as the histochemical marker distinguishing this organelle or microbody such differences should be interpreted with a cautionary note[17].

Zusammenfassung. Mikrokörperchen mit Einfachmembranen wurden bei 4 Tage alten Kotyledonen von *Pisum sativum* studiert und für einen cytochemischen Katalase-Test elektronenmikroskopisch verwendet. Das granuläre Reaktionsprodukt aus dem 3, 3-diamino-benzidin Test für Katalase wurde ausschliesslich in den Einfachmembranen der Mikrokörperchen gefunden.

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Possibility of the Use of Some Physical-Chemical Agents for the Differentiation of Phages *Pseudomonas aeruginosa*

Some phages with a wide spectrum of effectiveness cannot be differentiated sufficiently by means of known criteria (spectrum of phages effectiveness, electron microscopy, serological group etc.) used at present[1–5]. In our former experiments we utilized the inactivation effect of UV-and X-ray radiation, acridine orange (AO) in darkness, photodynamic inactivation of AO, and hydroxylamine for the differentiation of a group of polyvalent staphylococcal phages, and we succeeded in the differentiation of some polyvalent phages by means of various inactivation effects of these physical-chemical agents[6–8]. In this study we tried to use these new tests for the differentiation of phages *Pseudomonas aeruginosa*.

Material and methods. Bacteriophages *Pseudomonas aeruginosa* P57[3], Pb and 7S were multiplicated on host bacteria PS 1 (phages P57[3]) and PS 2 (phages Pb and 7S). Bacteria were cultivated on the tryptone-yeast extract medium (15 g tryptone Oxoid, 7 g NaCl, 2 g yeast extract Oxoid; for solid media 15 g of agar per 1000 ml of liquid medium were used). When plating the phages, the method of the double-layer agar was used[4].

Irradiation of phages with UV-light was carried out in phosphate buffer. For irradiation a 15 W germicide fluorescent lamp Philips was used. The dose-rate was 20 ergs/sec/mm^2. At selected time intervals, irradiated phages were plated on Petri dishes together with the corresponding bacterial host. For X-ray irradiation the Chaul apparatus, Machlett's tube with the exposition of 8,200 rads/min was used. After irradiation the samples were plated together with bacteria on Petri dishes.

Photodynamic inactivation. The phage lysate was diluted 10 times with phosphate buffer (PH 7.0) and

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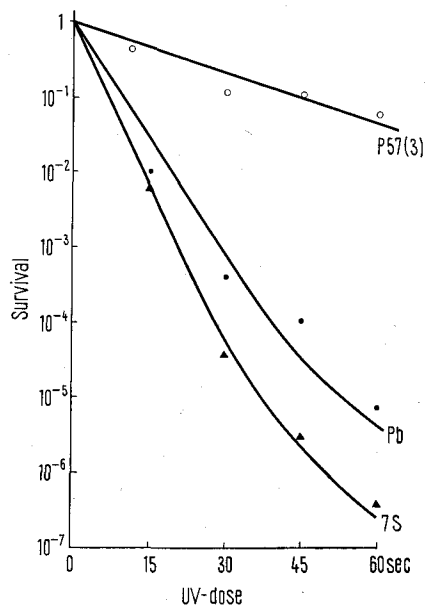

Fig. 1. Inactivation effect of UV-radiation on phages *Pseudomonas aeruginosa* P57[3], Pb and 7S.

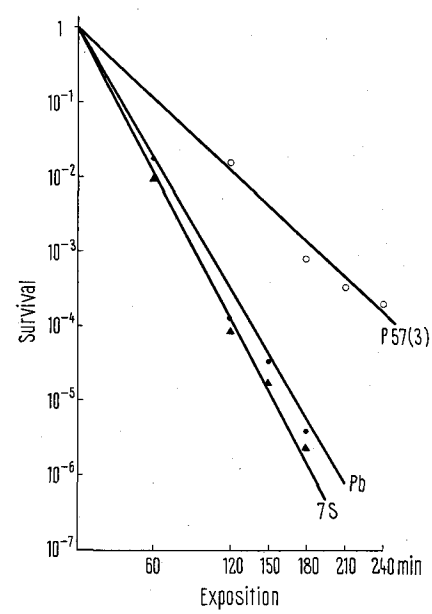

Fig. 2. Inactivation effect of X-ray radiation on phages *Pseudomonas aeruginosa* P57[3], Pb and 7S.

10 μg/ml AO (0.35 ml of diluted phage lysate + 0.15 ml of $10^{-4}M$ AO) was added under the orange light. The mixture of phage with the acridine dye was incubated in darkness at 30 °C for 60 min. Thereafter 0.2 ml of the mixture was diluted with 4.8 ml of buffer and irradiated in a crystallization dish with the 500 W Tungsramphot bulb at a distance of 20 cm (41 ergs/cm²/sec). During irradiation the 2-mm-thick layer of fluid in the crystallization dish was intensively agitated. Irradiated bacteriophages were plated to the bacterial host under the orange light and the inocculated dishes were cultivated at 37 °C for 18–20 h.

Dark inactivation. In darkness 0.3 ml of 10^{-3} *M* (1000 μg/ml) or 10^{-2} AO HCl (1,000 μg/ml) was added to 0.7 ml of phage lysate diluted 10 times with phosphate buffer. Phages were incubated together with the AO concentration mentioned in darkness at the temperature of 30 °C. At selected time intervals samples were taken and titrations were carried out under the orange light.

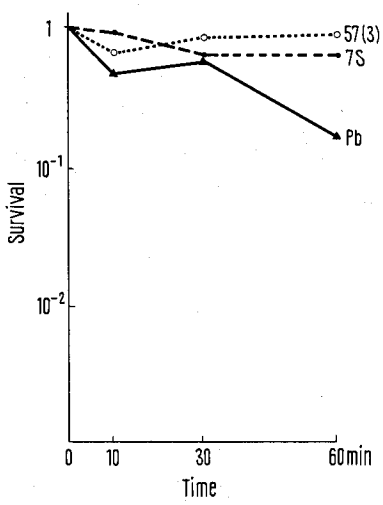

Fig. 3. Inactivation effect of acridine orange in darkness on phages *Pseudomonas aeruginosa* P57[3], Pb, and 7S.

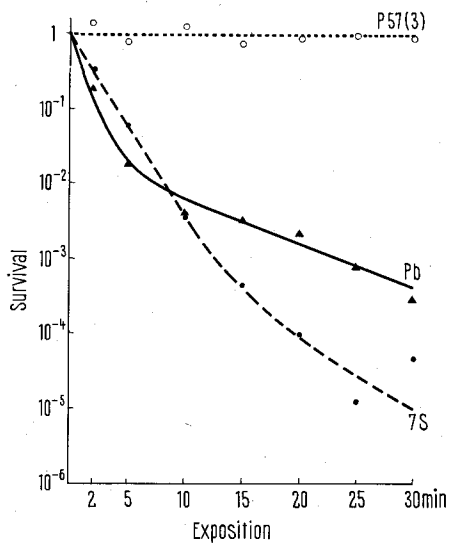

Fig. 4. Photodynamic effect of acridine orange on phages *Pseudomonas aeruginosa* P57[3], Pb and 7S.

Results and discussion. In our study we tried to utilize several physical-chemical agents of various inactivation effects for the differentiation of phages *Pseudomonas aeruginosa*. UV-radiation, X-ray radiation and AO (dark inactivation and photodynamic inactivation) were used. As we know the sensibility of phages to radiation is dependent on the structure and content of DNA and RNA. In Figure 1 we can see a great sensitivity of phages 7S and Pb to the inactivation effect of UV-radiation and a similar phenomenon can be observed after the inactivation of these phages by means of X-ray radiation (Figure 2). As all these phages contained either single-stranded DNA or RNA[9], it was possible to differentiate these phages from the phage P57[3] which contained a normal double-stranded DNA. All phages studied belonged to polyvalent phages, i.e. they had a wide spectrum of hosts. The sensibility of these phages to irradiation contributed to their basic differentiation.

On the other hand, the phages *Pseudomonas aeruginosa* were not sensitive to the dark inactivation with 100 μg/ml AO though in our previous studies we succeeded in differentiating well in this way various polyvalent staphylococcal phages[7,8] or phages belonging to different serological groups[6]. In darkness the *Pseudomonas* phages were not inactivated even with the 10 times increased concentration of AO (1,000 μg/ml) (Figure 3). The phage *Pseudomonas aeruginosa* P57[3] was resistent also to the photodynamic effect of 10 μg/ml AO, while the other phages studied (Pb and 7S) were considerably inactivated with this concentration of AO (Figure 4). An increased sensitivity of these phages to the photodynamic effect of AO can be dependent on the presence of single-stranded DNA and RNA, too[9]. During the photodynamic inactivation of bacteriophages with AO the breaks of one DNA strand obviously occur, and in phage *E. coli* T7 the formation of labile N-glycosidic bonds was also found[10]. For all that, the nature of lethal phage injuries induced by the photodynamic action of acridine dyes is not completely clear, and in some phages and in a number of viruses[11] it was found that their sensitivity or resistance to the photodynamic action of various dyes is not too dependent on the type of nucleic acids contained nor on the ratio of bases present. It seems that the sensitivity is more likely determined by the structure and the permeability of their protein coat or by the type of bond of their proteins to nucleic acids. For that reason probably the different sensitivity of phages *Pseudomonas aeruginosa* to the photodynamic inactivation with AO is not dependent only on the type of their nucleic acids. The definitive evaluation of the applicability of inactivation effect of AO for the differentiation of phages *Pseudomonas aeruginosa* will be possible only after the testing of a greater number of phage strains with various properties. However, if also the increased resistance of these phages to the inactivation effect of hydroxylamine (unpublished data) is taken into account, the observed slight inactivation effect of AO in darkness is not surprising because, among the bacterial representatives, the whole group Enterobacteriaceae is not the most sensitive to the inactivation effect of hydroxylamine[12]. With regard to favourable results obtained during the differentiation of phages[6–8]

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and bacteria[13] by means of some physical-chemical agents, further research and application of these agents can be considered as a promissing method.

Zusammenfassung. *Pseudomonas-aeruginosa*-Phagen wurden durch UV-, X-Strahlen und Afridinorange inaktiviert und die erzielte Wirkung auf die Differenzierung der DNS oder RNS enthaltenden Bacteriophagen untersucht. Es ergaben sich eine auffallend schwache Inaktivierung durch Akridinorange im Dunkelversuch und gegen Strahlenwirkung ausgesprochen sensible Phagen mit einfaseriger DNS und RNS.

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Über den Einbau von Dipeptiden in Ergotoxin-Alkaloide

Es konnte gezeigt werden, dass Valin, Leucin und Prolin weitgehend spezifisch in den Peptidteil von Ergocornin bzw. Ergokryptin inkorporiert werden[1–3]. Als unmittelbare Vorstufe des α-Hydroxy-α-aminosäure-Teils der Ergotoxine fungiert Valin[2,3]. Nach Agurell[4] soll die Ergotaminbildung via Lysergylalanin verlaufen. In Analogie dazu sollte bei den Ergotoxin-Alkaloiden Lysergylvalin eine obligatorische Zwischenstufe sein, an welche die restlichen Aminosäuren sukzessive oder aber ein Dipeptid angefügt werden. Unter Verwendung eines zellfreien Extraktes bzw. der «Replacement»-Technik konnten Abe et al.[5,6] nach Applikation von L-Leucyl-L-prolin-lactam-^{3}H, L-Leucyl-D-prolin-lactam-^{3}H sowie L-Phenylalanyl-L-prolin-lactam-^{3}H und L-Phenylalanyl-D prolin-lactam-^{3}H) radioaktive Peptidalkaloide nachweisen. Ein chemischer Abbau wurde nicht durchgeführt. Bei den D-Prolin enthaltenden Lactamen müsste also beim Einbau Konfigurationsumkehr stattgefunden haben.

Wir untersuchten die gleiche Problematik mit einem *Claviceps*-Stamm, der submers Ergocornin und Ergokryptin bildet. Die Ergebnisse sind in der Tabelle zusammengefasst. Falls ein spezifischer Einbau erfolgt, sollte nach Applikation von L-Valyl-L-leucin-U-^{14}C (I) und L-Leucyl-L-prolin-U-^{14}C-lactam (III) nur Ergokryptin und nach Fütterung von L-Valyl-L-prolin-U-^{14}C-lactam (II) nur Ergocornin markiert sein. Die drei Peptide ergaben spezifische Einbauraten von etwa 1%. Es waren jeweils beide Alkaloide radioaktiv. Die Radioaktivitätsverteilung im Peptidteil der Ergotoxine ergab, dass die applizierten Peptide vom Pilz gespalten wurden und die radioaktiven Aminosäuren sowohl in Ergocornin als auch in Ergokryptin inkorporiert worden sind. Nach Fütterung von I liessen sich 20% der Radioaktivität in der Proteinfraktion des Mycels nachweisen. Prolin aus der Fraktion der freien Aminosäuren des Mycels nach Applikation von II besass nach rigoroser Reinigung eine Gesamtradioaktivität von $1{,}02 \times 10^4$ dpm. Aus diesen Befunden ergibt sich, dass in *Claviceps* Dipeptide spaltende Fermente enthalten sind und zumindest in unserem Fall Dipeptide nicht als unmittelbare Vorstufen zur Synthese des Peptidteiles der Ergotoxine verwertet werden.

Experimentelles. Es wurde eine Selektante des *Claviceps purpurea*-Stammes Pepty 695 eingesetzt, Fermentation nach[3]. Markierte Verbindungen: L-Valyl-L-Leucin-U-

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Einbau von Dipeptiden in Ergotoxin-Alkaloide

Versuche	Ergocornin A	B	C	Ergokryptin A	B	C
Spezifische Radioaktivität (dpm/mMol)	1.02×10^6	$2{,}06 \times 10^6$	$2{,}13 \times 10^6$	$2{,}27 \times 10^6$	$1{,}7 \times 10^6$	$2{,}3 \times 10^6$
Spezifische Einbaurate	0,5%	1,2%	1,03%	1%	0,93%	1,1%
Abbau	Radioaktivitätsverteilung (%)					
Lysergsäure	n.b.	n.b.	n.b.	n.b.	2,6	n.b.
Dimethylpyruvat	4	16	4,4	10	3,4	7,5
Prolin	5	72	86	8,4	91,6	90
Leucin	—	—	—	80,5	3,4	5
Valin	44	4,9	4,8	—	—	—

n.b., nicht bestimmt.

Verfüttert wurden: A) 5,8 mg L-Valyl-L-leucin-U-^{14}C ($2{,}13 \times 10^8$ dpm/mMol), Dauer 17 h; B) 10,7 mg L-Leucyl-L-prolin-U-^{14}C-lactam ($2{,}02 \times 10^8$ dpm/mMol), Dauer 44 h; C) 18 mg L-Valyl-L-prolin-U-^{14}C-lactam ($2{,}07 \times 10^8$ dpm/mMol), Dauer 24 h.