the proteolytic enzymes, pepsin, trypsin, papain and aminopeptidase, but not carboxypeptidase, produced significant inactivation. Among the non-proteolytic enzymes studied, hyaluronidase and neuraminidase diminished activity, whereas ribonuclease and lysozyme were ineffective.

From these data, it is inferred that the active portions of ESF consist of both carbohydrate and polypeptide moieties. Susceptibility to aminopeptidase suggests the presence of an unblocked polypeptide approach to the active region from an N-terminal position. Resistance of the C-terminal position to carboxypeptidase could have occurred if this end were blocked by carbohydrate or by a slowly lysed peptide bond. Inactivation with neuraminidase indicates that the terminal carbohydrate derivative of human ESF is neuraminic acid in agreement with LOWY *et al.*[4] who noted that rabbit plasma ESF is also susceptible to neuraminidase.

Evidence that the effects of these enzymes were exerted directly upon ESF *in vitro* and were not due to depression of erythropoiesis within the recipient rats has also been obtained and will be included with complete details in a forthcoming paper.

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Negative-staining electron microscopy of proteins at pH values below their isoelectric points. Its application to hemocyanin

The negative-staining technique of BRENNER AND HORNE[1] is a very important method for studying the structure of protein molecules with the electron microscope. In this method contrast is obtained by the use of potassium phosphotungstate at pH 7.2. Because of the negative charge of the phosphotungstate ions, this method is applicable only at pH values above the isoelectric point, where the protein molecules also carry negative charges and thus do not take up the contrasting ion.

We are studying the structure of the molecules of *Helix pomatia* hemocyanins at different pH values and wished to extend this method below the isoelectric point. Uranyl acetate appeared to give the best result as a contrasting agent. It is clearly seen from Fig. 1 that uranyl ions do not interact with the protein. Uranyl acetate

seems to be amorphous and gives a high contrast. The projections of the molecules appear as circles with a diameter of 300 Å and squares with a side of 300 Å. Their substructure is clearly visible. It is to be expected that this method will be generally applicable to proteins and other macromolecules with a positive charge below pH 5.

A discussion of these results and those of other experiments on hemocyanins will be published shortly.

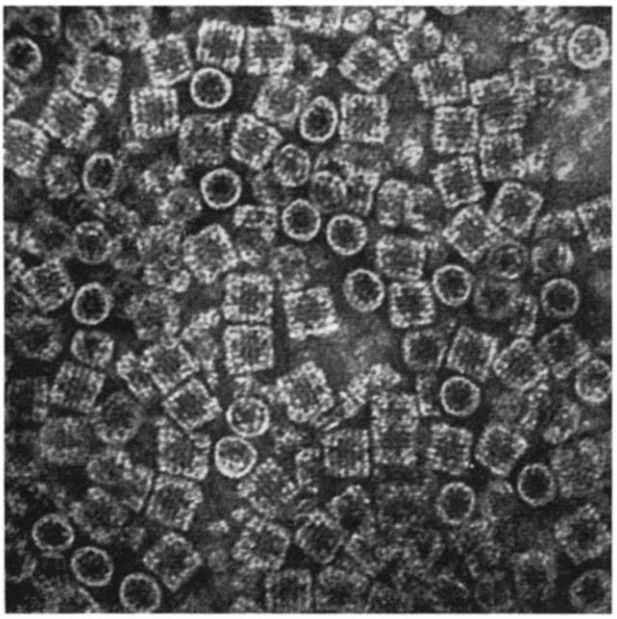

Fig. 1. Hemocyanin molecules (× 210,000). One droplet of the dialysed and centrifuged blood of *Helix pomatia* was mixed with 2 ml of a solution containing 0.5 % uranyl acetate and 0.1 % ammonium acetate, which was brought to a pH 4.5 by the addition of dilute acetic acid The mixture was immediately sprayed on a carbon-coated specimen grid and studied in the new Philips EM-200 electron microscope.

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