that also the number of CFU_c in mice is increased after administration of BM 12.531. Because of the bone marrow toxicity of many drugs used in cancer chemotherapy and of therapeutic radiation, BM 12.531 might be an efficient adjuvant in cancer chemotherapy. Moreover BM 12.531 might be a valuable drug after radiation accidents.

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High K^+ content explains the abolition of the action potential in amphibian sciatic nerve in vitro by *Lathyrus sativus* seed extract

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Summary. The action potential in amphibian sciatic nerve in vitro has been reported to be abolished by the topical application of *Lathyrus sativus* seed extract. We have confirmed this effect, but find that it is probably caused by the high K^+ content of such seed extracts and that organic neurotoxins are not implicated.

The consumption of *Lathyrus sativus* seed by man causes neurolathyrism[2,3]. A number of toxic substances occur in these seeds[4-6] and one of them, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), is believed to be the main cause of neurolathyrism, but the evidence is inconclusive[7]. A report that the action potential in toad sciatic nerve in vitro was abolished by the topical application of *L. sativus* seed extract[8] suggested a comparatively simple experimental system for evaluating the relative importance of the numerous neurotoxins in *L. sativus* seed.

Materials and methods. Frog sciatic nerves *(Rana pipiens)* were dissected and desheathed. Action potentials were recorded by placing the nerve on silver wire electrodes, and stimulating supramaximally. Nerves were bathed in Ringer solution containing NaCl (115 mM), KCl (2 mM), $CaCl_2$ (2 mM), glucose (11 mM) and Tris (10 mM) at 20 °C.

Ground, decorticized, *L. sativus* seed (200 g) was extracted in 1.2 l 30% v/v aqueous ethanol and heated for 2 h at 85 °C under reflux[8]. The mixture was cooled and centrifuged at 10,000 × g for 15 min at 20 °C; the supernatant fluid was concentrated to approximately 60 ml and dialyzed against 3 changes of 350 ml water. The dialysate was freeze-dried and dissolved in water to give a final volume of 16 ml.

Results. When frog sciatic nerves were bathed in a 5% v/v dilution of this extract with Ringer solution (equivalent to 0.625 g seed/ml), the action potential was abolished within 30 min, while control nerves, bathed in Ringer solution alone, were unaffected. Affected nerves recovered after bathing in Ringer solution for 20 min. The concentrations of some ions in the seed extract were determined by flame photometry; the concentrations of β-ODAP and the corresponding α-isomer derived from it[9] were determined by quantitative paper electrophoresis at pH 1.9[9,10]. The results are shown in table 1.

The $[K^+]$ of the diluted seed extract was sufficiently high to suggest that it might abolish the nerve impulse[11] and this was confirmed by bathing nerves in Ringer solution containing a $[K^+]$ concentration which simulated that in the diluted seed extract.

To investigate further whether K^+ or organic neurotoxins were responsible for the activity of the seed extract, a number of solutions was prepared and tested on the nerve preparation. K^+ was removed from a sample of seed extract by precipitation with sodium cobaltinitrite at 0 °C. A

Table 1. Content of some substances in decorticized *L. sativus* seed

Substance	Content in seed (m moles/kg) ± SEM (n)	Mean concentration (mM) in 5% v/v dilution of seed extract in Ringer solution
Na^+	< 0.08 (3) (below detection)	115
K^+	92.0 ± 0.07 (3)	57
Mg^{2+}	9.70 ± 0.01 (3)	6.0
Ca^{2+}	1.00 ± 0.01 (3)	2.5
Mn^{2+}	0.13 (1)	0.08
β-ODAP	20.0 ± 0.20 (4)	12.5
α-ODAP	6.80 ± 1.80 (4)	4.2

Table 2. Effect of diluted seed extract, or solutions simulating its composition, on the action potential in frog sciatic nerve in vitro

Component in Ringer solution	Effect on the action potential after 30 min at 20 °C
1. Seed extract (0.625 g seed/ml)	Reversibly abolished
2. Seed extract (ashed)	Reversibly abolished
3. Seed extract (sodium cobaltinitrite treated)	None
4. KCl (57 mM)	Reversibly abolished
5. KCl (57 mM) (sodium cobaltinitrite treated)	None
6. α-ODAP (4.2 mM)	None
7. β-ODAP (12.5 mM)	None
8. α-ODAP (4.2 mM) plus β-ODAP (12.5 mM)	None
9. $MgCl_2$ (6.0 mM) plus $MnCl_2$ (0.08 mM)	None

KCl solution which simulated the $[K^+]$ concentration of the seed extract (1.15 M) was treated similarly. (The $[K^+]$ of solutions following such treatment was < 1 mM.) Solutions of α- and β-ODAP (from *L. sativus* seed[12]), and $MgCl_2$ and $MnCl_2$ were prepared in Ringer solution, to simulate the concentrations of these substances in the diluted seed extract. A sample of seed extract was ashed to remove organic material and diluted as before with Ringer solution. Each solution was tested on at least 3 different nerve preparations (table 2).

Discussion. The results suggest that the high K^+ content of the *L. sativus* seed extract was the major cause of the abolition of the action potential in frog sciatic nerve, but do not exclude the possibility that sodium cobaltinitrite removed a neurotoxic substance as well as K^+. This is unlikely as similar results were obtained when K^+ was removed with sodium perchlorate (results not shown). The activity of the ashed extract excludes a synergistic action of an organic neurotoxin with K^+. The results also exclude the direct involvement of Mn^{2+} (Sadasivan et al.[13]) and Mg^{2+}, of α- and β-ODAP, and presumably the other known neurotoxins in *L. sativus* seed. Although we have confirmed the previously reported findings[8], no evidence was obtained that *L. sativus* seed contains a neurotoxin which effects the activity of amphibian peripheral nerve in vitro following topical application. Our own and previous findings therefore are probably solely attributable to the effects of the high K^+ content of the seed extract.

Norris et al.[8] also reported that an i.v. bolus of 10–15 ml of *L. sativus* seed extract (approximately 1.8 M K^+ from our calculations) caused electrocardiographic changes, bradycardia, a fall in blood pressure and death in dogs. In monkeys an i.v. bolus of 15–20 ml of crude *L. sativus* seed extract (approximately 0.5 M K^+ from our calculations) was reported to cause stupor, coma and death[6]. The established effects of a high circulating $[K^+]$ on cardiac function[14] makes it likely that these effects too were mediated mainly by the the very large amounts of K^+ which the seed extracts contained.

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Possible mutagenic activity of saccharin

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Summary. A mutagenic effect of saccharin in Chinese hamsters, using the in vivo SCE test, was observed when massive overdoses were administered; cyclamate was not mutagenic.

Mutagenic effects of saccharin have been reported for several years. Some authors regard not the saccharin itself, but contaminants present in the preparations used, as responsible for such effects. According to a review of the National Academy of Sciences panel on saccharin, 21 mutagenicity studies on saccharin available until the end of 1978 yielded 16 negative and 5 positive results[1]. Carcinogenetic effects (carcinoma of the bladder in male rats[2]) can be regarded as being consistent with the positive mutagenicity findings. The following conclusions have been drawn from a compilation and evaluation of the results of 17 mutagenicity studies on saccharin up to 1975[3]:

Saccharin is a weak mutagen:
- at very high doses in *Salmonella;*
- at moderate doses in *Drosophila;*
- at medium to very high doses in mice.

Saccharin was found to have a low chromosome-breaking effect in plant roots and in Chinese hamster cells, but in these and other test systems doubtful and negative results were obtained as well. Altogether there is an indication that saccharin is capable of chromosome-breaking rather than of inducing point mutations.

A much more sensitive test than the chromosome aberration test for the detection of mutagenic substances is the sister chromatid exchange (SCE) test. After 2 cell cycles, the chromatids of mammalian chromosomes are stained differentially in most cells in the presence of 5-bromodeoxyuridine (BUdR), a base analogue of thymidine. SCEs appear as reciprocal exchanges of DNA between the chromatids. The number of SCEs increases depending on the mutagenic dose added. Using this test, a mutagenic effect of saccharin at relatively high concentrations was verified recently[4]; this study revealed also strongly different degrees of sensitivity between the cell types used (human lymphocytes and CHO cells), a phenomenon observed earlier in other test systems[5].

For 2 years, the SCE test has also been carried out as an in vivo test in small mammals[6,7], and since 1978 in an improved, standardized form[8]. Using this new test we examined the question of a possible mutagenic effect of saccharin. Chinese hamsters (10–11 weeks old, average weight 30 g) were implanted s.c. with 50-mg BUdR tablets, 2 h later they received sodium saccharin (pure quality C. Roth, D-7500 Karlsruhe) by stomach tube (40%, dissolved in water) and after 24 h 1 mg/kg colcemid. After 26 h the animals were sacrificed to obtain cell suspensions from the femur bone marrow[9]. The results are shown in the table.