them with each other than by comparing them with epinephrine as a common standard. The results of both methods indicate that the per cent loss of intestine-inhibitory potency of phenylephrine which results from demethylation is of the same order as that observed when the methyl group is removed from epinephrine(5). Thus removal of the methyl group has the same effect on intestine-inhibitory potency whether the para OH group is present as in the case of epinephrine or absent as in the case of phenylephrine, and the amount of loss of potency is slight relative to the effect of removing any of the hydroxyl groups.

Summary. (1) The effects of dl-phenylephrine and dl-norphenylephrine on motility of isolated strips of rabbit intestine were studied. Comparisons of their actions were made with the action of l-epinephrine and with each other to obtain relative potency of these phenylethylamine derivatives as inhibitors of intestinal smooth muscle. (2) The potency of dl-norphenylephrine as an inhiibtor of isolated rabbit intestine is approximately one-half that of dl-phenylephrine.

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Immune Hemolysis: Determination of Values for Optimal and Maximal Sensitization. Comparison in Several Hemolytic Systems. (24779)

HAROLD S. GOODMAN (Introduced by S. G. A. Alivisatos)
Mount Sinai Medical Research Fn., Chicago, Ill.

The role of Complement (C′) in the mechanism of immune hemolysis has received a great deal of study. Yet, relatively little attention has been devoted to the other participant of immune hemolysis, namely the sensitized cell. Recent studies by Talmage and co-workers(1) have afforded some insight as to the role played by the sensitizing antibody in immune hemolysis. We observed(2) striking differences in amount of C′ required to produce comparable lysis in various hemolytic systems, sensitizing the cells with optimal amounts of hemolysins and using the identical C′. Further, the number of hemolytic units required for optimal sensitization also differed from one system to another. These findings were characteristic of the particular system and were apparently reflections of differences in immunological properties of the sensitized cell. It therefore seemed feasible to use such information in characterization of immune hemolytic systems. The practical aspects of the concept of optimal sensitization in immune hemolysis were presented by Osler, Strauss and Mayer(3). Determination of amount of hemolysin required for optimal sensitization was used for selecting suitable hemolysins and determining amount of hemolysin which should be used for sensitization. By refining the original method it was possible to obtain a more exact determination of this value. It was found that amount of antibody required for optimal sensitization did not necessarily represent maximal amount of antibody capable of combining with the cells. Therefore, it was also possible to obtain a value for maximal sensitization, based upon hemolytic absorptive capacity of the cell. In the present communication we determined values for optimal and maximal sensitization in each of 4 hemolytic systems and have shown the relationship of these values to C′ activity. *Materials and methods* were essentially the same as previously described(2). Certain modifications and additional procedures are given below: *Complement.* Lyophilized guinea pig C′ (Sharpe and Dohme) was reconstituted and absorbed once with washed and packed hu-

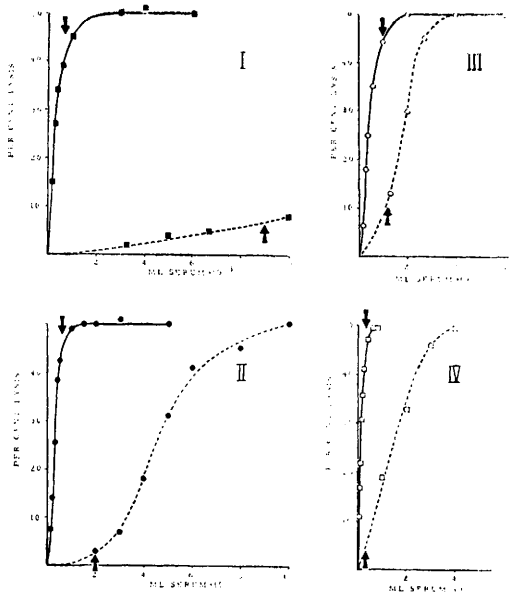


FIG. 1. Determination of amount of antiserum required for optimal and maximal sensitization. Solid line = optimal sensitization and upper arrow indicates point of optimal sensitization. Broken line = maximal sensitization and lower arrow indicates point of maximal sensitization. Hemolytic systems tested: I. Rabbit anti-sheep and sheep cells = ■. II. Human anti-A and sheep cells = ●. III. Human anti-A and A cells = ○. IV. Rabbit anti-sheep and A cells = □.

man A erythrocytes (20% of the original volume at 0°C). The C′ was stored at −20°C in 1 ml amounts. Sheep cells were preserved with ACD solution(4) and used within 2 weeks. *Determination of amount of antiserum required for optimal sensitization.* A preliminary determination was made according to the method of Osler *et al.*(3) using serial dilution of hemolysin to sensitize a constant amount of erythrocytes in the presence of 1 $C'H_{50}$ unit of C′. Based upon the preliminary test, concentrations of serum were selected from linear portions of the curve (Fig. 1) and the determination was repeated. These values, from the 2 portions of the curve, when plotted on double-log paper, gave a value related to the point of optimal sensitization. *Determination of amount of antiserum required for maximal sensitization.* The methodology was quite similar to that used in above determination; however, our purpose was to determine amount of hemolytic activity capable of being absorbed by a standard cell suspension. Five-tenth ml amounts of the appropriate cell suspension, *e.g.*, one-half the volume of concentration of cells used in the hemolytic system, were sensitized with varying concentrations of antiserum and made up to 3 ml with saline (veronal buffered, pH 7.4). Sensitization was carried out at room temperature for 30 minutes with frequent mixing. The tubes were centrifuged, decanted and the supernatant tested for hemolytic activity in a system with appropriate cells and an amount of C′ equivalent to 1 $C'H_{50}$ unit for the particular system tested. The value of the appropriate function for the per cent hemolysis $\left(\frac{Y}{1-Y}\right)$ in each tube was plotted against the log of corresponding amount of serum on semi-log paper, and the value for maximal sensitization was determined from the point where the linear portion of this curve crosses the Y axis. Values were corrected for dilution (÷ 3) and cell concentration (× 2). *Complement titration.* The C′ titer was determined in the presence of optimal amount of hemolysin. Titrations were carried out simultaneously and with identical C′ for all 4 systems. *Hemolysin titration.* In each system 2 $C'H_{50}$ units of C′ were used in titration of hemolysin. Because optimal amount of C′ cannot be added as in the case of hemolysin, the hemolysin titer represents a value which is relative and extremely sensitive (5) to concentration of C′. For this reason the number of $C'H_{50}$ units added was critical.

Results. Four hemolytic systems which differ markedly in amount of C′ required for hemolysis were compared. These systems were: 1) rabbit anti-sheep and sheep cells, 2) human anti-A and sheep cells, 3) human anti-A and A cells and 4) rabbit anti-sheep and A cells. Table I gives the summary of data obtained for each of these systems. Calculations for derived values are indicated in the Table. Figs. 1-3 present these data graphically.

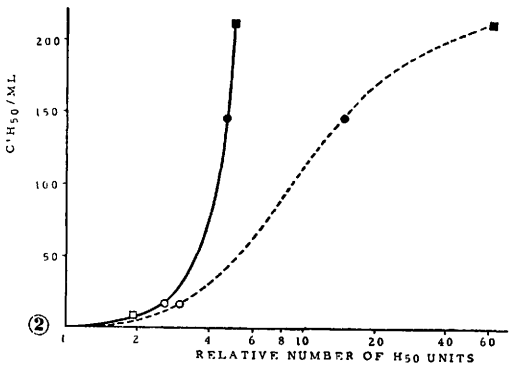
Data in Fig. 1 were used to obtain values for optimal and maximal sensitization. Arrows indicate values for optimal and maximal sensitization, determined as described in the methods, and are placed in curves so that amount of serum required for each may be visually compared. Differences in amount of

TABLE I. Titration and Derived Values Obtained for Each of the 4 Hemolytic Systems.

	$C'H_{50}$/ml*	H_{50}/ml†	Opt. sens., doses/ml	Max. sens., doses/ml	No. H_{50} units required for sensitization Opt.	Max.	Ratio: Opt./ml Max./ml
Sheep hemolysin and sheep cells	210	6,750	1,350	111	5.0	61	12.2
Human anti-A and sheep cells	145	72	15.4	5.0	4.7	14.4	3.1
Human anti-A and A cells	16	26	10.0	8.6	2.6	3.0	1.2
Sheep hemolysin and A cells	8	56	29.4	29.4	1.9	1.9	1.0
Calculations	A	B	C	D	B/C	B/D	C/D

* $C'H_{50}$ titers determined with optimally sensitized cells.
† H_{50} " " in presence of 2 $C'H_{50}$ units of C'.

serum required for optimal and maximal sensitization in each system are apparent by comparing horizontal distance between 2 arrows in each of the individual systems.



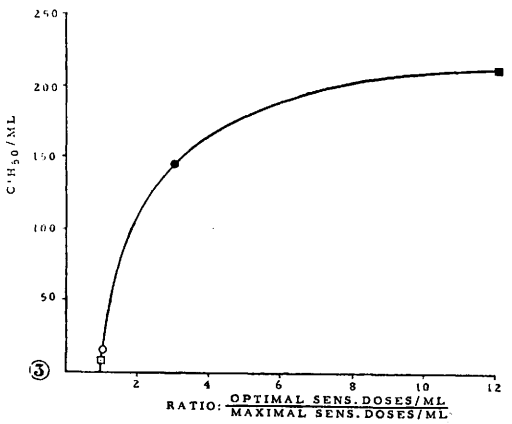


FIG. 2. Relationship of number of H_{50} units required for optimal and maximal sensitization to C' activity. Symbols same as in Fig. 1.

FIG. 3. Relationship of optimal/maximal sensitization to C' activity. Symbols same as in Fig. 1.

In Fig. 2 amounts of serum required for optimal and maximal sensitization in each system are expressed in terms of corresponding amount of hemolytic activity (number of H_{50} units) and correlated to C' activity (number of $C'H_{50}$ units/ml). When optimal and maximal sensitization are expressed in this fashion a definite relationship exists between C' activity and number of hemolytic units required for optimal sensitization, and similarly between C' activity and number of hemolytic units required for maximal sensitization.

A further relationship between optimal and maximal sensitization and C' activity is expressed in Fig. 3. Amounts of serum required for maximal and optimal sensitization in each system are given in terms of a ratio, which is an approximate expression of degree of antibody saturation required for optimal sensitization. When amount of antibody required for optimal sensitization approaches amount of antibody required for maximal sensitization, there is a corresponding decrease in C' activity. When relatively little antibody is required for optimal sensitization, as compared to amount required for maximal sensitization, an upper limit is approached beyond which an increase in this ratio has a progressively decreasing effect on C' activity.

Discussion. The reciprocal relationship of C' and hemolysin, as illustrated by Eagle(6), is of particular importance in considering the mechanism of immune hemolysis. Although, as suggested by Talmage(1), a bimolecular antibody-complex is required to initiate he-

molysis, the greater the number of such complexes/cell the greater the opportunity will be for C′ to combine with such an antibody complex. Consequently, if many complexes are present/cell, less C′ will be required to produce hemolysis. Therefore, it follows that concentration of C′ required for lysis in a particular hemolytic system is a measure of efficiency of sensitization of the cell.

Each of the hemolytic systems in which we have compared C′ activity to amounts of serum required for optimal and maximal sensitization were subject to accumulative effect of various factors which influence sensitization of the cell. The fact that a correlation exists between amount of C′ required for lysis and relative amounts of antibody required for both optimal and maximal sensitization, when expressed in hemolytic activity, indicates that certain general basic factors are involved in sensitization of the cell.

In considering possible factors which influence sensitization of the cell, those which determine the number of antigen-antibody reactions/cell would be of basic consideration. The number of antigenic sites on the cell would be a fundamental limiting factor, which determines the opportunity of antibody to combine with the cell. Specificity of the antibody would effect the opportunity for antigen-antibody reactions to occur, by involving antigenic sites of different specificities. In addition, antibodies which differ in their hemolytic efficiency, but with the same specificity(7), would effect sensitization due to their competition for the same antigenic sites.

Summary. Methods are given for determination of amount of serum required for optimal and maximal sensitization of a hemolytic system. These values were determined for each of 4 hemolytic systems which differ in amounts of C′ required for lysis. When amounts of serum required for optimal and maximal sensitization in each system were expressed in hemolytic units, a correlation was found between these values and C′ activity. This correlation indicated that certain general basic factors are involved in sensitization of the cell by antibody. Possible factors and their role in cell sensitization are discussed.

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Enzymatic Digestion of Rabbit Globulin.* (24780)

FRANK WHITEHOUSE, JR. AND GWEN ULRICH (Introduced by W. J. Nungester)
Dept. of Bacteriology, University of Michigan Medical School, Ann Arbor

Antibodies can be enzymatically digested under favorable conditions so that antibody activity is maintained even though molecular weight is greatly reduced. Much work has been done with digestion of horse globulins but relatively little with rabbit globulins. An abbreviated summary of enzymatic digestion of rabbit antibodies is as follows. Pepsin or papain destroyed antiurease activity while trypsin did not(1). All typhoid "O" and most "H" agglutinins were destroyed by trypsin(2), although it was later found that "H" agglutinins became increasingly resistant to digestion as immunization progressed(3). Trypsin digestion of agglutinins, heated either alone or in presence of albumin, resulted in release of agglutinating antibodies postulated to be reduced in size(4). Following digestion

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