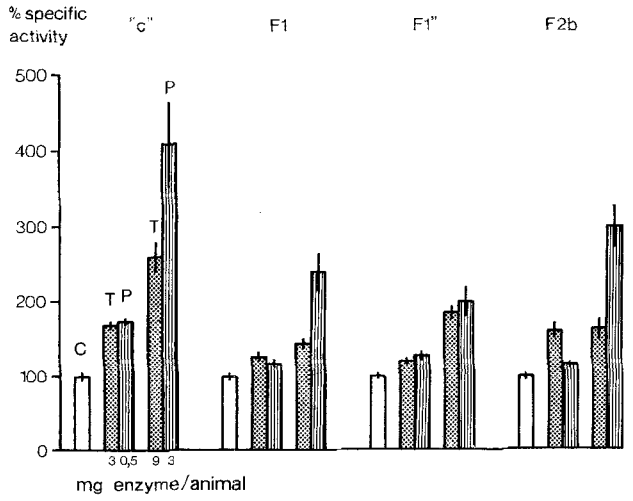

Stimulation of incorporation of ^{32}P orthophosphate into histone fractions F1, F1″ and F2b and into fraction 'c' after injection of trypsin and papain. Indicated amounts of enzymes were dissolved in 1 ml 0.9% NaCl and injected i.p. into 3-month-old male Sprague-Dawley rats (controls obtained the same amount of 0.9% NaCl). 16 h later animals were injected i.p. with 1 ml (2.5 mCi) ^{32}P, as described earlier[16]. 1 h thereafter animals were sacrificed. Livers were excized, histones extracted and separated on 15% polyacrylamide gels and specific activity was determined[16]. C, control; T, trypsin treated; P, papain treated. Values represent arithmetic means of 3 determinations ± S.D.

tumor therapy and consisting primarily of pancreatic extracts and papayotin. The phosphorylation of other nuclear protein fractions, however, is not significantly influenced by these agents.

Earlier results suggested that gene derepression induced by the action of proteolytic enzymes was caused by a degradation of nuclear proteins[13,14]. However, stimulated phosphorylation, as described in the present paper, could be sufficient to result in changes of the tertiary structure of those proteins[15], giving rise to stimulated DNA synthesis and cell proliferation.

Results. Intraperitoneal injections of proteolytic enzymes or enzyme preparations, including papain, trypsin and 'Wobe-Mugos', result in a significant stimulation of phosphate incorporation into rat liver histones F1, F1″, and F2b. The stimulation of DNA synthesis and cell multiplication by these agents, described in earlier reports, might be a consequence of stimulated phosphate uptake by nuclear proteins.

Zusammenfassung. Die i.p. Injektion der proteolytischen Enzyme Papain und Trypsin, sowie des Enzympräparates Wobe-Mugos, bewirkt eine signifikante Steigerung des Phosphateinbaues in die Histone F1, F1″ und 2Fb der Rattenleber. Die früher beobachtete Stimulierung der DNS-Synthese und Zellproliferation durch proteolytische Enzyme könnte die Folge einer Steigerung der Phosphorylierung von Kernproteinen sein.

K. Letnansky and F. Seelich

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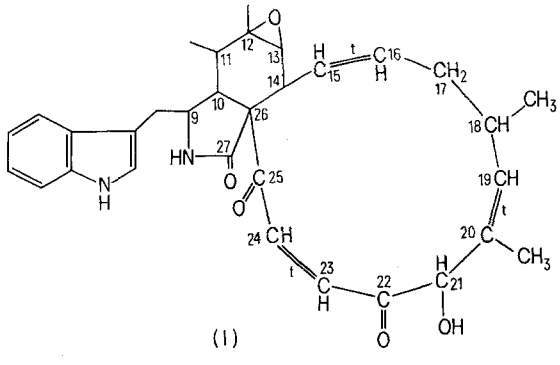
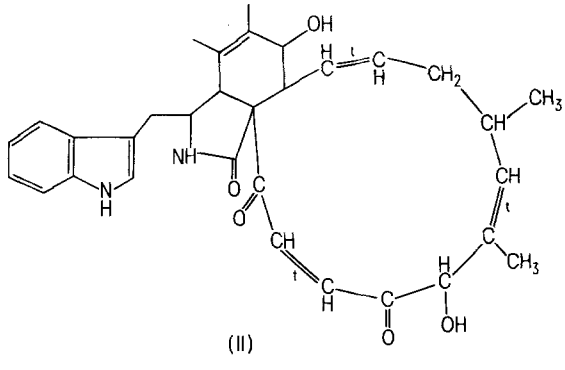
11 December 1974.

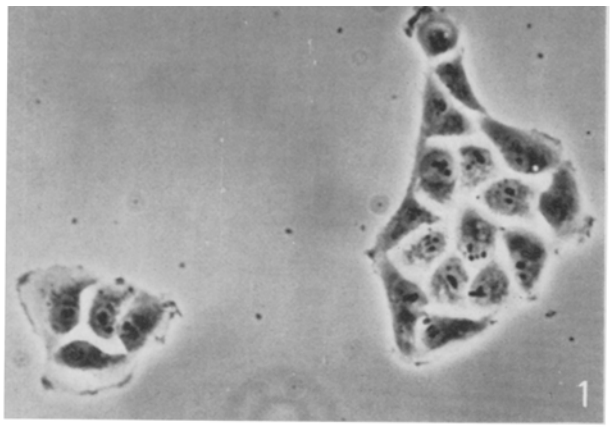
Cytotoxicity of New Cytochalasans from *Chaetomium globosum*

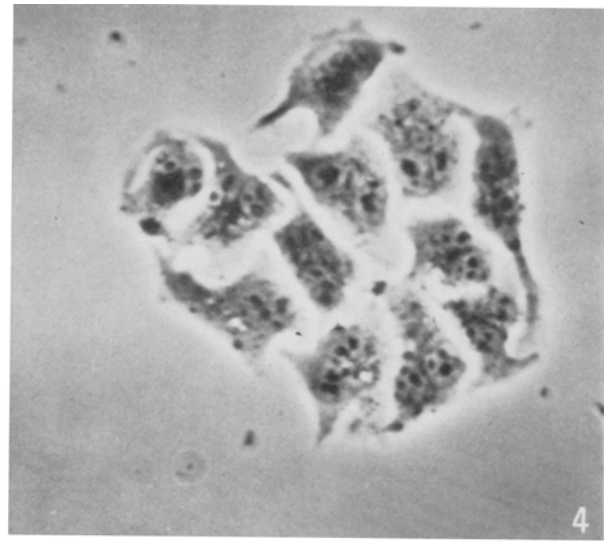
In the course of the screening tests for detecting mycotoxin-producing fungi isolated from foodstuffs, crude extracts of the mycelium and the culture filtrates from all tested isolates of *Chaetomium globosum* Kunz ex Fries were noticed to cause polynucleation and multipolar division of HeLa cells and also acute toxicity in mice[1-3]. Because of these dramatic biological effects, we conducted further chemical and biological investigations. The mycelium of fungi grown in liquid media or on rice was extracted by chloroform. The extract was separated by chromatographic methods which led the isolation of 6 metabolites showing the peculiar effect on HeLa cells; chaetoglobosin A, mp 168–170°, B, mp 186–187°, C, mp 259–261°, D, mp 216°, E, mp 279–280°, and F, mp 177–178°. These compounds showed closely related physical properties, suggesting a common molecular framework. The molecular formulae, $C_{32}H_{36}O_5N_2$ for A, B, C and D, and $C_{32}H_{38}O_5N_2$ for E and F, were established by high resolution mass spectrometry. The structures of A and B have been proposed preliminarily as I and II, chiefly from the spectroscopic data[4].

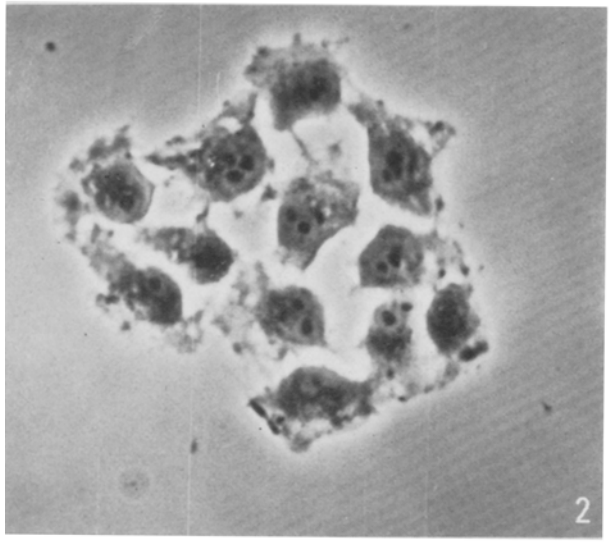
The structures suggested that chaetoglobosins belong to cytochalasans, a series of cytostatically active mold metabolites such as phomins from *Phoma* sp., cytochalasins

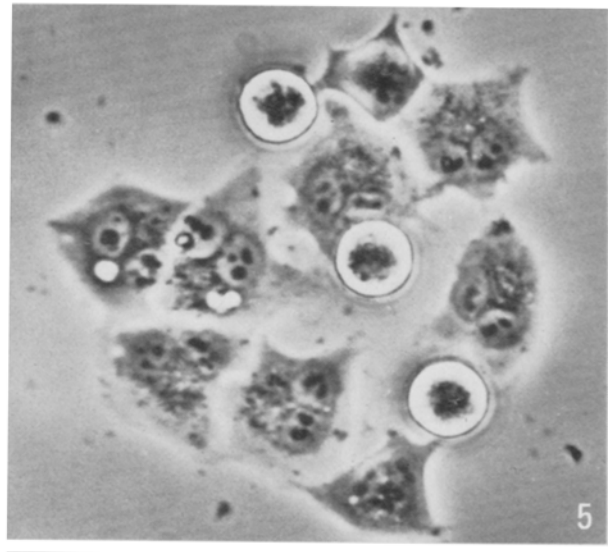


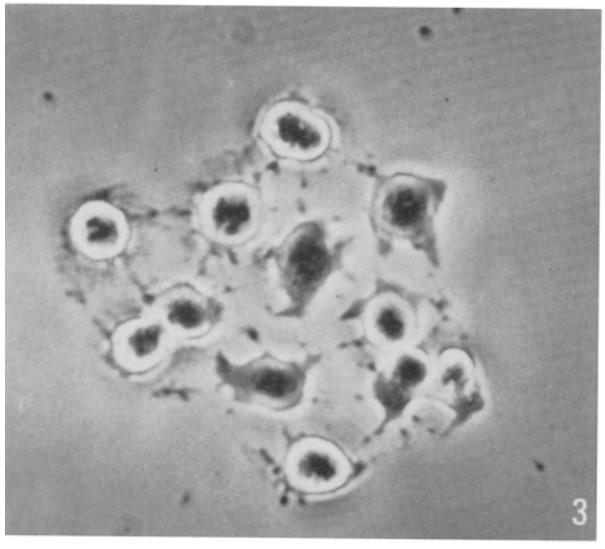












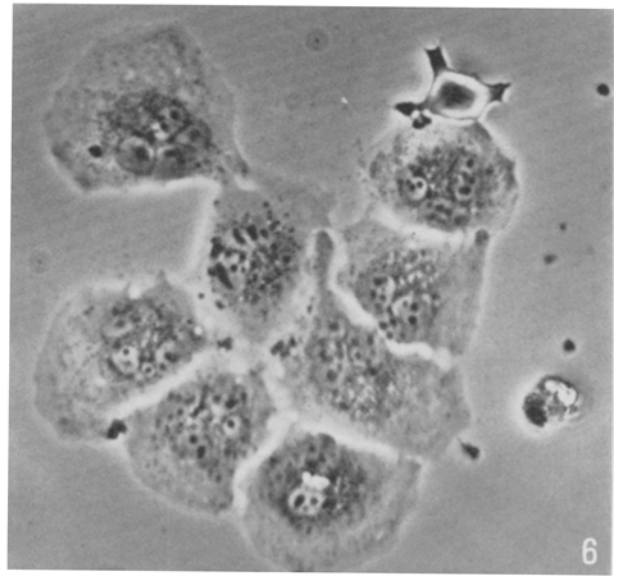


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Fig. 1-8. Cinemicrographs of HeLa cells treated with mycelial extract of *Chaetomium globosum* (NHL 2718) at 100 μg/ml for 3 days and then transferred to control medium (Phase contrast). 1. HeLa cells before the treatment. 2. Immediately after the addition of the extract. The cytoplasm becomes bizarre. 3. At 6 h the cells are rounded and the cytoplasmic protrusions are remarkable. 4. At 24 h the cytoplasm spread on the glass surface. Each cell contains 2 nuclei. 5. At 44 h the cells became enlarged. 3 cells are in multipolar mitosis. 6. At 48 h giant cells with 4 nuclei are characteristically observed. 7. Seven h after the removal of the extract. A mitosis follows the cytoplasmic cleavage. 8. Eight hours after the removal of the extract. The cell which underwent mitosis in Figure 7 becomes 4 cells with 2 nuclei.

Cytotoxic degree of chaetoglobosins and cytochalasins on HeLa cells

			32	10	3.2	1.0	0.32 μg/ml
Chaetoglobosin	A		4	4	1		
	B		4	3.5	0.5		
	C		3.5	1	0.5		
	D		4	4	2		
	E		4	4	2		
	F		4	2	1		
	A	monoacetate	4	2	1		
	B	diacetate	4	2	0.5		
Cytochalasin	A	(dehydro-phomin)	4	4	2	0.5	0
	B	(phomin)	4	3.5	2.5	1	0
	C		3.5	3.5	3.5	3	3
	D	(zygo-sporin A)	3.5	3	3	2.5	1

Samples were dissolved in dimethyl sulfoxide at 10 mg/ml and diluted in the medium. Cells on cover-glasses were treated for 3 days. Degree of cytotoxicity was estimated on a scale ranging '0' (little cellular damage) through '4' (complete cytolysis) by observing the stained cover-glasses. '2' denoted approximate 50% growth-inhibitory dose[1,2].

from *Helminthosporium dematioideum*, *Metarrhizium anisopliae*, *Rosellinia necatrix* and *Aspergillus clavatus*, and zygosporins from *Zygosporium masonii*[5]. In chaetoglobosins, the benzyl group in cytochalasans so far reported is replaced by 3-indolyl group; i.e. chaetoglobosins are formed from a tryptophan unit instead of phenylalanine in other cytochalasans.

About cytochalasans, especially phomin (cytochalasin B), an extensive study has been conducted because of the multiple biological activities[6]. Thus, it is of great interest to investigate the biological effects of chaetoglobosins, a new group of cytochalasans. For culture cell experiments, HeLa cells grown in Eagle's minimum essential medium supplemented with 10% calf serum were used. The cells were grown on cover-glasses, treated with the samples, fixed with Carnoy's fixative and stained with hematoxylin and eosin. Cytotoxic degree and cytomorphological changes were recorded on these preparations.

When the cells were treated with 3.2% of the filtrate from one isolate of *Chaetomium globosum* (NHL 2718), binucleate cells were observed in about 1/3 of the cells after 24 h, and all cells became polynucleate after 48 h. It is remarkable that the observed mitotic cells underwent tetrapolar division resulting in 4 nucleate cells. Thus, after 72 h multinuclear cells with 4 or 8 nuclei, on some occasions with 6 nuclei, were demonstrated. The cells became very large and the size of nuclei in each cell was almost equal. Multipolar division was characteristically observed. When the concentration of the filtrate was raised to 10%, the cells detached from the glass surface after 24 h, making further observation impossible.

The sequences of the affection were precisely analyzed by taking cinemicrography, using the mycelial extract of *Chaetomium globosum* (NHL 2718). Immediately after the addition of the sample, the cellular configuration became bizarre and the condition continued for more than 12 h (Figures 2 and 3). After about 18 hours the nuclei began to divide. But without subsequent cytoplasmic cleavage, the cytoplasm gradually extended on the glass surface to form binucleate cells (Figure 4). At about 48 h, after another interphase period, second division of binucleate cells occurred (Figure 5). Both nuclei in one cell entered the mitotic phase simultaneously and underwent tetrapolar division. Again no cytoplasmic division occurred following the nuclear division, giving rise to a giant cell with 4 nuclei of equal size (Figure 6). When the culture kept for 3 days in the presence of the sample was washed with saline and transferred to control

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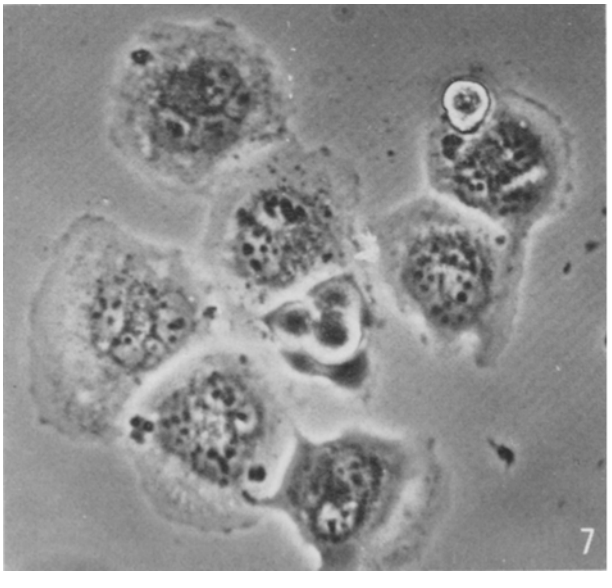

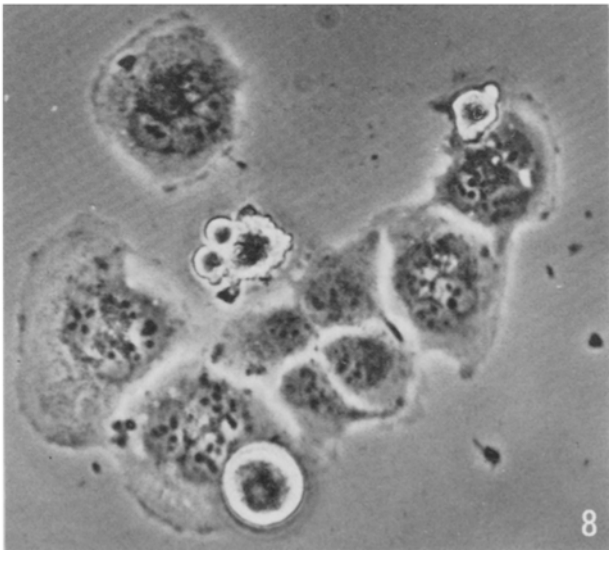

medium, subsequent multipolar separation of chromosomes (not to be exactly enumerated but probably octapolar division) was followed on this occasion by cytoplasmic cleavage, but not to 8 cells (Figure 7). Thus, the result of the division raised 3 to 4 cells with 1, 2 or more nuclei, respectively (Figure 8).

The chaetoglobosins isolated in crystalline form were tested by cover-glass cultures (Table). After 3 days' treatment, HeLa cells were lethally affected by all chaetoglobosins at 32 µg/ml. Chaetoglobin C was less toxic and the approximate 50% growth inhibitory dose was about 20 µg/ml. Those of others were between 3.2 and 10 µg/ml. All these crystals induced the particular multinuclear cells which had been induced by crude extracts of the fungus. When the authentic cytochalasins were tested by the same method as above (Table), 50% growth inhibitory doses were about 1 to 3 µg/ml, that is, cytochalasins were a little more effective than chaetoglobosins. Morphologically, quite similar characteristic changes to chaetoglobosins were elicited by the samples, suggesting that chaetoglobosins and cytochalasins are similar in the biological activities on cultured cells. With chaetoglobosins cytoplasmic cleavage might also be inhibited after normal nuclear division because of the impairment of microfilament function as precisely reported in the case of cytochalasins.

When extracts of the fungus or moldy rice were injected i.p. or s.c. into DDD strain of mice, the resultant effects were rapid and the mice died within a few minutes or hours. Histological examination of various organs revealed scarce lesion. Mice which passed through this crisis period, survived without any prominent late effect. LD_{50} of chaetoglobosin A by a single s.c. injection was estimated as 6.5 and 17.8 mg/kg body weight (Behrens) for 5-week-old male and female mice, respectively. The reported LD_{50} values of cytochalasin E in rats are 2.6 and 9.1 mg/kg body weight by a single i.p. or oral administration, respectively[7].

Further chemical and biological works are now in progress and the details will be presented elsewhere.

Zusammenfassung. Nachweis, dass $CHCl_3$-Rohextrakte aus Myzelien und Filtraten der Zuchtmedien von *Chaetomium globosum* Kunze ex Fries einen akuten toxischen Effect auf HeLa-Zellen in vitro, sowie auf Mäuse zeigten. Chaetoglobosine, die aus Mycelium-Rohextrakt isoliert wurden und chemisch zu den Cytochalasanen gehören, induzierten in HeLa-Zellen eine Polynukleierung und multipolare Kernverteilung. Auch beim Chaetogobosin wird eine Mikrofilament-Schädigung angenommen.

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Influence de la nature du milieu de croissance sur la réduction des stéroïdes par les actinomycétales

Les Actinomycétales (*Nocardia, Mycobacterium*) peuvent oxyder et réduire les stéroïdes. Alors que les voies métaboliques d'oxydation des stéroïdes sont assez bien connues[1-3], les réductions de doubles liaisons des stéroïdes, notamment en position 1, 4, 6 et 7[4-10], ont été observées sans qu'il ait été possible de les relier au catabolisme oxydatif des stéroïdes. D'autre part, peu de réductases de stéroïdes ont été étudiées chez les Actinomycétales. A notre connaissance seules la $\Delta 1$ réductase de *Mycobacterium globiforme*[11] et la $\Delta 4$ réductase de *Nocardia corallina*[12] ont été mises en évidence dans les extraits acellulaires. Quant aux facteurs qui orientent le métabolisme des stéroïdes dans le sens oxydatif ou réducteur, ils étaient jusqu'à présent obscurs. Dans une publication antérieure[13] nous avions signalé l'influence de la composition du milieu de croissance des cellules de *N. corallina* sur l'apparition de la $\Delta 4$ réductase. Nous rapportons ici l'étude des facteurs qui conditionnent la réduction en 4 des $\Delta 4$ céto-3 stéroïdes par *N. corallina*.

N. corallina est cultivée dans les conditions décrites précédemment[10] dans 1 l de milieu A contenant 3 g de peptone, 1 g d'extrait de levure et 30 g de glucose, jusqu'en phase stationnaire (85 h) et en présence de 200 mg de progestérone (I). Après acidification, extraction au chloroforme, puis chromatographie sur couches minces de silicagel on obtient 78 mg de 5α-pregnane-3, 20-dione (II) dont les caractéristiques correspondent à celles de la littérature[14,15]: F = 197–200 °C; $[\alpha]_D$ + 128° ($CHCl_3$); IR $\nu_{CHCl_3}^{max}$ 1720 et 1732 cm^{-1}; SM *m/e* 316 (M^+). En l'absence d'α, α'-dipyridyle qui est un inhibiteur d'hydroxylases[16,17] aucun métabolite oxydé ne s'accumule. Dans un milieu B, identique au milieu A, mais ne contenant que 5 g/l de glucose, après 85 h de culture, on obtient seulement 9 mg de II à partir de 200 mg de I.

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