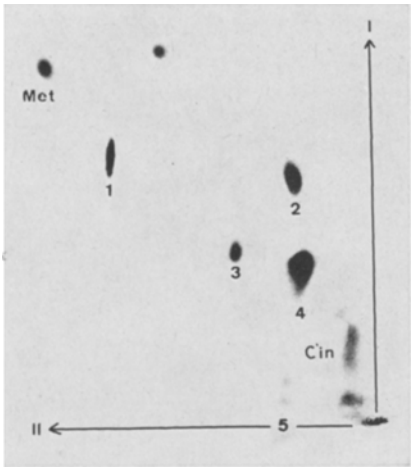

Fig. 3. Autoradiographie eines Dünnschichtchromatogramms der Aminosäurenfraktion des Petiolaextrakts. Met, Methionin; C'in, Cystin/Cystein; 1, 2, 3, 4, 5, primäre ^{35}S-Amine. I. Dimension: Butanol-Aceton-Diäthylamin-Wasser (10:10:2:5). II. Dimension: Isopropanol-Ameisensäure-Wasser (20:1:5).

Die Resultate deuten darauf hin, dass der toxische Schwefelwasserstoff im Blatt durch Oxidation oder Bindung entgiftet wird. Transportiert werden im Phloem die im Blatt gebildeten Verbindungen. Die anorganischen Oxidationsprodukte stehen der Pflanze als Schwefelquelle zur Verfügung[10]. Der an primäre Amine gebundene Schwefel scheint weitgehend die toxischen Eigenschaften zu verlieren. Selbst bei grösserem H_2S-Angebot ($> 0{,}75$ mg/100 ml) stirbt nur das direkt vom Schwefelwasserstoff betroffene Organ ab[11]. Offenbar besitzen die Pflanzen für geringe Mengen von Schwefelwasserstoff einen Entgiftungsmechanismus. Die Bedeutung der «unnatürlichen» Schwefelverbindungen im Stoffwechsel der Pflanze ist noch nicht geklärt. Sie ist das Ziel unserer Untersuchungen[12].

Summary. Some unnatural sulphur amino acids can be detected in the phloem of the vascular bundles from *Phaseolus vulgaris* after the application of $H_2{}^{35}S$ to the primary leaves.

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Activation of Virus Production by DMSO in *C. albicans* Experimentally Infected with Polyoma-DNA[1]

The propagation of mammalian virions in Protista was described by KOVÁCS et al.[2–4]. The present report is concerned with the experimental transfer of a viral infection to the yeast-like fungus, *Candida*, by using the genome of Polyoma Virus (PyV). This is the confirmation of earlier work done by BAYREUTHER et al.[5] who used a different experimental system, namely *B. subtilis* as a host. The enhancing effect of dimethyl sulphoxide (DMSO)[6] on the infectivity of the isolated DNA of PyV was investigated[7,8] and also will be presented.

Materials and methods. C. albicans was isolated from the vaginal discharge of a patient and carried through several hundred partially anaerobic passages in this laboratory. Technical details of the cultivation in submerged cultures, infection with virus and processing of the harvested cells were published[2–4] together with growth, purification and quantitation of the large plaque variant of the T strain of PyV[4]. The DNA of the virus was extracted by the method of GIERER and SCHRAMM[9] from partially purified supernatant of infected secondary cultures of mouse embryo cells. The criteria of a good preparation were: A typical UV-absorption curve, low DNase-labile infectivity, negative serologic reactions, such as neutralization, hemagglutination (HA) and inhibition of hemagglutination (HI). After infection these tests gave positive results and there was no DNase lability, but CPE was demonstrated.

Results. A typical experiment is illustrated in the Table. Approximately 6×10^6 cells were washed once and taken up in 0.3 ml phosphate buffered saline (PBS) mixed with Py-DNA, all precooled, and left to interact for 6 min at 2 °C. Following incubation, the cells and medium were separated and titrated[3,4]. Addition of DMSO during or

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Effect of DMSO on infectivity of PyV-DNA in *C. albicans*

Experiment, medium (incubation 4 h/37 °C)	Inoculum (no HAU/system)	Final yield as HAU/system	Serologic test of cell homogenates
Exp 74-A, medium 1415 + sucrose	0.5 ml PyV-DNA	1100	HI = + (1:8/0.2 ml)
Exp 74-B, medium 1415 + sucrose	+ 5% DMSO after adsorption	2100	HI = + (1:16/0.2 ml)
Exp 74-C, medium 1415 + sucrose	60% DMSO at adsorption	5970	HI = + (1:32/0.2 ml)
Control, medium 1415 + sucrose	0.5 ml heterolog. DNA + 60% DMSO	nil	nil

after the adsorption period enhanced the HA production. There was linearity with the increase of DMSO concentration and virus yield, the optimum being 60 vol./100 administered during adsorption. The addition of more than optimum DMSO concentration did not have further activator effect, thus is in agreement with others who worked with the RNA of poliovirus[6].

Discussion and conclusions. The positive serologic reactions and infectivity tests prove that the progeny is indeed PyV originating from the isolated genome used as inoculum. The biosynthesis was enhanced considerably by DMSO. The mechanism of action of this chemical is not yet clarified[8]. Our results confirm, in a different system the findings of BAYREUTHER et al.[5] hitherto not reproduced by others, emphasizing the universality of the DNA code[10] and demonstrating the penetration of the free viral genome during the initiation of the infective process in Protista[2-5,7,8]. Details, including infectivity titrations and findings with EMC-RNA will be published separately.

Zusammenfassung. Die Infektion von Schimmelpilzen, *C. albicans*, durch Polyomavirus mit isolierter Polyoma-DNS und die aktivierende Wirkung von DMSO werden beschrieben.

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Isoenzyme of Some Dehydrogenases and Nonspecific Esterase in Experimental Virus Hepatitis in Mice

Some biochemical characteristics and the morphology of the development of experimental virus hepatitis in mice have been described in our previous papers[1,2]. The results concerning alkaline phosphatase, LDH and GOT suggest that some of their multiple forms (isoenzymes) originating in liver cell cytoplasm and subcellular particles are delivered to the blood from liver cells damaged to different degrees.

The present report deals with some qualitative changes of isoenzymes of LDH (lactate dehydrogenase, i.e. L-lactate: NAD oxidoreductase, EC 1.1.1.27), MDH (malate dehydrogenase, i.e. L-malate: NAD oxidoreductase, EC 1.1.1.37), ICDH (isocitric dehydrogenase, i.e. threo-D_s-isocitrate: NADP oxidoreductase [decarboxylating], EC 1.1.1.42), GlDH (glutamic dehydrogenase, i.e. L-glutamate: NAD(P) oxidoreductase [deaminating], EC 1.4.1.3), G-6-PDH (glucose 6-phosphate dehydrogenase, i.e. D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) and nonspecific esterase during a slightly mitigated virus hepatitis in mice. LDH isoenzymes are used as well-known indicators of development of the infection. A strain of hepatotropic ectromelia virus, derived from the so-called Motol virus[1-4] was used. The virus was propagated in mouse fibroblast cells and partially purified by the method of end-point dilution.

Adult Swiss albino mice (strain 'H') weighing 21–23 g, fed a standard diet and water ad libitum were given i.p. injections of 0.1 ml containing approximatinelly $10^2 LD_{50}$ virus in phosphate buffer or only with vehicle (control group). 8 mice were sacrified each day by decapitation up to 7 days after the inoculation. Enzyme changes in the liver and blood plasma were analyzed separately in each mouse. Blood was collected in Heparin (Spofa) in order to obtain non-hemolyzed plasma. The liver was removed, washed with cold saline, dried with filter paper, weighed and homogenized for 40 sec in $4 \times 10^{-2} M$ veronal buffer at pH 8.3 (w/v) at 0 °C using a glass Potter-Elvehjem homogenizer (600 *g*). Sodium deoxycholate (Koch-Light) was added to a final concentration of 0.2%. The mixture was rehomogenized, stored at + 4 °C for 10 min and centrifuged for 30 min at 20,000 *g* at 4 °C. The supernatant was used for spectrophotometric determination of the total activity of enzymes and for electrophoresis. A quantitative micromodification of electrophoresis in agar gel[5] was employed. Electrophoresis was carried out in 1.5% purified Bacto-Agar (Difco) in $4 \times 10^{-2} M$ veronal buffer at pH 8.3 and ionic strength of 0.3 for 60 min at + 3 °C, 30 V/cm and 4 mA/cm. After separation, the electrophoreograms were incubated at 37 °C for 60 min (or for 20 min when determining LDH and esterase). The incubation mixtures for LDH, MDH and GlDH contained $10^{-1} M$ sodium lactate (Spofa), $5 \times 10^{-2} M$ sodium malate (Lachema) and 5×10^{-2} sodium L-glutamate (Koch-Light) as substrates respectively, and $2 \times 10^{-4} M$ PMS (N-methyl-phenazonium methosulfate, Calbiochem), $2 \times 10^{-3} M$ NAD (Boehringer), $1.5 \times 10^{-3} M$ NBMT (2-/*p*-nitrophenyl/-5-phenyl-3-/3, 3'-dimethoxy-4-diphenylyl/-tetrazolium chloride)[6]. The ICDH assay mixture contained $2.7 \times 10^{-3} M$ DL-isocitric acid trisodium salt (Koch-Light), $2 \times 10^{-4} M$ PMS, $2.5 \times 10^{-3} M$ NBMT, $1.8 \times 10^{-3} M$ NADP (Boehringer), and $10^{-2} M$ $MnCl_2$. The G-6-PDH mixture contained: $1.3 \times 10^{-3} M$ D-glucose-6-phosphate disodium salt (Calbiochem), $8 \times 10^{-4} M$ PMS, $7.5 \times 10^{-4} M$ NBMT, $2.3 \times 10^{-3} M$ NADP, and $5.5 \times 10^{-2} M$ $MgCl_2$. The esterase mixture consisted of $5.3 \times 10^{-4} M$ 2-naphthyl acetate (Lachema), and 0.1% Echtblausalz BB (Hoechst). $4 \times 10^{-2} M$ veronal buffer at pH 8.3 was used in all reactions. After incubation the electrophoreograms were washed with 3% acetic acid. It should be pointed out here that a better separation was achieved at the higher voltage as compared with previous experiments[2] using a prolonged time of electrophoresis. In addition, a different agar batch was used.

The infection culminates 7 days after inoculation, i.e. 1–2 days later, as compared with results obtained if using larger doses of the virus[1,2]. The morphological characteristics of the liver were analogous with those already described[1]. Total activities of all blood plasma enzymes studied increases considerably, beginning on day 3–5 and culminating at 7 days. The changes in total activities of liver extracts were not significant. The changes in isoenzymes originating as a result of damaged liver are

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