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# Serum Complement Levels in the Albino Rat: Effects of Periodic Bleedings.* (28477)

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Increased interest in serum complement (C′) is shown by the recent publication of several reviews on the subject(1-5). Although rat serum contains C′ of relatively high hemolytic activity(6), most studies concerned with changes in C′ levels have employed man or the guinea pig as subjects(3,4). For many experimental investigations the rat is a more suitable test animal than the guinea pig. In the current study the distribution of hemolytic C′ activity in 106 normal, albino rats and the effects of bleeding at weekly intervals were investigated.

*Materials and methods. Animals.* Adult, male Sprague-Dawley rats weighing approximately 400 g were used. The rats were caged singly and maintained on a diet of Purina Laboratory Chow and tap water *ad lib.*

*Periodic bleeding.* The rats were bled by cardiac puncture, under light ether anesthesia, at weekly intervals for a total of 7 bleedings. At each bleeding 1.3 ml of blood per 100 g of body weight were removed, the sample representing approximately 20% of total blood volume(7).

*Complement.* Hemolytic C′ activity was determined by a modification of the procedure of Kent *et al.*(8). The changes consisted of the use of tris-buffer(9), a final volume of 4.0 ml and reading at 550 m$\mu$ in a Coleman Jr. spectrophotometer.

*Chemical and hematologic analyses.* Total serum protein, hemoglobin and hematocrit values were determined by methods previously reported(10). Analysis of the data was made by standard statistical procedures employing the *t* test(11). Electrophoretic analyses by a filter paper technique(12) were done on 2 pools of serum from each bleeding. Each pool was composed of an aliquot from half of the serum samples.

*Results.* The histogram (Fig. 1) showing frequency distribution of C′ levels represents the cumulative results of baseline bleeding data over a period of 2 years. None of the rats had been subjected to any experimental procedures prior to the initial bleeding.

A summary of general, hematologic, protein and C′ data is presented in Table I. The rats continued to gain weight over the period of observation. Hematocrit and hemoglobin levels fluctuated with the latter values showing greater variability. However, at the final bleeding both were in the normal range. The effects of blood loss on total serum proteins were most pronounced following the initial bleeding. Serum C′ levels were remarkably stable throughout the experiment. A significant decrease occurred only at the fourth

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TABLE I. Effects of Periodic Bleedings on Serum Complement Levels.

| Determination | Bleeding No. 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| No. of rats | 20 | 20 | 18 | 17 | 16 | 13 | 13 |
| Wt (g) | 400 ± 2.2 | 412 ± 2.5† | 427 ± 3.7† | 439 ± 3.7† | 445 ± 5.0† | 459 ± 4.3† | 467 ± 3.9† |
| Hematocrit (%) | 45 ± .4 | 43 ± .4† | 45 ± .5 | 43 ± .4† | 44 ± .6 | 45 ± .3 | 44 ± .5 |
| Hemoglobin (g %) | 14.2 ± .10 | 13.2 ± .11† | 13.8 ± .21 | 13.3 ± .18† | 13.4 ± .14† | 13.4 ± .18† | 14.3 ± .21 |
| Total serum protein (g %) | 5.7 ± .09 | 5.3 ± .07† | 5.5 ± .06 | 5.7 ± .05 | 5.5 ± .06 | 5.6 ± .05 | 5.4 ± .06* |
| Complement ($C'H_{50}$) | 45 ± 1.4 | 48 ± .9 | 45 ± 1.3 | 37 ± 1.0† | 43 ± 1.2 | 45 ± 2.7 | 50 ± 2.4 |

Findings include standard error of mean. Statistically significant differences from baseline values are indicated: * P = <.05 >.01, † P = <.01.

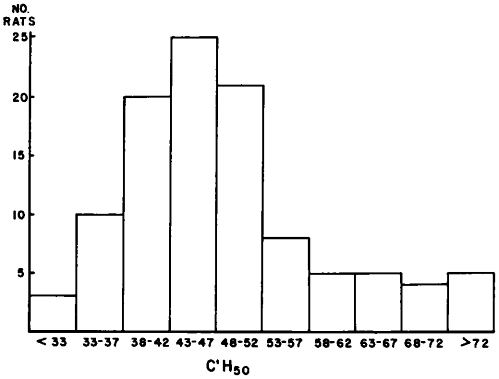


FIG. 1. Histogram depicting frequency distribution of hemolytic complement activity ($C'H_{50}$) for 106 normal, male rats. Mean 48, Median 46, Standard Error ± 1.2, Standard Deviation ± 11.3, Range 20-80.

bleeding. Several animals died from the effects of the repeated hemorrhages (Table I). However, for each bleeding there were no significant differences in results between rats that survived the entire series and those that succumbed.

Results of electrophoretic analyses are shown in Table II. Albumin and the $\alpha$-globulins exhibited the greatest divergences from baseline values. Albumin and $\alpha_2$-globulin concentrations were markedly decreased and increased, respectively, at the final bleeding.

*Discussion.* Rats have been primarily employed in C′ studies concerned with species comparisons and with the interchangeability of components(3,4,6,14). Our results provide some estimate of the variability that may be encountered. The skewness in the frequency distribution of C′ levels (Fig. 1) is indicative of an asymmetric population (11). Although the rats which comprised the sample were of the same sex and were relatively uniform with respect to age, weight, and diet fed, some departure from a normal distribution was observed. It should be noted, however, that the colony of origin was not highly inbred and that the samples were collected over a 2-year period. Mean C′ values for man and the guinea pig by this method were $75C'H_{50}$ and $350C'H_{50}$, respectively. Relative C′ levels for man, rat and guinea pig were thus 1:0.6:4, ratios intermediate between those reported by Levy(6) and by Reader(14).

TABLE II. Effects of Periodic Bleedings on Distribution of Serum Proteins.

| | Bleeding No. | | | | | | |
|---|---|---|---|---|---|---|---|
| Determinations | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Albumin, g % | 2.0 | 1.8 | 2.1 | 2.2 | 1.7 | 2.0 | 1.5 |
| $\alpha_1$-globulin, g % | 1.4 | 1.3 | 1.4 | 1.5 | 1.2 | 1.4 | 1.2 |
| $\alpha_2$-globulin, g % | .9 | .8 | .7 | .6 | 1.1 | .8 | 1.2 |
| $\beta$-globulin, g % | 1.0 | 1.0 | 1.0 | 1.1 | 1.1 | 1.1 | 1.2 |
| $\gamma$-globulin, g % | .4 | .4 | .3 | .3 | .4 | .3 | .3 |
| A/G ratio | .54 | .51 | .62 | .63 | .45 | .56 | .38 |

The current results with respect to periodic bleedings confirm and extend an early observation of Ecker and Rees(13). Based on the results obtained with a limited number of guinea pigs and with a less sensitive technique of measurement, they concluded that bleeding caused only a transient alteration in serum C′ activity.

A comparison of the C′ data with results of electrophoretic analyses revealed some correlation between C′ activity and concentration of the $\alpha_2$-globulin fraction. Both C′ activity and $\alpha_2$-globulin levels were lowest and highest at the 4th and 7th bleedings, respectively. The proteins comprising the C′ complex, however, represent such a small percentage of the total serum proteins that this relationship may be coincidental and requires confirmation by more sensitive analytical methods. The restoration of C′ to normal levels in the interval between bleedings is indicative of a rapid rate of synthesis and may reflect a conservation of factors involved in native and acquired resistance to infectious agents. It is concluded that samples comprising 20% of the total blood volume may be obtained from adult, male rats at weekly intervals without any marked alterations in hemolytic C′ activity.

*Summary*. The distribution of hemolytic C′ activity in 106 normal, adult, male rats and the effects of bleeding at weekly intervals for a period of 6 weeks were investigated. A mean value of 48 $\pm$ 11.3 (S.D.) $C'H_{50}$ units was obtained for the normal group. A transient decrease in C′ levels occurred only at the 4th bleeding. It was concluded that samples comprising 20% of the total blood volume may be obtained at weekly intervals without marked alterations in hemolytic C′ activity.

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