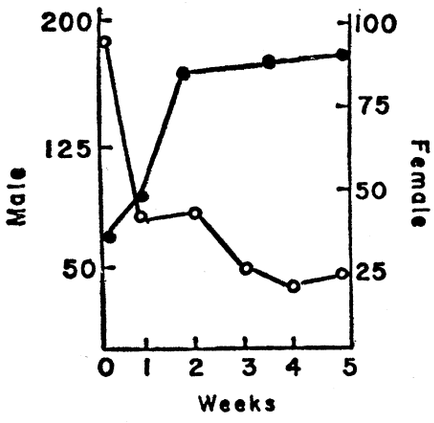

Fig. 2. The depression of serum C′ in male mice by diethylstilbestrol and the elevation of serum C′ in female mice by testosterone. ○——○, Males injected (sc) with a weekly dose of 1.5 mg diethylstilbestrol in two or three injections per week. Average of seven mice determined each point. ●——●, Females injected (sc) with a weekly dose of 0.75 mg of testosterone in two or three injections per week. Average of 11 mice determined each point. *Abscissa*: Weeks of treatment. *Ordinate*: Mean titers ($C'H_{50}$ units per milliliter).

and the non-inbred CF-1 mice of C′ genotype *Hc′/Hc′* (*8*).

As shown in Fig. 1, the subcutaneous (sc) injection of 10 mg of hydrocortisone in saline (Cortef Acetate, Upjohn, or Hydrocortone Acetate, Merck, Sharp & Dohme) caused a decrease in serum C′ which was most pronounced at 2 to 4 weeks. Many deaths occurred in the treated mice, and the normal mouse pathogen *Corynebacterium kutscheri* was frequently cultured from tissues of these animals. Survivors eventually regained normal C′ titers. Experiments in which 2.5 to 25 mg of hydrocortisone were injected demonstrated that, with smaller doses, less depression of C′ was induced and, with larger doses, the mice succumbed too rapidly for an effect on C′ to be noted.

Results of experiments in which male mice were "feminized" and female mice were "masculinized" are shown in Fig. 2. Male mice injected (sc) with weekly doses of 1.5 mg of diethylstilbestrol in ethyl oleate (Lilly) manifested a depression in C′. Conversely, female mice injected (sc) with 0.75 mg of testosterone propionate in sesame oil (Perandren, Ciba) per week showed a threefold increase in serum C′ titer.

Comparable numbers of female mice injected (sc) with diethylstilbestrol and males injected (sc) with testosterone showed no such consistent change. Mice of both sexes injected (sc) with 0.06 ml of sterile saline per week were also studied. Repeated bleeding of the saline-injected group of male mice results in some decrease in the serum C′, but not to the marked extent shown in the hydrocortisone- or testosterone-treated males. Hematocrits and total serum protein levels determined on samples from control groups and groups treated with sex hormones showed no change in the course of the experiment.

Our data do not indicate whether the C′ alterations were due to primary effects of the administered hormones, or to secondary, more generalized influences upon host metabolism. One might speculate upon the relation of the anti-inflammatory properties of hydrocortisone to its anticomplementary effect. That components of the C′3 complex augment certain inflammatory responses (*9*) gains particular interest in view of the report of Weintraub *et al.* that some steroidal effects involve the C′3 complex (*7*). It remains to be determined whether the one or more C′ components involved in sex hormonal alteration of C′ are the same ones affected during hydrocortison treatment.

The ease with which mouse C′ is influenced by exogenous hormones indicates the usefulness of mice for further studies of complement function.

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Mouse Complement: Influence of Sex Hormones on Its Activity

Abstract. *Sex hormones influence the hemolytic activity of one or more of the late-acting components of complement measured in the presence of trisodium ethylenediaminetetraacetate. The titers of the late-acting components in the serums of male mice, normally tenfold higher than those of females, fell after castration, becoming about the same as those of females. The titers of the serums from females rose after these mice were castrated, but castration did not affect the activities of the first, second, and fourth components of complement. Serums of normal and castrated mice of both sexes treated with testosterone showed increased late-acting component activity, whereas the estrogen caused decreased activity. Treatment in vitro of mouse serum with these hormones had no effect on the activity of late-acting components.*

The hemolytic complement (C′) activity of the serum of male mice is greater than that of female mice (*1*, *2*). This difference is due to one or more of the C′ components which act after C′1, C′2, and C′4 (*2*). In an accompanying report, Caren and Rosenberg present data on the influence of hydrocortisone, diethylstilbestrol, and testosterone on the whole C′ activity in mice (*3*). In this report, we present evidence that the amount of at least one of the late-acting components of mouse complement (C'^{m}) is influenced by sex hormones. Hemolytic activity of these late-acting components was determined by measuring the capacity of mouse serum in the presence of 0.01*M* trisodium ethylenediaminetetraacetate (C'^{m}-EDTA) to lyse sheep erythrocytes (E) sensitized with Forssman antibody (A) and containing guinea pig C′4 and C′2 on their surface ($EAC'4^{gp},2a^{gp}$). Before 1958 it was thought that the lysis of EAC′4,2a by C′-EDTA was due to a single C′ component identified as C′3. It is now known that the activity of C′-EDTA is due to several factors, perhaps as many as six (*4*). Serums lacking any

one of these factors are incapable of lysing EAC′4,2a.

To test the hypothesis that sex hormones affect C'^{m} activity, we castrated weanling and adult Balb/c mice of both sexes. At intervals after castration, individual mice were bled and killed by heart puncture, and their serums were tested for C'^{m}-EDTA activity. Controls consisted of mice from which only one gonad was removed, mice which were sham castrated, and untreated mice (Fig. 1). As these mice matured there was a rise in the C'^{m}-EDTA activity of the serums of control animals. The C'^{m}-EDTA activity of the serums of castrated mice stayed at a low level throughout the course of the experiment. The results of a similar experiment with adult male mice are shown in Fig. 2. By 1 week after castration, their serums contained significantly less C'^{m}-EDTA activity than those of the controls; and, like the serums of the castrated weanling males, their C'^{m}-EDTA activity remained low during the period of observation (4 months) and was about the same as the low C'^{m}-EDTA activity normally found in the serums of control adult females. The C'^{m}-EDTA activity of serums from castrated females (weanling or adult) did not change significantly during the first 60 days following castration, after which there was a slight but definite rise.

The thesis that there is a relation between sex hormones and complement was further tested by the administration of testosterone and estrogen (*5*) to castrated and normal mice of both sexes. Four days after a single injection of testosterone there was an increase in the C'^{m}-EDTA activity of the serums of castrated male mice. By the 8th day, C'^{m}-EDTA reached one-half that of normal male adults, but by the 17th day C'^{m}-EDTA activity fell to that observed before hormone treatment.

Table 1. C'^{m}-EDTA titers of serum of mice treated with testosterone or estrogen for 6 to 8 weeks. C'^{m}-EDTA titrations were performed on individual mouse serum as follows: 0.2 ml portions of $EAC'4^{gp},2a^{gp}$ containing 3×10^{7} cells were mixed with equal volumes of varying dilutions of mouse serum in 0.01*M* EDTA buffer. Each mixture was incubated, with frequent mechanical agitation, for 90 minutes at 37°C. Then 3.0 ml of 0.01*M* EDTA buffer were added, and the cells were collected by centrifugation. The supernatant fluid was discarded, the sedimented cells were lysed by addition of 3.0 ml of distilled water, and the optical density of each lysate was measured at 412 mμ. Titers (C'^{m}-EDTA-H_{50}) are expressed as the reciprocal of the dilution of mouse serum giving 50 percent lysis.

Mice			Titer (C'^{m}-EDTA-H_{50})	
Type		No.	Av.	Range
*N ♂	*Testo.	6	19.0	13.2–21.0
N ♂	Vehicle	6	8.6	5.0–13.5
*C ♂	Testo.	6	13.0	8.8–17.3
C ♂	Vehicle	6	3.0	2.1– 4.1
N ♀	Testo.	6	8.8	6.2–14.3
N ♀	Vehicle	6	2.1	1.8– 2.4
N ♂	*Estra.	4	3.9	2.0– 6.2
N ♂	Oil	4	10.0	7.8–13.0
C ♀	Estra.	4	1.8	1.5– 2.0
C ♀	Oil	4		1.5– 2.2

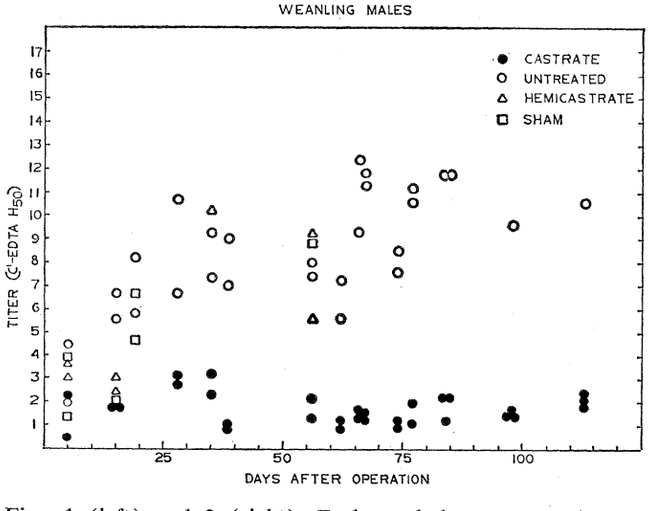
* N, Normal; C, castrated; Testo., testosterone; Estra., estradiol.

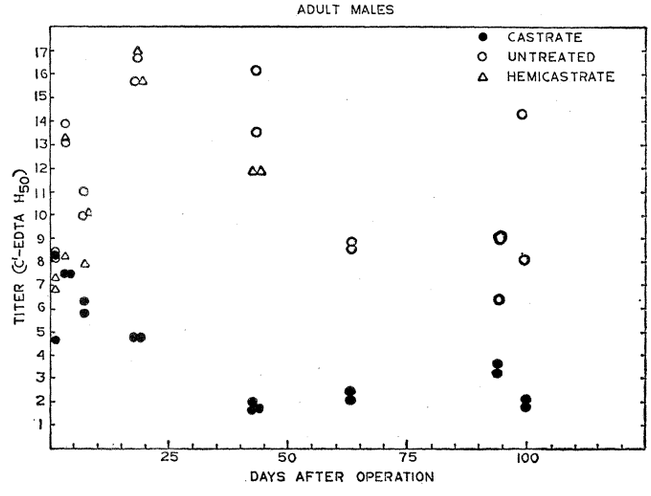
Normal and castrated male and female mice received weekly injections of testosterone. The C'^{m}-EDTA activity of their serums was increased. The serums of a similar group of animals receiving estrogen instead of testosterone had reduced C'^{m}-EDTA activity (Table 1). Testosterone had no effect in vitro on the C'^{m}-EDTA activity of the serum of normal females or castrated males. Estrogen had no effect in vitro on the C'^{m}-EDTA activity of the serum from normal males. Furthermore, there was no reduction in the C'^{m}-EDTA activity of the serum of normal males when mixed with the serum of either normal females or castrated males. It is therefore unlikely that the low C'^{m}-EDTA activity of the serum of normal females and castrated males is due to the presence of an inhibitor.

Several inbred strains of mice (such as, DBA/2, B10D2 "old" line) have a genetically determined deficiency of at least one complement component (*2, 6*). It has been reported that these animals lack a component which may be analogous to human C′5 (*7*). Despite this genetic C′ deficiency these animals are capable of normal Arthus, homograft, and passive cutaneous anaphylaxis reactions and possess normal phagocytic activity and herd immunity (*8*).

Terry *et al.* (*2*) have shown that the serums of C′-deficient mice contain normal levels of C′1, C′2, and C′4 whether the deficiency is sex-related or genetically determined. We have found that the serums of normal and castrated Balb/c mice also contain normal amounts of C′1, C′2, and C′4. Furthermore, observations in our laboratory indicate that the complement component, or components, missing from mice with the genetic deficiency are not the same as the component or components affected by sex hormones.

One genetically C′-deficient strain of mice (DBA/2) is unusually susceptible to carcinogen-induced leukemia (*9*), carcinogen-induced tolerance to tumor allografts (*10*), and to death from





Figs. 1 (left) and 2 (right). Each symbol represents the results of a single C'^{m}-EDTA titration of the serum from an individual mouse. For experimental details of C'^{m}-EDTA titration, see Table 1.

Plasmodium berghei infection (*11*). In each case, females or castrates are more susceptible than normal males. The possibility exists that the increased susceptibility of these animals is related to a deficiency of at least two late-acting components of complement. These observations suggest that mice lacking more than one component of complement may be useful for studies on the in vivo functions of complement.

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Antigenic Differences in the Surfaces of Hyphae and Rhizoids in Allomyces

Abstract. *Immunofluorescent techniques have demonstrated a difference in surface components of hyphae and rhizoids of* Allomyces macrogynus. *An antigenic component detected on the rhizoidal surface may be present, but masked, in the hyphal-wall matrix material. The system also allows visualization of the hyphal wall during aging, when changes from a smooth to a fissured surface are noted, and differences in adsorptive properties occur.*

Many Phycomycetes produce rhizoids as outgrowths of a thallus or of hyphal filaments. These rhizoids are thought to serve for support and, particularly in the case of some of the chyridiaceous fungi where they penetrate the host cells, as absorptive organs. Despite their wide occurrence little is known of their composition or structure. Aronson and Preston (*1*) have published electron micrographs of rhizoids of *Allomyces macrogynus* prepared by chemical treatment, which show microfibrils that are continuous with those in the wall of the parent hyphal segment. Analysis of purified cell wall preparations of this organism has disclosed the presence of components other than the *N*-acetylglucosamine resulting from hydrolysis of chitin microfibrils (*2, 3*). There has been no evidence to indicate that the composition of rhizoid walls differs from that of hyphal ones. When mention has been made of walls of rhizoids they have been analyzed together with hyphal walls and assumed to have the same composition. However, using immunofluorescent techniques, we have shown that differences in the composition of the walls of those two structures do exist in *A. macrogynus*.

Cell wall extracts containing one or two antigens were used to absorb portions of an antiserum which had been produced against whole mycelium. Young plants of *A. macrogynus* were incubated in absorbed antiserum, washed several times, and treated with the same antiserum, unabsorbed but labeled with fluorescein isothiocyanate. The results of such a procedure can be seen in Fig. 1 where only the rhizoids show attachment of the fluorescein-labeled antiserum.

The rabbit antiserum was prepared by injecting rabbits beneath the scapulae with ground lyophilized vegetative mycelium mixed with Freund's adjuvant. We used serum from a single bleeding; it showed at least seven precipitin lines when tested by the Ouchterlony technique with fresh mycelial extracts. Labeled antiserum was prepared from a globulin fraction of the specific serum by the method of Riggs *et al.* (*4*). Fluorescein-labeled goat antiserum to rabbit globulin (*5*) was used in some tests.

Table 1. Fluorescent antibody labeling patterns of 2-day-old mycelium of *Allomyces macrogynus*.

Antiserum treatment of mycelium		Fluorescence	
First	Second	Hyphae	Rhizoids
Absorbed	Labeled	−	+
Labeled	None	+	+
Unabsorbed	Labeled goat*	+	+
Labeled goat	None	−	−
Unabsorbed	Labeled	−	+†
Absorbed	Labeled goat*	+	+

* Goat antiserum to rabbit globulin. † Weak positive.

Various procedures have been employed to release antigens from the cell walls, including enzymatic digestion and treatment with sodium deoxycholate. As many as three antigens were detected in such extracts and each can be obtained by more than one extraction procedure. Preparation of the cell walls by mechanical means and a preliminary chemical analysis of antigen-containing fractions have been described (*2, 6*).

Two different absorbed antiserum preparations were used in these experiments: One (I) was absorbed with a heated enzymatic wall digest which contained one antigen, and the other (II) was absorbed with a digest containing two antigens, one being common to both extracts. Both preparations gave identical results in the labeling patterns obtained. Absorptions were carried out by mixing equal volumes of the antiserum and the antigen solution and incubating the mixture at 4°C for 48 hours (I) or 1 week (II). Precipitates were removed by centrifugation, and the supernatants were tested for excess antigen or antibody. Preparation I contained excess antigen; preparation II gave no detectable precipitin lines in the agar diffusion tests with either antiserum or antigen. Figure 4 shows a double diffusion test prepared with one of the wall digests and several of the serum samples used.

Two- or three-day-old plants grown from zoospores in Emerson broth were used for most experiments. Plants grown