biotic cochromatographed with these phospholipids near the solvent front (Fig. 1). Mixtures of polymyxin with PE, CL and PS did not exhibit this behaviour. PE and PG isolated from *S. typhimurium* showed identical properties like the commercial phospholipids. These results were checked by extraction experiments with the phospholipids dissolved in chloroform and polymyxin B dissolved in aqueous 0.14 M sodium chloride pH 6.9. Again, only PA and PG were able to transfer the radioactive PX derivatives from the aqueous into the organic phase (see Table). The chloroform layer containing PX and PG was optically clear. The use of fluorescent mono-*N*-dimethylaminonaphthalenesulfonyl polymyxin B [8] confirmed that the PX-PG complex was not deposited at the glass walls of the reaction tube but was evenly distributed in the organic phase.

Table. Phospholipid-dependent extractability of mono-*N*-acetyl-^{14}C-polymyxin B (14.7 μCi/μmole) from the aqueous into the organic phase. 0.1 ml of chloroform containing 25 μg (phosphatidic acid, phosphatidylserine, phosphatidylethanolamine) or 30 μg (phosphatidylglycerol, cardiolopin) of phospholipids were overlaid with 0.12 ml of 0.14 M sodium chloride pH 6.9 containing 4 nmoles (about 5 μg) of radioactive polymyxin B derivatives. The two phases were mixed for 15 sec at 20 °C with rapid stirring and separated by 15 min centrifugation at 2500 rpm. 10 μl Portions of the clear phases were counted for their content of radioactive polymyxin B.

Phospholipid in chloroform layer	^{14}C-Polymyxin B derivative [cpm] in 10 μl of	
	aqueous phase	organic phase
none	4 900	130
phosphatidic acid	1 270	2 760
phosphatidylglycerol	395	3 800
phosphatidylserine	4 720	400
cardiolipin	5 560	490
phosphatidylethanolamine	5 070	350

These properties make the system amenable to spectroscopic investigations, *e. g.* nuclear magnetic resonance, infrared and fluorescence spectroscopy. A detailed quantitative analysis of the physicochemical properties of the polymyxin-phosphatidylglycerol complex is in progress in our laboratory.

Whereas phosphatidic acid is only a trace component of the membrane phospholipids in *S. typhimurium* [5], phosphatidylglycerol makes up 17% of the phospholipids in the outer membrane and 33% in the plasma membrane [9]. Since polymyxin B has to penetrate the outer membrane before it gains access to the plasma membrane [10], this could occur *via* the phosphatidylglycerol-polymyxin B complex and/or the complex with the lipid A region of lipopolysaccharide as described in an accompanying paper [11]. The presence of these compounds in the outer membrane of Gram-negative bacteria would therefore be a reasonable explanation for the specific action of polymyxin against these organisms.

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6,8-Dihydroxypurine in the Hen's Egg Yolk

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6,8-Dihydroxypurine, egg yolk, purine metabolism

During an investigation on nucleic acid catabolism in chicken eggs, we were able to identify several purine and pyrimidine derivatives in white and yolk [1]. Isolation of these products from yolk (Leghorn) was achieved by dialysis against ammonia solutions (pH 9.0 – 9.2). Further separation and purification was done as described for the products isolated from white [1]. One of the peaks obtained after chromatography on Dowex 2 × 8 (acetate) and gel filtration on Sephadex G-10 shows an UV spectrum resembling that of hypoxanthine. Paper chromatography in different solvent systems revealed the presence of at least two products, one of them being hypoxanthine (evidence for the presence of hypoxanthine was obtained by means of UV spectroscopy and paper chromatography). The purification of the other product was achieved by paper chromatography with isopropanol/H_2O/NH_3 (28%) : 85/15/1.3 [2]. The elution volume on Sephadex G-10 sug-

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gested a purine derivative. A spectrophotometric titration carried out in the range pH 2 to pH 12 reveals two pK values: 7.9 and 10.5. The isosbestic points are rather sharp, indicating only a low contamination by other UV absorbing substances. The obtained spectra were identical with those of 6,8-dihydroxypurine [3]. The product was then compared with an authentic sample of 6,8-dihydroxypurine (Aldrich).

A spectrophotometric titration of this product gave the same pK values, isosbestic points and other UV characteristics as those obtained for the product isolated from yolk. Further evidence for the identity of both samples comes from paper chromatography in six different solvent system and from two dimensional thin layer chromatography on cellulose [4]. Paper electrophoresis carried out at pH 1.9 and at pH 10 confirms the identity. The observed spectral changes after UV irradiation at pH 4.6 (0.01 M acetate) and pH 9.2 (0.01 M carbonate) are identical for both samples. UV spectra and R_F values do not change after treatment with 1 N HCl for 1 hour at 100 °C, neither for 6,8-dihydroxypurine nor for the product isolated from yolk.

The role of 6,8-dihydroxypurine in the yolk of chicken eggs is not elucidated. Catabolizing enzymes that may account for it as a degradation product of other purines were not yet found. Bergmann and Dikstein [3] considered it as a possible intermediate in the oxidation of hypoxanthine to uric acid, although they did not prove this. They also showed that 6,8-dihydroxypurine is a substrate for the xanthine oxidase from milk. On the other hand, Wyngaarden and Dunn [5] found that 8-hydroxyadenine is the major intermediate in the enzymic oxydation of adenine to 2,8-dihydroxyadenine.

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