vitro technique[23]. Concentrations of 10 ppm peptide in rumen liquor significantly increased the digestion of starch following a 24-h incubation (about 1.9%), but it decreased with more than 100 ppm. In the case of cellulose, 100 ppm and 500 ppm increased the digestion about 7.6% and 7.5%, but decreased the activity of rumen microorganisms in higher concentration.

When subjected to quantitative amino acid analysis, crystalline paracelsin exhibits non-stoichiometric amino acid ratios which indicates its microheterogeneity. Using HPLC it could be separated in 3 main components, paracelsin A, B and C (fig.). After vigorous hydrolysis of isolated components the amino acid composition could be determined (table 2). Paracelsin cannot be esterified by diazomethane, indicating that Glx is present as Gln. The occurrence of Phol was proved by UV-spectroscopy (maxima at 253, 260, 262, 265 and 269 nm), 2-dimensional TLC of a hydrolysate (1st direction: n-butanol-acetic acid-water 40:10:10, v/v; 2nd direction: phenol-water 75:25, w/w) and comparison with synthetic Phol, and ^{13}C NMR-spectroscopy (62.89 MHz, in $^{12}C,^{2}H$-methanol): δppm = 38.1 (Phol-Cβ), 54.2 (Phol-C_a), 64.8 (CH_2OH); phenyl: 127.2 (C-4), 129.2 (C-3,5), 130.5 (C-2,6), 139.8 (C-1). The blocking of the N-terminal amino acid by an acetyl group could be demonstrated unambiguously, using methods which were developed for sequencing trichotoxin and related peptide antibiotics[24].

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Transfection assay: A new test system for studying mutation induction

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Summary. A new test system for the detection of mutagens and carcinogens is presented. It is based on the observation that mutations in a gene coding for a repressor protein destroy the ability of ϕ105 phage to lysogenize *Bacillus subtilis* cells. A clear instead of a turbid plaque is formed. To exclude cellular influence on the reaction of a mutagen with DNA, purified ϕ105 DNA was used. The biological properties of the treated DNA in a transfection system were assayed.

A variety of test systems have been developed in the past to detect the mutagenic potential of physical and chemical agents[2-4]. Recently, a bacteriophage system for detecting substances that are potentially carcinogenic has been described[5]. In this test, infectious nucleic acids isolated from either MS2 or from ϕX174 bacteriophages, treated with test compound, were incubated with *Escherichia coli* spheroplasts and assayed for their plaque-forming capability. This is a valuable test, because of its directness. A similar test system using purified DNA of a bacteriophage as an indicator for the interaction of a mutagen with genetic information is described in the present communication as a simple and sensitive test to detect agents which may be mutagenic per se.

Material and methods. Bacteria and phage: A derivative of *Bacillus subtilis* 168, strain BR 95 (try^{-2}, phe^{-1}, ilv A1) and the lysogenic strain BR 95 (ϕ105) together with the temperate *B. subtilis* phage ϕ105 were kindly provided by Dr Rutberg.

Growth and preparation of competent cells. For the preparation of competent cells, 100 ml of tryptone broth were inoculated with *B. subtilis* strain BR 95 from Tryptose Blood Agar Base (TBAB, Difco) plates. Cells were grown in a shaker bath at 37 °C to an absorption at 600 nm of 1.2.

Competence medium in which the efficiency of cells for transfection (competence) is increased, was inoculated at the ratio of 1:10 with the bacterial stock suspension and shaken at 37 °C for 4 h. These competent cells were then either frozen with glycerol (30% final concentration) in liquid nitrogen or immediately centrifuged and re-suspended in the transformation medium[6].

Isolation of vegetative ϕ105 DNA. Phage ϕ105 DNA was isolated from Mitomycin induced lysogenic *B. subtilis* BR 95 (ϕ105), as described by Rutberg et al.[7]. The concentration of this vegetative phage ϕ105 DNA was determined spectrophotometrically. In an average experiment a DNA stock solution of 400–500 µg/ml is obtained. This solution can be stored for more than 6 months at 4 °C without loss of transfection activity. Transfection efficiency was determined by scoring infectious centers using varous dilutions of ϕ105 DNA.

Treatment of transfecting DNA with mutagens. The DNA stock solution is diluted to approximately 40 µg/ml with 0.05 M phosphate buffer, pH 7.4. A typical reaction mixture with a total volume of 1 ml contained 0.5 ml of DNA and 0.5 ml of the mutagen in either 0.05 M phosphate buffer, 0.2 M sodium borate (pH 7.4), distilled water or dimethyl sulfoxide. All solutions were freshly prepared and used immediately. DNA treatment was terminated either directly by 10^{-1} dilution in 0.05 M phosphate buffer or by the addition of a stopping mixture followed by dilution. The stopping mixture for alkylating agents was 0.1 M sodium thiosulfate in 0.05 M phosphate buffer. Hydroxylamine was destroyed by the addition of 1 M sodium chloride in 0.05 M Tris/HCl, pH 7.5 containing 10% acetone. The reaction mixture was then kept in ice for 30 min. 0.1 ml of this mixture was used in the transfection assay.

Transfection assay. A typical transfection assay contained 0.1 ml of vegetative ϕ105 DNA (either treated with a mutagen or untreated as a control) and 0.9 ml of competent bacteria. DNA uptake by the cells takes place within 30 min at 37 °C with gentle shaking. Aliquots of 0.1 ml were removed and added either directly or after appropriate dilution in saline (0.05 M phosphate, 0.075 M sodium chloride) to 2.5 ml of liquefied top-layer agar[8] and 0.1 ml of an overnight culture of BR 95 in tryptone broth and plated on min-CH agar plates[9]. Incubation for 24 h at 37 °C gives turbid plaques 1–2 mm in diameter. Clear plaque mutants are clearly visible in a lawn of up to 10^5 turbid plaques.

Observations. We tested 14 chemicals, carcinogens as well as non-carcinogens (table). A broad range of doses of all chemicals (with a constant time period for ϕ105 DNA treatment) was utilized to check for the existence of a dose relationship.

Ten of the chemicals assayed showed a dose relationship. With few exceptions, both carcinogens and non-carcinogens were found to be mutagenic in this test system. Adrimycin, reported to be mutagenic in the Salmonella assay system, did not show any mutation induction in our test, whereas hydroxylamine was non-mutagenic in the Ames test but mutagenic in this test system. Among the 14 chemicals tested, N-methyl-N-nitro-N-nitrosoguanidine (NMG) and methyl methane sulfonate (MMS) were found to be highly mutagenic but 9-amino acridine and sodium azide were weak mutagens.

We also measured the lethal effect of the mutagen treatment by dividing the titer of treated plaques (Nt) by the titer of control plaques (No). N-Nitroso methyl urea, NMG and MMS were found to be highly lethal with Nt/No values ranging from 0.03 to 0.06.

Discussion. In this communication we describe an easy, fast inexpensive and reproducible test to detect agents with toxic and mutagenic effects. This test can readily be standardized. Once transfecting DNA has been isolated and late logarithmic cultures of recipient and indicator cells have been prepared and frozen, the test requires only treatment of DNA, growth of competent cells for 4 h (or use of frozen competent cells), treatment of competent cells with DNA, and plating.

Showing the effect of the 14 chemicals on ∅105 DNA in transfection assay

Chemicals	Group of chemicals*	Time of DNA treatment (min)	Final concentration of mutagens in reaction mixture giving maximum mutations	Mutation/10^3 transfectants (average of 5 experiments)	Inactivations Nt/No (average of 5 experiments)	Dose relationship (exist or not)	Carcinogenic or non-carcinogenic
9-Aminoacridine	A	30	5×10^{-6} M	0.10	0.10	No	?
Bis-(2-chloroethyl)amine	B	30	5×10^{-4} M	0.6	0.17	Yes	?
Benzo-(a)-pyrene	C	60	5×10^{-4} M	-	-	-	+
Ethyl methane sulfonate	D	180	5×10^{-1} M	0.72	0.15	Yes	+
Methyl methane sulfonate	D	30	5×10^{-1} M	2.6	0.03	Yes	+
Diethyl sulfate	D	60	5×10^{-3} M	0.4	0.43	Yes	+ (weak)
4-Nitrobiphenyl	E	30	5×10^{-2} M	0.6	0.6	Yes	+
N-Nitrosomethylurea	F	30	5×10^{-3} M	0.9	0.06	Yes	+
N-Methyl-N-nitro-N-nitrosoguanidine	F	30	0.01 M	3.5	0.06	Yes	+
Adrimycin-Hcl	G	30	5×10^{-6} M	-	0.12	-	+
Mitomycin-C	G	30	5×10^{-4} M	-	0.04	-	?
Sodium azide	H	30	5×10^{-3} M	0.12	0.2	No	-
Hydroxylamine	H	60	1 M	0.65	0.2	Yes	-
Azaserine	I	30	5×10^{-4} M	0.35	0.4	Yes	+

*A, Aromatic amines; B, alkyl halides; C, polycyclic aromatics; D, esters and epioxide; E, nitroaromatics; F, nitrosamines; G, antibiotics; H, nitrogen compound; I, diazo compounds; Nt, titer of treated plaques; No, titer of control plaques. Benzo-(a)-pyrene (activated) gave mutation per 10^3 transfectants = 0.15 (work in progress).

Most important, in the system reported here, we test for the effect of mutagen on a regulator protein, a repressor determining lysogeny of phage $\phi 105$ in *B. subtilis*. This protein is not required for phage growth but rather for control of certain functions, and viable mutants are easily found.

The other important advantage of the transfection assay is its insensitivity towards any cyto-toxicity of the agent to be tested, since the chemical can be removed from the reaction mixture after the incubation period.

A limited number of chemical covering 9 major groups of chemicals showing positive results are indicative of the sensitivity and suitability of this test system. Experiments are in progress using a number of other chemicals to further confirm the usefulness of this test system.

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Cytogenetic evidence for hemizygosity at the thymidine kinase locus in P388 mouse lymphoma cells

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Summary. A detailed cytogenetic investigation was carried out on P388 mouse lymphoma cells. The cells have a mean chromosome number of 36.86 with a mode and median of 37 chromosomes. G-banding analysis of 12 spreads revealed a total of 15 marker chromosomes with chromosome 11, the determinant of thymidine kinase, being present only in single copy per cell. It is therefore concluded that the P388 cell line is hemizygous at the thymidine kinase locus. Thymidine kinase activities were assayed in P388 cells and two other malignant cell lines, clone 707 Friend mouse leukaemia cells and L5178Y mouse lymphoma cells. No clear relationship was observed between enzyme activity and gene dosage.

Resistance in cultured mammalian cells to thymidine analogues, e.g., bromodeoxyuridine (BrdU) or iododeoxyuridine (IdU) is brought about by deficiency of the enzyme, thymidine kinase. As thymidine kinase is autosomally linked in mammals, mutations are required in both copies of the relevant chromosome in order for BrdU (and IdU) resistance to occur. It therefore follows that the spontaneous frequency of cells resistant to either BrdU or (IdU) is usually very low e.g. 10^{-10} in mouse L cells[2] and 10^{-9} in hamster V79 cells[3].

Two exceptions to the observation and expectation of low spontaneous frequencies of cell lines resistant to halogenated thymidine analogues are clone 707 Friend mouse leukemia cells and P388 mouse lymphoma cells. Both cell lines have high spontaneous mutation frequencies to thymidine kinase deficiency: 5×10^{-5} in clone 707 Friend cells[4] and

Table 1. Results of G-banding analysis of P388 cells

Chromosome	% of cells containing specific chromosome	Mean No. of copies per cell	Chromosome	% of cells containing specific chromosome	Mean No. of copies per cell
1	100	1.0	M 1	100.0	1.0
2	100	1.92	M 2	100.0	1.0
3	100	2.0	M 3	100.0	1.08
4	100	1.42	M 4	75.0	0.92
5	100	1.83	M 5	41.67	0.42
6	100	1.08	M 6	91.67	0.92
7	100	2.0	M 7	100.0	1.0
8	100	1.0	M 8	83.33	1.42
9	100	1.33	M 9	25.0	0.25
10	100	1.17	M 10	8.33	0.08
11	100	1.0	M 11	8.33	0.08
12	100	1.08	M 12	8.33	0.08
13	100	1.33	M 13	8.33	0.08
14	100	1.08	M 14	16.67	0.25
15	100	1.67	M 15	8.33	0.08
16	100	1.17			
17	100	1.42			
18	100	1.75			
19	100	1.17			
X	0	0			
Y	0	0			