bility of the procedure, as indicated by the standard error of quadruplicate determinations, is also shown. The extraction technique effectively separates betahistine from normally interfering serum constituents since determinations with normal serum give little or no response. The major metabolite of betahistine, 2-(2 amino ethyl)pyridine[4,5], which has similar pharmacologic activity[3,6], was also detected by this technique. About 45% of the serum betahistine is reproducibly extracted regardless of drug concentration (table) over a range of 0.6–6.0 µg/ml.

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Mammalian spot test with moxnidazole, a 5-nitroimidazole[1]

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Summary. Moxnidazole [3-(5-nitro-1-methyl-2-imidazolyl)-methyleneamino-5-morpholinomethyl-2-oxazolidinone, HCl], known to be mutagenic in microbial tests and *Drosophila,* induced genetic alterations in somatic cells of mice. Ethyl methanesulfonate (EMS) served as positive control.

The mammalian spot test, an in vivo method for the detection of genetic alterations - especially point mutations - in somatic cells of mice[2], has been shown to be sensitive to mutagens with different modes of action[2–6]. According to this method, embryos which are heterozygous to 4 different recessive coat-color genes are treated in utero during the 7th to the 10th day of fetal development. If the treatment leads to an alteration or to a loss of the wild type allele of one of the genes under study in a pigment precursor cell, a colour spot in the adult coat will result.

A number of 5-nitroimidazoles have mutagenic properties[7–12], and additionally metronidazole was shown to be carcinogenic in mice[13]. While moxnidazole was found to be mutagenic for *Salmonella typhimurium* TA 1538, *Escherichia coli* WP_2, WP_2 uvrA$^-$ (unpublished results), 343/113[14], *Neurospora crassa* heterokaryon H 12[14] and *Drosophila* (inducing recessive sex-linked lethals)[15], it was negative in the dominant lethal assay in male mice (unpublished results). The purpose of the present experiment was to investigate whether moxnidazole can induce point mutations in mammals in vivo.

Materials and methods. Embryos of the genotype *a/a; b/+; $c^{ch}p$/++; d se/++; s/+* (black coat, dark eyes) were produced by mating 9-week-old virgin females of the inbred C57BL/6J/BOM-spf strain (*a/a,* otherwise wild type) to fertile males of the rotation bred T-stock (*a/a* = nonagouti; *b/b* = brown; *$c^{ch}p/c^{ch}p$* = chinchilla and pink-eyed dilution; *d se/d se* = dilute and short ear; *s/s* = piebald spotting). The randomly selected pregnant females were given 1000 mg moxnidazole/kg b. wt (Schering AG, Berlin/Bergkamen) 3 times as a microcristalline suspension in physiological saline by gavage 8, 9 and 10 days after observation of the vaginal plug (day 1). Control females were given physiological saline at 10 ml/kg in the same manner. EMS (Ferak, Berlin, batch 7306) (100 mg/kg) dissolved in physiological saline served as positive control and was injected at the same days i.p. Litters were checked for colour spots once a week between 2 and 5 weeks of age. Examination of spots was performed with the naked eye. Differentiation between midventral white, white-gray and light-gray spots was possible by fluorescence-microscopy[2]. According to Fahrig[2], white and white-gray spots result from pigment cell death rather than from genetic alterations, and therefore only non-white spots were considered to be of genetic origin.

Results and discussion. The frequency of colour spots in negative and positive (EMS) control animals, as well as in moxnidazole treated mice, is summarized in the table. Compared to the negative control, EMS and moxnidazole induced a significant increase ($p < 0.01$) in brownish and grayish spots. Out of 235 offspring, 13 offspring (5.5%) with non-white spots were derived from 10 EMS treated females. In comparison, out of a total number of 255 offspring, non-white spots occurred in 9 (3.5%) derived from 8 females given moxnidazole, whereas none of the 275 offspring of the control animals showed similar spots. In the moxnidazole group, 1 animal showed 2 clearly separated spots. In addition to the non-white spots, 4 of the

The effect of moxnidazole and EMS in the mammalian spot test

Treatment	Dosage (mg/kg) (route and number of doses)	Females Treated	With litters surviving to observation	Offspring Surviving to observation (average litter size)	With mid-ventral white spots	With white-gray spots	With spots of brownish or grayish color
Negative control	0 (p.o. 3)	65	42	275 (6.6)	1	0	0
Moxnidazole	1,000 (p.o. 3)	70	38	255 (6.7)	4	0	9*
Positive control (EMS)	100 (i.p. 3)	67	38	235 (6.2)	17	1	13*

*$p < 0.01$ vs control. Fisher's exact test (one-tailed).

EMS treated animals showed 1 white midventral spot. In the moxnidazole group, this could be observed in only 1 animal. All together 17 offspring with midventral white spots were found in the EMS group (additionally 1 with a midventral white-gray spot), 4 in the moxnidazole group and only 1 in the control animals. Main positions for colour spots for the EMS/moxnidazole group were in percent: head 0/20; back and sides 85/40; ventral 15/40. The low spontaneous frequency, the position and distribution of the spots are in good agreement with published data[2,6].

The results presented show that moxnidazole induces not only point mutations in microbes and *Drosophila* but also genetic alterations in mammalian somatic cells in vivo. Since mutagens of different types of action including a 5-nitroimidazole have proved to be active in the mammalian spot test, it can be expected that this test system will become a suitable procedure for routine testing of environmental mutagens and possibly carcinogens.

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Amnesic effects of intravenous diazepam and lorazepam

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Summary. The amnesic effects of 2 benzodiazepine drugs, diazepam (Valium) and lorazepam (Ativan), have been investigated. Some of the effects were similar to those of certain clinical amnesic syndromes. The effects were more extensive than previous work has indicated.

Amnesic effects have been reported[5–8] following i.v. injection of the benzodiazepines, diazepam (Valium) and lorazepam (Ativan). The effects of lorazepam have previously been investigated in a clinical setting under preoperative conditions, so that extensive testing of the subjects has not been possible. The present experiment was designed to show whether the amnesic effects of these 2 drugs are similar to those of certain clinical amnesic syndromes[9–11]. If they are, a more effective analysis of the syndromes becomes possible, since the onset and duration of the amnesia is then under experimental control and the same subjects can be tested with and without the drug. In addition, the drugs may be of value in analysing the neural basis of memory using experimental animals.

Material and methods. 2 cycles of memory tests (described below) were used to divide 27 medical student volunteers into 3 equal matched groups. 3 days later, on the day of injection, there were 10 further cycles. After cycle 1, either 2 ml of solution containing 7.5 mg diazepam or 3.0 mg lorazepam, or 2 ml of normal saline was given by slow i.v. injection. A double-blind procedure was employed. A cycle comprised: a) A list of 10 words from a single category, such as flowers, projected on to a screen for 20 sec. At the same time the words were spoken from prerecorded tape. The list was followed by 6 random digits. The task was immediate, ordered recall of the digits followed by free recall of the words. Recall was written in a booklet. b) Word completion. The 1st 3 letters of 10 5-letter words were provided in the booklet. After an initial attempt to write down the solutions, these were projected (feedback) and a 2nd attempt was made on another page of the booklet. c) Picture recognition. 2 colour-slides of landscapes were presented for later recognition. Following cycles 1–5 and 6–10 the materials presented in the previous 5 cycles were retested (delayed tests). Free recall of the word lists was cued by presenting the category names, e.g. 'flowers'. The recall test was followed by a recognition test in which the words were mixed with an equal number of new words from the same categories. Each word was rated as new or old on a 4-category scale. From the rating data, the A-index[12,13] was calculated: if recognition were all-or-none, this index would be equivalent to the proportion of original items genuinely recognized. Recognition of the pictures was evaluated by a similar method. New booklets were used for the retest of word completions. Immediately after the delayed tests, simple reaction times to an auditory stimulus were measured. Cycles 1–5 were before lunch and at 12-min intervals, apart from a double interval between cycles 1 and 2 for the injection. Cycles 6–10 were at 30-min intervals and commenced 200 min after injection. Thus the effects of the drugs were studied over a 5.5-h period. The results for 2 lorazepam subjects and 1 diazepam subject who failed to complete the experiment were discarded.

Results and discussion. Our major findings for the injection day were as follows: 1. With the exception of 1 lorazepam subject, immediate recall of digits was good on all cycles, with over 90% of sequences wholly correct. 2. Substantial impairment was found in the free recall of words in both immediate postdigit recall (figure, a) and delayed recall (figure, b). For cycles 2–5 combined, the 2 drug groups were significantly worse than the saline group both in the immediate and in the delayed tests ($p < 0.001$, based on one-way analysis of variance). For cycles 6–10 combined, the impairments were significant only for the lorazepam group ($p < 0.001$). The peak effect with diazepam on cycle 2, about 12 min after injection, is consistent with the findings of previous studies[5,6,8]. 3. Delayed recall of cycle 1 (figure, b) was better by the drug groups, although only the superiority of the diazepam to the saline group was significant ($p < 0.05$). This finding can be attributed to lowered