We are indebted to Prof. C. D. Darlington and Dr. Robert Brown for helpful discussion, and to Dr. E. M. Deeley for providing facilities at the Wheatstone Physics Laboratory, King's College, London.

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Reduction of the Viscosity of a Potassium Hyaluronate Solution by Ultra-violet Irradiation

In experiments on the immediate effects of ultraviolet irradiation on connective tissue we have demonstrated a marked reduction of the viscosity of potassium hyaluronate solutions, the reduction being as pronounced as that obtained with hyaluronidase. The effect has been determined in vitro by the viscosimetric method described in 1948 by Dalgaard-Mikkelsen and Kvorning¹

As radiation source we used a Philips (R^*) high-pressure mercury lamp $(HP \ 125 \ W)$ without the glass filtering bulb. The total intensity of radiation was approximately 95 ergs/mm.²/sec. In Fig. 1 are shown values for viscosity and irradiation time.

No significant reduction in viscosity was observed after the interposition of a Chance (R^*) glass filter OX 1, but considerable reduction was obtained with an OX 7 filter; therefore, the active wave-lengths must be less than 300 mµ.

In the experiments made so far we have observed no effects due to the presence or absence of physically



Fig. 1. Variation of viscosity of potassium hyaluronate solutions during ultra-violet irradiation (phosphate buffer, pH 6.8). A, Control solution stored at room temperature in darkness; B, irradiated solutions; C, solution treated with excess hyal-uronidase

absorbed oxygen. The addition of light-sensitizing substances has, in contrast to the observations of Castellani², accelerated the reduction of viscosity only slightly. Ultra-violet irradiation has no effect on the viscosity of a chondroitine sulphate solution under similar conditions.

By the method for measuring the spreading of solutions in subcutaneous tissue of mouse skin³, found a markedly increased spread of saline (0.9 per cent) in skin irradiated on the inside for 1 min. immediately after the animals were killed. The effect corresponds closely to that obtained by adding hyaluronidase to the saline.

Using the method for demonstrating increased capillary permeability (intracutaneous injection of test solutions into guinea pigs immediately after intravenous injection with pontamine sky blue), we found no difference between the effect of an irradiated and an untreated hyaluronate. Therefore the vascular phenomena in the inflammation caused by ultraviolet light are probably not directly due to substances formed from hyaluronic acid.

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March 12.

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Polymerization-Depolymerization of Tobacco Mosaic Virus Protein

Knight and Lauffer¹ reported that nucleic acid-free protein, obtained by degrading tobacco mosaic virus in alkaline solutions, tended to aggregate to form rapidly sedimenting particles. Schramm² showed that nucleic acid-free rod-like particles resembling tobacco mosaic virus but void of activity could be obtained by allowing the protein, isolated after mild alkaline treatment of the virus, to aggregate. The structure of these nucleic acid-free protein rods has been shown by Franklin⁸ to be similar to that of the intact virus, except for the absence of nucleic acid. In other words, the protein sub-units are arranged in a giant helix around a hollow core.

It was pointed out by Knight and Lauffer¹ in 1942 that the aggregation of the alkaline degradation product of tobacco mosaic virus was accelerated by a rise in temperature. Harrington and Schachman⁴ showed that at 0° C. protein with a sedimentation coefficient of about 4 S was obtained when tobacco mosaic virus was treated at pH 9.8; however, when the reaction was carried out at 25°, nucleic acid-free protein particles with a much higher sedimentation coefficient were obtained. Harrington and Schachman showed that some of these larger particles were produced by polymerizing the 4 S component.

We have now discovered that protein prepared by the degradation of tobacco mosaic virus at pH 10.3, purified by means of electrophoresis and high-speed contrifugation, and placed in tenth ionic strength phosphate buffer at \hat{p} H 6.5, become polymerized at room temperature and depolymerized in the cold.

Results for optical density shown for pH 6.5 in Table 1 and for viscosity shown in Table 2 demon-