Australia). Total protein was determined by digestion of the membrane fraction with 10% sodium deoxycholate before the addition of biuret reagent[25]. Collagenase type IV, SP, Trypan blue, PNP, UDPGA, D-saccharic acid-1,4-lactone, Triton X-100 were purchased from Sigma Chem. Co., USA, and monotest LDH opt., test UV from Boehringer GmbH, Mannheim, FRG. Sodium deoxycholate came from Fluka AG, Switzerland.

Results and discussion. The results presented in table 2 indicated that protein concentration in the hepatocytes increased significantly after SP pretreatment irrespective of the dose, and that the increase was about 46%. On the other hand UDP-glucuronyltransferase activity estimated by the rate of PNP glucuronidation, showed an increase that seemed to be proportional to the SP dose when data were expressed per 10^6 cells. The increase observed was 67% when the daily SP dose was 120 µmoles/kg, and 136% when the daily SP dose was 240 µmoles/kg, respectively. When data were expressed per mg of protein, enzyme activity was only significantly increased (60%) after the administration of the higher dose of SP.

Glucuronidation is probably the most important truly detoxicatory process, and UDPG-glucuronyltransferase is the enzyme, or enzyme system responsible for glucuronylating a wide variety of endogenous and exogenous compounds[26]. The glucuronidation of PNP has been extensively used as an estimation of enzyme activity in liver by using microsomes, homogenates or isolated hepatocytes[23,26,27]. Data obtained from single cell experiments can, in a sense, prepare us for the results of the in vivo experiments[12].

The viability of isolated cells and electron microscopy studies show that the preparation used in this investigation was suited to our purpose. However, artificially high levels of UDPGA[28] had to be added since preliminary experiments showed no glucuronidation of PNP in the absence of UDPGA. Furthermore the addition of the beta-glucuronidase inhibitor, 1,4-saccharolactone, prevented the hydrolysis of some of the conjugated product[12]. The results described in this paper demonstrate that SP treatment increase protein concentration and enzyme activity in the isolated cells, and that the increase in enzyme activity apparently is related to SP dose. In previous as yet unpublished studies we observed that increased enzyme activity in - presumably fully - digitonin-activated homogenates from SP-treated rats was unrelated to the SP dose. Thus we conclude that SP may induce the enzyme system involved in PNP glucuronidation in the hepatocyte, and that isolated cells are suitable for detecting the effect of potential inducers of such a process.

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Paracelsin, a peptide antibiotic containing α-aminoisobutyric acid, isolated from *Trichoderma reesei* Simmons. Part A*

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Summary. A peptide antibiotic has been isolated from *Trichoderma reesei* QM 9414. Although crystalline and uniform in TLC, this antibiotic could be resolved by HPLC into 3 sequence analogues. The close relationship to alamethicin was proved by chemical and spectroscopic methods, and the formation of ion-conducting pores in lipid bilayers.

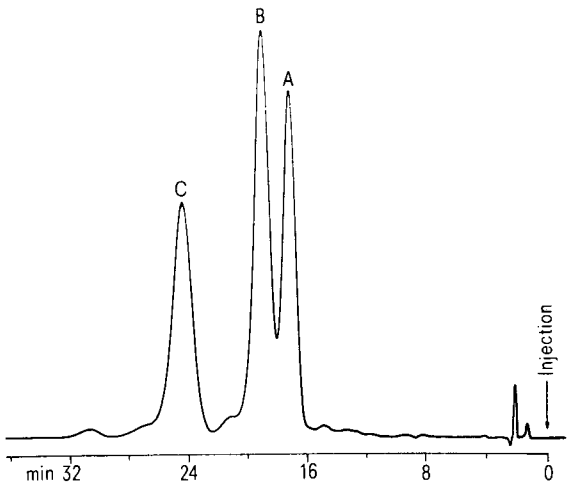
In recent years the structure of several peptides with the following properties has been clarified[2]: a) molecular masses between 1600 and 2000, b) a high content (up to 50%) of α-aminoisobutyric acid (Aib, 2-methylalanine), c) some contain isovaline (Iva, 2-ethylalanine), d) N-termini acetylated, and C-termini linked with phenylalaninol (Phol) or, as in the so far unique case of trichotoxin[3,4], with valinol (Vol). With the exception of Iva, which is D[5,6] both

the amino alcohols and amino acids are in the L configuration[7]. Besides the antibiotic activities[8,9], the chemistry[10–13] as well as the ability of some of the peptides to induce characteristic voltage-dependent ion conduction in lipid bilayer membranes[14,15] is of particular interest.

In the following a description will be given of the isolation and properties of a new member of this class of peptides from cultures of *Trichoderma reesei (viride)* QM 9414[16–18] which will be called paracelsin[19]. The content of a 100-l fermenter filled with Raulin-Thom medium[20] and adjusted to pH 4 with HCl prior to sterilization was incubated with the content of 8 400-ml flasks precultivated with *T. reesei* on a rotary shaker for 8 days at a temperature at 25 °C. The fermentation was carried out by stirring (250 rpm) and aeration (2×10^3 l/h) for 7 days at a temperature at 25 °C. The production of antibiotic was followed by extraction of 50-ml aliquots of the filtered culture broth 3 times with 50 ml each of ethyl acetate, washing the combined organic layers once with water and evaporation to dryness in a rotary evaporator in vacuo. The residue obtained was then hydrolyzed for 3 h at 130 °C in a mixture of concentrated HCl/propionic acid 1:1 (v/v) and subjected to quantitative amino acid analysis. The content of Aib, and therefore the formation of peptide, reached a maximum of 191 mmoles Aib/100 l after 5 days and decreased to 96 mmoles Aib/100 l after 7 days. (Remarkably, this fast and specific method of the extraction with organic solvents and subsequent hydrolysis indicated the formation of Aib peptides during solid phase fermentation of *T. reesei* on bagasse and other cellulose-containing substrates.) The mycelium was separated by being filtered through several layers of linen and yielded 2092 g of wet mycelium which corresponds to 302 g of dry mycel. The wet mycelium was digested for 24 h each with 4 l of methanol, methanol-chloroform 1:1 and methanol-chloroform 4:1 (v/v), and the organic solvents separated at intervals by centrifugation. All organic supernatants were combined and evaporated to dryness, resulting in 66 g of brown oil which has not yet been worked up, but still contains significant amounts of paracelsin as is indicated by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The pale yellow culture broth (approximately 100 l) was extracted in portions of 20 l 3 times with 4 l of ethyl acetate, and twice with 4 l of n-butanol-ethyl acetate 1:1.

Evaporation of the combined organic layers in vacuo yielded 252 g of dark brown oil. It was dissolved in 400 ml methanol and slowly added to 4 l ether while continuously being stirred. The peptide precipitated was isolated by centrifugation, yielding 10 g of a brown powder. It was dissolved in 50 ml n-butanol and during constant stirring was slowly dropped into 1 l ether, yielding 8 g of pale brown powder. All mother liquors which still contained peptide were combined and stored for further utilization. 1 g of the crude peptide obtained was dissolved in 3 ml methanol and chromatographed on a Sephadex LH-20 column (120×2 cm) with methanol. Fractions of 10 ml were collected and the elution of peptide visualized on TLC by spraying with water and, after drying, with chlorine/o-tolidine (precoated TLC plates, silica gel 60 F 254, Merck No. 5729, solvent system: chloroform-methanol-water-acetic acid 65:25:4:3, v/v). Paracelsin: $R_f=0.36$; for comparison: alamethicin: $R_f=0.44$; trichotoxin: $R_f=0.38$. Suitable fractions containing the major component were combined and evaporated to dryness yielding 780 mg of pale yellow solid. This material was dissolved in 10 ml chloroform-methanol 7:3 and chromatographed in this solvent on a silica gel column (61×2 cm, silica gel 70–230 mesh, Merck No. 7734). The elution of peptide was monitored as decribed above, yielding 500 ml solid. Repeated chromatography of the remaining 7 g material resulted in 4 g solid, showing only traces of impurities in TLC. It could be crystallized from 50% aqueous methanol. This material forms voltage-dependent ion-conducting pores in lipid bilayer membranes very similar to alamethicin[21] and exhibits antibiotic activities mainly against gram positive bacteria (table 1).

The influence of paracelsin on rumen fermentation was tested by determination of the gas production using an in



Separation of crystalline paracelsin in components A, B and C by HPLC. Relative peak areas (%): A = 28.1, B = 40.8, C = 31.1; instrumentation: Perkin-Elmer LC series 3B, spectrophotometric detector LC-75, recorder 561 and integrator M3B; column: Perkin-Elmer C18 SIL-X-5, 5 µm, 250×4.6 mm; eluant: methanol-isopropanol-water 45:20:35, v/v, isocratic operation; flow rate: 1.5 ml/min; pressure: 37 MPa; temperature: ambient (25 °C); detection: 200 nm, 0.16 AUFS; chart speed: 0.5 cm/min; sensitivity recorder: 10 mV; injection: 250 µg peptide in 5 µl methanol.

Table 1. In vitro antibacterial activities of paracelsin

Test organisms	Zone of inhibition, diameters (mm)
Bacillus subtilis	23
Micrococcus luteus	30
Staphylococcus aureus	16
Streptococcus faecalis	18
Streptococcus lactis	17
Streptococcus thermophilus	14

No activity under the conditions reported was found against *Candida albicans, Escherichia coli, Proteus vulgaris, Serratia marcescens, Salmonella typhimurium, Pseudomonas aeruginosa* and *Enterobacter aerogenes*. Test conditions: paper discs (9 mm diameter) were dipped in a solution of paracelsin (25 mg crystals dissolved in 1 ml ethanol and 4 ml water), dried and transferred to dishes filled with 20 ml Grove and Randall medium No. 1[21] and seeded with test organisms (0.1 ml of a suspension containing approximately 10^6 organisms/ml). Zones of inhibition were measured after 20 h at 37 °C.

Table 2. Amino acid composition of paracelsin (PC) A, B and C after repeated resolution by HPLC

	Acetyl	Glx	Pro	Gly	Ala	Aib	Val	Leu	Phol
PC A	1	3	1	1	3	9	2	0	1
PC B	1	3	1	1	3	9	1	1	1
PC C	1	3	1	1	2	10	2	0	1

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.