spleen occurring after pertussis vaccination. The average relative spleen-weight found in the P-LCM group shows no significant deviations as compared to the control group, thus the effect of *B. pertussis* vaccine to cause spleen hypertrophy and that of the LCM virus to induce atrophy in this organ fail to become manifested in this case (Figure 2). According to our results, the simultaneously introduced *B. pertussis* vaccine and LCM virus influence each other's effect.

Accordingly, the course of intracerebral LCM virus infection shows the same changes in mice treated with *B. pertussis* vaccine as in immune depressive states provoked by other methods[8,10–21]. Our results support the data which suggest that pertussis vaccine treatment reduces the cellular immune response to heterologous antigen being simultaneously present in the organism.

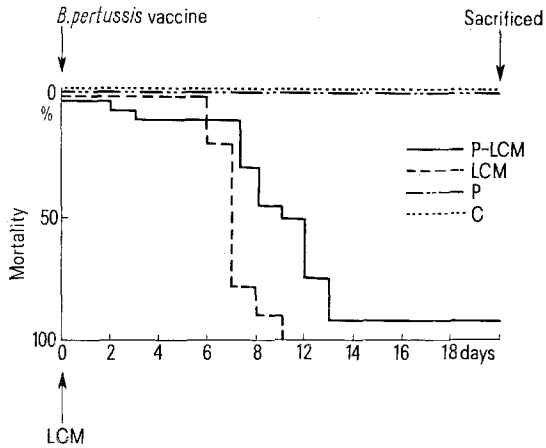

Fig. 1. Mortality rate in the individual mice-groups.

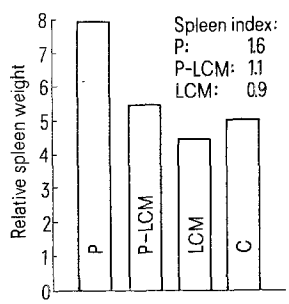

Fig. 2. The average relative spleen-weight on the 7 to 11th day of the experiment. Spleen index: P, 1.62; P-LCM, 1.12; LCM, 0.9.

Zusammenfassung. Gleichzeitig verabreichte *Bordetella pertussis* Vakzine und lymphozytäre Choriomeningitis Virus bewirkt keine Splenomegalie und auch keine lymphozytäre Choriomeningitis.

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Inhibition of Induction of Group A Bacteriocins of *Serratia marcescens* by Rifampin

The rifamycin antibiotics (rifampin) are known to specifically inhibit the bacterial enzyme deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase[1,2]; thus, rifampin has been employed to study various aspects of bacterial RNA and protein synthesis. Because the rifamycins are such specific inhibitors of bacterial DNA-dependent RNA polymerase, they have been used to determine the role of the drug-sensitive host RNA polymerase during the intracellular replication of a number of bacterial viruses; DNA phages (e.g., T4 and λ) were found to be sensitive to rifampin throughout replication[3,4]. The growth of the RNA phages f2 and $Q\beta$ was not inhibited when rifampin was added 4 to 5 min after infection; however, when the drug was added before or immediately after phage infection, viral replication was markedly suppressed[5,6]. Very recently it has been shown that the major pathway whereby phage inhibit host syntheses requires protein synthesis; on the other hand, the inhibition of host syntheses by phage ghosts was not affected by inhibitors of protein synthesis (puromycin, rifampin, and chloramphenicol)[7].

We recently developed a technique for typing clinical isolates of *Serratia marcescens*[8], based on sensitivity to 10 selected group A bacteriocins[9]. Meanwhile we have determined that these 10 bacteriocins are phage tails that consist of cores only (subgroup I) or of tails that are made up of cores and contractile sheaths (subgroup II)[10]. During the course of our investigations concerning the properties of these bacteriocins, rifampin was employed in attempts to answer the following three questions: 1. Does rifampin inhibit the induction of these bacteriocins by mitomycin C, i.e., does induction require functioning host cell DNA-dependent RNA polymerase? 2. Does rifampin inhibit the killing activity of these bacteriocins, i.e., is the

Table I. Inhibition of induction of group A bacteriocins of *S. marcescens* by rifampin

Time (h) following addition of mitomycin C	Bacteriocin No.	Bacteriocinogenic cells exposed to	
		Rifampin (50 µg/ml)	None (control)
6	5	0[a]	40[a]
	16	0	80
19	5	0	160
	16	0	160

[a] Killing units/0.05 ml.

Table II. Failure of rifampin to inhibit induction of group A bacteriocins in rifampin-resistant bacteriocinogenic *S. marcescens* cells

Bacteriocin No.	Bacteriocinogenic cells sensitive (S) or resistant (R) to rifampin	Bacteriocinogenic cells exposed to	
		Rifampin (100 µg/ml)	None (control)
5	S	0[a]	80[a]
16	S	0	80
5	R	80	80
16	R	80	80

[a] Killing units/0.05 ml.

killing activity of these bacteriocins dependent upon host cell protein synthesis? 3. Does rifampin render bacteriocinogenic cells susceptible to the killing action of homologous bacteriocins, i.e., does exposure of bacteriocinogenic cells to rifampin result in immunity breakdown[11–14]?

Bacteriocinogenic *S. marcescens* isolates No. 5 (subgroup I) and 16 (subgroup II) were employed in all experiments; they were induced with 1 µg/ml of mitomycin C (Sigma Chemical Co., St. Louis, Missouri) in Tryptic Soy broth (TSB; Difco) at 33°C, as described before[8]. The non-bacteriocinogenic *S. marcescens* isolates No. 1 and 9 served as the respective indicator (i) organisms. The bacteriocins were titrated as described previously[8]; titers are expressed in terms of killing units/0.05 ml. Rifampin was a gift of the Ciba Pharmaceutical Co., Summit, New Jersey. A stock solution of 2,000 µg/ml of rifampin in methanol and sterile distilled water was prepared and stored in the dark at 4°C. For control purposes, rifampin-resistant variants of all 4 *S. marcescens* isolates were obtained through successive passages of the growths in TBS containing 25, 50, 100, and 200 µg/ml of rifampin.

First it was determined that rifampin at concentrations of 50, 25, 12.5, and 6.25 µg/ml did not induce bacteriocins No. 5 and 16. When the bacteriocinogenic isolates No. 5 and 16 were exposed to 50 µg/ml of rifampin for 10 min[7], after which the cells were washed twice in TSB and exposed to mitomycin C, there was no inhibition of induction. However, when rifampin had not been removed prior to the addition of mitomycin C, there was complete inhibition of induction (Table I). On the other hand, rifampin-resistant bacteriocinogenic cells could be induced with mitomycin C in the presence of 100 µg/ml of rifampin (Table II).

It was found that exposure of indicator cells to 50 and 100 µg/ml of rifampin for 30 min prior to the addition of crude lysates of bacteriocins No. 5 and 16 did not result in a larger number of survivors as compared with the numbers of survivors among untreated control indicator cells and rifampin-resistant variants; a representative experiment is shown in Table III.

Finally, it was attempted to ascertain whether rifampin abolished the immunity of bacteriocinogenic *S. marcescens* cells to their own group A bacteriocins. It was observed that crude lysates of bacteriocins with titers of 640 killing units/0.05 ml overcame infection immunity in these cells, killing roughly 80% of the cell populations, whereas bacteriocinogenic cells were refractory to the killing action of bacteriocin lysates with titers of 160 killing units/0.05 ml. Exposure of bacteriocinogenic cells to rifampin (50 and 100 µg/ml, 30 min) prior to the addition of bacteriocins (crude lysates adjusted to 160 killing units/0.05 ml) had no effect upon the refractoriness of the bacteriocinogenic cells to the killing activity of their own group A bacteriocins.

The results obtained permitted the following conclusions. Induction of group A bacteriocins in *S. marcescens* by mitomycin C appears to depend upon functioning host cell DNA-dependent RNA-polymerase. The killing action of these phage tail-like bacteriocins apparently is independent of protein synthesis by susceptible indicator cells, a finding in agreement with that of DUCKWORTH[7], who had observed that phage ghosts exerted their killing action in the absence of host cell protein synthesis. Our

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Table III. Killing action of group A bacteriocin No. 5 against *S. marcescens* indicator isolate No. 1 in the presence or absence of rifampin

Time (min) following exposure to bacteriocin	Bacteriocin No. 5 added to cells of *S. marcescens* indicator (i) isolate No. 1			
	1 iS[a]	1 iS + 100 µg/ml rifampin	1 iR[a] + 100 µg/ml rifampin	1 iS (control)
0	1.3×10^4 [b]	1.3×10^4	1.4×10^4	1.3×10^4
10	2.0×10^1	5.0×10^1	1.0×10^1	—
30	1.5×10^1	1.5×10^1	0	1.3×10^4

[a] S denotes sensitive to rifampin, R indicates resistant to rifampin. [b] Number of colony-forming units/ml (survivors).

finding that high-titered bacteriocin preparations could overcome the immunity of bacteriocinogenic cells to their own bacteriocins confirms previous observations[11–13]. The failure of rifampin to abolish the immunity of exposed bacteriocinogenic cells to their own group A bacteriocins was probably due to the fact, that these cells had synthesized adequate amounts of immunity susbtance[14] before their exposure to rifampin. Conceivably the catabolic turnover of this immunity substance is rather slow, so that sufficient amounts of this inhibitor were stlil present after exposure of the bacteriocinogenic cells to rifampin.

Zusammenfassung. Rifampin blockierte die Induktion von Gruppe A Bakteriozinen in bakteriozinogenen *Serratia marcescens* Zellen; demnach scheint die Induktion dieser Bakteriozine auf funktionstüchtiger, DNS-abhängiger RNS-Polymerase der Wirtszellen zu beruhen. Rifampin hatte keinen Einfluss auf die Empfindlichkeit von Indikatorzellen auf Gruppe A Bakteriozine; demzufolge scheint die bakterizide Wirkung dieser Bakteriozine unabhängig von Proteinsynthese durch Indikatorzellen zu sein.

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PRO EXPERIMENTIS

Thin Layer Chromatography of Metal Ions on a New Synthetic Inorganic Ion-Exchanger

Ion-exchange thin layer chromatography of inorganic species has been reviewed by LEDERER[1]. However, very few attempts have been made with the use of synthetic inorganic ion-exchangers in thin layer chromatography[2–4]. QURESHI et al.[5–11] have recently synthesized a few new inorganic ion-exchangers and papers impregnated with some of these ion-exchangers have been used successfully in chromatographic separation of metal ions[12–15]. As far as we are aware, stannic arsenate a new synthetic inorganic ion-exchanger[11] has not been used for thin layer chromatography of metal ions. The present work was therefore undertaken to study the chromatographic behaviour of 38 metal ions on combined thin layers of stannic arsenate gel and silica gel using 15 buffer solutions. As a result, a number of useful and interesting separations have been developed, and some important separations are summarized in this paper.

Experimental. Apparatus: A 'Quickfit' thin layer apparatus was used. Development was performed in 22×24 cm circular glass tanks using the ascending method.

Reagents: Chemicals and solvents were either E. Merck or Riedel. Silica gel GF (E. Merck) was used.

Preparation of ion-exchanger plates: 0.05 *M* solutions of stannic chloride and sodium arsenate were mixed together in volume ratio (3:2). The mixture was continuously stirred during which a white gel was formed, the pH of the gel was adjusted to 1 using 6 *M* nitric acid. This gel was left overnight. The gel was washed 3 or 4 times with distilled water. The supernated liquid was removed completely. Now 50 cm^3 of this wet gel was mixed with 14 g of Silica gel GF. This slurry was used to coat 5 clean glass plates (20×20 cm) using the Quickfit apparatus preset to give an applied layer 0.25 mm thick. These plates were dried in a hot oven at 105 °–110 °C for 1 h and then were stored in an oven at room temperature, after that they were used as such.

Cation solutions: 0.2 *M* solution of chlorides, nitrates or sulphates of most of the cations were prepared in 0.2 *M* solution of the corresponding acids. Antimony(III) and Bismuth(III) chlorides were prepared (0.1 *M*) in 30% (v/v) HCl solution. Selenium dioxide was dissolved in water and made alkaline with 1 *M* KOH solution. Ceric sulphate was prepared in 3 *N* H_2SO_4. Mercuric nitrate solution was prepared in 0.5 *N* HNO_3, As_2O_3 and $Be(NO_3)_2$ were dissolved in 1% HNO_3, and 1% solution of the Gold(III) chloride was prepared in 4 *M* hydrochloric acid.

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