

centrations of urea might interfere with proteolytic bacteria; but tests on this point must await the isolation of the organism concerned.

A detailed account of our experiments will shortly be published elsewhere.

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<sup>1</sup> Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C., *J. Amer. Dent. Assoc.*, **33**, 695 (1946).

<sup>2</sup> Kesel, R. G., O'Donnell, J. F., Wach, E. C., and Kirch, E. R., *Amer. J. Orthodontics*, **33**, 80 (1947).

<sup>3</sup> Kesel, R. G., *J. Dent. Res.*, **27**, 244 (1948).

<sup>4</sup> Conway, E. J., and O'Malley, E., *Biochem. J.*, **36**, 655 (1942).

<sup>5</sup> Friedemann, T. E., and Graesser, J. B., *J. Biol. Chem.*, **100**, 291 (1933).

<sup>6</sup> Henschel, C. J., and Lieber, L., *J. Dent. Res.*, **28**, 248 (1949).

<sup>7</sup> Kato, N., quoted by Baldwin, E., "Dynamic Aspects of Biochemistry" (Cambridge, 1947).

### Hæmolytic Complement and Ascorbic Acid

IN a recent communication, Traina<sup>1</sup> described the anti-hæmolytic effect of vitamin C in complement and saponin hæmolysis systems. Investigations conducted thirteen years ago<sup>2</sup> showed that while ascorbic acid in high concentration had an inhibitory effect on complement activity, hæmolysis by complement was enhanced by low concentrations.

The method of complement estimation employed<sup>3</sup> consisted in the estimation of the time for complete hæmolysis (the speed of hæmolysis is a function of the complement concentration) of sensitized sheep red cells incubated at 37° C. in Dreyer agglutination tubes in a hæmolytic system consisting of:

- 0.3-2 ml. 0.9 per cent sodium chloride. *x* ml. ascorbic acid solution, 5 mgm./ml. buffered to pH 7.2 (*x* varied from 0 to 0.2 ml.).
- 0.05 ml. 3 per cent sheep red cell suspension (standardized to a hæmoglobin content of 1 gm. per 100 ml.), sensitized with 5 M.H.D. amboceptor.
- 0.05 ml. fresh guinea-pig serum (ascorbic acid content ranged from 0.8-1.28 mgm. per 100 ml.).

The following results were representative:

Mgm. ascorbic acid added to system	Hæmolysis time (in sec.)	% of initial hæmolytic time
0.0	90	100
0.1	82	110
0.2	72	125
0.4	78	115
0.5	80	113
0.6	88	102
0.8	95	95
1.0	105	86

Estimation of the degree of hæmolysis by complement at varying time intervals yielded similar results. Dehydro-ascorbic acid was found to be without effect.

Further, incubation of fresh guinea-pig serum with ascorbic acid added up to 5 mgm./ml. serum under oil resulted in increased complement activity for a period up to seven days; higher concentrations produced complete inhibition within twenty-four hours.

Ecker *et al.*<sup>4</sup>, estimating the complement titre for initial and 50 per cent hæmolysis in sera of normal and scorbutic guinea pigs to which ascorbic acid had been added, arrived at very similar conclusions. A correlation was also demonstrated between the ascorbic acid content of guinea-pig sera and their

complement activity, which was optimal at a concentration of 1 mgm. ascorbic acid per 100 ml. serum.

It would therefore appear that the *in vitro* action of ascorbic acid, in amounts that are physiological, is one of enhancement of the hæmolytic effect of complement.

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<sup>1</sup> Traina, V., *Nature*, **164**, 843 (1949).

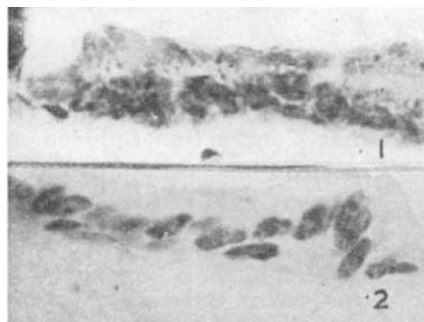
<sup>2</sup> Bernstein, R. E., Studies on Hæmolytic Complement, M.Sc. thesis, University of the Witwatersrand (1937).

<sup>3</sup> Osborn, T. W. B., "Complement or Alexin" (Oxford Univ. Press, 1937).

<sup>4</sup> Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, **34**, 19 (1938).

### Basophilia in the Rodent Prostate

DURING the course of a cytological investigation of the prostate which is being carried out by one of us (P.L.H.D.), a strong and characteristic basophilia of the epithelial cells of the adult male rat and guinea-pig prostate was noticed after formol-Zenker fixation and staining with Methyl-Green Pyronin<sup>1</sup>. This basophilia (Fig. 1) is found throughout the cytoplasm except in the 'clear' area on the luminal side of the nucleus described by Moore, Price and Gallagher<sup>2</sup>. (This 'clear' area is the region in which the Golgi element probably occurs.) The basophilic material appears to be of the nature of minute granules.



(1) Control preparation showing basal nuclei with surrounding basophilia separated from luminal basophilia by the 'clear' areas.  
(2) Similar section after incubation with ribonuclease, showing absence of basophilic staining.

Both preparations are 5- $\mu$  sections of adult male guinea-pig prostate, stained back to back with Pyronin-Methyl Green

In view of the recent interest in cytoplasmic basophilia, and its frequent association with the presence of nucleic acids<sup>3</sup> and the relation between the latter and protein synthesis<sup>4</sup>, it was decided to investigate whether this basophilia could be removed by digestion of sections with crystalline ribonuclease, according to the technique of Davidson and Waymouth<sup>5</sup>, but using McIlvaine's disodium phosphate and citric acid buffer at pH 7.

One set of sections was incubated for  $\frac{1}{2}$  hr. in the buffer alone and the other incubated for the same time in the buffered ribonuclease solution (0.04 mgm. enzyme per c.c.) at 37° C. The control and experimental sections were stained back to back in Pyronin and Methyl Green, when it was found that the basophilia had been destroyed in those sections treated with the enzyme (Fig. 2).