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ESTIMATION OF THE HAEMOLYTIC ACTIVITIES OF CAMEL COMPLEMENT

By

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SUMMARY

109 serum samples from apparently healthy Sudanese camels were screended for total haemolytic complement activity. Four gorups of animals were categorized according to age and sex into group I: males over 7 years; group 2: males between 4.5-5 years; group 3: females over 7 years and group 4: females between 4.5-5 years. In this work, the sensitized horse erythrocytes (SHRBCs) proved to be the optimal indicator system for the estimation of camel complement haemolytic activity (CH_{50}) with a percentage of haemolysis ranged between 13.3 - 46.2. Sensitized sheep erythrocytes (SSRBCs) did not give any detectable absorbencies. Only undiluted camel sera showed reasonable percentages of haemolysis ranged between 44.5-76.6, as these sera in the diluted form could not show any readable absorbencies. The average level of CH_{50} units were ranging between 0.25-2.94 in group 1; 0.44-1.66 in group 2; 0.50-2.13 in group 3 and 2.1 in group 4. The total average of CH_{50} units in females sera showed slightly higher CH_{50} values than those in males sera, with average CH_{50} units of 1.176 and 0.989 respectively.

INTRODUCTION

Current knowledge about the complement (C) system originated with Pfeiffer and Issaeff observation in 1849, that fresh immune serum obtained from guinea pig (G.P.) immunized with *Vibrio cholera* lysed the organism specifically. During the last decade, it has become apparent that antibody (Ab) per se is biologically greaty ineffective unless aided by effector system, the complement constitutes one of them as an effector C' present in blood serum (Muller-Eberhard, 1968).

In those early days, (C')was considered to be a single entity. Today, however, the (C') system consists of at least 20 chemically and immunologically distinct plasma proteins, 9 major components being concerned with the classical pathway of (C') activation. The second pathway of (C') activation is called alternative pathway, its individual proteins are normally present in the circulation as functionally active molecules, representing only 15% of plasma globulin fractions (Cooper, 1982).

Complement activity plays an important role in the inflammation and defense against invading pathogens (Frank, 1979). Some intermediates of complement cascade, which are released during its activity, C' 3a and C' 5a behave as anaphylatoxins. The released histamine increases vascular permeability and thereby aids in the emigration of phagocytic leukocytes into tissues, passage of antibodies from the blood to tissues and dilution of any bacterial toxins in tissue (Atkinson and Frank, 1980). In addition, (C') activation is responsible for adherence of bacteria to phagocytic cells (Frank, 1979).

While all the above mentioned mechanisms and events are well known in several animal species and man (Barta and Hubbert, 1978), very little knowledge is presently available about (C') activity in camel serum (Bhatnagar et al., 1987), and because of the possible contribution of functioning (C') cascade in camel sera, an effort

has been to orient the present work into the following objectives: 1- Evaluation of the validity of certain haemolytic system for estimation of CH_{50} in camel sera; 2- Determination of the optimal dilutions to be used for estimation of camel C'; 3- Estimation of CH_{50} in camel sera using statistical devices.

MATERIAL AND METHODS

-Animals:

Chinchilla rabbits (2-3 kg weight) were purchased and kept under conventional environmental conditions. These rabbits were mainly used for the preparation of anti-S RBCs (Sheep RBCs) and anti-H RBCs (Horse RBCs).

-Sheep and horse red blood cells:

Blood was collected in equal volumes of Alsever's solution and kept at 4°C for at least 2-5 days to get rid of the weak fragile red cells. This blood was mainly used for preparation of anti-SRBCs and anti-HRBCs hyperimmune sera.

- Sera and other biologics:

a- Pooled cattle sera: Sera collected and separated from 15 slaughtered animals in Cairo abattoir were pooled and served as a positive control complement source to be used with SHRBCs (sensitized Horse RBCs). Sensitization of horse RBCs with the rabbit anti-HRBCs was carried out according to Miller et al. (1991).

b- Pooled camel sera: Sera were collected from 15 slaughtered animals in Cairo abattoir and used in different dilutions to set up the readable concentration of complement (C') in camel sera.

c- Camel serum samples: 109 serum samples were collected from healthy Sudanese camels. 87 sampels represented male camels over 7 years, 11 samples were taken from males between 4.5-5 years, 10 samples were from females over 7 years and a single female sample (4.5-5 years).

d- Guinea pig complement: Lyophilized ampoules of Gp C' was kindly obtained from Prof. Dr. Salah Abdel Karim Selim, Dept. of Microbiology, Fac. Vet. Med., Cairo Univ. This complement was reconstituted and used in the standard haemolytic system at a dilution of 1:30.

- Preparation of hyperimmune sera against SRBCs and HRBCs:

- Hyperimmunization of rabbits with SRBCs and HBRCs was carried out according to the procedures described by Miller et al. (1991).

- Titration of the rabbit anti-SRBCs and anti-HRBCs

- Rabbit hyperimmune sera were first inactivated by heating at 56°C for 30 mins. to exclude its natural complement activity.

- Different dilutions of this rabbit haemolysin were setup according to Shaaban (1992). SRBCs and HRBCs were incubated first with their respective haemolysin at 37°C for 15 mins. Negative controls were represented by SRBCs and HRBCs incubated only with diluting buffer. Finally 1 ml volumes of 1:30 dilution of Gp C' were added to all RBCs-haemolysin mixtures, followed by incubation at 37°C for 1 hr. and finally centrifuged at 500 xg for 5 mins. The absorbencies of the supernatants were measured at an OD 541 nm and the relative percentage of haemolysis (Y) in each dilution was calculated according to the following formula:

$$Y = \frac{\text{Absorbance of the test supernatant}}{\text{Absorbance of 100\% haemolysis supernatant}} \times 100$$

- Estimation of 50% haemolytic complement activity (CH_{50}) of camel sera:

The procedures adopted by Miller et al. (1991) were applied with certain modification as described by Shaaban (1992) The final CH_{50} of (C') in collected camel sera was estimated with the help of the statistical calculation reported by

Amer (1989).

RESULTS

- **Evaluation of the indicator system for estimation of the total haemolytic complement activity in camel sera:**

SSRBCs and SHRBCs were used comparatively in this work as an indicator system. It has been found that the highest dilution of rabbit anti-SRBCs and rabbit anti-HRBCs that represent one unit were 1:1000 and 1:3000 respectively. For comparison G. pig serum at a dilution 1:30 and undiluted pooled cattle serum has been tilized as a control with their corresponding standard haemolytic system namely SSRBCs and SHRBCs respectively. Table 1 reveals clearly that the SSRBCs could not give any readable absorbencies related to H process when used with undiluted pooled camel serum (% of haemolysis ranged between 0-0.01) if compared with the readable absrbencies given by GP complement (% of haemolysis ranged between 24.4-55.5). The same table shows that the SHRBCs could give a readable absorbencies with the undiluted pooled

Table (1): The validity of sheep and horse haemolytic systems for estimation of CH_{50} in camel serum.

Serum \ Tube No.	1	2	3	4	5	6 (SB)	7 (CB)	8 100% Haemolysis
Undil., pooled camel serum with SSRBCs	0.099* 0.0%** -	0.88 0.0%	0.103 0.0%	0.140 0.0%	0.220 0.01%	0.109	0.100 (-)°	1.046 -
Undil., pooled cattle serum with SSRBCs	0.067* 0.0%**	0.077 0.0%	0.074 0.0%	0.081 0.0%	0.099 0.0%	0.115	0.057 (0.172)°	0.988 (0.931)
Guinea pig complement 1:30 with SSRBCs	0.695* 28.4%** 0.395***	0.764 35.9% 0.560	0.856 45.8% 0.845	N.D N.D N.D	0.944 55.4% 1.242	0.272	0.161 (0.433)°	1.084 (0.923)
Undil., pooled camel serum with SHRBCs	0.289* 13.3%** 0.153***	0.425 23.8% 0.312	0.431 26% 0.351	0.579 39.1% 0.642	0.659 46.2% 0.859	0.086	0.053 (0.139)°	1.178 (1.125)
Undil., pooled cattle serum with SHRBCs	0.788* 72.0%** 2.571***	0.805 73.9% 2.821	0.806 74.0% 2.846	0.853 79.2% 3.808	0.956 90.7% 9.753	0.095	0.044 (0.139)°	0.945 (0.901)

* Absorbances obtained by the readings of the supernatents in a LKB spectrophotometer at OD 541 nm.

** Percentage of haemolysis obtained through the following equation:

$$Y = \frac{\text{corrected absorbance of each dilution}}{\text{corrected absorbance of 100\% haemolysis tube}} \times 100$$

N.B.: See methods for corrected absorbances.

*** The figure (y/l.y) represents the logarithmic value of the percentage of haemolysis** (Y). These values originally obtained from the method described by kabat and Mayer (1961).

° The value represents the summation of the readings obtained by the SB and CB values. This correction factor is used for calculation of the corrected reading obtained by the different serum dilutions.

N.D. Not determined. CB: Cell/Buffer, SB: Serum/Buffer

Table (2): Estimation of CH_{50} in different dilutions of camel serum.

Serum \ Tube No.	1	2	3	4	5	6 (SB)	7 (CB)	8 100% Haemolysis
Pooled undil. camel serum withSHRBCs	0.652* 44.5%** 0.802***	0.742 53.3% 1,141	N.D N.D N.D.	0.917 70.6% 2.401	0.979 78.7% 3.292	0.110 (0.200)*	0.090	1.06 (1.016)
Pooled, dil. 1:10 camel serum with SHRBCs	0.078* 0.0%**	0.087 0.0%	0.090 0.0%	0.101 0.0%	0.115 0.0%	0.050 (0.138)*	0.088	1.257 (1.169)
Pooled, dil. 1:30 camel serum with SHRBCs	0.068* 0.0%**	0.065 0.0%	0.067 0.0%	0.068 0.0%	0.075 0.0%	0.044 (0.095)*	0.051	1.181 (1.130)
Pooled, dil. 1:60 camel serum with SHRBCs	0.070* 0.0%**	0.068 0.0%	0.069 0.0%	0.069 0.0%	0.069 0.0%	0.043 (0.101)*	0.058	1.162 (1.104)

* See table (1)
** See table (1)
*** See table (1)
N.B. Not determined.
* The value represents the summation of the readings obtained by the SB and CB values. This correction factor is used for calculating the corrected reading obtained by the different serum dilutions.

camel serum (% of haemolysis ranged between 13.3-46.2), as well as with undiluted pooled cattle serum (% of haemolysis ranged between 72-90.7).

- **Determination of CH_{50} using different dilutions of fresh pooled camel serum with SHRBCs as an indicator system:**

For the determination of the optimal dilution of camel sera which can be used for the estimation of CH_{50}, fresh pooled camel sera were tested for this purpose at different dilutions viz: undiluted, 1:10, 1:30 and 1:60 with SHRBCs as an optimal haemolytic system. Table 2 throws a clear spot of light on the readable absorbencies given only by the undiluted camel sera (% of haemolysis ranged between 44.5-76.7). Unlike, the other different dilutions tested could not show any readable haemolysis with SHRBCs.

- **Estimation of total haemolytic complement activity in camel sera:**

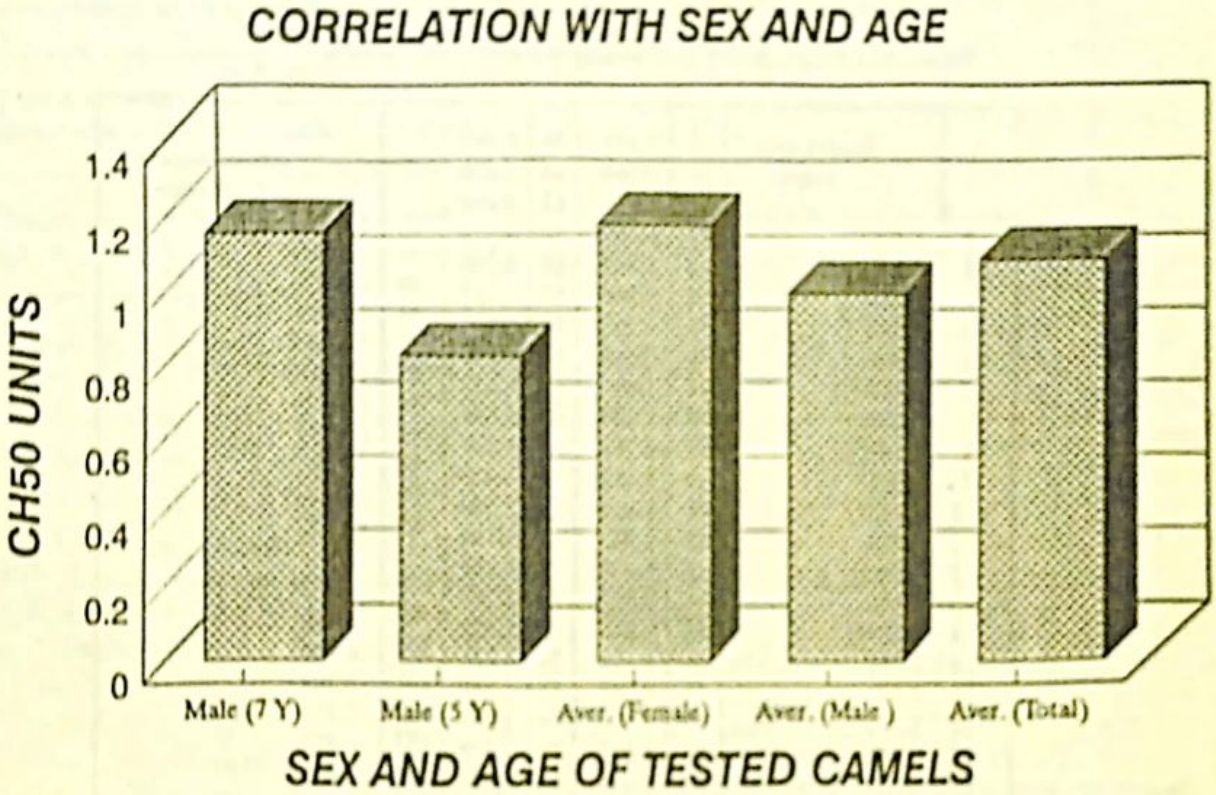
Following the statistical equation described by Amer (1989) by which the CH_{50} was specifically determined for each tested individual serum sample. Table 3 documents the CH_{50} units calculated for 109 serum samples and categorized as follows: Group 1 (G1)= males over 7 years (87 samples); Group 2 (G2)= males between 4.5-5 years (11 samples); Group 3 (G3)= females over 7 years (10 samples); Group 4 (G4) = female between 4.5-5 years (Only one sample). It can be noticed from Fig. 1, that the level of CH_{50} units of 1.155 and 0.823 units respectively. The total average level of CH_{50} units in females sera show slight increase than that in males with CH_{50} units of 1.176 and 0.989 respectively. The average level of CH_{50} units in camel sera (male and female) is 1.082.

Table (3): CH_{50} units for individually collected camel serum.

	(males over 7 years)	29	1.600	59	0.769		(males between 4.5-5 years)
		30	1.300	60	1.470		
		31	1.920	61	0.606		
1	1.428	32	8.333*	62	0.230	88	0.465
2	0.041	33	1.942	63	0.769	89	1.250
3	1.333	34	0.909	64	1.358	90	0.500
4	0.800	35	5.263*	65	0.667	91	0.444
5	0.606	36	0.300	66	0.760	92	0.476
6	1.143	37	1.000	67	0.833	93	1.666
7	2.083	38	1.330	68	0.606	94	0.769
8	1.143	39	0.769	69	1.111	95	0.952
9	1.053	40	1.492	70	1.430	96	0.900
10	1.212	41	1.429	71	1.300	97	0.800
11	1.250	42	2.083	72	1.100	98	0.833
12	1.140	43	0.470	73	1.250		(females over 7 years)
13	1.176	44	0.952	74	1.290		
14	5.555*	45	1.515	75	1.333	99	0.850
15	1.786	46	1.111	76	0.571	100	0.900
16	0.300	47	0.769	77	1.309	101	0.769
17	1.574	48	0.500	78	1.053	102	1.176
18	0.952	49	1.053	79	1.379	103	0.972
19	1.724	50	0.869	80	0.588	104	5.812*
20	1.212	51	0.791	81	0.526	105	0.502
21	5.263*	52	1.000	82	1.250	106	2.130
22	1.470	53	1.212	83	1.250	107	1.111
23	1.100	54	0.256	84	1.470	108	1.300
24	2.941	55	0.909	85	1.562		(females between 4.5-5 years)
25	2.941	56	1.309	86	1.043		
26	1.400	57	1.250	87	0.357	109	2.100
27	0.500	58	0.833				
28	1.250						

N.B. Samples marked by astriks represent deviated figures obtained through work fallicity and excluded from the calculations.

Fig. 1 LEVELS OF SERUM COMPLEMENT IN CAMELS



DISCUSSION

Complement activity plays an important role in inflammation and the defense against invading pathogens (Frank, 1979). Due to the existence of the two activation pathways, the complement system can function in the immune as well as the non-immune organism (Atkisnson and Frank, 1980), therefore, a functioning complement system was found particularly in the early stage of infection when antibodies are not available. Both alternative and the classical pathways of activation lead to the deposition of activated C^3 the key component of the system, on the surface of the invading pathogens (Muller-Eberhard, 1975). This activation is responsible for adherence of bacteria to the phagocytic cells (Frank, 1979).

One of the major factors found affecting the evaluation of the complement activity in the serum of certain species of animals, is the choice of an appropriate target erythrocytes. Variation in the ability of C' to lyse antibody coated erythrocyte have been recognized (Houle and Hoffmann, 1987).

For estimation of the (C') activity in camel sera, it was necessary first to determine the optimal haemolytic system which should be used as a combination of haemolysin and RBCs of several animal species. In our preliminary experiments, we found that the optimal dilution of rabbit anti-HRBCs and rabbit anti-SRBCs that represent two units and which was used in the final test for CH_{50} wee 1:1500 and 1:500 respectively. Table 1 showed that SHRBCs proved itself as the optimal indicator system for estimation of camel C', where a percentage of haemolysis ranged between 13.3 to 46.2 was obtained with the undiluted pooled camel serum. These results support previous findings reported by Pflauder and Moro (1907); Barta and Hubbert (1978); Boulard and Bencharif (1984) and Jain and Goel (1989), where they proved the unsuitability of SSRBCs as an indicator system. The introduction of SHRBCs as indicator system for the estimation of bovine complement encouraged us to compare such a type of RBCs for estimating the haemolytic C' activity of camel (Houle and Hoffmann, 1987).

Table 2 documents an important finding giving the readable absorbencies measuring the haemolytic activity of camel C', only in the undiluted form with a percentage of haemolysis ranged between 44.5 to 76.7. This relative low level of C' activity in camel sera was found to be in agreement with the work published by Bhatnagar et al. (1987), who screened about 25 serum samples collected from apparently healthy adult dromedaries and recorded low levels of haemolytic C' activity.

The obtained CH_{50} units depicted in Table 3, clearly show significant differences in the level of camel (C') between males over 7 years old (with average CH_{50} of 1.15) and males between 4-4.5 years old (with average CH_{50} of (0.82). In the different famale groups, a significant difference in CH_{50} was not detectable. Comparing these results with those obtained by Bhatnagar et al. (1987), who documented a C' activity in camel sera (7.6 units), we can suggest that these authors were perhaps using the statistical equation of Von Krogh (1916) with other different conversion factors. These discrepancies in the values of CH_{50} obtained in this work and others might encourage us to recommend further investigation to compare all those previously evaluated statistical procedures using more samples of camel sera to be a basis for an accurate method which could be used for estimation of CH_{50} in camel sera.

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