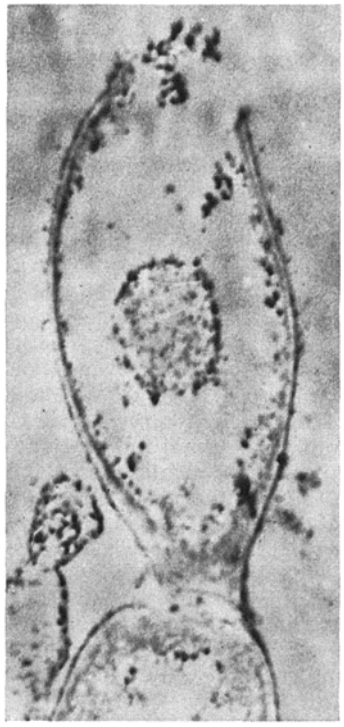
Feinstruktur von Pilzzellen. II. Endogene Konidienbildung bei *Penicillium cyclopium*

Die Konidien werden bei den meisten Penicillien exogen durch Bildung von Querwänden an der Spitze der Phialiden abgegliedert. Es sind aber einige wenige Fälle beschrieben (RAPER und THOM[1]), die zeigen, dass die Vorbildung der Konidien auch im Innern der Phialide erfolgen kann; so z.B. bei *Penicillium digitatum* und einem Stamm von *P. tardum* (NRRL 1129).



Längsschnitt durch eine Phialide von *Penicillium cyclopium* Stamm 114 mit «Prokonidie» im Innern. × 10000.

Im Rahmen von elektronenmikroskopischen Strukturuntersuchungen an Konidien und am Konidien-bildenden Apparat von Penicillien konnte wiederholt festgestellt werden, dass bei *P. cyclopium* Stamm 114, der von geräuchertem Speck isoliert wurde, in den Phialiden «Prokonidien» in verschiedenen Entwicklungsstadien vorkamen. Die Präparationstechnik ist in Mitteilung 1 (FRANK[2]) beschrieben. Die Figur zeigt einen typischen Fall dieser Art. Lichtmikrokopisch gelang es nicht, den Vorgang zu verfolgen. Es war auch nicht möglich, die bei RAPER und THOM[1] gezeigte trichterförmige Phialidenmündung wie bei *P. tardum* zu sehen. Die Phialiden waren, der Diagnose entsprechend, flaschenförmig mit einem scharf abgesetzten Ende.

Summary. On investigation by electron microscope of the phialides of a strain of *Penicillium cyclopium* endogenous layed proconidia could often be observed.

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Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe (Deutschland), 20. Februar 1967.

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Influence of Heavy Water (D_2O) on the Multiplication of Adeno and Mengo Virus

We have reported[1,2] that the growth of poliomyelitis virus (a small RNA virus) is enhanced when this virus is permitted to multiply in tissue culture cells maintained in 20–50% D_2O. This effect has been confirmed in another laboratory[3]. On the other hand, the growth of a DNA virus, SV_{40}, was found to be inhibited in D_2O[4]. In addition the effect of D_2O on the growth of 3 other RNA viruses was studied. The growth of western equine encephalitis was significantly inhibited in D_2O[5], whereas rubella and rabies viruses were unaffected[6].

This communication concerns our experiments with Mengo virus (a small RNA-containing virus) and adenovirus-7 which is a DNA virus.

The Grider strain of adenovirus-7 (obtained from Dr. A. J. GIRARDI of this institute) was grown in human embryo kidney (HEK) cells and the pool of virus used had a titer of 2×10^7 plaque-forming units (PFU)/ml. The Mengo virus pool (37 AL-3) was obtained from Dr. B. BROWNSTEIN of this institute and, after 3 passages in L cells, had a titer of 2.5×10^8 PFU/ml.

Both HEK and L cells were grown in Eagle's medium in Earle's balanced salt solution (BSS) containing 10% calf serum. The medium used for cell maintenance was identical except that it contained 2% calf serum. The medium used for the growth and maintenance of cells contained the following antibiotics: streptomycin, 100 µg/ml; penicillin, 100 U/ml; mycostatin, 200 U/ml; and kanomycin 166 µg/ml.

HEK and L cells were used throughout the study. One million HEK cells were planted in 60 mm plastic Petri dishes and were maintained at 37 °C in an atmosphere of 4% CO_2 in air. Confluent monolayers formed after 1 week.

L cell cultures were maintained in one liter bottles and subcultured in 60 mm plastic Petri dishes seeded with

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Influence of 50% deuterium oxide (D_2O) upon replication of adenovirus-7 in HEK cells and of mengo virus in L cells

Time (h)	Adenovirus-7			Mengo		
	No. of experiments	D_2O	H_2O	No. of experiments	D_2O	H_2O
1	–	–	–	4	4.6 ± 0.65[a]	4.4 ± 0.62
2	4	4.3 ± 0.17	4.2 ± 0.01	–	–	–
8	3	4.0 ± 0.14	4.0 ± 0.15	5	6.4 ± 0.20	7.5 ± 0.22[b]
12	–	–	–	1	9.0	8.8
18	2	4.9 ± 0.15	6.0 ± 0.25	–	–	–
24	5	5.3 ± 0.32	6.5 ± 0.30[c]	4	8.6 ± 0.25	8.7 ± 0.29
48	6	6.0 ± 0.34	7.0 ± 0.27[c]	1	8.2	8.3

[a] Virus titers in Log_{10} units ± the standard error. [b] $0.001 < p < 0.01$. [c] $0.01 < p < 0.05$.

2×10^6 cells/dish. Confluent monolayers formed after 2 days at 37 °C.

HEK cells maintained in plastic Petri dishes were used for the plaque assay of adenovirus. Growth medium was removed from confluent monolayers and virus (0.2 ml/monolayer) diluted in Hanks' BSS was allowed to adsorb for 2 h at 37 °C. The overlay medium consisted of 1% Noble's agar and Eagle's medium in Earle's BSS supplemented with 10% calf serum and 0.11% bovine albumin. Five ml of this overlay were added initially, followed by 3 ml on day 5 and 3 ml on day 9. The final overlay contained neutral red at a concentration of 1:10,000. Plaques were counted from day 10 to day 15.

For the plaque assay of Mengo virus, confluent L cell monolayers were used. Virus was allowed to adsorb for 1 h at 37 °C. The overlay medium for L cells was the same as that used for HEK cells, but the regimen included only 2 overlays, the initial overlay of 5 ml followed on day 4 by an additional overlay of 3 ml containing neutral red. Plaques were counted from day 5 to day 8.

One-step virus growth experiments were performed as follows: growth medium was removed from confluent cultures of HEK or L cells, which were then infected with either virus at a virus-to-cell ratio of between 3 and 10 to 1. After 1 (Mengo) or 2 (adenovirus) h at 37 °C, with occasional agitation of the infected cultures, the monolayers were washed 3 times with 3.6 ml of Hanks' BSS; an overlay of 4 ml of maintenance medium (protonated or containing 50% D_2O) was then added. At the times specified, the cells were scraped into the overlay medium. Aliquots were frozen and thawed 3 times prior to titration on primary HEK cells, using the plaque technique.

The results obtained with the 2 viruses are summarized in the Table. The replication of adenovirus-7 in HEK cells was lower in medium containing D_2O than in that containing H_2O, as shown by the yields of virus in the 2 media at 18, 24 and 48 h postinfection. The titers listed at the earlier times (2 and 8 h) represent eclipsed virus, prior to multiplication. The rate of multiplication of Mengo virus was initially slower in deuterated medium, but at 24 and 48 h there was no difference between the titer of virus grown in either H_2O or D_2O. Thus, although virus replication was slowed in D_2O, the final yields of Mengo virus per infected cell were the same in both media.

From the studies reported here and from our earlier work it can be seen that the growth of the 2 DNA-containing viruses tested was definitely inhibited by D_2O. The effect of D_2O on RNA-containing viruses varied, depending on the virus. Western equine encephalitis virus was inhibited by D_2O, whereas the growth of poliomyelitis virus was enhanced. There was no effect of D_2O on the growth of Mengo, rabies or rubella viruses. Further studies must be made to determine whether the variation in the effects of D_2O is mediated by cellular factors such as differences in permeability, access to cellular nutrients, influence on nucleic acid metabolism or an effect on specific enzymes[7].

Zusammenfassung. Der Einfluss von Deuteriumoxyd (50%) auf die Vermehrung von Mengo-Virus (kleiner RNS-Virus) und Adeno-7-Virus (ein DNS-Virus) in L-Zellen wurde untersucht. Nach 8 h im H_2O-Medium ist der Titer vom Mengo-Virus signifikant erhöht. 24 h nach Infektion sind die Titer von Mengo-Virus in H_2O und D_2O enthaltenden Medien identisch. Der Adenovirustiter in H_2O enthaltendem Medium ist 24 und 48 h nach Infektion signifikant höher als im Medium mit D_2O.

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