Complement Levels in Mammals.* (24427)

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While investigating the level of complement in sera of patients with Hodgkin's disease and patients with carcinoma, the complement level in various experimental animals was determined so that these animal sera could be used to find complement-fixing antibodies. The level of serum complement for human beings and guinea pigs has been determined many times. Information about other species is limited, and determinations by a well-tested method such as that of Mayer and coworkers (1) are nonexistent. Since this method shows good precision and reproducibility(2), it has been employed with some simplifications for measuring mammalian complement, and it is useful whenever C′ levels determined in different laboratories are to be compared.

Materials and methods. (a) Sheep cells obtained commercially† were stored in Alsever's solution. They were washed, standardized, and sensitized as previously described(3) to give a 1.25% sensitized cell suspension in barbital-buffered saline with added Mg. (b) Hemolysin was obtained commercially† and titered as described(3). (c) Sera were obtained from blood allowed to clot for 2 hours at room temperature, rimmed, spun at 1200 g at 1°C for 10 minutes. Serum was removed and spun again under the same conditions for 30 minutes more to remove residual red cells. Complement titrations were carried out within 6 hours on this serum; simultaneously small portions were sealed in glass ampoules and stored in dry ice at minus 70°C. (d) A Coleman model 6 spectrophotometer was used. Cuvettes were Wasserman tubes, 16 x 100 mm, selected for uniformity and marked so that the same optical density was obtained with $CuSO_4$ solution as described (4). All operations were carried out in these tubes so that no transfers were made once all the reagents had been combined. These tubes gave the same results as 40 ml centrifuge tubes recommended previously(1,2) and were considerably more convenient. (e) All glassware was washed in trisodium phosphate solution, rinsed in tap water, distilled water, then allowed to remain overnight in distilled water, and dried. Duplicate analyses carried out both in tubes washed in dichromate-sulfuric acid and tubes washed in phosphate agreed within experimental error. (f) Complement titration was done as follows: 1) Human sera and sera from rabbit, hamster, rat and dog were diluted 1:100 with barbital-buffered saline; guinea pig serum 1:500; and at least three tubes set up for each sample. 2) Two milliliters of sensitized sheep cells were added to each tube. 3) Buffered saline was added to each tube in an amount calculated to give a final total volume of 7.5 ml when all reagents were present. 4) Usually 2.0, 2.5 or 3.0 ml of the serum dilution were added to the appropriate tube. 5) A blank of cells and saline only, as well as 2 duplicate tubes of cells plus water, corresponding to 100% hemolysis, were set up simultaneously. 6) Tubes were stoppered, shaken, incubated at 37°C for 40 minutes with frequent shaking, centrifuged at 1200 g for 10 minutes and optical density read at 550 mu. 7) Per cent hemolysis in each tube, milliliters of serum giving 50% hemolysis, and the units of C′ per milliliter were calculated as described previously(3). 8) Mouse serum was used undiluted and with one-fifth of the above quantities of cells and buffer in a total volume of 1.5 ml. Cuvettes used were 10 x 75 mm. Milliliters giving 50% hemolysis were multiplied by 5. Human sera assayed as described above gave the same values when repeated using this "micro" method.

Results for different species are shown in Table I. For mice, values were obtained graphically from the von Krogh equation(3) in its logarithmic form: $\text{Log } x = \log k + \frac{1}{n} \log \left(\frac{y}{1-y} \right)$ where x is milliliters of serum used, y is degree of hemolysis (100 y = %

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TABLE I. Complement Levels in Mammals.

Species	No.	C'_{50} units/ml	Range
Normal humans	65	39.2 ± 4.1*	25.4 – 48.4
Guinea pig	11	262 ±36	218 –341
Dog	7	35.7 ± 7.9	21.7 – 47.3
Hamster, Chinese *(Cricetelus griseus)*	6	40.6 ± 7.7	32.8 – 55.1
Rat (Long-Evans)	10	32.4 ± 8.4	18.6 – 49.8
Rabbit (New Zealand White)	11	8.8 ± 1.7	6.5 – 11.6
Mouse, C3H	9	.67± .38	.39– 1.5

* Stand. dev.

hemolysis) and k and n are constants. When $\log x$ is plotted against $\log \left(\frac{y}{1-y} \right)$ a straight line results, and its slope is $\frac{1}{n}$. C′ values for all other species were calculated using the von Krogh factors(3). All species except the C3H mice gave a slope of 0.2 ± 0.02 as indicated previously(1) for humans and guinea pigs. Normal C3H mice gave slopes of 0.38, 0.43, 0.59, 0.81 on four different occasions.

One human normal serum titered 8 times on the same day gave a value of 42.9 u/ml with a standard deviation of 1.1. Another one titered 5 times gave 40.2 ± 1.4. Storage at dry ice temperature did not affect C′ titers of normal or pathological sera for at least 2 months; by 6 to 9 months titers had decreased about 15%. Titers on freshly drawn, unfrozen serum were the same as on those portions frozen immediately after shedding and assayed the next day. Complement titers in 10 normal humans determined at different times on freshly drawn samples during the course of a year were constant within 10%.

Of interest is the fact that the mouse, previously reported(5) to be void of or very low in C′, does have a readily measurable amount. Furthermore, the same hemolytic system used to assay human C′ can be used on the mouse without any modifications, contrary to the report of McGhee(5), who had to modify the system in order to find measurable amounts of C′ in Swiss albino mice. Since it has been shown(1) that hemolytic titers will vary greatly with the same number of sheep cells used, the system volume, and ionic concentrations, it is important when making comparisons that the same system be used throughout. We have also used this hemolytic system for measuring the fixing of guinea pig complement in the presence of antibody-antigen reactions.

Relative C′ levels found by Reader(6) were in the ratio of 1:1:4 for humans, rats and guinea pig, compared to 1:0.8:7 in this study. Larin *et al.*(7) found a ratio of 1:4 for dogs and guinea pigs compared to 1:7 found here. Values for humans and guinea pigs in this work agree well with those reported by investigators using the same technic(1,2).

Summary. 1. A sensitive, reproducible method capable of measuring complement levels in 7 different mammalian species is described. This method is a modification of the technic of Mayer and co-workers(1). 2. In sera of hamster, rat and dog the hemolytic complement activity is about the same as in that of normal humans; in guinea pig serum it is 7 times greater; in rabbit serum it is one-fourth of that in humans and in mice it is one-sixtieth, although readily measurable. 3. The slope of the hemolytic curve for C3H mice differs from that of all other species examined and also varies for individual mice.

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