45. STUDIES ON DIFFUSING FACTORS

8. THE REDUCTION OF THE VISCOSITY OF VITREOUS HUMOUR PREPARATIONS BY ASCORBIC ACID AND SOME DIAZO COMPOUNDS

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THE work of McClean & Hale [1940] and of Favilli [1940] indicates that the viscosity of vitreous humour preparation is decreased not only by the diffusing factors of biological origin, but also by some compounds known to increase the area of spread of indicators when tested for diffusing activity. The two types of compounds examined have been ascorbic acid and diazo compounds.

It has been established [Madinaveitia & Quibell, 1940] that, under certain conditions, the concentration of testicular extract or snake venom required to bring about a given change of the viscosity of a preparation of vitreous humour is inversely proportional to the time required for this change to take place. The effect of the concentration of ascorbic acid on the time required to decrease by a given amount the viscosity of a vitreous humour preparation does not follow such a simple law as testicular extracts of snake venom (Fig. 1). It is therefore unlikely that the mechanism of the reaction in which the viscosity is lost is the same in the case of ascorbic acid as in those of preparations from testicle or snake venom.

The spreading properties of diazobenzene-*p*-sulphonic acid and of some proteins coupled with it have been known for some time [Claude, 1933; 1935]. As reported by Favilli [1940] diazobenzene-*p*-sulphonic acid also causes a reduction in the viscosity of mucin preparations. The same effect has now been observed with diazotized aniline. Aniline, sulphanilic acid and sodium nitrite are without effect. When diazobenzenesulphonic acid is coupled with either dimethylaniline or with phenol the resulting dyestuff has no effect on the viscosity of vitreous humour preparations.

It is difficult to picture the type of reaction which occurs between the diazo compound and the viscous material which is of a polysaccharide nature, built up of acetyl glucosamine and uronic acid residues [Palmer *et al.* 1937]. Phenylhydrazine reacting with α -aminocarbonyl compounds will form osazones [Fischer, 1893], in some cases even when the nitrogen is substituted [Jacob & Madinaveitia, 1937]. Similarly it has been found in these Laboratories by Miss M. East that phenylhydrazine reacts with *N*-acetyl glucosamine yielding glucosazone. A slow reduction of the viscosity of vitreous humour preparations is observed when phenylhydrazine acts on them. In the reacting mixture the presence of diazo compounds can be detected. Since the reaction is greatly accelerated by H_2O_2 (Fig. 2) it seems likely that small amounts of diazobenzene formed by the oxidation of phenylhydrazine are responsible for the decrease in the viscosity. Neither semicarbazide nor hydroxylamine have any effect on the viscosity of vitreous humour preparations so that the action of phenylhydrazine cannot be due merely to its property of reacting with carbonyl groups.

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Fig. 1. Effect of the concentration of ascorbic acid on the rate of decrease of the viscosity of vitreous humour preparations.

Action of phenylhydrazine and H₂O₂ on the viscosity of vitreous humour.

EXPERIMENTAL

Action of ascorbic acid. Ascorbic acid (1.7 g.) dissolved in *M* citrate buffer pH 4.7 (15 ml.) was partially neutralized with *M* NaOH (5 ml.). Dilutions of the solution in *M* citrate buffer (1 ml.) were allowed to react with a solution of a vitreous humour acetone powder (4 ml.) in *M*/5 citrate buffer having *M* NaCl in a viscosimeter in a thermostat (40°). The technique used for following the fall of viscosity was that previously described [Madinaveitia & Quibell, 1940]. The results obtained are plotted in Fig. 1.

Action of diazobenzene-p-sulphonic acid. An ice-cold suspension of the diazo compound in water (2 ml.) was mixed with chilled *M* NaOH (2 ml.) and *M* citrate buffer pH 4.7 (2 ml.) was added to the mixture. This solution (1 ml.) was further diluted with citrate buffer which was *M*/2 in NaCl. A solution of a vitreous humour preparation (see above) (5 ml.) was allowed to react with the diluted solution of the diazo compound (1 ml.) at 25°.

Action of aniline and diazobenzene. A vitreous humour solution as used in the previous experiment (5 ml.) was mixed with *M*/5 aniline hydrochloride (0.5 ml.). The viscosity remained unchanged for 20 min., when *M*/5 NaNO₂ (1 ml.) was added. The viscosity now decreased at a speed comparable with that observed with diazobenzene-*p*-sulphonic acid.

Action of phenylhydrazine. Freshly distilled phenylhydrazine (1 ml.) was dissolved in *M* citrate buffer pH 4.7 (29 ml.). A sample of this solution (1 ml.) was allowed to react with a solution of vitreous humour acetone powder (5 ml.) in buffered *M* NaCl at 25°. The viscosity slowly decreased. After 1 hr. a small amount (0.05 ml.) was removed and acidified with HCl. On addition of a solution of α -naphthylamine in HCl a pink colour developed. A control test with phenylhydrazine solution alone was negative.

A buffered solution of vitreous humour (5 ml.) was mixed with the above phenylhydrazine solution (1 ml.). After the mixture had stood for 2 hr. at 25° H₂O₂ (0.1 ml. 30 %) was added. Now the viscosity rapidly decreased (Fig. 2). The same vitreous humour solution (5 ml.) was mixed with 30 % H₂O₂ (0.5 ml.). The viscosity of the mixture did not decrease with time, but when phenylhydrazine solution (1 ml.) was added the viscosity at once started to decrease (Fig. 2).

SUMMARY

The observations of McClean & Hale [1940] and of Favilli [1940] that ascorbic acid and diazo compounds produce a gradual diminution of the viscosity of mucin preparations have been confirmed. The effect of the concentration of ascorbic acid on the time required to bring about a given decrease of the viscosity of vitreous humour preparations does not follow the same law as testicular extracts or snake venom. Phenylhydrazine has the effect of lowering the viscosity of mucins in a similar way. The action is much more marked in the presence of H₂O₂. Other carbonyl reagents are inactive.

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