eter and 1-cm cells.) Over 400 particles were measured from fields in two electron micrographs from the same polymeric preparations. From the proportionality constants obtained for the weight-average molecular lengths (proportional to weights) of the preparation (164 mμ) and the observed turbidity at given wavelengths, the turbidity for a monodisperse system of particles of 271 mμ length (the length of TMV[6]) was calculated.

With this information it is of interest to see how well the spectrum of TMV (pH 7.3) can be accounted for. In the figure we have shown by the circles the sum of the spectra of RNA (pH 7.3), present to the extent of 5.1% in TMV[7], and polymerized X-protein, and, in the uppermost curve, the sum of the spectrum of RNA (the small scatter by RNA has been neglected[1]) and of a calculated spectrum for X-protein polymerized to the full length of TMV. The agreement between the spectrum of TMV and the calculated spectrum is satisfactory above 260 mμ wavelength. The over correction below 260 mμ probably results from the approximations used.

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The reactivity of O-methylisourea towards the protein of tobacco-mosaic virus

O-Methylisourea reacts with the ε-amino group of lysine of proteins converting these to homoarginine residues[1,8]. This reaction is found to be quantitative with human serum albumin[1], bovine serum albumin[2,3], chymotrypsinogen[4], insulin[5], ribonuclease[6], growth hormone, lactogenic hormone and lysozyme[7]. The free α-amino group of proteins was generally found not to react with the reagent[8] with the exception of insulin where the terminal amino groups became partly substituted[5]. The reaction with the single lysine residue in insulin has also been reported to be incomplete and only three of the four lysine residues in α-corticotropin were found to react. Similarly, only 75% of the ε-amino groups of fibrinogen has been found to react with the reagent[9].

The protein of tobacco-mosaic virus (TMV) contains two lysine residues per molecule of the protein[10–12] but reaction of the protein with fluorodinitrobenzene (FDNB) has been reported to yield only one mole of ε-DNP-lysine[13]. This has also been found to be the case with the tryptic hydrolyzate of TMV protein[14]. This, naturally, has raised the question whether the ε-amino group which did not react

with FDNB was involved in secondary peptide-chain branching. An alternate possibility, however, of this group being unavailable to the bulky reagent molecule due to steric reasons had to be considered. Hence the reactivity of the ε-amino groups in the protein toward a smaller reagent molecule, O-methylisourea, has now been examined.

TMV protein[15] was reacted with 1000-fold excess of the reagent under conditions otherwise similar to those employed for chymotrypsinogen[4] and the reaction product isolated by dialysis and lyophilization. Samples of the protein were hydrolyzed with 6 *N* HCl at 108° for 24 h and the content of lysine and homoarginine present in the hydrolyzates determined by ion-exchange chromatography[16,4]. The results of these experiments were averaged and are summarized in Table I.

TABLE I

CONTENT OF LYSINE AND HOMOARGININE IN TMV PROTEIN AND GUANIDATED TMV PROTEIN

Sample	*Reaction conditions*	*moles amino acid/18,270 g protein*	
		Lysine	*Homoarginine*
TMV Protein	Untreated	2.02	nil
TMV Protein	pH 10.5, 5°, 5 days	1.98	0.08
Heat-denatured TMV Protein*	pH 10.5, 5°, 5 days	1.02	1.01
Urea-denatured TMV Protein**	pH 10.5, 5°, 5 days	1.03	0.88
Heat-denatured TMV Protein in 8 *M* Urea***	pH 10.5, 5°, 5 days	0.93	0.99

* Prior to reaction the protein in solution (1 %) at pH 7.0 was heated at 100° for 5 min.
** Reaction carried out in the presence of 8 *M* urea.
*** After heat denaturation of the protein, reaction carried out in the presence of 8 *M* urea. The presence of the urea prevents the immediate precipitation of the protein on addition of O-methylisourea.

These data indicate that in the native TMV protein neither of the two lysine residues react with the reagent, while in the heat- and urea-denatured samples the equivalent of one lysine reacts to yield homoarginine. It is to be noted that at pH 10.5 in the cold, TMV protein stays largely native[17]. In the reaction with FDNB when one of the two lysines react to yield ε-DNP-lysine, the conditions of reaction are such as to cause the protein to be denatured rapidly. It was of some interest to ascertain whether in the guanidated derivative the single lysine which had not reacted with O-methylisourea would react with FDNB. For this purpose the guanidated derivative obtained from urea-denatured TMV protein was dinitrophenylated. The product on hydrolysis yielded 0.20 mole ε-DNP-lysine/mole protein, while, from the homoarginine content of the derivative, the anticipated value would be 0.12 mole. Thus the evidence indicates that the single ε-amino group in the protein which reacts with FDNB is also the group which participates in the reaction with O-methylisourea. The structure of the TMV protein around the second lysine residue possibly involves some unusual linkages.

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Effect of chondroitin on the uptake of radioactive sulphate into chondroitin sulphate

D'ABRAMO AND LIPMANN[1] have found that adenosine-3'-phosphate-5'-phosphosulphate (PAPS) is formed by an enzyme system present in particle-free extracts of chick-embryo condyles. By using ^{35}S-labelled PAPS they showed that the sulphate group was incorporated into chondroitin sulphate (CSA). A synthesis of CSA *de novo* from the component parts was demonstrated by the incorporation of radioactive acetate into CSA by this extract. The stage at which sulphation occurs in the biosynthesis of CSA is as yet unknown. MEYER and his co-workers[2,3] have suggested that the polysaccharide is formed first and then sulphated. This hypothesis was based on the isolation from cornea of the sulphate-free polysaccharide chondroitin[2], which they suggested was the immediate precursor of CSA. It was obviously of interest, then, to investigate the effect of added chondroitin on the biosynthesis of CSA.

CSA was isolated from bovine trachea by the method of BERA, FOSTER AND STACEY[4] and further purified by treatment with Sevag's Reagent and fractionation with alcohol from calcium acetate buffer, pH 5. The fraction precipitated by 30% (v/v) alcohol was removed and the fraction precipitated by 40% (v/v) alcohol was converted to the CETAVLON salt[6], the latter dissolved in 10% KCl and the free polysaccharide precipitated with alcohol. This was dissolved in water, dialysed, the solution passed through a column of Dowex 50, K form, and lyophilised (Found: N, 2.11; S, 5.03. Calc. for $C_{14}H_{19}NSO_{14}K_2 \cdot 4H_2O$: N, 2.3; S, 5.3). Chondroitin (K salt) was prepared from this material by the desulphation method of KANTOR AND SCHUBERT[5]. (Found: N, 2.73; S, 0.31. Calc. for $C_{14}H_{20}NO_{11}K \cdot 2H_2O$: N, 3.1; S, 0). Metachromasia was virtually absent when solutions of the product were tested with methylene blue[5] by measuring the absorption at 665 mμ.

The enzyme used in the study was a high-speed supernatant prepared from condyles of 15-day-old chick embryos by extraction with 0.01 *M* phosphate saline (1 ml per embryo)[1]. PAPS was prepared by incubation of adenosine triphosphate (ATP), $MgCl_2$, enzyme and tris(hydroxymethyl)aminomethane(Tris), pH 8.2, with