availability. Bunnell[8] has shown that the availability of α-tocopherol in lucerne to be only about 30 per cent.

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[1] Eggitt, P. W. R., and Ward, L. D., *J. Sci. Food Agric.*, **6**, 329 (1955).
[2] Green ,J., *et al.*, *J. Sci. Food Agric.*, **6**, 274 (1955).
[3] Dju, M. Y., Quaife, M. L., and Harris, P. L., *Amer. J. Physiol.*, **160**, 259 (1950).
[4] Swick, R. W., and Baumann, C. A., *Anal. Chem.*, **24**, 758 (1952).
[5] Brown, F., *Biochem. J.*, **51**, 237 (1952).
[6] Ames, S. R., *Poultry Sci.*, **35**, 145 (1956).
[7] Ames, S. R., University of Maryland, Proc. Nut. Conf., March 1957.
[8] Bunnell, R. H., *Poultry Sci.*, **36**, 413 (1957).

Normal Components of Guinea Pig Serum which may cause Antigens to show an Apparent Anti-complementary Effect

Normal guinea pig serum is the most common source of complement for the complement-fixation test, and may be used fresh or, more commonly, preserved either by freeze-drying or by Richardson's method[1]. Such 'complement' is used in the presence of a great variety of antigens, and the possibility of a reaction between components of this serum other than complement and the antigen is usually either ignored or allowed for as an anti-complementary effect of the antigen acting alone.

To investigate such reactions, normal guinea pig serum was obtained by cardiac puncture from thirty animals, pooled, and preserved by Richardson's method. Following titration this serum was diluted with barbitone buffer solution, so as to contain 12·5 *MHD*50 units (minimal hæmolytic dose giving 50 per cent hæmolysis) per unit volume of 0·5 ml.

1 mgm. 'Zymosan' (Fleischmann Laboratories, Stamford, Conn., U.S.A.)[2] was added to each of two aliquots of 2 ml. of this standardized serum, and these, together with two untreated aliquots, were then incubated at 17° C. for 30 min. One of the aliquots that had been treated with 'Zymosan' was then centrifuged at 3,000 r.p.m. for 3 min. at 0° C. The supernatant fluid was decanted off and recentrifuged to ensure that all traces of the 'Zymosan' had been removed. To the final supernatant fluid 0·5 ml. barbitone buffer solution and a further 1 mgm. 'Zymosan' were added. 0·5 ml. of a 1 : 15 solution of Wassermann antigen (Burroughs Wellcome, alcoholic extract of sheep heart with cholesterol) in barbitone buffer solution was added to one of the aliquots that had not previously been treated with 'Zymosan', and also 0·5 ml. of the barbitone buffer solution to the remaining two aliquots. All four aliquots were then incubated at 37° C. for 15 min.

The amount of complement remaining after the various treatments was determined by titration. The technique used for this titration involved a serial dilution of the four solutions containing the residual complement, with a log dilution factor of 0·125. When 0·5 ml. amounts of each dilution were incubated at 37° C. for 60 min. with 2 ml. of a 0·5 per cent suspension of sheep red cells optimally sensitized with anti-sheep red cell hæmolysin (Burroughs Wellcome), several tubes were obtained within the zone of partial hæmolysis. The degree of hæmolysis in each tube of the titration was estimated by means of an EEL portable colorimeter, and the titre calculated graphically[3].

Pillemer and his co-workers have demonstrated that normal guinea pig serum contains 'Properdin'[4], and this has been shown to combine with components of preparations from a very great variety of plant and animal sources, for example, yeasts ('Zymosan'), bacteria, inulin, dextrans, gastric mucin, viruses, protozoa, trematodes, etc.[5–8]. When such preparations are used as antigens, the complexes so formed are capable of fixing complement by specifically inactivating the third component, at temperatures above 18° C. Below 18° C., however, although the complex is formed, it does not inactivate complement[4]. A factor which will fix complement in the presence of the Wassermann antigen is known to occur in the normal serum of many adult animals, although guinea pig serum has been reputed not to possess this property[9].

Table 1. The Percentage of the Initial Complement remaining following the Various Treatments

Treatment of guinea pig serum		Residual complement as percentage of initial complement (per cent)
Treated with 'Zymosan'	'Zymosan-Properdin' complex removed	46
	'Zymosan-Properdin' complex remains	23
Not treated with 'Zymosan'	Wassermann antigen added	21
	Wassermann antigen not added	63

The results presented in Table 1 show that the amount of complement fixed by the 'Properdin-Zymosan' complex, or by the Wassermann system was markedly greater than the loss of complement activity occurring during the process of incubation, or due to the presence of 'Zymosan' after most of the 'Properdin' has been removed. In the presence of an excess of antigen, this activity will vary with the amount of guinea pig serum present, independently of its complement content, unlike a true anti-complementary effect. Any allowance to be made for this effect, in addition to that made for any anti-complementary action of the antigen, must therefore be determined using the same amount of guinea pig serum as is to be used to provide the complement for the fixation tests in question.

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[1] Richardson, G. M., *Lancet*, ii, 696 (1941).
[2] Pillemer, L., and Ecker, E. E., *J. Biol. Chem.*, **137**, 139 (1941).
[3] Mayer, M. M., Eaton, B. B., and Heidelberger, M., *J. Immunol.*, **53**, 31 (1946).
[4] Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., and Wardlaw, A. C., *Science*, **120**, 279 (1954).
[5] Pillemer, L., Schoenberg, M. D., Blum, L., and Wurz, L., *Science*, **122**, 545 (1955).
[6] McNall, E. G., *Proc. Soc. Exp. Biol., N.Y.*, **94**, 399 (1957).
[7] Gronroos, P., *Ann. Med. Exp. Fenn.* (Helsinki), **33**, 310 (1955).
[8] Kagan, I. G., Rice Inst. Pamph., **45**, 157 (1958) (quoting Iralu, V., unpublished).
[9] Mackie, T. J., and Watson, H. F., *J. Hyg.* (Camb.), **25**, 176 (1926).