Fig. 1. Crystals of *Pseudomonas* cytochrome₅₅₁. ($\times 800$)

amount of distilled water. After the re-addition of a trace of sodium dithionite, solid ammonium sulphate was added to give slight turbidity, with frequent adjustments of the pH to 7-8 with ammonia. The solution gradually increased in turbidity and the needle-like crystals shown in Fig. 1 began to appear after a few hours storage. The addition of the ammonium sulphate and the storage should be carried out at almost the same temperature, since the solubility of *P*-cytochrome₅₅₁ in ammonium sulphate solution increases greatly as the temperature decreases.

Twice recrystallized *P*-cytochrome₅₅₁ had the absorption spectrum shown in Fig. 2, and more than two recrystallizations did not increase its purity.

Fig. 2. Absorption spectrum of crystalline *Pseudomonas* cytochrome₅₅₁. —, Oxidized form; ---, dithionite-reduced form. The numerals in the figure are the absorption maxima

Compared with crystalline cytochrome *c* prepared from bovine heart muscle^{4,5} the α -absorption peak was at a slightly longer wave-length (about 0.5 $m\mu$). The twice recrystallized sample of *P*-cytochrome₅₅₁ was homogeneous both by electrophoresis and by ultracentrifugal analysis.

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Action of Micrococcal Phosphodiesterase on Tobacco Mosaic Virus Nucleic Acid

Cunningham, Catlin and de Garihe¹ were the first to report the occurrence of a calcium-activated nuclease in the culture medium of *Micrococcus pyogenes* var. *aureus*. These authors partially purified the enzyme and studied its action on deoxyribonucleic acid. In the complete digest they reported the presence of mononucleoside 3'-phosphates and also oligonucleotides. The latter were not identified except for one dinucleotide, deoxyadenylyl-(3'-5')-deoxycytidine 3'-phosphate². Recently, it was further demonstrated that this enzyme, when activated with calcium, hydrolyses ribonucleic acid³.

This enzyme has been purified in this laboratory a hundredfold and its action on tobacco mosaic virus nucleic acid was studied. Our enzyme preparations also require calcium ion for activation. Among the reaction products formed after exhaustive digestion of tobacco mosaic virus nucleic acid with this enzyme the following mono- and di-nucleotides have so far been identified: adenosine 3'-phosphate; guanosine 3'-phosphate; cytidine 3'-phosphate; uridine 3'-phosphate; adenylyl-(3'-5')-cytidine 3'-phosphate; adenylyl-(3'-5')-guanosine 3'-phosphate; uridylyl-(3'-5')-guanosine 3'-phosphate; and uridylyl-(3'-5')-uridine 3'-phosphate; adenylyl-(3'-5')-uridine 3'-phosphate; and uridylyl-(3'-5')-cytidine 3'-phosphate. This enzyme has no action on purine or pyrimidine cyclic nucleotides, and hence the possibility of its cleavage of ribonucleic acid via transphosphorylation can be excluded. Full details of this investigation will be published elsewhere.

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Isolation of $\Delta^{5,7,9}$ -Estratrienol-3-one-17 from the Urine of Pregnant Mares

$\Delta^{5,7,9}$ -ESTRATRIENOL-3-ONE-17 was isolated from pregnant mares' urine in 1940 by Heard and Hoffman¹. These workers obtained approximately 160 mgm. of pure material per 10,000 gallons of urine, that is, about 3.8 μ gm./l., but stated that this did not represent the total quantity excreted. Since no other isolations of $\Delta^{5,7,9}$ -estratrienol-3-one-17 from pregnant mares' urine have been reported in the literature, we believe that it is of interest to record that we have found the material to be present in pregnant mares' urine in relatively large quantities (approximately 430 gm. of $\Delta^{5,7,9}$ -estratrienol-3-one-17 per 10,000 gallons of urine, that is, about 10 mgm./l.) and that we have isolated the material readily in the pure state.

A concentrate, representative of mares' urine collected from approximately the fourth to ninth month of pregnancy, was prepared by methods previously outlined by us². The concentrate was