The Properdin System in Mice.* (26211)

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Properdin, a naturally occurring serum protein described by Pillemer *et al.*(1) has been implicated as a factor of importance in the natural resistance of man and other mammalian species to infection. Properdin, in conjunction with Mg^{++} and all 4 components of complement constitutes the properdin system. Evidence(1-7) indicates that this humoral system participates in destruction of certain bacteria and protozoa, inactivates certain viruses and is involved in resistance or susceptibility of experimental animals to infection. "Properdin by itself is not bactericidal, and it has been shown to be without bactericidal effect, even in concentrations 100 times its concentration in serum"(8). Among the serums of a variety of species assayed for properdin activity, the laboratory rat shows highest activity and the laboratory albino mouse ranks second with a serum properdin level of 10-20 units/ml(1).

The absence of bactericidal activity of normal mouse (albino *Mus musculus*) serum has been reported(9-11). The apparent inconsistency of high properdin levels but absence of serum bactericidal activity can be resolved if one recalls that mouse serum is deficient in one or more components of complement (C')(12-14). Since all fractions of C' are reported to be necessary for properdin activity, this lack could account for the failure of mouse serum to be bactericidal.

The following studies were based on 2 hypotheses. First, if the properdin system is, indeed, concerned with humoral mechanisms of host resistance and susceptibility, then parenteral injection of C' into the mouse, which has high properdin levels, may alter the animal's ability to withstand bacterial infection. Second, addition of C' to mouse serum which is lacking certain C' components but is high in properdin content, should result in demonstration of bactericidal activity *in vitro*.

Materials and methods. 1. *Bactericidal test.* The procedure for testing *in vitro* bactericidal activity was to add a 0.2 ml aliquot of a suitable dilution of *Klebsiella pneumoniae* or *Salmonella typhimurium* to mouse serum or a mixture of mouse serum and guinea pig serum (C') to give a final volume of 2.0 ml. Inocula of 1,000 to 100,000 organisms/ml were used; aluminum capped tubes containing these inocula were incubated in a 37°C water bath for 1.5 hours. Pour plates were then prepared using 1 ml aliquots of appropriate dilutions of the incubated serum-organism suspension. These plates were then incubated at 37°C for 24 to 36 hours and colony counts made.

2. *Complement.* Fresh guinea pig serum, which has the highest C' activity of any laboratory animal(12) served as the source of C' throughout these studies.

Results. Preliminary work showed that when groups of mice were subjected to total body x-irradiation, a procedure well known to produce infection and bacteremia of enteric origin, and then treated with C' (guinea pig serum), antibiotics, or a combination of these materials, that increase in survival resulted only among antibiotic treated animals. Table I summarizes these treatments and results obtained. Although injections of complement failed to increase survival of x-irradiated mice, the results could not be taken as evidence that this failure was expressing the failure of C' to interact with properdin since mouse serum properdin levels have been reported to be markedly decreased following x-irradiation exposure(15).

Experiments were, therefore, conducted to determine if C' (0.1 or 0.2 ml guinea pig serum) injected subcutaneously could reduce mortality by activating the properdin system. The test system chosen were normal mice

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TABLE I. Effect of Antibiotic and/or Complement Treatment* on Survival of Irradiated Mice.

X-radiation dose (r)	30-day mortality			
	Saline (stress control)	Complement	Antibiotic mixture	Antibiotics and complement
	%	%	%	%
350	4/26—15	3/20—15	1/20— 5	9/20—45
400	10/23—43	12/20—60	4/20—20	9/20—45
450	24/25—96	17/20—85	12/20—60	13/20—65

* Treatment consisted of daily inj. of 0.1 ml C′ intraper., 5 mg streptomycin and 5000 u penicillin, or combinations thereof for 2 wk and thereafter every other day for 10 days; saline and antibiotics inj. subcut., nuchal area (0.1 ml).

challenged with either *K. pneumoniae* or *S. typhimurium*. The results presented in Table II record the failure of C′ to increase survival of mice infected with *K. pneumoniae*; similar results were obtained with *S. typhimurium*. Both organisms were shown to be susceptible to *in vitro* bactericidal activity of either rat or rabbit serum. Nevertheless, these results cannot be accepted as evidence that C′ failed to activate the properdin system since with this *in vivo* system it is impossible to exclude the interaction of other intrinsic mechanisms which might be contributing to the observed results, *e.g.*, inactivation of either C′ or properdin.

In an effort to evaluate this latter possibility, an isolated *in vitro* system was employed to test the second working hypothesis; *i.e.*, that addition of C′ to normal mouse serum would restore bactericidal activity *in vitro*. Table III records results of a typical experiment. It appears at first glance that C′ failed to render mouse sera bactericidal against the test organism, *S. typhimurium*.

TABLE II. Effect of Complement Injections on Survival of Mice Infected with *Klebsiella pneumoniae*.

Group*	Complement treatment	Mortality, %
I	No treatment (organism challenge control)	52
II	0.1 ml subcut. (nuchal area) injections of C′ twice daily for 1 wk; first C′ inj. preceded challenge by 12 hr	48
III	0.2 ml *idem*	64
IV	0.1 ml subcut. (nuchal area) injections of C′ twice daily for 3 days prior to and 7 days following challenge	60
V	0.2 ml *idem*	56

* Each group contained 25 animals.

However, upon further analysis it is apparent that mouse serum, in the concentrations employed, is anticomplementary (compare line 3 with line 6). Therefore, the conclusion that C′ fails to restore bactericidal activity in the mouse may be unjustified. That is, some substance(s) in mouse sera may have destroyed the added C′ which was to react with the properdin present in the system and result in bactericidal activity.

TABLE III. Failure of An Experiment Designed to Test Bactericidal Activity of Mouse Serum against *Salmonella typhimurium** Due to Anticomplementary Activity.

Tube	PSS (ml)	C′ (ml)	Mouse serum‡ (ml)	Bactericidal activity† (samples) 1	2	3	4	5
1	1.8	0	0	m	m	m	m	m
2	1.7	.1	0	m	m	m	m	m
3	1.3	0.5	0	+	+	+	+	+
4	0	0	1.8	m	m	m	m	m
5	0	.1	1.7	m	m	m	m	m
6	0	.5	1.3	—	—	—	—	—

* Prior to incubation a 0.2 ml broth suspension of organisms was added to the test system to give an initial avg concentration of 880 organisms/ml with a range of 780 to 920 organisms/ml.

† A sample was negative (—) if there was no reduction of initial number of organisms or if multiplication (m) took place. Positive (+) bactericidal activity was reported if bacterial count was reduced by at least 50%.

‡ Each sample consisted of pooled serum from 5 mice.

An effort was made, therefore, to determine maximum amount of complement (guinea pig serum) which was not bactericidal in order that a maximum concentration of complement could be added to this *in vitro* system. At the same time maximum amount of mouse serum which would lack anticomplementary activity under the test conditions was determined. It was found that 0.1 ml of pooled undiluted guinea pig serum in the

TABLE IV. Failure of Complement To Render Mouse Sera Bactericidal.

Tube	C′ (ml)	Serum* (ml)	PSS (ml)	No. of surviving organisms†	
				K. pneumoniae	*S. typhimurium*
1	.1	—	1.7	17,100	11,500
2	.1	.4 (mouse)	1.3	16,500	10,600
3	.2	—	1.6	13,200	2,300
4	.2	.4 (mouse)	1.2	14,300	3,500
5	—	.4 (")	1.4	18,000	10,800
6	—	—	1.8	17,600	10,200
7	—	.4 (rat)	1.4	2,100	0
8	—	.4 (rabbit)	1.4	3,400	0

* Mouse serum sample consisted of pooled serum from 5 mice.

† Prior to incubation a 0.2 ml broth suspension of organisms was added to each tube to yield an initial concentration of approximately 17,000 *K. pneumoniae* organisms/ml or 10,000 *S. typhimurium* organisms/ml.

final test volume of 2.0 ml was uniformly non-bactericidal. Likewise, from a pool, 0.4 ml of undiluted mouse serum in a final volume of 2.0 ml (final dilution 1:5) was the largest amount which could be routinely used without exhibiting anticomplementary activity.

With this information a number of experiments were carried out to determine whether a mixture of these concentrations of C′ and mouse serum would yield bactericidal activity. The experimental results were uniformly negative. Table IV presents results of a typical experiment. Mouse serum exhibits no bactericidal activity (tube 5) whereas both control rat and rabbit sera are markedly bactericidal for either *K. pneumoniae* or *S. typhimurium* (tubes 7 and 8). Addition of 0.1 ml of C′ (guinea pig serum) failed to restore bactericidal activity to mouse serum (tube 2). Guinea pig C′, 0.2 ml, possessed bactericidal activity (tube 3) and apparent restoration of such activity to mouse serum (tube 4) must be attributed to the bactericidal activity of the added C′.

Discussion. We have been unable to adduce evidence in support of either of the proposed working hypotheses. Injection of C′ into the laboratory albino mouse failed to alter ability of the available strain of animals to withstand bacterial infection, and addition of C′ to mouse serum failed to induce bactericidal activity *in vitro*.

The experimental design had inherent limitations and certain factors require additional comment. Questions to be considered are: 1. Was amount of C′ added in these tests sufficient for activation of the mouse properdin system? C′ assays revealed that the pooled guinea pig sera (the source of C′ in these experiments) used never had a titer of less than 330 units/ml. Therefore, in the *in vivo* studies, mice received either 33 or 66 units of C′ twice daily. With *in vitro* bactericidal tests, at least 16.5 units were added per tube. It is of interest that Kornfeld(16, 17) reported that addition of 0.2 ml of 1:5 or 1:10 diluted guinea pig serum to 0.3 ml of serially diluted mouse serum would yield bactericidal action with normal mouse serum. 2. In the *in vivo* studies, were succeeding injections of C′ inactivated by formation of anti-complement antibody from preceding injections? Since whole guinea pig serum was utilized as the source of complement, antibody would be expected to develop. Whether rate of formation was rapid enough to yield significant amounts of antibody resulting in partial or complete inactivation of C′ is open to speculation. No overt signs of untoward reactions to C′ injections were observed among the experimental animals. 3. The routine procedure utilized in testing bactericidal activity always results in a final dilution of 1:5 for mouse serum. Could the dilution factor be critical in the observed failure to obtain bactericidal activity? Undoubtedly this dilution may have decreased bactericidal activity. It should, however, be noted that rat and rabbit control sera, when diluted 1:10, still retained bactericidal activity.

Although the data presented cannot be taken as definitive evidence that the proper-

din system is not significant in the natural defenses of the mouse, the results obtained do not contribute evidence to support the suggested importance of this system.

Summary. Injection of complement (guinea pig serum) into the laboratory albino mouse failed to alter capacity of these animals to withstand parenterally induced bacterial infection with *K. pneumoniae* or *S. typhimurium*. Addition of complement to mouse serum failed to induce bactericidal activity *in vitro*.

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Total Exchangeable Potassium in Systemic Lupus Erythematosus with Reference to "Triamcinolone Myopathy".* (26212)

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Muscular weakness and atrophy have been described following prolonged use of adrenal steroids, particularly triamcinolone(1,2). This has been referred to as triamcinolone myopathy(3). Histologic changes in the muscles of such patients were relatively minimal and consisted of scattered areas of muscle vacuolization and proliferation of sarcolemmal muscle. Increased creatine synthesis and decreased ability to store this substance have been suggested as a cause of the creatinuria observed in these patients. Potassium loss also has been considered as a possible pathogenic factor although serum potassium levels have been normal in triamcinolone myopathy.

To elucidate the effect of adrenal steroids on total body potassium stores, 2 groups of patients with systemic lupus erythematosus (SLE) were studied. One group of patients had a mild form of the disease and received only symptomatic therapy with salicylates and antimalarials. The other group was severely affected and had been treated with various adrenal corticoids.

Total exchangeable potassium (Ke) was determined according to the method of Corsa *et al.*(4). Radioactive potassium (K-42) was given orally in tracer doses of 300-500 μc.‡ The specific activity of K-42 was .2-.4 mc/mg so that only a minute amount of stable inorganic potassium was administered. Radioactive potassium (K-42) and stable potassium (K-39) concentrations were deter-

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