With the aid of ^{14}C-UCA, the products of the reaction in the presence and absence of dye have been investigated. In the absence of dye, the product of the reaction appeared to be L-formamidinoglutaric acid as judged by its behavior upon ionophoresis on paper and its conversion to L-glutamic acid. In the presence of dye, a new substance was found which behaved upon ionophoresis as a neutral molecule at pH 2 and as a divalent anion at pH 10. The oxidized nature (relative to urocanic acid) of this compound was demonstrated by its conversion to α-ketoglutaric acid upon treatment with acid. These properties suggest that the compound formed in the presence of dye is 5-imidazolone-4-acrylic acid. We have previously suggested[3,7] that in the absence of dye the product of urocanase action is 5-imidazolone-4-propionic acid.

Although our results indicate that an oxidation and subsequent reduction are involved in UCA degradation, the absence of an easily dissociable cofactor (which could act as an electron carrier) makes it likely that a single protein catalyzes both reactions. It appears that urocanase oxidizes UCA and that oxidized UCA is then either reduced without detachment from the enzyme or, in the presence of dye, liberated as imidazolone acrylic acid. In summary, we suggest the following mechanism for urocanase action:

$$\text{urocanate} + \text{enzyme} \longrightarrow [\text{oxidized urocanate} \cdot \text{reduced enzyme}] \qquad (1)$$

$$[\text{oxidized urocanate} \cdot \text{reduced enzyme}] \longrightarrow \text{imidazolone propionic acid} + \text{enzyme} \qquad (2a)$$

or in the presence of dye:

$$[\text{oxidized urocanate} \cdot \text{reduced enzyme}] + \text{dye} \longrightarrow \text{imidazolone acrylic acid} + \text{reduced dye} + \text{enzyme} \qquad (2b)$$

We have also observed that urocanase prepared from histidine-adapted *Pseudomonas fluorescens*[8] required no easily dissociable cofactor and catalyzed dye reduction in the presence of UCA, results which suggest that the mechanism of action of the bacterial enzyme is similar to that of mammalian urocanase.

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1 A. MILLER AND H. WAELSCH, *Arch. Biochem. Biophys.*, 63 (1956) 263.
2 H. TABOR AND J. C. RABINOWITZ, *J. Am. Chem. Soc.*, 78 (1956) 5705.
3 B. A. BOREK AND H. WAELSCH, *J. Biol. Chem.*, 205 (1953) 459.
4 H. TABOR AND A. H. MEHLER, *J. Biol. Chem.*, 210 (1954) 559.
5 B. MAGASANIK AND H. R. BOWSER, *J. Biol. Chem.*, 213 (1955) 571.
6 A. H. MEHLER AND H. TABOR, *J. Biol. Chem.*, 201 (1953) 775.
7 H. WAELSCH AND A. MILLER, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, Johns Hopkins Press, Baltimore, 1955, p. 407.
8 H. TABOR AND O. HAYAISHI, *J. Biol. Chem.*, 194 (1952) 171.
9 R. E. BASFORD AND F. M. HUENNEKENS, *J. Am. Chem. Soc.*, 77 (1955) 3873.

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Chromatographic purification of T2r bacteriophage

The recent development of cellulose ion-exchange resins[1] which permit the chromatography of nucleic acids from various sources[2,3,4] suggested that such resins would be of use in the purification of nucleoproteins, such as bacteriophage, and bacteriophage nucleic acids. Successful fractionation of Tobacco Mosaic Virus has been reported[5].

Preliminary experiments showed that it is possible to chromatograph the components of lysed cultures of *Escherichia coli* and, therefore, the following method was devised for the preparation of T2r from such lysates. *E. coli* B/1,5 was grown in 1 l of nutrient broth (Difco) containing 0.4% glucose and 0.5% sodium chloride to a concentration of $5 \cdot 10^8$ cells/ml. The culture was then infected with T2r bacteriophage in a multiplicity of one. After lysis had occurred the culture was dialysed against 20 l of water overnight. There was no decrease in the virus titre after dialysis. To the dialysed suspension was added 10 g of ECTEOLA-SF cellulose ion-exchange resin,

prepared as described by PETERSON AND SOBER[1]. After thorough mixing the resin was filtered off and the filtrate discarded. The resin was then stirred with 100 ml of 0.01 *M* NaCl in 0.01 *M* phosphate buffer at pH 7.0, filtered, and the filtrate was again discarded. The treated resin was then extracted with 100 ml of 0.2 *M* NaCl in buffer, the filtrate was centrifuged at 20,000 g for 40 min and the sediment resuspended in a convenient volume of buffer. Although this suspension was found to contain the bacteriophage in yields of 25–80 % of the total virus in the original lysate, only about 1 % of the total protein and 10–20 % of the total nucleic acid were present. Experiments are in progress to determine the cause of the lower yield of virus. Further elution of the resin with increasing concentrations of NaCl did not result in any significant increase in the recovery of virus.

The virus preparation was slightly turbid, the ratio *E* 260 mμ: *E* 650 mμ being 80:1. Hydrolysis and paper chromatography[6] of the preparation was performed and the only bases found were adenine, guanine, thymine and 5-hydroxymethyl-cytosine, indicating that *E. coli* ribo- and deoxyribonucleic acids were not present in detectable amounts. It is not possible directly to measure contamination of the virus preparation by *E. coli* protein. However, the low protein content of the preparation compared with the starting material, and the fact that soluble proteins of the bacterium do not sediment under the conditions used to sediment the virus, assure that contamination by non-viral protein is low. The virus preparation was rechromatographed on ECTEOLA–SF resin before and after it had been subjected to osmotic shock[7]. Fig. 1 shows that the live virus is eluted as a single peak with a maximum at 0.14 *M* NaCl, and with no evidence of heterogeneity. The virus peak is parallelled by a nucleic acid peak; a smaller nucleic acid peak is at 0.4 *M* NaCl. The split virus, which possessed less than 1 % of the original titre, showed a single nucleic acid peak at 0.4 *M* NaCl. Thus it would appear that the only chromatographically detectable contaminant in the virus preparation was free virus nucleic acid, which was probably released in the second chromatographic procedure. Similar results were obtained with other preparations of ECTEOLA–SF, except that the peaks were displaced.

Preliminary experiments show that the protein component of the split virus is eluted at approximately the same concentration of NaCl as in live phage, indicating that the chromatographic properties of live virus are determined by its protein component.

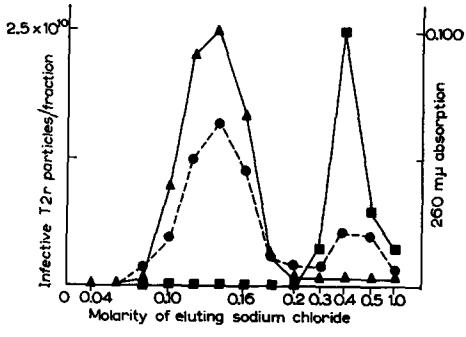

Fig. 1. Chromatography of infectious and osmotically shocked T2r bacteriophage on a 0.5 g column of ECTEOLA–SF resin. Material was adsorbed onto the column and eluted with 5 ml aliquots of sodium chloride solution of the indicated molarity. ▲—▲ Infectious T2r particles; ●---● Nucleic acid of infectious T2r particles; ■—■ Nucleic acid of osmotically shocked T2r particles.

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[1] E. A. PETERSON AND H. A. SOBER, *J. Am. Chem. Soc.*, 78 (1956) 751.
[2] A. BENDICH, J. R. FRESCO, H. S. ROSENKRANZ AND S. M. BEISER, *ibid.*, 77 (1955) 3671.
[3] D. F. BRADLEY AND A. RICH, *ibid.*, 78 (1956) 5898.
[4] L. ASTRACHAN, E. VOLKIN AND M. H. JOHNS, *ibid.*, 79 (1957) 130.
[5] B. COMMONER, J. A. LIPINCOTT, G. B. SHEARER, E. E. RICHMAN AND J. WU, *Nature*, 178 (1956) 767.
[6] G. R. WYATT AND S. S. COHEN, *Nature*, 170 (1952) 1072.
[7] T. F. ANDERSON, C. RAPPAPORT AND N. A. MUSCATINE, *Ann. inst. Pasteur*, 84 (1953) 5.

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