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INDIRECT QUANTITATIVE COMPLEMENT CONSUMPTION ASSAY

Introduction

ONE of the major advances in the area of complement fixation has been the development of improved complement fixation procedures for the detection and estimation of antigen and antibody. The main stream of contribution has been based on 50% hemolytic unit of complement ($C'H_{50}$), which has been defined as the reciprocal of the volume of antiserum which fixes 50 out of 100 $C'H_{50}$ employed in the optimally reactive dilution of antigen[1]. However, the unit thus defined has relevance with reference to the test system used and has not been conducive to be considered on absolute terms. In addition, one or both of the antigen or antiserum are often anticomplementary and give rise to non-specific complement fixation. The above definition does not clearly exclude these events of complement consumption. In our work, we have established a test system which gives rise to quantitative complement fixation analysis and this is conducive to a definition of complement fixation unit, which, though not absolute, is qcantitative. In wost immunological systems, the antigen is often anticomplementary; the antibody is sometimes anticomplementary and at times both antigen and the antibody have anticomplementary activity. In such cases, the use of our system makes feasible quantitation of complement consumption due to antigen alone, due to antibody and due to antigen plus antibody and thereby we can determine the specific consumption due to antigen antibody interaction.

Materials and Method

Diluent : Isotonic Veronal buffer with 0·0005 M $MgCl_2$, 0·00015 M $Cacl_2$ and 0·1% Bovine Serum Albumin.

Complement (C) : Lyophilized guinea pig serum, obtained from Cappel Laboratories. The lyophilized complement was reconstituted to the original volume using the diluent purchased from Cappel Laboratories. Aliquot volumes were stored at –20° C.

Hemolytic antibody (*A*) : Rabbit antisheep erythrocyte serum was purchased from Colorado Serum Company Laboratories.

Sheep Erythrocytes (E) : Sheep Red Blood Cells (SRBC) preserved in Alsevers solution was obtained from Colorado Serum Company Laboratories.

Well washed SRBC are suspended 2% in diluent. Hemolysin is diluted 1 : 32 to 1 : 8192 by serial dilution. To each tube is added an equal amount of Sheep Red Blood Cells to obtain a 1% suspension. After incubation for 30 minutes at room temperature, one agglution unit of sheep cell hemolysin titration is determined (Table I).

Quantitative complement fixation analyses were performed as follows. Four sets of serial dilution of complement were incubated overnight at 4° C: one set with known amount of antigen in all the tubes; the second set with appropriate dilutions of antibody; the third set with both antigen and antibody in the same dilutions as used in the first two sets; and the fourth set with diluent as control. The next day, to all the tubes were dispensed a fixed amount of sensitized (SSRBC) to one agglutinating unit and reaction mixtures were incubated for 30 minutes at 37° C. At

TABLE I

Determination of units of agglutination of 1% SRBC titrated against anti-SRBC

	Dilution of anti-SRBC						
	1:32	1:64	1:228	1:256	1:512	1:1024	1:2048
SRBC + anti-SRBC	+	+	+	+	⊕	–	–
Units of Agglutination	16	8	4	2	1	½	¼

⊕ indicated 1 agglutinating unit.

TABLE II

Determination of units of complement

Reaction Mixture	Dilution of Guinea-pig Complement							
	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
C′ + Ag + SSRBC	+	+	+	+	+	⊕	–	–
C′ + Ab + SSRBC	+	+	+	+	+	+	⊕	–
C′ + Ag + Ab + SSRBC	+	+	⊕	–	–	–	–	–
C′ + diluent + SSRBC	+	+	+	+	+	+	⊕	–
Units of Complement	64	32	16	8	4	2	1	½

⊕ indicated positive for 50% hemolysis.

the end of the incubation period hemolytic activity giving rise to at least 50% lysis of SSRBC was determined.

Quantitation

We define 1 unit of complement as the least amount of complement required to lyse 50% of 1% SRBC sensitized to 1 agglutinating unit. As shown in Table II, this unit is determined from the fourth series of tubes which have C′ as control by finding 50% hemolytic activity. Similarly, we can determine the end points of the other three series of tubes as illustrated in Table II.

The specific complement fixation due to antigen antibody reaction $= C_{tot} - C'_{Ag} - C'_{Ab}$ where, C'_{tot}, C'_{Ag} and C'_{Ab} are units of complement consumed in the presence of antigen plus antibody, antigen alone, and antibody alone, respectively. As per Table II, for example, specific complement consumption due to antigen-antibody interaction = (16–1) – (2–) – (1–1) = 14. For the sake of convenience serial dilutions of complement have been considered. Instead, increasing dilutions of complement may be used to determine intermediate units of complement.

Discussion

The definition of one unit of complement helps in quantitating the amount of complement consumed by specific antigen antibody interaction. This aids in several assays where the antigen is anticomplementary, particularly in the case of tumor antigens. In defining the unit of complement, we have taken in account one agglutinating unit of SRBC which also quantities of amount of antibody coating the indicator SRBC. This ensures reproducibility of the amount of antibody on SRBC for all tests and hence assures uniformity in indicator cell sensitivity and standardization of Sheep Red Blood Cell lysis.

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POST HARVEST INDUCED CHANGES OF DIOSGENIN IN COSTUS SPECIOSUS SIMS

COSTUS speciosus Sims (Family—Zingiberaceae) is considered to be the potent source of diosgenin[1,2]. Sarin[3] *et al.* found that diosgenin content of this plant is lowest in the dormant rhizomes (0·63%) and highest at early flowering stage (2·61%). The post-harvest increase of 5 to 15% in the steroidal sapogenin from fresh tuber of Dioscorea belizensis on its incubation with water under defined condition have been reported by Blunden and Hardman[4]. Incubation of the harvested tuber of Dioscorea deltoidea and whole seeds of Trogonella foenumgraecum, wih indole 3 acetic acid and gibberelic acid increased the sapogenin yield upto 35%[5].