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The Interrelation of Corresponding Complement Components of Man and Guinea Pig.*

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A question of considerable interest is whether or not the corresponding components of human and guinea-pig complements are interchangeable or "mutually substitutive." The identity of corresponding components of the two species has been an assumption in at least two important studies on complement by Hegedüs and Greiner[1] and Heidelberger and Mayer,[2] but Ecker, Pillemer, and Seifter,[3] reported that: "(1) Human C'1 can replace guinea-pig C'1, but guinea-pig C'1 cannot effectively replace human C'1. (2) Human C'2 cannot effectively replace guinea-pig C'2, but guinea-pig C'2 can replace human C'2. (3) Human C'3 can replace guinea-pig C'3, and guinea-pig C'3 can replace human C'3. (4) Human C'4 can replace guinea-pig C'4, but guinea-pig C'4 cannot replace human C'4."

In these conclusions, drawn from experiments with specifically inactivated complements of both species, the word "effectively" was used as a modifier, because the reasons for the failure of replacement were not understood, and it was thought that anti-complementary effects of the testing fractions (specifically inactivated complements) might be implicated. In a dissertation from this laboratory presented in May, 1944[4] it was pointed out that, "in light of data accumulated in the present research of the effective concentrations of the components of human serum, the experiments on which the above conclusions were based may be re-examined, more reliable conclusions drawn, and further experiments suggested." From the study of the effective concentrations of the individual complement components, it was concluded that the amounts of certain components used in the original experiments were in some cases too small, particularly in those instances in which effective substitution of the corresponding components was not achieved. Accordingly, another series of experiments, using larger amounts of the indicated components, was conducted. In addition, the availability of more highly purified complement fractions made it possible to attack the problem more directly than had been true previously. A series of experiments was then conducted by use of single components of the complement of one species with the specifically inactivated complements of the other species.

Methods. Specifically inactivated guinea-pig complements were prepared by the methods summarized in the review by Ecker and Pillemer.[5] Specifically inactivated human complements were prepared, as described by Ecker, Pillemer, and Seifter.[3] Purified fractions of guinea-pig complement were prepared by ammonium sulfate fractionation, according to the methods of Pillemer, Ecker, Oncley, and Cohn[6]; and of those of human complement by the methods summarized by Ecker, Seifter, and Dozois.[7]

Complement was titrated by the method of complete hemolysis,[3] a unit of complement (or the unit equivalent volume of a specifically inactivated complement) being defined as the

* Aided by a grant from the Commonwealth Fund.

1 Hegedüs, A., and Greiner, H., *Z. Immunitätsforsch.*, 1938, **92**, 1.

2 Heidelberger, M., and Mayer, M., *J. Exp. Med.*, 1942, **75**, 285.

3 Ecker, E. E., Pillemer, L., and Seifter, S., *J. Immunol.*, 1943, **47**, 181.

4 Seifter, S., Dissertation, Western Reserve University, 1944.

5 Ecker, E. E., and Pillemer, L., *Ann. N. Y. Acad. Sc.*, 1942, **43**, 63.

6 Pillemer, L., Ecker, E. E., Oncley, J. L., and Cohn, E. J., *J. Exp. Med.*, 1941, **74**, 297.

7 Ecker, E. E., Seifter, S., and Dozois, T. F., *J. Lab. and Clin. Med.*, 1945, **30**, 39.

TABLE I.
The Mutual Substitution of Corresponding Components of Human and Guinea-pig Complements as Demonstrated with Specifically Inactivated Complements.

Guinea-pig				Human				%
C'1	C'2	C'3	C'4	C'1	C'2	C'3	C'4	hemolysis
	X	X	X	X	X		X	100
X		X	X		X	X	X	55*
X	X		X	X		X	X	95
X	X	X		X		X	X	80
X	X		X		X	X	X	75*
X	X		X	X		X	X	95
	X	X	X	X	X		X	100
	X	X	X	X	X	X		90†

* Six units of specifically inactivated human complement used.
† Two units of specifically inactivated guinea-pig complement used.
X indicates the presence of the component designated in the column heading.

TABLE II.
The Substitution of Purified Fractions of Human and Guinea-pig Complements.

Guinea-pig				Human				%
C'1	C'2	C'3	C'4	C'1	C'2	C'3	C'4	hemolysis
	X	X	X	X				95
X		X	X		X			55
X	X	X					X	100
X					X	X	X	55
	X	X		X		X	X	100
	X	X		X	X		X	80

minimal amount of normal serum required to hemolyze completely the test dose of sensitized sheep red blood cells.

The Interrelation of Specifically Inactivated Complements of Man and Guinea-pig. Table I shows the degree of hemolysis of the standard dose of sensitized sheep red cells obtained by the action of various mixtures of specifically inactivated complements of man and guinea-pig. Unless otherwise noted, single unit amounts, as defined above, were used. The table is so constructed that the results may be conceived of as guinea-pig components substituting in a human complement system or *vice versa.* In each case, if sufficient amounts of the specifically inactivated complements were used, a significant degree of hemolysis was obtained, demonstrating the capacity of the corresponding components of one system to substitute effectively in the other system. Results not shown in the table again demonstrated that only corresponding components can replace one another; that is, a mixture of complements, similarly inactivated, of the two species produced no hemolysis of the sheep cells.

The Substitution of Purified Complement Fractions. Table II shows the degree of hemolysis obtained with the use of various mixtures of the specifically inactivated complements of man or guinea-pig and the available purified fractions of guinea-pig or human complement. The guinea-pig fraction containing C'2 also had some C'3 activity, and so was used to test the capacity of these components for completing human systems lacking C'2 and C'3 respectively. Again the results show that corresponding components of the two systems are "mutually substitutive."

Summary and Conclusions. Under the proper conditions of concentration, all of the corresponding complement components of man and guinea-pig are mutually substitutive. This represents a revision of the view previously expressed[3] that only certain substitutions are effective. Nevertheless, it is to be borne in mind that in those cases in which specifically inactivated complements of the two species are used together, the full effect of component substitution may be inhibited or concealed. Such an aberration may be due to (a) the increased concentration of anti-complementary substances in these fractions, particularly in the globulin concentrates, or (b) the disarrange-

ment of the complement complex in the course of specific inactivation by the recommended procedures.

It is pertinent to report that the corresponding components of rabbit and human complements have also been found to be mutually substitutive.[8]

Since submitting this article we have become acquainted with an excellent paper by O. G. Bier, G. Leyton, M. M. Mayer, and M. Heidelberger which appears in the May, 1945, issue of the *J. Exp. Med.* These authors demonstrate that corresponding components of guinea pig and human complements are mutually substitutive; and emphasize that testing reagents for complement components must contain an excess of the desired components and must be used in dilutions which ensure absence of anti-complementary effects. Further, though it came to our attention too late for incorporation in the present paper, the suggestion of Bier *et al.* that C′ component titers be expressed solely in units is constructive, and will be observed in our subsequent publications.

[8] Dozois, T. F., Seifter, S., and Ecker, E. E., in preparation.

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Rapid and Submerged Growth of Mycobacteria in Liquid Media.

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Attempts to obtain rapid and diffuse growth of virulent tubercle bacilli have led us to recognize that certain complex lipids exert a remarkable stimulatory effect on the multiplication of mycobacteria. Particularly striking results have been obtained with the following materials (a) phosphatide fractions prepared from egg yolk, cattle brain, human erythrocytes and soya bean.* (b) synthetic non-ionic surface active agents consisting of esters of long chain fatty acids and of polyhydric alcohols.†

The growth promoting effect of these substances has been tested with 5 strains of saprophytic mycobacteria, 5 virulent avian strains, 1 bovine strain (Ravenel) and 2 human strains (H37 RV and Jamaica No. 22). The results indicate that the different groups of mycobacteria (saprophytic, avian, bovine, and human) differ greatly in their optimal requirements with reference to the lipids mentioned above; on the other hand, within one given group, the different strains tested have exhibited a striking similarity of behavior. The avian strains are only little affected by the phosphatide preparations but are greatly stimulated by the fatty acid esters; on the contrary the bovine and human strains respond remarkably well to the different phosphatides but are inhibited by minute concentrations of the non-ionic surface active agents. The following table illustrates the composition of satisfactory media prepared by adding to Long's medium different concentrations of soya bean phosphatide and of a polyoxyalkylene derivative of sorbitan monostearate (Tween 60).‡

Mycobacteria can be made to grow in the lipid media without the necessity of floating

* The brain and red cell phosphatides were prepared by Dr. Jordi Folch-Pi. The soya bean phosphatide was obtained through the courtesy of Dr. A. Scharf of Associated Concentrates, Inc., Atlanta, Georgia.

† A variety of these synthetic non-ionic surface active agents are produced by the Atlas Powder Company, Wilmington, Delaware, and by the Glyco Products Co., Inc., Brooklyn, N. Y. Although the present report deals only with the product marketed under the name of Tween 60 by the former company, similar results have been obtained with a number of other long chain fatty acid esters of polyhydric alcohols.

‡ Tween 60 is directly dispersible in water. The soya bean phosphatide was dissolved in ether and dispersed from ether solution into water. Both these products can be autoclaved with Long's medium.