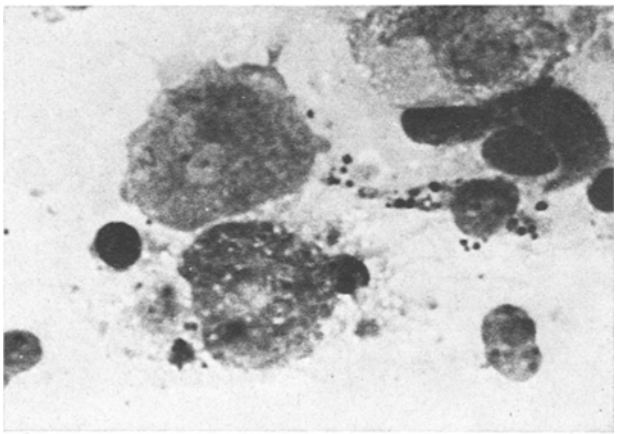
evident at the 48th, 72nd and 96th h after the injection of the cytostatic drug, respectively at the 72nd, 96th and 120th h after the administration of trypan blue. (2) Giant histiocytes present both nucleus and cytoplasm of increased diameter; their size is twice, three or more times larger than that of normal histiocytes. 10, 15 and 16% of the histiocytes showed, 48, 72 and 96 h respectively after the administration of cyclophosphamide, a nuclear diameter larger than 20 μ. 2% of them showed a nuclear diameter larger than 30 μ. According to our observations the mean nuclear diameter of the normal histiocytes of the bone marrow is 8.5 ± 1.3 μ large. (3) The histiocytes so transformed show still incorporated particles in their cytoplasm.

These findings support the assumption that histiocytes of the bone marrow can undergo giant cellular patterns after treatment with antimitotic drugs, likewise it has been observed by BASERGA and MARINONE[1] for myeloid cells (megamyeloid cells).



A giant histiocyte (lower, on the left) compared with a megamyeloid cell (upper, on the left) and with a normal histiocyte. Bone marrow. May-Grünwald-Giemsa stain. × 1200.

Since it is known that the reticuloendothelial system is sharing in the immunological response[12–16], it is conceivable that the antimitotic drugs may bring about an immunosuppressive effect by damaging the histiocytes too.

Riassunto. Nel midollo osseo di topi intossicati con dosi elevate di ciclofosfamide compaiono elementi giganti, che, per la capacità fagocitaria di cui sono dotati, appaiono appartenere al sistema istiocitario (istiociti giganti).

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Enhancement of Haemagglutinin Production in Polyoma Virus-Infected Candida by a Defined Medium and Urethan

The transfer of polyoma virus (PyV) to *B. subtilis* with the infectious DNA was described by BAYREUTHER et al.[1]. We were not only able to verify their findings[2–4] but to propagate EMC virion[5,6] and PyV[7], as well as viral-RNA[5] and viral-DNA[4], in intact yeasts and Tetrahymena. During these assays the stimulation of virus production by Urethan was observed[3,8]. This effect can be substantially increased with further technical refinement, such as the use of the defined medium of HEALY et al.[9]. The great increase in PyV hemagglutinins is described in this communication. More details will be published separately[8].

Materials and methods. *C. albicans* was isolated from a patient and carried axenically in a natural medium[10,5] at 28 °C. PyV was the large plaque variant propagated in mouse embryo cells[4,7] and it went through 30 passages in yeast before being used as inoculum. 0.2 ml cell homogenate[6] containing 2048 HAU (approximately 10^3 PFU) was added to 0.3 ml log phase yeast culture (10^6 cells) 1 h adsorption was allowed at 27 or 37 °C, under constant agitation in a Dubnoff water-bath at 90 rpm. To remove the unadsorbed PyV, 5 washings were made with 2 ml PBS each, followed by centrifugation. After 1 h interaction the system was brought to 50 ml with natural[10] or defined medium[9], the latter containing 0.15 *M* sucrose.

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Results. Preliminary assays demonstrated the enhancing effect of elevated temperatures, thus 37 °C was generally used. Higher hemagglutinin yield resulted when Urethan was incorporated into defined medium as illustrated in Table I, part a. During these 4-h experiments there was 26% difference in HAU between the 2 systems. The increase in mortality and the decrease in budding rates during short incubation was about the same in both; however, more cell-associated hemagglutinins were found with defined medium. The progeny was identified as PyV by HI test carried out with rabbit antiserum. Longer experiments gave more emphasis to the above findings (Table I, part b). There was approximately 2.8-fold rise in the mortality rate of the infected population, but no significant difference in budding as compared with that of the control. The level of these parameters is almost equal in the experimental system indicating a stillstand of the population growth. The control which received inactivated instead of live PyV, exhibited about 3 times higher cell multiplication than death-rate. The yield in viral hemagglutinins was 19 times the input and 21 times the adsorbed inoculum. HI was strongly positive. Urethan did not bring much change in the cell biological and population data as illustrated in Table I, part c. The final difference in cell counts between infected and control was 18%. This was slightly less than the system lacking Urethan. However, the HAU were almost doubled as compared with the parallel culture without the carcinogen and the progeny was identified with Py-antiserum. The search for the cause of this phenomenon is illustrated in Table II recording preliminary findings in Polyoma-DNA infected *C. albicans*. The systems without Urethan did not exhibit significant cell population changes, but a relatively good HAU yield, mainly cell-associated, being identified by HI as PyV. The increase in HA was 625-fold and this was almost doubled when defined medium was used. Urethan did not cause any HAU increase, but the budding increased, relative to infected 1. In Urethan-Medium 1415 assays, there was about 10% higher HAU yield than with complete nutrient alone. The budding rate declined slightly relative to infected 3. The HA was virus specific, being inhibited by PyV-antiserum.

Discussion. The findings are novel, reproducible and their biological significance may be manifold. The stimulation of the oncogenic PyV by Urethan may have some bearings on the genesis of experimental tumors. The Candida parasitizing organs of high cancer incidence may carry and transmit tumor viruses or viral nucleic acids. The association of carcinogens with oncogenic virus or viral genome may be an ever-present possibility. Experiments with Urethan and PyV or other viruses were carried out mainly in vivo[11,12] revealing a virus-activating

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Table I. Hemagglutinin production of *C. albicans* inoculated with PyV

Cells, counts Incubation, 37 °C	Inoculum HAU/System	Mortality %	Budding %	Final titer Distribution	Serolog. tests Increase[a]
Part a *Infected 1:* 2.28×10^5 cells/ml 4 h, complete M	PyV, 0.2 ml 2048 50 mg Urethan	6.29	6.82	*11.800 HAU* M: 67% C: 33%	HI: posit. (1:128) 5.7×
Infected 2: 2.6×10^5 cells/ml 4 h, defined M	Same	6.94	8.48	*15.640* M: 51% C: 49%	HI: posit. (1:256) 7.6×
Part b *Infected 3:* 1.18×10^7 cells/ml 24 h, defined M	PyV, 0.2 ml 2048 No Urethan	8.90	9.11	*34.137* M: 24% C: 76%	HI: posit. (1:1024) 19× (21×)
Control: 1.44×10^7 cells/ml 24 h, defined M Difference in counts I/Co: 19%	Inactivated PyV, 0.2 ml No HA	3.20	9.40	nil	nil
Part c *Infected 4:* 1.40×10^7 cells/ml 24 h, defined M	PyV, 0.2 ml 2048 50 mg Urethan	9.21	9.41	*73.000* M: 28% C: 72%	HI: posit. (1:2048) 35× (38×)
Control: 1.50×10^7 cells/ml 24 h, defined M Difference in counts I/Co: 18%	Inactivated PyV, 0.2 ml 50 mg Urethan	3.25	10.15	nil	nil

Part a. Exp. 73-A: Parallel systems with Urethan for comparison of the 2 media. The average of 10 cytometer cell counts is tabulated; the viability was measured by dye exclusion test with Trypan Blue (6). Adsorption was 1 h at 27 °C. Hemagglutination was carried out with freshly washed guinea-pig RBC, overnight at 4 °C. Total HAU per system and % distribution between medium (M) and cells (C) is recorded. *Part b.* Exp. 71-A: Defined medium 1415[9] with 0.15 *M* sucrose in long incubation, without Urethan. Control had a token inoculum with heat inactivated PyV. *Part c.* Exp. 72: Effect of Urethan when incorporated into the defined medium[9]. [a] Increase over input inoculum (over 'ecclipsed', in brackets). Abbreviations: HA, hemagglutination; HAU, hemagglutinating units; HI, inhibition of HA; I, infected; Co, control.

Table II. Hemagglutinin production by *C. albicans* infected with DNA of PyV

Cells, counts Incubation, 37 °C	Inoculum/System	Mortality %	Budding %	Final titer Distribution	Serolog. tests Increase
Infected[a]: 2.19×10^6 cells/ml 4 h, complete M	Py-DNA 0.5 ml	3.52	7.44	*625 HAU* M: 60% C: 40%	HI: posit. (1:8) 625×
Infected[b]: 2.69×10^6 cells/ml 4 h, defined M	Same	4.53	7.39	*1075 HAU* M: 58% C: 42%	HI: posit. (1:16) 1075×
Infected[c]: 2.66×10^6 cells/ml 4 h, complete M	Same + 50 mg Urethan	3.03	8.91	*590 HAU* M: 59% C: 41%	HI: posit. (1:16) 590×
Infected[d]: 2.19×10^6 cells/ml 4 h, defined M	Same + 50 mg Urethan	3.41	8.00	*1135 HAU* M: 55% C: 45%	HI: posit. (1:32) 1135×

[a] Exp. 74-A: 0.5 ml PyV-DNA + 0.3 ml yeast, approximately 10^6 cells, 6 min interaction at 2 °C, then reincubated with 50 ml growth medium for 4 h at 37 °C. Further processing like Table I. [b] Similar to above, but with medium 1415_9 + 0.15 *M* sucrose. [c] Exp. 74: Similar to 74-A[a] but 50 mg Urethan in the growth medium or [d] in medium 1415 + 0.15 *M* sucrose. Polyoma DNA was extracted with hot phenol and 1 *M* NaCl., ether and N_2 and did not give HA and HI reaction before the inoculation of Candida (OD_{260} was 0.026/0.3 ml and was infectious to mouse embryo cells).

effect of the carcinogen. Investigations in vitro are scarce or irrelevant[13,14], although a virus activation theory was put forward to explain Urethan carcinogenesis[15]. Its effect on the nucleic acid metabolism of the host, in agreement with others[16], is doubtful. The slight virus production by Urethan in Candida-Py-DNA assays may not be significant. The tumorigenic action of Urethan was thought to be connected with its slow evacuation and insufficient metabolism in young mice[17]. The dose was chosen on basis of in vivo data[15,16]. In our assays Urethan stimulation was observed only in defined medium[9] and sucrose, lacking macromolecules. The high amino acid, vitamin and carbohydrate content of this mixture may be the enhancing factor in virus production of Protista[3], further increased by Urethan through some unknown mechanism. The alkalinity cannot be a cause, since adjusting complete medium to pH 7.6 had no similar effect[8]. HA test was used for the quantitation of PyV. This was not taken as synonimous with infection, although a good correlation was demonstrated between HA and infectivity[3,5,7]. Serologic findings underline these results, especially with Py-DNA, which gave negative reactions before inoculation, but after incubation. HA and HI tests became positive, suggesting the production of Py-virion[3,4].

Zusammenfassung. Das onkogene Polyomavirus kann sich im Pilz *C. albicans* vermehren und die Virusausbeute wird durch Urethanzugabe erhöht.

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Experimental Brain Tumours in Dogs

Brain tumours have been induced by different methods in mice, rats and rabbits[1]. But experimental neurosurgery demands brain tumours in animals larger than rodents. Recently we succeeded in producing such neoplasms in dogs after administration of 1-methyl-1-nitrosourea (MNU). MNU was introduced into experimental neuro-oncology by Druckrey et al.[2].

Ten mongrel dogs, aged 4 months to 3 years, received 20 mg MNU/kg body weight monthly by i.v. injections of freshly prepared solution of MNU in sterile physiologic saline with phosphate buffer (pH 4.2). 4 dogs developed brain tumours (Table). Histologically the tumours resemble intracerebral sarcomas or multiforme glioblastomas. All of them show signs of malignancy: infiltrative