

pigment cells it is not essential to destroy or completely remove the neural portion of the retina. If a segment of the fully differentiated or regenerating neural retina becomes detached as an elevated fold it may survive. In the arch beneath it the retinal pigment cells immediately give rise to a secondary neural retina.

In 59 experiments segments of the eye wall containing the retinal pigment layer were im-

planted in the vitreous chamber of recipient eyes from which the lens had been removed. They also showed the development of neural retina from the pigment layer. It is therefore concluded that the regenerating neural portion of the retina in the adult urodele eye has its only source of origin from the cells of the retinal pigment layer.

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Enhancement of Hemolytic Activity of Complement by Polyethylene Glycols.* (17440)

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In the course of an investigation designed to test the ability of various substances to prevent the deterioration of complement, it was found that certain of the polyethylene glycols greatly enhance the hemolytic activity of complement. The polyethylene glycols, a series of polymers with the general formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_x\text{CH}_2\text{OH}$, are soluble in water and in many of the aromatic hydrocarbons. The compounds with average molecular weight up to 700 are liquids; those with average molecular weight above 1000 are wax-like solids, sold under the trade name "Carbowax".[†] This paper constitutes a preliminary report on the effect of the polyethylene glycol carbowax-4000 on the hemolytic activity of complement, as determined by complement titrations in the presence and absence of this compound.

Procedure. Complement titrations were carried out according to the 50% hemolysis end-point method developed by Mayer *et al.*,¹ and Kent *et al.*² The reaction mixtures, 20

ml in volume, had the following composition: 1) 1 part complement, the dilution so adjusted as to yield final dilution values ranging from 1:3300 to 1:950; 2) 1 part standardized suspension of sensitized erythrocytes; 3) either 2 parts of buffered saline,³ or 2 parts of an 8% solution of carbowax-4000 in buffered saline. The sensitized erythrocytes were well mixed with either buffered saline or carbowax-4000 solution, and then the complement added dropwise with constant agitation. All components were kept in ice-water until time of incubation. The reaction mixtures thus prepared were incubated at 37°C for 60 minutes, with frequent agitation. After centrifugation of the mixtures, readings of per cent hemolysis in the supernates were made at 5500 Å in a Coleman spectrophotometer, an optical density of .500 representing complete hemolysis. Titrations were carried out in duplicate. Controls were set up of 1) sensitized erythrocytes and carbowax-4000, 2) unsensitized erythrocytes and carbowax-4000, and 3) complement, unsensitized erythrocytes and carbowax-4000.

Results and discussion. None of the control tests showed any hemolysis. The results of the titrations are shown in Fig. 1, in which

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† Carbon and Carbide Chemicals Corporation, New York.

¹ Mayer, M. M., Eaton, B. B., and Heidelberger, M., *J. Immunol.*, 1946, **53**, 31.

² Kent, J. F., Bukantz, S. C., and Rein, C. R., *J. Immunol.*, 1946, **53**, 37.

³ Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberger, M., *J. Exp. Med.*, 1946, **84**, 535.

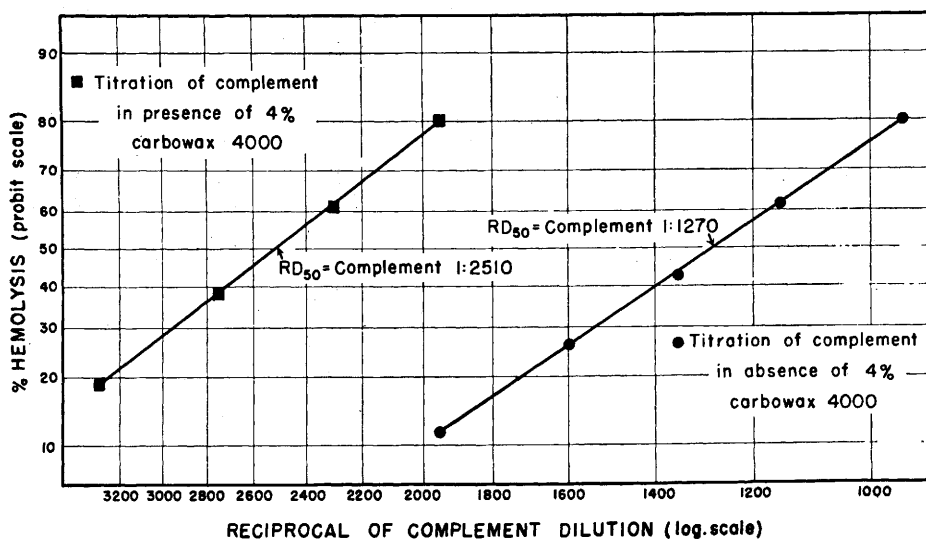


FIG. 1.

Titration of complement in the presence and absence of 4% carbowax-4000. The complement dilutions referred to on the abscissa represent final dilutions, not the dilutions added to the reaction mixture.

the probit of the percent hemolysis is expressed as a function of the log of the dilution of complement, in the presence and absence of 4% carbowax-4000. The dilutions of complement, with or without 4% carbowax-4000, calculated to yield a required per cent hemolysis, as determined from the curves of Fig. 1, are set forth in Table I. A striking difference in the ability of a given dilution of complement to lyse sensitized erythrocytes in the presence or absence of carbowax-4000 is evident. Within the range of values for per cent hemolysis which can be used with assurance in this technic, the two curves over-

lap only at a complement dilution of 1:1950. At this point, complement alone gives 11.5% hemolysis; complement and 4% carbowax-4000 together, give 79.9% hemolysis, an increase of nearly 700%. The RD_{50} unit (that amount of complement required to lyse 50% of the sensitized erythrocytes) in the absence and presence of 4% carbowax-4000 is 1:1270 and 1:2510 respectively. The fact that only approximately half the usual amount of complement is necessary for 50% hemolysis, with 4% carbowax-4000 present, may be of practical importance for complement-fixation tests, a possibility which is being investigated. The other polyethylene glycols are being tested for similar activity, and a detailed study of the general reaction described in this paper is under way. At present no explanation of the mechanism of the enhanced hemolytic activity of complement in the presence of polyethylene glycols can be offered, though it is perhaps worthwhile to speculate on a possible relation between the fat-solvent nature of these compounds and the lipoidal components of the erythrocytic membrane.

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TABLE I.

Comparison of Reciprocals of Dilutions of Complement Calculated to Give a Required Per Cent Hemolysis in Presence and Absence of 4% Carbowax-4000.

% hemolysis	Reciprocal of dilution of complement	
	Without carbowax	With carbowax
10	2000	3750
20	1710	3260
30	1525	2960
40	1390	2720
50	1270	2510
60	1165	2330
70	1065	2140
80	950	1950