injection (0.1 ml per 25 g mouse) of $CH_3{}^{203}HgCl$ (0.4 mg Hg/kg) in 5 mM Na_2CO_3 on day 0. Three 8-week-old $C129F_1$ male mice were similarly dosed (0.4 mg Hg/kg). $CH_3{}^{203}HgCl$ (specific activity 1.55 mCi/mg Hg, purity >92%) was purchased from New England Nuclear and was mixed with nonradioactive (ethanol-recrystalized, purity >99%) methylmercuric chloride (K&K Labs, Plainfield, NJ). Total body radioactive mercury was determined immediately after dosing, and again on day 8 before autopsy, with a whole body gamma counter, by placing each mouse in the 70×130 mm center well of a 135 mm diameter × 160 mm deep thallium activated sodium iodide crystal. Kidneys were counted in a Packard Series 5000 autogamma spectrometer. Mercury body burdens and kidney mercury concentrations were calculated after correction for radioactive decay and are expressed as nanograms of mercury, Fractions of Initial Dose (F.I.D.) or Fractions of Initial Concentration (F.I.C.):

$$\text{F.I.D.} = B_t \div B_0 \quad \text{F.I.C.} = \frac{B_t \div W_t}{B_0 \div W_0} \quad \text{(or)} \quad \frac{K_t \div KW_t}{B_0 \div W_0}.$$

B_0 and B_t = total body radioactivity on day 0 or day t; W_0 and W_t = body wt on day 0 or day t; K_t = total radioactivity of kidneys on day t; KW_t = total weight of kidneys on day t. Results are recorded in the table and show that 8 days after the methylmercury dose, adult male mouse kidneys contain approximately twice as much radioactive mercury per g wet wt as do kidneys of similarly dosed adult females.

To confirm this observation using another stock of mice, 4 female and 4 male 16-week-old strain 129 mice were each similarly dosed i.p. with $CH_3{}^{203}HgCl$ (0.4 mg Hg/kg; specific activity, 1.71 mCi/mg; purity >99%). Total body counts were determined on day 0 and on day 5 before the mice were autopsied. Kidneys were counted as described for the previous experiment. Results are shown in the table. t-tests were performed to test the hypothesis that there were no significant differences in kidney mercury concentration between males and females in terms of retained whole body total mercury content (F.I.D.), whole body mercury concentrations (F.I.C.) or kidney mercury concentrations (F.I.C.). Probability values for the stated hypothesis are listed in the table. It can be seen that though no significant sexual differences were observed in terms of whole body mercury content (F.I.D.), and whole body mercury concentrations (F.I.C.), highly significant differences were found between male and female kidney mercury concentrations (F.I.C.).

A 2fold sex difference has been observed in the amount of alcohol dehydrogenase contained in kidneys of adult male mice compared to those of adult females[2]. Sex differences in activities of mouse kidney glucuronidase have also been reported[3]. Our results reveal that adult male and female mouse kineys concentrate mercury differently.

We are currently examining sex correlated differences in metabolic processing of mercury compounds in terms of mouse strain, stage of development, cellular localization and hormonal stimulation. These observations could help in defining biochemical details of renal excretion of different forms of mercury. A better understanding of factors influencing excretion of mercury compounds is expected to aid in evaluation of hazards of exposure to different mercury compounds and in design of treatment methods for accelerating excretion of mercury compounds.

Whole body retention and kidney concentrations of mercury in adult male and female mice

Mouse strain	Sex	F.I.D. Whole body $\bar{x}$ (SE)	F.I.C. Whole body $\bar{x}$ (SE)	F.I.C. Kidney $\bar{x}$ (SE)
$C129F_1$ (day 8)	Male	0.495 (0.031)	0.481 (0.021)	5.33 (0.40)
	Female	0.507 (0.019)	0.507 (0.020)	3.34 (0.20)
t-test result (p)		(0.76)	(0.42)	(0.011)
129 (day 5)	Male	0.704 (0.013)	0.683 (0.019)	7.47 (0.16)
	Female	0.691 (0.006)	0.691 (0.001)	3.57 (0.15)
t-test result (p)		(0.39)	(0.68)	(0.000002)

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Free radical intermediates produced by autoxidation of 1,8-dihydroxy-9-anthrone (dithranol) in pyridine

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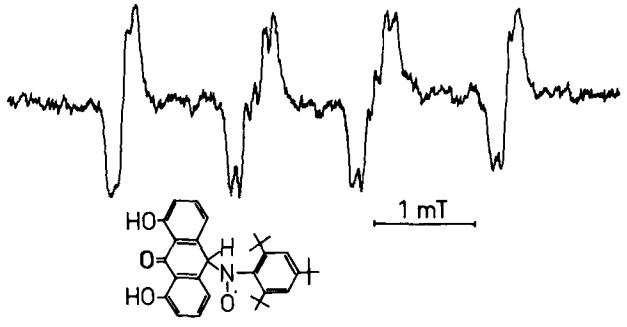
Summary. The autoxidation of the antipsoriatic agent dithranol, monitored by an ESR-spectrometer, proceeds through several free radical intermediates. The initial radical, attacking a bulky spin trapping agent in a sterically comparatively hindered constellation, may be the active therapeutic form of dithranol.

Dithranol (anthralin, anthranol, Cignolin®) has been the mainstay in the topical treatment of psoriasis since 1916[1,2]. Indirect evidence has accumulated that dithranol has to be activated before its final oxidation to the therapeutically inactive anthraquinone and dimeric products[3], and it has been postulated that a free radical mechanism is involved[4]. We have proved this by electron spin resonance (ESR) and spin trapping techniques.

The autoxidation of dithranol (~1 mmole/l) in pyridine solution in the presence of 2,4,6-tri-tert-butylnitrosobenzene (TBNB) (~3 mmoles/l), known as an effective difunctional spin trapping agent[5,6], gave the ESR spectrum shown in the figure. It indicates the presence of one TBNB adduct with the g-value 2.0066 and the hyperfine splitting constants $a_N = 1{,}20$ mT (1 N), $a_{H(\beta)} = 1{,}36$ mT (1 H), and $a_{H(m)} = 0.075$ mT (2 H), as verified by computer simulation. These data are in accordance with literature[5,6] values for a nitroxyl-type adduct so that the illustrated spectrum is assignable to a trapped 1,8-dihydroxy-9-anthrone radical in which the attacked site is the 10-position (see figure). It is

interesting that in spite of its bulky t-butyl groups TBNB acts as a spin trapping agent for such a large radical. It could be expected that the adduct would prefer the sterically less hindered anilino-type structure. This radical may be the active therapeutic form of dithranol. Swanbeck and Thyresson have calculated that dithranol interacts with DNA by intercalation between every 8th base-pair of the double-stranded DNA molecule[7].



The ESR-spectrum of the trapped dithranol radical produced by autoxidation in pyridine. The spectrum was recorded 4 min after dissolution. A Varian E-4 ESR-spectrometer with 100-kHz field modulation was used.

In dilute solutions containing oxygen, the adduct radical is stable enough for ESR-measurement. Within few hours it converts, however, into several different radicals, not trapped by TBNB. The ESR-spectra of these radicals appear in succession at $g = 2.0050$. In more concentrated (~ 3 mmoles/l) dithranol solutions, their formation rate increases and very strong ESR-signals are recorded. The last ESR-spectrum recorded after completion of the oxidation is a very intense singlet, and the corresponding solution has acquired a dark-brown color similar to the 'dithranol brown' well-known to the dermatologists[3]. The work is continued with identification of the latter radicals.

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Influence of treatment duration on audiogenic seizure susceptibility during barbiturate withdrawal in rats

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Summary. Barbiturate withdrawal seizure susceptibility in rats increased with increasing duration of treatment during a 15-day treatment period in which the animals were given an i.p. dose of sodium barbital every 12 h. This method of producing dependence has clear advantages over previously described methods.

In the study of barbiturate dependence phenomena investigators have put forth considerable effort in producing drug dependence in animals, sometimes requiring weeks or months of treatment[1-4]. In these experiments sodium barbital was administered to the animals in their drinking water. Although this method of administration of drug is a relatively simple procedure, it results in some variation in barbiturate blood levels produced[4] since it is impossible to measure and adjust for the amount of drug which is lost by spillage and dribbling or variations in fluid intake by the animals. Since some degree of dependence has been reported after short term treatment of animals with barbiturates[5], the present experiment was conducted to determine the relationship between barbital treatment duration and withdrawal-induced audiogenic seizure susceptibility.

Method. Male rats of Sprague-Dawley descent weighing 200 g (±20 g) at initiation of treatment, and which had been previously screened to be nonresponsive to audiogenic seizure stimulus were used in the study.

Audiogenic response score (ARS) was measured by the method of Jobe et al.[6], in which animals were exposed to high intensity sound (approximately 120 db) produced by 2 electric fire bells inside a testing chamber approximately 40 cm in diameter. In this method the score is determined by visual observation and depends on the number of running fits which characteristically precede the convulsion, and the extent of clonus and/or tonus during the convulsive phase of the seizure. The possible scores range from 1 to 9, with a score of 1 being assigned to a seizure consisting of running fits only, with no convulsive episode. A score of 9 represents a seizure consisting of 1 running fit terminated by a tonic convulsion involving full tonus of the torso and all limbs of the animal.

Test animals received i.p. injections of sodium barbital, 150 mg/kg every 12 h for treatment periods of 5, 10, or 15 days. Control animals received a comparable regimen of normal saline. At the end of the treatment period, injec-

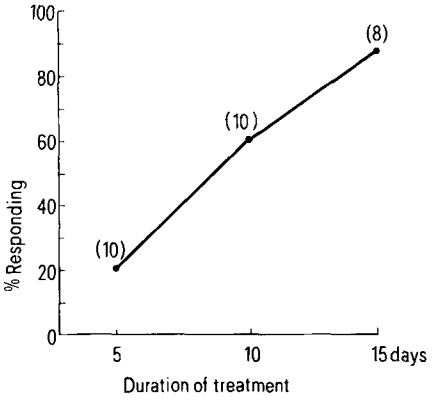

Fig. 1. Relationship between treatment duration and percent of animals responsing (exhibiting any degree of audiogenic seizure susceptibility)during withdrawal. Numbers in parentheses indicate the number of animals tested. Normal saline-treated controls were tested for each time period and found to be unresponsive to the stimulus (see text).