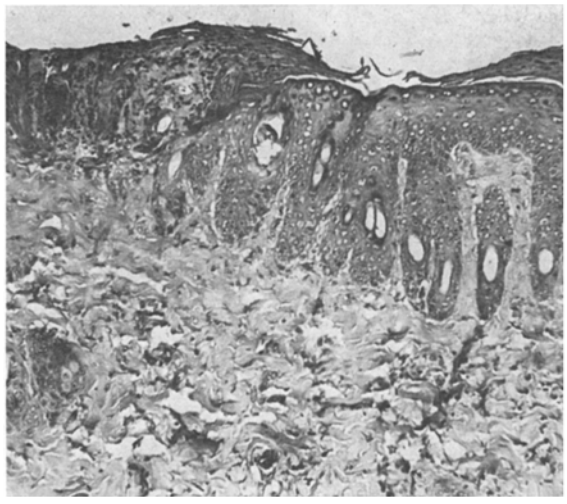

Fig. 2. Intra-epidermal as well as dermal abscess filled in with polymorphonuclear cells. Mild acanthosis seen with elongated rete ridges and cells of epidermis at some places appeared necrosed (25 paintings) × 100.

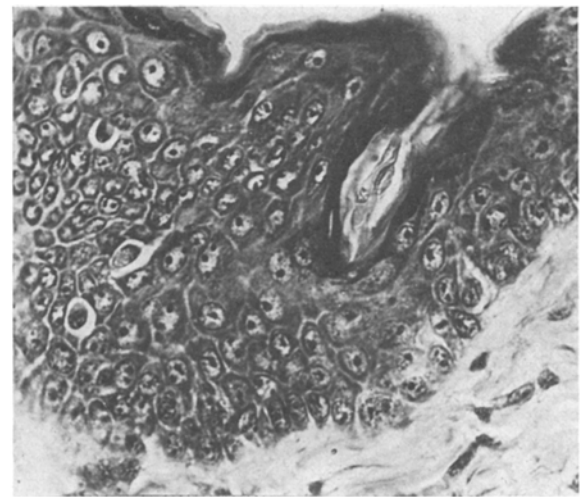

Fig. 3. Vacuolization and multinucleate condition of the cells of malphighian layer (25 paintings). × 400.

25 days has shown vacuolization and multinucleate condition of the cells of malpighian layer (Figure 3).

The danger of absorption of lindane is increased with prolonged handling of powders and emulsions. Higher dermal toxicity of lindane in lipoid solvents is especially noteworthy[2]. Tolerance to progressively increased concentrations of lindane has not been observed. Reports are available both for and against the safety of lindane. The comparatively quick excretion of the compound by the renal system acts as a protective measure against the cumulative effects of the compound, particularly after repeated exposure. Cases of dermatitis and urticaria in humans are also recorded in relation to lindane exposures. These symptoms of allergy are, however, manifested in individuals who are susceptible to this compound[3].

Findings reported here are of interest because of their possible relationship to the cases of occupational poisoning, due to such exposure under tropical conditions. These abnormalities are not reported here as an indication that the picture in man would be similar, but the information gathered from these studies indicates the possible types of skin damage due to insecticides[10].

Zusammenfassung. Langfristig wiederholte Lindan-Applikation ruft bei der Abinoratte histopathologische Hautveränderungen hervor: Hyperkeratinisation, intraepidermale und dermale Abszesse, Infiltration polymorphkerniger Zellen sowie Vakuolisation und Vielkernigkeit in der Malpighi-Schicht.

T. S. S. Dikshith, Prakash Chandra and K. K. Datta

Industrial Toxicology Research Centre, Chattar Manzil Palace, Lucknow (India), 28 November 1972.

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The Effect of Spironolactone on Acute Toxicity and Liver Damage in Mice Induced by Cerium Chloride

Cerium belongs to the light lantanons. The greater part of i.v. administered cerium quickly accumulates in the hepatic tissue. The maximal concentration, about 70% of the administered dose, is obtained a few hours after administration[1]. In large doses, cerium causes fatty infiltration and necrosis in the liver. The structure of hepatic endoplasmic reticulum is changed[2], and an inhibition of drug metabolism in rat liver has been established[3]. Arvela and Kärki[4] found that phenobarbital impairs the inhibitory effect of cerium on drug metabolism. We were interested in finding out if and to what extent the spironolactone pre-treatment, which induces drug metabolism in the liver[5], prevents the acute toxicity and liver damage induced by cerium chloride ($CeCl_3 \cdot 6H_2O$).

Material and methods. Altogether, 134 female NMRI mice were used in the experiment. The initial weight of the animals was about 30 g (ranging between 26–36 g), and the average age was about 3 months. During the experiment, the animals received normal laboratory pellet food and water ad libitum; the temperature varied be-

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Table I. The effect of spironolactone pre-treatment on acute toxicity in female mice induced by 2 different doses of cerium chloride

Dose	Mortality Control	Spironolactone	Significance
12 mg/kg	55% (22)	10% (20)	$p < 0.01$
15 mg/kg	93% (28)	63% (24)	$p < 0.025$

The figures indicate percentage mortality. Number of animals in parentheses. Statistical analysis was carried out using the X^2-test.

Table II. The effect of spironolactone pre-treatment on the hepatic lesion induced by 15 mg/kg cerium chloride in female mice

Days after cerium injection	Control	Spironolactone	Significance
1	1.0 ± 0.0	0.4 ± 0.2	$p < 0.05$
2	2.1 ± 0.2	1.1 ± 0.3	$p < 0.01$

Each group consist of 10 mice. Results expressed as mean $\pm$ SE. Statistical analysis was carried out using Student's t-test.

tween 22 °C and 24 °C, and the moisture between 48% and 65%.

In the first part of the experiment, 94 mice were divided into 2 groups: 44 animals received 100 mg/kg spironolactone p.o. twice daily in 2% carboxy methyl cellulose (CMC) (5ml/kg) for 3 days. The other group, 50 animals (control), received only 2% CMC (5 ml/kg). On the 4th day, both the test and the control group were given a toxic dose of cerium chloride (12 or 15 mg/kg i.v.) in physiological saline solution (pH 5.1). The animals were kept under observation for 7 days, and the mortality was registered.

In the histological part of the experiment, 40 mice were divided into 4 groups, 10 in each. 2 groups received 100 mg/kg spironolactone p.o. in CMC (5 ml/kg) twice daily for 3 days. The other 2 groups (control) received only CMC (5 ml/kg). On the 4th day all animals received 15 mg/kg cerium chloride i.v. The animals were decapitated on the 1st and the 2nd day after the cerium injection. The degree of hepatic lesion was estimated macroscopically on the basis of the following scale: 0, no changes; 1, small, separate, pale focuses; 2, wide, uniform, pale areas; 3, wide, uniform, haemorrhagic areas. 2 pieces of liver were immediately taken in longitudinal sections from the central lobe of the liver. 1 piece was frozen in CO_2-ice (−70 °C), cut in a cryostat (−20 °C) and stained with scarlet-red to demonstrate fat. The other piece was fixed in neutral formalin, embedded in paraffin and stained with van Gieson and Hematoxylin-Eosin for histological examination. All 40 animals were examined microscopically.

Results. Most of the animals died on the 3rd and 4th day after cerium injection. The difference in mortality between the control group and the pre-treated group is statistically significant both in the 12 mg/kg and 15 mg/kg cerium dosage groups $p < 0.01$ and $p < 0.025$ respectively. The results are given in Table I.

In the histological part of the experiment, the difference in macroscopic mean value of hepatic lesions 1 day after the cerium injection between the control group and the spironolactone pre-treated group was statistically significant ($p < 0.05$). 2 days after cerium injection, the mean values also different significantly ($p < 0.01$). The results are given in Table II.

The microscopical investigation fully corroborated the results of the macroscopical observations. In gradus 0 the greater part of hepatic tissue was normal. A few single cells were necrotic. In gradus 1 focuses of unchanged hepatic cells were detected. Necrotic tissue also in focuses with nuclei could be seen in their proximity. Occasionally cells with 2 nuclei, and some mitotic figures were observed. Clearly necrotic areas consisted of a few cells. Gradus 2 consisted of unchanged and necrotic tissue in equal quantities. Nuclei were unstained and the cytoplasm seemed more solid than usual. The unchanged areas were situated around capillaries. Leucocytes as well as mitotic figures were found in moderate or abundant amounts. In gradus 3 areas of uniform haemorrhagic necrosis could be seen, often covering the whole sample. A small amount of polymorphonuclear leucocytes were found among the erythrocytes. Haemorrhagic areas were wide, overlapping the necrotic tissue. The fatty infiltration always coincided with the tissue necrosis.

Discussion. The results show that pre-treatment with spironolactone decreases the acute lethal toxicity induced by cerium in mice. Cerium differs in this way from carbon tetrachloride (CCl_4) and white phosphorus. It has been observed earlier that the induction of the drug-metabolizing enzyme system in the liver increases the toxicity of CCl_4[6], but does not affect the toxicity of white phosphorus[7].

The pre-treatment with spironolactone also decreased the liver damage induced by cerium chloride. It is obvious that the decrease of mortality depends on the less extensive liver damage in the spironolactone pre-treated animals. The greater part of i.v. administered cerium chloride is taken up by the liver tissue where it is retained for at least 14 days[1,8]. According to MAGNUSSON[2], the secretion of cerium in bile is very small. The possible increase in bile flow caused by spironolactone treatment does not explain the decreased hepatotoxicity. BJONDAHL et al.[1] found that the Ce^{144} output in feces did no differ between the control groups and the spironolactone prereated groups. The mild diuretic effect of spironolactone treatment did not speed up the Ce^{144} output in urine either.

Earlier investigations have shown that cerium causes changes in the liver endoplasmic reticulum[2] and impairs the activity of the drug-metabolizing enzymes[3]. ARVELA and KÄRKI[4] found that the pre-treatment with phenobarbital had a normalizing effect on the cerium-induced changes in the metabolism of rat liver.

It is known that spironolactone increases the amount of smooth endoplasmic reticulum in the liver[9] and speeds up the drug metabolism[5]. Probably the increased amount of smooth endoplasmic reticulum caused by spironolactone pre-treatment decreases the hepatotoxicity induced by cerium.

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Zusammenfassung. Vorbehandlung von 3 Tagen mit Spironolacton verringert die Mortalität und die Leberdegeneration, die durch i.v. Gabe einer toxischen Dosis Cerium Chlorid verursacht wird.

K. Bjondahl, M. Möttönen and L. Nieminen

Research Center, Lääke-Medipolar, SF–20360 Turku 36 (Finland) and Department of Forensic Medicine, University of Turku, SF–20520 Turku 52 (Finland),

29 November 1972.

Antiandrogenic Potency of Drugs, Evaluated by Urinary Enzyme Excretion Technique

Among the numerous enzymatic activities in urine, only acid phosphatase is related to male genital secretions and to androgenic activity in man and laboratory animals[1,2]. Acid phosphatase activity in urine derives partly from serum and partly from prostatic secretions[3]. As a consequence, urinary acid phosphatase activity in women and in female laboratory animals is significantly lower than in males.

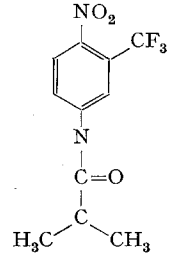
In the following paper, a new antiandrogenic compound, 4′-nitro-3′-trifluoromethyl-isobutyranilide[4] (Formula), was evaluated by studying the changes in urinary enzymatic activities in rats. In addition to acid phosphatase, alkaline phosphatase and 'leucine aminopeptidase' activities were determined.

Material and methods. 25 male rats of the strain FW 49 Biberach were used, weighing 200 ± 20 g at the start of the experiments. The animals were kept in metabolic cages for collecting 24-h urine specimens. At the beginning of each collection period, 10.0 ml of water were administered orally to produce maximal diuresis. After determination of control values (mean of 2 consecutive days), 20 animals were fed daily 0.2 g/kg of the antiandrogenic compound, suspended in 10.0 ml of water. On the 1st, 8th and 15th day of the experiment, urines were collected. 5 controls received water only over the same period of time. On the 15th day of the experiment, all animals were killed and serum was prepared for the determination of acid phosphatase activity.

Urinary acid phosphatase (SP), alkaline phosphatase (AP), and 'leucine aminopeptidase' (LAP) activities in rats following the administration of nitro-trifluoromethyl-isobutyranilide

	mU SP	mU AP	mU LAP
Start	93.6 ± 14.7	313.0 ± 34.7	96.2 ± 13.3
1st day	53.1 ± 16.4	254.9 ± 54.2	89.1 ± 17.4
8th day	37.4 ± 10.5[a]	403.8 ± 82.1	94.3 ± 16.4
15th day	36.7 ± 12.3[a]	286.9 ± 44.3	82.7 ± 15.3

[a] $P < 0.01$ vs. control (start). All values refer to total 24-h excretion.



The structural formula of 4′-nitro-3′-trifluoromethyl-isobutyranilide.

In the urines, acid phosphatase (SP; EC 3.1.3.2), alkaline phosphatase (AP; EC 3.1.3.1) and 'leucine aminopeptidase' (LAP; EC 3.4.1.2) activities were determined according to the methods published previously[3,5]. All enzymatic activities were calculated on the total 24-hours urine output.

Results. In the controls, no significant changes in urinary enzymatic activities were observed. In the rats under antiandrogenic medication, urinary AP- and LAP-activities were not altered significantly, whereas SP-activity revealed a statistically significant decrease. Details are compiled in the Table. SP-activity of serum amounted to 9.6 mU in the controls and to 10.2 mU in the animals under antiandrogenic treatment ($P > 0.1$).

Discussion. In rats, castration is accompanied by a marked drop in SP-activity in the prostate[6]; a similar effect is exerted by estrogens and antiandrogens[6,7]. The decrease in urinary SP-activity in rats under antiandrogenic medication observed in this study, may be attributed to a drug-induced drop of prostatic enzyme levels. Changes in prostatic alkaline phosphatase and aminopeptidase which were demonstrated under similar conditions[6] did not take any significant influence on urinary enzymatic activities.

For screening antiandrogenic properties of new compounds, determinations of urinary SP-activity might be useful as they provide reliable results with minimal technical effort. Comparative studies might be suitable for evaluating the relative efficacy of various antiandrogens.

Zusammenfassung. Bei Ratten konnte nach der Verabreichung von Nitrotrifluoromethylisobutyranilid, einer neuen antiandrogen wirksamen Substanz, ein signifikanter Abfall der sauren Phosphatase-Aktivität im Harn festgestellt werden. Andere Harnenzymaktivitäten (alkalische Phosphatase, «Leucinaminopeptidase») blieben unbeeinflusst. Die Aktivität der sauren Phosphatase im Serum zeigte nach zweiwöchiger Verabreichung des Antiandrogens keine signifikante Veränderung. Die Bestimmung der sauren Phosphatase-Aktivität im Harn eignet sich demnach zum einfachen «Screening» antiandrogener Wirkungen.

W. P. Raab and Claudia Mörth

Vienna University Medical School, Department of Medical Chemistry, Währingerstrasse 10, A–1090 Wien (Austria),

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