to decrease in the case of the acetate buffer mixture and acetic acid, but it did not change from that of the control in the case of the sodium acetate. The inhibiting effect of acetic acid on the protoplasm might have persisted in this case because the acetic acid in the vacuole served as a reserve supply by diffusing into the protoplasm after the cells had been transferred from these solutions into the dye solution, while that of the sodium acetate did not persist because there was no storage of sodium acetate or acetic acid in the vacuole.

Some of these experiments seem to indicate that though a decrease in the rate of penetration of a basic dye into the vacuole may take place at the same time the pH value of the sap is decreased, such results do not necessarily discredit the theory that a basic dye enters the vacuole much more readily in form of free base than in form of salt. If we assume that the inhibiting effect of acetic acid on the protoplasm exceeds the accelerating effect on the vacuolar sap, and that the rate of penetration of dye into the vacuole is controlled in this case by the diffusion of dye in form of free base from protoplasm into the vacuole, then a decrease in the pH value or some other alteration in the protoplasm corresponding to a decrease in the pH value of the sap might very well bring about a decrease in the rate of penetration of the dye into the vacuole.

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A Method of Proteolytic Enzyme Titration.

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In 1906 Müller and Jochmann[1] proposed a simple method of determining the presence of proteolytic enzymes in biological fluids by erosion of the surface of a Loeffler serum plate under a drop of the fluid. Later this technique was modified by the use of a gelatin plate dyed with carmine, but at best it was a + or — method, probably because the substrate was not sufficiently uniform to permit quantitative estimates of enzyme action.

If a photographic plate, or film, is fogged on both sides by equal exposures, is developed, fixed, washed, and dried, it presents a gelatin surface and texture of sufficient uniformity for quantitative tests. Proteolytic enzymes erode the surface and free the included silver,

so that the light transmission of the exposed area increases as digestion proceeds, and can be estimated relatively by colorimetry, or directly by a calibrated thermopile and galvanometer. The method is as follows:

Small paraffined chambers are filled with trypsin or pepsin solution so that the meniscus projects above the rim. Face down over each chamber is placed a small square of the photographic plate, which has been previously brought to the proper pH concentration in phosphate buffer solution and allowed to dry without rinsing. Due to the inverted position of the gelatin layer the products of proteolysis and the released silver fall away from the surface, and interfere less with the progress of the reaction than as if they accumulated there. Temperature is carefully controlled, and plates are removed at timed intervals, rinsed quickly in cold water, immersed in a bath of the opposite reaction to arrest enzyme action, and dried rapidly before a fan.

The successive plates will be found to present areas of decreasing density. A quantitative estimate of the progress of the reaction is made with a Klett or other Duboscqre type colorimeter. On one side, under the cup stand, is placed a square of the photographic plate from which the silver emulsion has been removed. The gelatin-silver suspension from this piece of plate, dissolved off in hot 25 per cent glycerine solution (to maintain the silver longer in even suspension) is poured into this cup, as a variable comparison standard against which the eroded areas of the experimental plates, under the other cup (filled with 25 per cent glycerine solution) are read. A piece of the same plate which has been soaked in the enzyme solvent and dried is used for the control, or initial density reading.

The match is exact, since specimens of the same materials intercept the light on both sides of the colorimeter, and readings may be made repeatedly within 1 or 2 per cent. Preliminary titrations of trypsin solutions have given exponential curves, and seem to indicate a mononuclear reaction. The method is perhaps not as sensitive as the viscosity method of Northrop and Hussey,[2] but with more concentrated enzyme solutions it gives rapid determinations with an error of less than 5 per cent.

This is a preliminary report.

[1] Müller, Edw., and Jochmann, G., *Munch. Med. Woch.*, 1906, xxix, 1393.

[2] Northrop, J. H., and Hussey, R. G., *J. Gen. Physiol.*, 1923, v, 353.