Table 1.

	Spectra in acid max	min	250/260	280/260	Spectra in alkali max	min	250/260	280/260
Substance I	266	237	0.76	0.82	275	244	0.79	1.16
Substance II	261	237	0.63	0.13	261		0.76	0.17

1 × 8 column in the formate form by gradient elution with the formic acid-ammonium formate system of Hurlbert *et al.* A slight modification was introduced as the elution prodecure was started with very dilute formic acid (0.001 M) in the reservoir.

In addition to amino acid complexes containing adenine nucleotides, two other complexes with amino acids were isolated which had U.V. spectra different from any of the usual purines and pyrimidines. Both were eluted early in the fractionation procedure. One (substance I) appeared earlier than any of the known nucleotides, the other (substance II) together with CMP-5' or in a fraction following immediately after CMP-5'.

In paper chromatography both substances moved as single spots in five different solvents. In most solvents the R_F values exceeded those of the known nucleotides. No change in the mobility of the U.V. absorbing substances was observed after hydrolysis for 18 h in N HCl at 120°C. The two substances were homogeneous and negatively charged at pH 7.0 as indicated by electrophoresis in phosphate buffer.

Whereas the unhydrolyzed material was ninhydrine negative, hydrolysis in 6 N HCl gave for both substances ninhydrine positive spots corresponding to the position of alanine, serine, aspartic acid, glutamic acid, glycine and lysine in two dimensional paper chromatography. Small amounts of these amino acids also split off when the unhydrolyzed material was subjected to paper chromatography. None of the substances contained phosphate. One of them (substance I) could be reduced with sodium amalgam and the reduced material gave positive test for ribose.

The two substances exhibited a slight difference in the electrophoretic mobility at acid pH values and pronounced differences in the spectra values as shown in Table 1.

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1. Jonsen, J., Laland, S., Smith-Kielland, I. and Sömme, R. *Acta Chem. Scand.* **13** (1959) 838.

Biologically Active Fractions from *Haemophilus Pertussis*

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Cells from *H. pertussis*, cultivated in the 500 litres scale, were disintegrated in the frozen state in the Hughes press. The disintegrate was extracted by a series of aqueous salt solutions. The supernatant was highly neurotoxic and dermatonecrotic. The neurotoxin was purified by column electrophoresis on a stabilized medium (320 cm columns). Due to the presence of acid polyelectrolytes the conditions for a purification of the neurotoxin on cellulose ion exchangers are unfavourable. They were consequently removed by electrophoresis before chromatography. The neurotoxin occurs in the same pK region (around 7.4) as a blue coloured protein. There exist dermatonecrotic proteins in other pK regions. The neurotoxin is reasonably stable only in its isoelectric region.

The neurotoxin has been purified to a MLD per 10 g mouse of 10 μg. Further purification is tried.

The residue after extractions forms very stable suspensions after the salts have been removed. It can be spun down at 100 000 *g*. It is salted out by monovalents salts. It can be solubilised again by either removing the salts or by making it 2 M with regard to urea. The residue contains two lipid fractions, protein and (amino-)polysaccharide. It is nontoxic up to at least 2 mg per 10 g mouse, and protects mice against 1 000 MLD of *H. pertussis* down to at least 50 μg per 10 g mouse. Electron microscopy shows that it consists primarily of bacterial cell walls. A *Haemophilus pertussis* immunogen has thus been found that shows no toxic activity on mice even in high dosages.

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