Complement Levels in Normal Anaesthetised South African Pigs

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SUMMARY

This article presents a method for determining total complement in pigs. The importance of using a standard reference serum is emphasised and a comparison between the complement titre of heparinised and clotted blood is presented. The method evaluated demonstrates good reproducibility and is now in routine use in our laboratory.

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The measurement of total complement in porcine serum was studied during the course of a programme of experimental pig liver transplantation and homologous isolated perfusion of the pig liver. Although many other animals have been studied,[1] no method has heretofore been described for porcine complement. In addition, levels of serum complement had not previously been recorded in experimental porcine liver transplantation or homologous isolated porcine liver perfusion. Hence, baseline normal values were established for this laboratory and the technique of measurement of total porcine serum complement by haemolytic assay was developed.

MATERIALS AND METHODS

Pigs weighing between 20 and 40 kg (2 - 4 months of age) obtained from local farms were of pure Landrace or Landrace X Large White stock.

After 24 hours' starvation, anaesthesia was induced either with halothane, nitrous oxide and oxygen or by intravenous pentothal, nitrous oxide and oxygen, and transplantation or hepatectomy was performed. The subsequent experiments have been described elsewhere.[2,3]

Collection of Serum

Blood samples were taken immediately after induction of anaesthesia and insertion of intravenous sampling cannulae. Venous blood was allowed to clot at room temperature but occasionally heparinised blood (heparin concentration 0,05 mg/ml) was used. The specimens were stored at −20°C and the complement determinations were usually performed within 72 hours.

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Diluent

All reagents were diluted and sheep red cells suspended in veronal-buffered saline at pH 7,3, according to the method of Kabat and Mayer.[4]

Haemolytic System

Sheep blood, drawn aseptically, was preserved at 2 - 5°C in an equal volume of sterile modified Alsever's solution, and was allowed to age for one week prior to use. The red cells were washed three times in diluent and diluted to make approximately 5% suspension. The suspension was then standardised photometrically, an optical density of 0,700 corresponding approximately in terms of haemoglobin concentration to 1×10^9 erythrocytes per ml of cell suspension. Once standardised, the suspension was further diluted 1 in 4 with diluent, thus yielding $2,5 \times 10^8$ cells per ml of suspension.

The haemolytic antibody used was glycerinated anti-sheep cell serum (Wellcome brand Wassermann reagent) which was diluted in cold diluent.

When required, the cells were sensitised by incubating with an equal volume of haemolytic antibody at 37°C for 30 minutes and the sensitised cells were used immediately.

Standard Complement

A standard reference preparation of complement was used throughout this study. Fresh pig serum, in 3,0-ml volumes, was stored at −20°C and used within two months.

Procedure

Fresh pig serum was initially diluted to 1 in 25 diluent. After suitable serial dilutions had been made, 1,8 ml of sensitised cells were added to give a final reaction volume of 4,5 ml in each instance. In the reaction system described, a degree of lysis ranging between 10% and 90% was obtained, using 7 tubes.

Two control tubes were included. In one (representing zero haemolysis) the serum dilution was replaced by 2,7 ml diluent and 1,8 ml sensitised cells. In the other (representing 100% haemolysis) 1,8 ml of a 1:40 dilution of fresh guinea pig complement was added to 0,9 ml diluent and 1,8 ml cells. The entire reaction mixture was

set up in a bath of melting ice in order to retard the action of complement until all the tubes had been prepared.

When prepared, the tubes were incubated in a water bath at 37°C for 30 minutes, with occasional mixing of the contents to keep the cells in uniform suspension. At the end of the incubation period, the tubes were centrifuged at 2 000 rpm for 5 minutes to remove unlysed cells. The clear supernatant fluids were analysed photometrically for oxyhaemoglobin at a wave length of 541 nm in a Beckman DU spectrophotometer. The amount of haemolysis produced in each tube was expressed as a percentage of that occurring in the control tube (100%).

By relating the degree of haemolysis to the corresponding serum dilution, a sigmoid relationship was obtained (Fig. 1).

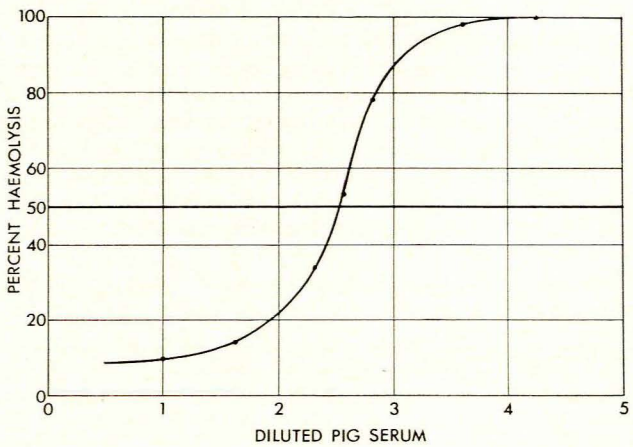

Fig. 1. Sigmoid relationship between percentage haemolysis and serum dilution.

For a mathematical description of the sigmoid response curve, the equation of Von Krogh is commonly employed:[4]

$$x = k\left(\frac{y}{1-y}\right)^{1n},$$

where x represents the amount of complement and y the degree of lysis. The constant k is the 50% unit of complement. The exponent $1/n$, which determines the shape of the sigmoid curve, depends on experimental conditions, but usually a value of 0,2 is applicable.

Logarithmic transformation of the Von Krogh equation furnishes a function which is convenient for evaluation of experimental results:

$$\text{Log } x = \log k + 1/n \log \frac{y}{1-y}.$$

If log x is plotted against log $\frac{y}{1-y}$ the equation describes a straight line of intercept as shown in Fig. 2.

It has been found useful to represent all results graphically as outlined, using log 3 cycles × probability graph paper (Chart Well, Ref. 5575). The best line was fitted to the experimental points and the 50% lytic dose read from the graph. The complement titre was then recorded as the reciprocal of the final serum concentration.

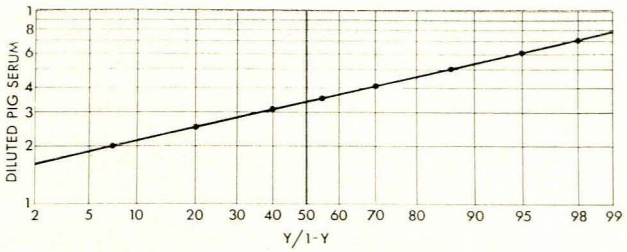

Fig. 2. Logarithmic plot of x against $y/1\text{-}y$ where x is ml of diluted complement and y the degree of lysis.

The 50% haemolytic unit of complement, designated $C'H_{50}$, is defined as the quantity of complement (contained in 1 ml of undiluted serum) required for 50% haemolysis. This is an arbitrary unit since its magnitude depends on a variety of experimental conditions.

RESULTS

All statistical analyses were made with an electronic desk computer (Wang 700 series) which was programmed to give mean, variance, standard deviation, 2 × standard deviation, coefficient of variation, 95% confidence limits and t values on any given set of figures.

For pig sera, it was found to be convenient to make an initial dilution of 1 in 25 in veronal-buffered saline. At lower or higher initial dilutions, the degree of lysis was either too diminished or increased for good evaluation of experimental results. Values of complement activity at different serum dilutions are given in Table I.

TABLE I. COMPLEMENT ACTIVITY AND SERUM DILUTION

Serum dilution	$C'H_{50}$ units	Average $C'H_{50}$ units*
1 in 20	**130**	**131**
1 in 25	**133**	**131**
1 in 30	**141**	**138**
1 in 35	**145**	**147**
1 in 40	**150**	**149**
1 in 45	**153**	**153**

* Mean of 7 single-point analyses calculated by the Von Krogh equation.

Consistent with results of Walton and Ellis,[5] significant variations were observed in repeated assays on a single pig serum sample with different batches of sheep cells. Values with one such sample varied from 110 to 139 $C'H_{50}$ units, depending on the batch of sheep cells used. To overcome this difficulty, a standard reference complement was run in parallel with each batch of titrations and all values obtained were corrected by referring to this standard value. The value of this standard serum was calculated by taking the mean of 18 estimations using 3 different batches of sheep cells (Table II).

A study of the effect of heparin on the complement titre was also performed and results were in accordance with those of Garratty.[6] The differences in complement activity between clotted and heparinised samples were

TABLE II. VALUES OBTAINED FOR TRIPLICATE ESTIMATIONS ON EACH OF 6 AMPOULES OF FRESH PIG SERUM

	Complement activity ($C'H_{50}$ units/ml serum)						Sheep cells
Estimations	Ampoule A	Ampoule B	Ampoule C	Ampoule D	Ampoule E	Ampoule F	
1	120	110	120	120	117	120	Batch S 1
2	120	134	139	134	124	124	Batch S 2
3	116	129	129	124	124	129	Batch S 3

Statistical analysis of values obtained in Table II:

Mean = 124,05.
Variance = 52,29.
Standard deviation = 7,23.
2 x standard deviation = 14,46.
Coefficient of variation = 5,82.
95% confidence limits = 109,59 and 138,51.

TABLE III. COMPLEMENT TITRES OF CLOTTED AND HEPARINISED PIG BLOOD

	Complement activity ($C'H_{50}$ units per ml)	
Sample	Clotted blood	Heparinised blood
1	188	190
2	214	214
3	129	130
4	211	204
5	135	135
6	136	141
7	86	90
8	71	71
9	113	114
10	100	93
11	124	130
12	151	160
13	136	140
14	151	143
15	124	113
16	103	89
17	102	98
18	90	112
19	103	103
20	130	128

negligible (Table III) ($P<0,1$) and heparin was only anti-complementary when more than 5 units/ml were present.

In this study, 94 complement titre determinations were performed on normal anaesthetised South African pigs. A mean value of 129,6 ± 40,7 was obtained.

It is difficult to compare these results in full with those of other workers because complement activity is so dependent upon the relative concentration of the individual reagents and the total volume used in titrations. No published data on complement levels in South African pigs are available for comparison and it was felt that these data would be of value to other workers.

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