was found in any of the samples. None of the compounds was further characterized.

TABLE I

Tube number	Ratios of absorbance	
	250 mμ/260 mμ	280 mμ/260 mμ
36	0.8	1.1
41	0.77	1.1
45	0.75	1.4
49	0.77	1.1
56	0.75	0.64
63	0.66	0.30
66	0.92	0.21
78	0.97	0.37
83	0.87	0.51
85	0.96	0.57
91	0.89	0.36
101	0.93	0.6
108	0.96	0.72

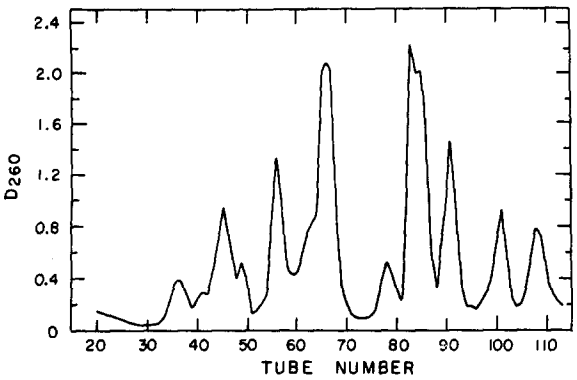

Fig. 1. Chromatography of a boiled, aqueous extract of mouse mast-cell tumor on ECTEOLA formate.

The procedure employed in this experiment was chosen arbitrarily, and no attempt has been made to modify it. It is anticipated, however, that good chromatographic results would be obtained with buffers of even lower molarities at pH's approaching neutrality. Since ECTEOLA has a pK of approximately 7 (ref. [3]) even the most firmly bound compounds should be readily eluted, without recourse to solutions of high molarity, merely by increasing the pH of the eluting buffer. In any event, the above observations are sufficient to indicate that chromatography on ECTEOLA formate is a useful procedure for the separation of complex mixtures of nucleotides.

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Scattering of light by tobacco-mosaic virus and X-protein from infected tobacco plants

In a study of the inactivation of tobacco-mosaic virus (TMV) by u.v. light the fraction of absorbed light which is not scattered was estimated from the absorption by ribonucleic acid (RNA) from TMV and TMV-protein[1]. The scattering of light by TMV arises from the enormous size of the elementary particle or molecule[2]. A more direct way of estimating the scattering would be to compare the absorbance of two solutions, one of which would consist of a low-molecular-weight protein and the other would contain

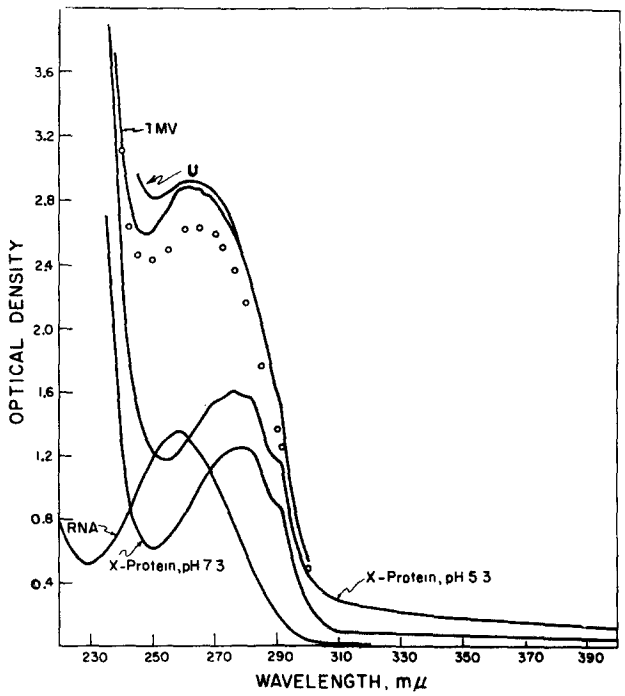

Fig. 1. U.v.-absorption spectra of tobacco-mosaic virus (1.054 mg/ml), X-protein at pH 7.3 and at pH 5.3 (1.0 mg/ml), and RNA (0.054 mg/ml). Circles are sums of points on the RNA curve and the X-protein (pH 5.3) curve. Uppermost curve (U) sums that of RNA and a hypothetical curve for X-protein fully polymerized to rods of 271 mμ length.

the protein polymerized into rods of the length and width of TMV itself. The difference would represent the turbidity due to scattered light, provided reabsorption of scattered light is neglected and provided the refractive index of the rods is the same as that of the virus. (As will be seen from the results to be described, these provisos are reasonable). A suitable protein for such a study is X-protein from infected tobacco plants. It is monomeric at pH 7.3 and polymerized at pH 5.3 as rods 150 Å in diameter and with lengths more or less commensurate with TMV itself[3]. This protein is very closely related to TMV-protein, if not identical with it (the amino acid composition of TMV and X-protein are the same[4]), and can be copolymerized with RNA to give particles having morphological and biological properties indistinguishable from TMV reconstituted from RNA and TMV-protein.[5]

The absorption spectra of X-protein in monomeric and polymeric forms are shown in Fig. 1. As may be seen by the difference between the two curves, the scattering is appreciable at all wavelengths below 400 mμ. Electron micrographs of these solutions revealed that only a few per cent of the polymeric rods were as long as those of TMV, as was expected from previous results[3]. In order to estimate the scattering which would have resulted from the X-protein if all of this protein were of uniform rods equal in length to those of TMV, use was made, as a first approximation, of the well-known relationship between turbidity and weight-average molecular weight[6]. This is permissible with dilute solutions and with scattering measured in the forward direction[6]. (All spectra were obtained with a Cary recording spectrophotom-

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