Serum Complement Activity in Rat Recipients of Small and Massive Skin Allografts.* (31759)

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(Introduced by C. A. Stetson)
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Evidence has been accumulated from several recent studies that, in rats, massive skin allografts representing one-third or more of the body surface of the animal survive longer than smaller transplants(1-7). Prolonged survival of massive allografts was judged by gross or stereomicroscopic findings of the graft *in situ* and histological analysis of skin biopsy specimens. The nature of the mechanisms responsible for the temporary tolerance and the delayed rejection of such grafts is still uncertain.

The appearance of hemagglutinating and cytotoxic isoantibodies in the sera of BN and Lewis recipient rats after massive skin transplantation has been noted by Ballantyne and Stetson(7). Because the antibody response to the massive grafts appeared at the same time and virtually to the same degree as that to smaller grafts, these authors concluded that the prolonged longevity of massive grafts cannot be attributed to a possible immune paralysis of the recipient due to large doses of donor antigen.

Recent observations in man(8,10) and in various species of animals including rat(8), rabbit(9), and dog(10) imply participation of serum complement (C′) in the rejection process of transplanted tissue. The host C′ system, therefore, could be considered a limiting factor in the prolonged survival of massive allografts. Antibody reactions with the large amounts of antigenic substances in the massive grafts may have exhausted host C′ activity.

The present study employing the isogenic rats of brown Norway (BN) and Lewis strains was undertaken (1) to evaluate the effect of graft size upon the C′ activity, and (2) to ascertain whether an exhaustion or any other inactivation of the C′ system could be a limiting factor in the rejection reaction of transplants of massive dosage.

Methods and techniques. All orthotopic suprapannicular skin allografts were interchanged between female adult rats of 2 unrelated inbred strains, the brown Norway (BN) and albino Lewis, weighing between 140 and 270 g. The animals were obtained from Microbiological Associates, Bethesda, Md. Both donor-recipient strain combinations were of approximately similar size and weight during the reciprocal skin transplantation.

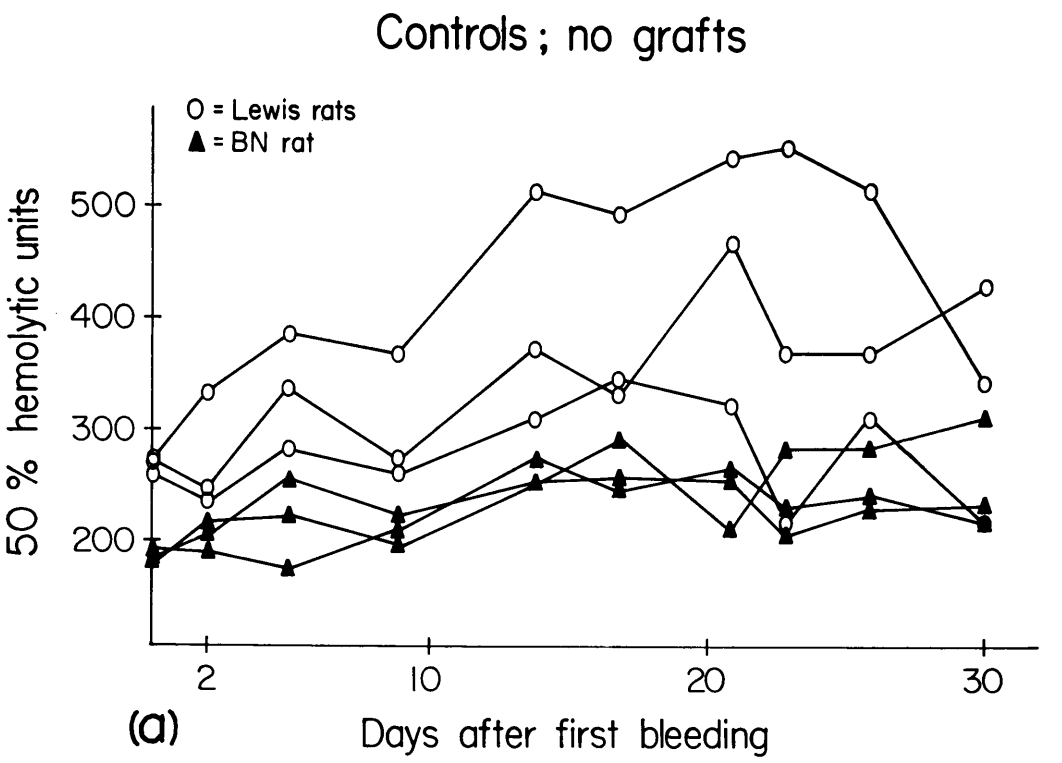
The surgical and bandaging procedures used for the 2 different sizes, small (1.3 × 1.3 cm) and massive (6 × 6 to 10 × 9 cm depending on the size of the donor-recipient combination) of full thickness skin allografts are similar to those described previously(7). The grafts reciprocally exchanged between the opposite strains at the time of grafting were consistently of similar dimensions.

In 2 control rats of each specific strain, massive skin isografts of similar size as described for the allografts were removed from the dorsum, rotated 180°, and interchanged between the isogenic pair. The grafts were maintained in the recipient sites with interrupted sutures.

Gross and stereomicroscopic observations at each change of dressings and at periodic time intervals provided an objective evaluation of the behavior and viability of the transplants (11,12). The time of skin allograft rejection was determined when skin stereomicroscopy indicated either cessation of blood circulation, vascular disruption, complete vascular breakdown or a combination of these changes in the graft vessels.

* This study was supported in part by USPHS grant AM 08986-02 and in part by a grant from the John A. Hartford Foundation.

† Milbank Memorial Fund Fellow.


Controls ; no grafts
O = Lewis rats
▲ = BN rat
50 % hemolytic units
500
400
300
200
2
10
20
30
(a)
Days after first bleeding

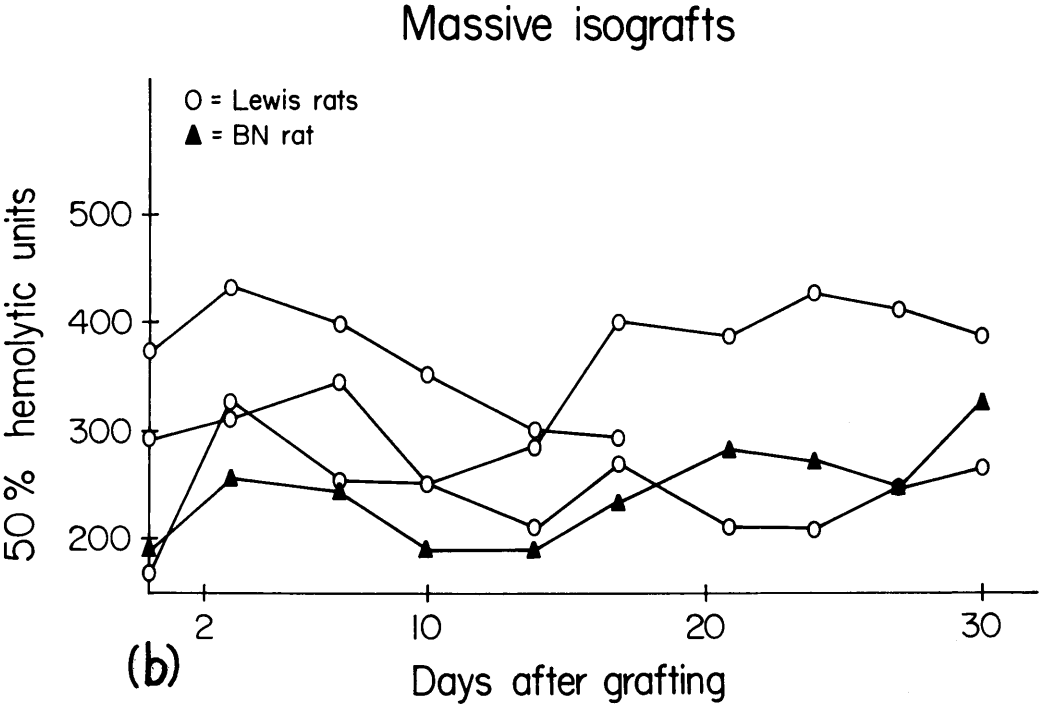

Massive isografts
O = Lewis rats
▲ = BN rat
50 % hemolytic units
500
400
300
200
2
10
20
30
(b)
Days after grafting

FIG. 1a. Serum complement of 3 BN and 3 Lewis rats which have never received skin transplants.

FIG. 1b. It is evident that the individual pattern of complement is not appreciably affected in recipients of massive skin isografts.

Blood was obtained either by cardiac punctures, from the tail veins or from the retro-orbital venous plexus at time intervals (Fig. 1a,b, 2a,b). The blood was clotted for 30 minutes at room temperature, one hour at 4°C, and the sera after centrifugation were stored in frozen state at −30°C until ready for the complement evaluation. For determination of the influence of graft size and allograft rejection reaction on serum complement activity, the titration method of Mayer (13) was employed and 50% hemolytic units (CH_{50}) were calculated graphically. To contrast with the hemolytic complement activity in the grafted animals, blood samples were also withdrawn at varying time intervals from 8 normal and untreated rats of each inbred line, thus reducing the likelihood of the effects of operative procedures and graft trauma or wound healing on the circulating serum complement.

Experimental results. Gross and stereomicroscopic observations. Postoperative observations showed that the small skin allografts in BN and Lewis recipients follow the previouly described behavior and survival of such grafts in the same isogenic strains by Steinmuller(14) and by Ballantyne and Stetson (7). The variation in the onset of the small allograft-rejection period was 9-12 days in 3 surviving Lewis hosts, with a mean survival time of 10.7 days, whereas the rejection time was 8-13 days in 4 BN recipients, with an average of 10 days.

In contrast to the small grafts, massive allografts exhibited prolonged longevity similar to that of massive transplants, previously described(1-7). Of the total of 8 Lewis hosts bearing massive BN grafts, 6 died before the entire course of events leading to the rejection of skin could be followed. The high mortality may be attributed to genetic deficiencies in this inbred line of animals and also to repeated bleedings which were necessary for the complement determinations. In 2 remaining animals, one graft survived for 15 days and the other for 24 days. On the other hand, all of the BN recipients, with one exception, survived past the rejection range of the massive Lewis grafts which was 18-21 days.

Massive isografts rapidly resumed the appearance and characteristics of normal skin and had a normal growth of their reversed hair. Normal appearance of the isografts in BN and Lewis rats continued to the 100th postoperative day, when the animals were sacrificed.

Serum complement activity. The mean complement activity from all animals per experimental group was expressed as an arithmetic mean of titrations of all serial blood from individual recipients in each group at varying time intervals as represented in Fig. 1a. As shown in Table I, the sera of BN recipients, independent of the presence or absence of skin transplants and also irrespective of the type or size of the donor skin, consistently demonstrated considerable less mean complement level than the Lewis line sera.

While the average complement activity appeared different in the two rat strains, the pattern of the complement titers in sera of individual animals during the period of 30 days after initial bleeding was similar. In the control groups of BN and Lewis rats which never received grafts (Table I), a slight initial rise of the serum C′ activity occurred in most of the animals (Fig. 1a), but during the subsequent days the pattern became irregular, while remaining persistently above the original level. In the control groups, the mean C′ activity of 48 individual blood samples collected during the 30-day period from 8 BN rats, was 259 CH_{50} units with a range of 168-430; for 8 Lewis animals it was 349 CH_{50} units from 50 samples with a range of 200-562.

There was no significant difference between the serum whole complement titer of the normal controls and the recipients of massive skin isografts (Table I). Further evidence showing that the presence of massive iso-

TABLE I. Serum Complement Activity in Sera of BN and Lewis Rats.

Size and type of grafts	Strain of rat recipient	No. of surviving hosts after skin rejection	Survival range of grafts in days	Serum complement titrations		
				Mean titer	Maximum	Minimum
No grafts, bleedings only	Lewis	8	—	349	562	200
Idem	BN	8	—	259	430	168
Massive isografts	Lewis	2	*	354	432	246
Idem	BN	2	*	246	330	186
Small allografts	Lewis	3	9–12	362	458	246
Idem	BN	4	8–13	223	345	163
Massive allografts	Lewis	2	15 & 24	330	475	246
Idem	BN	7	18–21	284	451	160

* Recipients were sacrificed 100 days after transplantation. At this time the isografts were still viable.

transplants has no appreciable effect on the normal range of C′ activity or the individual pattern of titers in individual recipients of both strains, is shown in Table I and Fig. 1b.

Skin allografts, whether small or massive, during the process of skin rejection reaction did not affect the average titer of C′ activity (Table I) or the activity pattern in the individual recipient (Fig. 2a & b). After transplantation of skin isografts or allografts, the initial rise in CH_{50} and subsequent irregular titers, which continued to persist above the preoperative level, has been observed in all BN and Lewis recipients with 2 exceptions (Fig. 2a). Even when the small or massive allografts were undergoing the process of active rejection reaction, there was no significant change in the complement activity when compared to that of untreated normal controls.

Discussion. In reviewing the literature it becomes apparent that little is understood of the factors which control the rejection reaction in the rat recipients of massive allografts, producing long-term survival of such grafts. Only recently have Ballantyne and Stetson (7) demonstrated evidence of circulating cytotoxic and hemagglutinating antibodies formed in response to massive allografts. The antibody response evoked by the massive transplant was similar to that observed when smaller grafts were used for the sensitizing stimulus. Thus, the authors concluded against "immunologic unresponsiveness" due to antigenic overloading as the underlying factor. Since the serologic data suggested that the temporary graft tolerance is not attributed to suppression of antibody formation or to neutralization of cytotoxic antibodies and hemagglutinins by excess antigen in the form of massive grafts, we turned to another immune factor, the serum complement system, in an attempt to elucidate the mechanisms responsible for longevity.

Complement is involved in the immune destruction of cells in a variety of systems, *i.e.*, hemolysis, bactericidal activity and cytotoxicity. The present study was initiated by *in vitro* observations of the isologous cytotoxic reaction of BN anti-Lewis lymphocyte sera. All known components of the complement system were involved in this reaction (15). As there is evidence indicating an active role of complement in allograft rejection (8-10), it has been considered that large amounts of antigen available in recipients of massive skin grafts may deplete the complement activity. It is evident, however, from the present findings after reciprocal skin transplantation between Lewis and BN rats that the longevity of massive grafts cannot be ascribed to an exhaustion or inactivation of the C′ system in the recipients.

The titers of serum C′ were consistently high following surgical procedure and throughout the duration of skin rejection in the re-

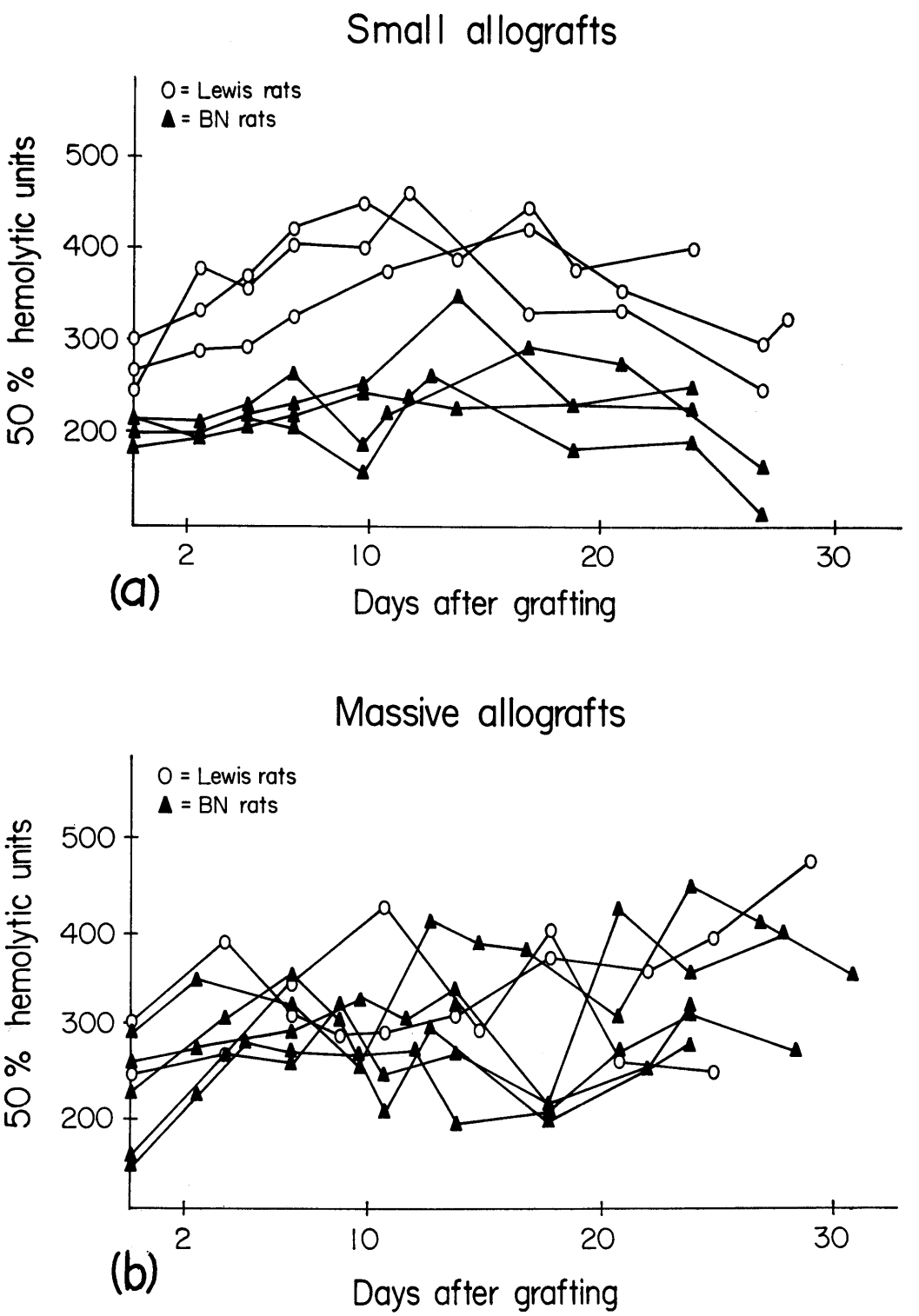


FIG. 2a and 2b. Note that levels of serum complement in BN and Lewis rats are not influenced by the size of the skin allografts, whether small (Fig. 2a) or massive (Fig. 2b).

cipients of small or massive grafts. Even when the massive grafts were rejected during the time period between 10 and 24 postoperative days as contrasted to 9 to 12 days for smaller grafts, the recipients of massive donor skin produced no apparent variation in serum complement levels. The present serologic data, therefore, substantiated previous studies (5,7) that the limiting factor in the prolonged survival of massive grafts may be operative at the effector level.

On the other hand, failure of the massive transplants to affect the overall C′ titer does not necessarily constitute evidence against the requirement of complement in the rejection process. The available data favoring C′ participation under various conditions suggest the involvement of only minute amounts; thus, the sera of human recipients of renal allografts indicate depressed C′ titers only when the highly sensitive method of C′2‡ determination is employed(8). The CH_{50} overall complement titer, as determined in this study, remains almost unaffected.

Our observations showing increased C′ titers during the first few blood collections warrant some comments. A similar pattern exhibiting elevated titer in rat recipients of small skin allo- and isografts has been reported previously(8); this serologic reaction has been tentatively interpreted as a result of a complicated response to ectopic foreign tissue. However, because in this study the bleedings undertaken in normal and unconditioned rats elicited a similar pattern of complement activity, the initial rise may reflect unknown consequences stimulated by bleedings, possibly an increased production of serum protein.

Summary. The complement activity was determined in sera of recipients after reciprocal skin transplantation between two isogenic strains of BN and Lewis rats. Small or massive allografts did not significantly affect the pattern or the level of overall complement activity, despite the fact that these animal sera are known to bind complement in the cytotoxic reaction *in vitro*. A slight initial rise of the serum complement activity has been noted in most of the animals. This rise is attributed to a possible increase in production of serum protein elicited by bleeding. The results have been interpreted to rule out either an exhaustion or an inactivation of the complement system as a causative factor in the longevity of massive grafts.

‡ C′2 = the second component of serum complement.

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Received October 25, 1966. P.S.E.B.M., 1967, v124.