tion of neural elements of the LH-release apparatus higher than the median eminence. The chief limitation would appear to be the fact that in some strains of rats (*e.g.* our "normal" strain derived from Osborne-Mendel stock) the persistent-estrous state often cannot quickly be induced until the animal is 6 months old.

Summary. Persistent estrus was induced in female rats by continuous illumination. Through "permanent" bipolar electrodes, stimulation was administered after recovery from anesthesia to either the amygdala (11 rats) or the septum pellucidum (1 rat). The latter and 5 of the former ovulated within 24 hours.

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"Transfer" of Complement from One Antigen-Antibody Complex to Another.*† (23481)

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It has been shown that some of the I^{131} labelled complement incorporated with antigen-antibody-I^{131} complement precipitates is released when these precipitates are resuspended in fresh sera containing active complement(1). This behavior was attributed to an equilibration between the I^{131} complement in the precipitates and the free complement present in the fresh sera. It was of interest to determine whether the complement components still maintain their original activities after the reaction with an immune complex. There is some evidence in the literature indicating that complement components once fixed may maintain some of the original ability to participate in the hemolysis of sensitized erythrocytes(2,3).

The purpose of the present investigation was to determine whether or not components of complement can "transfer" from one immune complex to an unrelated one and if so, what are the conditions which govern this "transfer." As an index of complement activity, use was made of the precipitation of soluble antigen-antibody complexes by complement(4,5). Antigen-antibody complexes (soluble or insoluble) containing complement were mixed with soluble I^{131} bovine serum albumin (I*BSA)-rabbit anti BSA complexes which were prepared in the region of excess antigen. The subsequent precipitation of the soluble I*BSA-anti BSA complexes was employed as a measure of the "transfer" of complement to these complexes. Although complement appears to be responsible for the precipitation of soluble immune complexes by normal sera, there is a possibility that other substances present in the sera may play a role. It has previously been inferred that

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nitrogenous materials other than complement may fix to immune precipitates(1).

Materials and methods. BSA was obtained from Armour and Co., lot No. 128-175, egg albumin (Ea) was prepared by the method of Kekwick and Cannan(6). Pneumococcal polysaccharide S III was kindly supplied by Dr. M. Heidelberger. I^{131} BSA (I*BSA) was prepared according to the method described by Talmage *et al.*(7). Sufficient counts were obtained in well type gamma counters to insure a maximum counting error of no greater than 5%. Anti BSA and anti Ea were prepared by intravenous injections of rabbits four times per week with neutral suspensions of alum precipitated protein as previously described(8). The globulin fraction of these antisera was precipitated at 40% saturation with ammonium sulfate, and dialyzed against 0.15 M NaCl until free of sulfate. The antibody content of the fractionated preparations of the anti BSA and anti Ea sera was 1.58 and 2.00 mg N per ml. respectively. These preparations were maintained as stock solutions and diluted for use. Rabbit anti S III, A66, was kindly supplied by Dr. M. Heidelberger. A pool of serum obtained from 30 guinea pigs was used as the source of complement. The serum was placed in a polyethylene container and stored in a dry ice chest at −50 to −70°C without preservatives. The serum was adjusted to pH 7.5 before use. The reagents and methods employed for the titration of the components of complement were those previously described(9,10). Sheep erythrocytes collected in Alsever's solution (11) were obtained from Carworth Farms, New City, N. Y. and the rabbit hemolysin was procured from Lederle Labs, lot No. 2409-132 A. The quantitative immunochemical procedures used were those developed by Heidelberger and co-workers(10). The total N in immune precipitates and in the various protein solutions was measured by the Markham modification of the micro Kjeldahl technic(12). Complement N was recorded as the difference between the total N precipitated (antibody + antigen + complement) in the presence of complement and the total N precipitated in the presence of 0.15 M NaCl or inactivated serum (antibody + antigen). Sera were inactivated by heating for one hour at 56°C. Two ml of guinea pig complement were reacted with mixtures of S III polysaccharide + anti S III and Ea + anti Ea formed at equivalence. The μg of total N in the S III-anti S III and Ea-anti Ea precipitates formed in the absence of complement were 110 and 105 respectively. The antigen-antibody-complement mixtures were incubated at 37°C for 30 minutes and then for various times (1 hour-6 days) at 0-3°C. After different periods of incubation the precipitates which formed were centrifuged and washed twice. Subsequent addition of the antibody-antigen-complement complexes to soluble I*BSA-anti BSA complexes which had been formed at 2 times equivalence and contained 14.5 μg of I*BSA N was performed in 2 different ways. One method consisted of resuspending the antigen-antibody-complement precipitates in the I*BSA-anti BSA complexes. The other involved dissolving the antigen - antibody - complement precipitates with excess antigen (either 2.15 mg S III or 14.70 mg Ea) at 37°C for 30 minutes, centrifuging the mixture at 0°C and adding the clarified solution to I*BSA-anti BSA soluble complexes. The soluble I*BSA-antiBSA complexes and the solutions of redissolved precipitates were present in a volume of 1 ml. These experiments were performed using veronal buffer pH 7.4(10) as a diluent both with and without added Ca^{++} and Mg^{++}. The total N precipitated by the transferred complement was also measured.

Results. The results in Table I show that

TABLE I. Precipitation of I*BSA-Anti BSA Soluble Complexes by Antigen-Antibody-Complement Complexes.

Antigen-antibody‡ complement Ppts.	C'N in Ppts., μg	I*BSA N Pptd. by Ag.-Ab.-C' Ppts.	I*BSA N Pptd. by dissociated Ag.-Ab.-C' Ppts.
S III-anti S III* (156.6 μg N)	51.2	1.6 (11)§	4.7 (32)
Ea-Anti Ea† (162.8 μg N)	60.8	2.6 (18)	3.0 (21)

* 2.15 mg S III used to dissociate precipitate.
† 2.35 mg Ea N " " " " .
‡ Used after 6 hr reaction time at 0-3°C.
§ The figures in parentheses are % of available I*BSA (14.5 μg N) which precipitated.

TABLE II. Dissociation of Antigen-Antibody-Complement Precipitates by Excess Antigen after Incubation at 0-3°C.

Time of incubation (0-3°C)	Total N Pptd. (μg) in presence of 2.0 ml of G.P. serum	2.0 ml 0.15 M NaCl	C'N Pptd., μg	Amt of antigen used to dissociate Ag.-Ab.-C' Ppts., mg	N in Ppts. (μg) after dissociations (a)	(b)	% dissociation of Ppts. (a)	(b)
S III-anti S III system								
3 hr	145.0	96.0	49.0	2.15	26.0	14.0	82	85
6 hr	153.4	99.2	54.2	"	28.2	18.2	82	82
24 hr	158.6	100.2	58.4	"	35.0	19.4	78	81
6 days	162.8	106.8	56.0	"	42.4	21.8	74	78
Ea-anti Ea system								
3 hr	151.8	108.2	43.6	14.70	64.6	8.2	58	82

(a) Precipitates initially formed in G.P. serum.
(b) " " " " 0.15 M NaCl.

complement from both antigen-antibody-complement precipitates and soluble antigen-antibody-complement complexes can precipitate soluble I*BSA-anti BSA complexes. The precipitation of these soluble complexes may be the result of the "transfer" of either free complement or complement in the form of antigen-antibody-complement complexes. However, it will be shown later that it is probably free complement which is transferred.

Before investigating the effect of the time of incubation of the antigen-antibody-complement complexes on the ability of complement to be transferred to a second antigen-antibody complex, it was necessary to study the resolution behavior of antigen-antibody precipitates with excess antigen. The results of the resolution of specific precipitates consisting of antibody, antigen and complement and of antibody and antigen (no complement) are given in Table II. The effect of the time of incubation of S III-anti S III precipitates (either with or without complement) at 0-3°C on resolution with excess antigen was not very pronounced. As the time of incubation of the precipitates was increased (from 3 hours to 6 days) they became only slightly more difficult to dissolve with excess S III. With the S III-anti S III system, the presence of complement had very little, if any, effect on the resolution of the precipitates by excess S III. However, with the Ea-anti Ea system, there was a pronounced effect of the presence of complement on the resolution of the precipitates by excess antigen. Eighty-two percent of the total precipitate formed in 0.15 M NaCl was dissolved with excess antigen, whereas, only 58% of the precipitate containing 43.6 μg of complement N was dissolved by the same amount of Ea.

The "transfer" of complement (nitrogen) from dissolved S III-anti S III-complement precipitates as evidenced by the precipitation of soluble I*BSA-anti BSA complexes is shown in Table III. This "transfer" of complement (nitrogen) was inhibited significantly by the addition of Ca^{++} and Mg^{++}. Precipitation of soluble I*BSA-anti BSA complexes occurred in the absence of Ca^{++} and Mg^{++}. Similar results were obtained with the dissociated Ea-anti Ea-complement precipitates (Table IV). When soluble I*BSA-anti BSA complexes were incubated with either S III-anti S III or Ea-anti Ea complexes which lacked complement there was no precipitation of I*BSA.

Precipitates containing complement had similar effects on the precipitation of soluble complexes as did the solutions of the dissolved antigen - antibody - complement precipitates (Tables III and IV). As mentioned above, this precipitation was also inhibited by Ca^{++} and Mg^{++}.

The S III-anti S III-complement precipitates obtained after 3 hours incubation at 0-3°C were dissolved with excess S III both in the presence and absence of Ca^{++} and Mg^{++} and were then analysed for component activities of complement. Only C'1 activity was found in the solutions of the dissolved precipitates. However, 3 times as much C'1 activity was found in the solution prepared in the ab-

TABLE III. Precipitation of Soluble I*BSA-Anti BSA Complexes by S III Polysaccharide-Anti S III-Complement Complexes.

		Dissociated Ppts.			Undissociated Ppts.	
	Time of incubation of S III-anti S III-C′ Ppts. (0-3°C)	Total N Pptd., μg	I*BSA N Pptd.,† μg	C′N Pptd.,* μg	Increase in N of S III-anti S III-C′ Ppts.,‡ μg	I*BSA N Pptd.,* μg
In absence of Ca^{++} and Mg^{++}	3 hr	21.8	2.7	6.9	13.2	3.3
	6 hr	37.6	4.4	12.7	15.2	3.4
	24 hr	43.4	4.9	16.5	16.2	3.3
	6 days	31.2	2.8	15.8	7.0	2.8
In presence of Ca^{++} (0.00015 M) and Mg^{++} (0.0005 M)	3 hr		.8		.0	.5
	6 hr		.7		.0	.9
	24 hr		1.3		.0	.5
	6 days		.2		.0	.3

* Obtained by subtracting the antibody N and I*BSA N Pptd. from the total N Pptd. An antibody N/antigen N ratio of 4.5 was used to determine antibody N Pptd.

† I*BSA-anti BSA soluble complexes contained 14.5 μg I*BSA N.

‡ Determined by difference in N content of S III-anti S III-C′ Ppts. before and after incubation with I*BSA-anti BSA soluble complexes.

sence of Ca^{++} and Mg^{++} than in the one prepared in the presence of the divalent cations.

Discussion. It has been observed that complement and possibly complement containing complexes can be transferred from one immune complex to an unrelated one. It is of interest that this phenomenon failed to take place in the presence of concentrations of Ca^{++} and Mg^{++} which have been shown to be necessary for the fixation of hemolytic complement(13-15). Mayer and co-workers(16-18) have postulated a sequence of steps involved in complement fixation and lysis of sensitized cells. They found that Ca^{++} was required for the fixation of C′1 and C′4 while Mg^{++} was required for the fixation of C′2. In the present studies it has been shown that the divalent cations Ca^{++} and/or Mg^{++} inhibited the ability of complement to be "transferred" from one antigen-antibody complex to another. From these results it appears that divalent cations are not only required for the fixation of guinea pig complement to antigen-antibody complexes, but may participate in maintaining a firm bond between complement and the complexes. Laporte *et al.*(19) postulated that complement may exist in the serum as a Ca-proteinate complex. When Ca^{++} was removed from serum they found that complement became more susceptible to inactivation at 37°C. It is possible that complement in the form of a Ca-proteinate maintains a stronger bond with antigen-antibody complexes than "cation free" complement. If this were true, it could readily explain the inhibiting effect of divalent cations on complement

TABLE IV. Precipitation of Soluble I*BSA-Anti BSA Complexes by Ea-Anti Ea-Complement Complexes.

	Total N Pptd., μg	I*BSA N Pptd.,† μg	C′N Pptd.,* μg	Increase in N of Ea-anti Ea-C′ Ppts.,‡ μg	I*BSA N Pptd.,* μg
In absence of Ca^{++} and Mg^{++}	9.7	1.4	3.4	13.0	2.9
In presence of Ca^{++} (0.00015 M) and Mg^{++} (0.0005 M)		.4		.0	.7

Ea-anti Ea precipitates were incubated for 3 hr at 0-3°C prior to dissociation and addition to soluble I*BSA-anti BSA complexes.

* Obtained by subtracting the antibody N and I*BSA N Pptd. from the total N Pptd. An antibody N/antigen N ratio of 4.5 was used to determine antibody N Pptd.

† I*BSA-anti BSA soluble complexes contained 14.5 μg I*BSA N.

‡ Determined by difference in N content of Ea-anti Ea-C′ Ppts. before and after incubation with I*BSA-anti BSA soluble complexes.

"transfer." It may well be that the precipitation of soluble I*BSA-anti BSA complexes in the presence of complement containing complexes is the result of the fixation of antigen-antibody-complement complexes by the soluble I*BSA-anti BSA complexes rather than the result of the "transfer" of complement from one complex to another. However, it appears that divalent cations would be more likely to inhibit the release of complement from immune precipitates than the fixation of complement. It was not determined whether both Ca^{++} and Mg^{++} were necessary to inhibit the "transfer" of complement from one complex to another. However, it appears from the work of Mayer and co-workers that Ca^{++} should be the responsible cation.

The complement released from the S III-anti S III-complement precipitates by excess S III was found to be hemolytically active C'1. This release of hemolytically active complement (C'1) was not surprising in view of the findings of Hill and Osler(3), who demonstrated a return of hemolytic activity after treatment of precipitates containing complement with 10% NaCl. These results are also in accord with the previously reported data(5) that the fixation of hemolytic complement by soluble complexes decreased with an increase in the concentrations of antigen added. Thus, the dissociation, by excess antigen, of precipitates containing complement should be expected to release complement. It is of interest that some of the complement (C'1) released in this manner still retained its hemolytic activity.

From the results presented in Table II, it appeared that complement which had fixed to Ea-anti Ea precipitates retarded the dissociation of the precipitates. This effect of complement on dissociation with excess antigen is similar to the "order of addition" effect reported by Neff and Becker(20). They found that when antibody was added to antigen and incubated for 2 days, less N was precipitated in the presence of complement than when the antigen was added to antibody. However, if the mixtures were incubated for a longer period of time (7 days), or the reaction performed in the presence of decomplemented sera this effect was diminished. The explanation advanced was that when antibody is added to antigen, the complexes are initially formed in the region of antigen excess and complete precipitation of antibody is not obtained until complete equilibrium is established. Neff and Becker showed that complement increased the time necessary for equilibrium to be reached. Likewise in the present experiments, complement inhibited the establishment of equilibrium when excess antigen was aded to Ea-anti Ea-complement precipitates. There are a number of reports in the literature demonstrating that complement retards the flocculation of antigen and antibody (21-23). This may also be attributed to the ability of complement to increase the time for equilibrium to be established.

Boyd(24) found that Ea-anti Ea precipitates incubated at 0°C for 10 months were dissolved with excess antigen to the same extent as "fresh" precipitates. He believed that this demonstrated that the strength of the bonds involved in antigen-antibody precipitates did not increase with time. The data in Table II demonstrate that S III-anti S III precipitates became only slightly more difficult to dissociate with excess S III as the time of incubation (1 hour-6 days at 0-3°C) was increased.

Summary. The "transfer" to soluble I*BSA-anti BSA complexes of guinea pig complement which had previously been fixed to S III-anti S III and Ea-anti Ea precipitates has been demonstrated. This "transfer" of complement was achieved if the complement containing precipitates were added directly to the complexes or first dissociated with excess antigen. It was found that Ca^{++} and Mg^{++} inhibited this "transfer" of complement. The only detectable component of complement released from antibody-antigen-complement precipitates by excess antigen was hemolytically active C'1. Complement which had been fixed to Ea-anti Ea precipitates retarded the establishment of the equilibrium between antibody and antigen when excess antigen was added to these precipitates. It was also shown that the time during which precipitates (antibody + antigen or antibody + antigen + complement) were incubated had only a minor effect on the rate of dissociation of the

precipitates with excess antigen.

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Hyperreactivity of *Coxiella burnetii* Infected Guinea Pigs to Subsequent Injections of Bacterial Endotoxins. (23482)

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Following Shwartzman's report(1) of a necropurpurogenic factor in meningococci, many workers noted that skin tissue could be prepared for the reaction by local infection with a number of microorganisms. Thus, *Bacillus anthracis*(2), *Mycobacterium tuberculosis*(3), *Haemophilus influenzae*(4), hemolytic streptococci(5), and even the virus of vaccinia(2,6) have been used to prepare animals for the local Shwartzman reaction. When animals with such skin infections were injected intravenously with a "provoking" dose of a culture filtrate from certain Gram-negative microorganisms capable of eliciting the Shwartzman reaction, hemorrhagic necrosis occurred at the infected skin site within a few hours. A generalized Shwartzman reaction has been produced in animals with systemic infections of *Vibrio cholera*(7), *Corynebacteria* and *Mycobacterium tuberculosis*(8), and Group A streptococci(9). The generalized Shwartzman reaction can also be consistently evoked by the intravenous injection of two doses of endotoxin from a number of Gram-negative bacteria. The most prominent pathological alteration seen in the classical generalized Shwartzman reaction has been reported to be bilateral renal cortical necrosis (5,10,11). While studying the interference of Brucella endotoxin on the course of a *Coxiella burnetii* infection in guinea pigs, a phenomenon was observed which appeared analogous to the generalized Shwartzman reaction. Guinea pigs infected with the rick-