of a rapidly migrating antigen displaying esterase properties (Fig. 4). This esterase was hardly visible in normal membrane extracts and not at all in the microsomal supernatant ("cell sap") of total liver. Extracting it from parallel agar strips and injecting it into rabbits gave rise to the formation of antiserums rendering only one esterase-active precipitate, when tested with microsomal extracts. This precipitate was electrophoretically identical with the rapidly migrating enzyme seen in Fig. 4.

The great similarity between rough and Sa membranes (Fig. 5) agrees well with electron microscopical and biochemical findings (*8*). It could be assumed that the antigen which is specific for the rough membranes could be of ribosomal origin. So far RNA has not been identified in this component.

It is not surprising to find a variety of microsomal esterases, since liver microsomes contain many hydrolytic enzymes (*10*). Although some of our esterases may represent multiple aggregates of the same protein or the same enzyme protein adsorbed to different carrier proteins, others such as e_4^t, e_5^k, and e_1^x most probably constitute different molecular entities. From the evidence at hand it cannot yet be stated to what extent they represent isoenzymes. Phenobarbital-stimulated induction of esterases hydrolyzing various drugs has been observed (*7*). However, the nature of our phenobarbital-induced esterase e_1^x is as yet unknown. The typical electrophoretic mobility of this enzyme makes it particularly suitable for separation and further biochemical studies.

An important finding of our study was the deviating composition of the extracts of the Sb membranes. Our findings are in agreement with those of Dallner who found that the enzymatic composition of this fraction was qualitatively and quantitatively different from that of the other submicrosomal fractions. The question of the subcellular localization of this fraction is unsettled, and it is not even known whether or not it is of parenchymal origin. However, the presence of two typical marker antigens (p_5 and p_6, Fig. 5) should greatly facilitate its further characterization.

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Steroids and Serum Complement in Mice: Influence of Hydrocortisone, Diethylstilbestrol, and Testosterone

Abstract. *Hydrocortisone depresses hemolytic complement in male and female mice. Testosterone causes increase of serum complement in female mice, and diethylstilbestrol causes decrease of serum complement in male mice, in each instance to activities approximating those found normally in the opposite sex. Male and female sex hormones have no effect, in the doses used, on the serum complement of male and female mice respectively.*

Complement (C′), a group of naturally occurring serum proteins, is believed to enhance immunological reactions. Among the endocrine organs which have been studied for an effect on the complement system are the hypophysis (*1*), the gonads (*2*), the thyroid gland (*1, 3*), and the adrenal gland (*1, 4*). No coherent picture emerges from these studies, and for good reason. Difficulties are encountered in measuring effects on the complement system, since it consists of at least nine distinct serum proteins, five of which have but recently been recognized. Changes in the concentration of one or more of these components may or may not be detected in any particular assay of complement.

There is a well-documented difference in amounts of C′ in male and female mice (*5, 6*). Since this difference suggests the possibility of a hormonal control of a C′ component determinative of the C′ titer, as measured by hemolytic assay, this species offers favorable material to study. We report on the effects of relatively large doses of an androgen, a synthetic estrogen, and an adrenocortical steroid on hemolytic complement action in mice. The accompanying report of Weintraub *et al.* (*7*) supplements our study and goes beyond it in attributing some of the observed effects to one or more of the late-acting C′ components.

Serum C′ levels are expressed as units of C′ per milliliter which yielded 50 percent lysis of a standard number of red blood cells sensitized with antibody (*5*). The dosage, vehicle, and route of administration of hormone are indicated for each experiment. The mice used were the inbred C57Bl/10-h-2d (new line) raised in this department

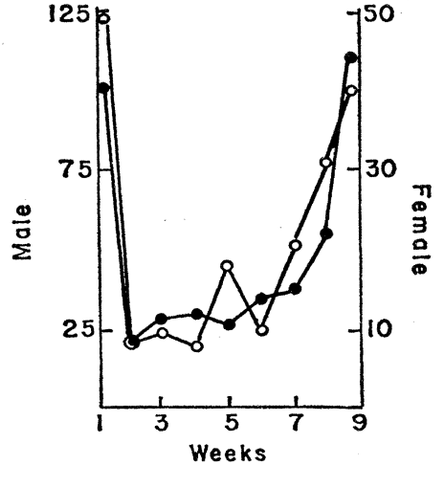

Fig. 1. The depression of serum C′ in male and female mice as a result of a single injection (sc) of 10 mg of hydrocortisone. *Abscissa*: Time in weeks after treatment. *Ordinate*: Mean titers ($C'H_{50}$ units per milliliter) in serum of male (left) and female (right) mice. ○——○, Average of six male mice determined each point; ●——●, average of three female mice determined each point.

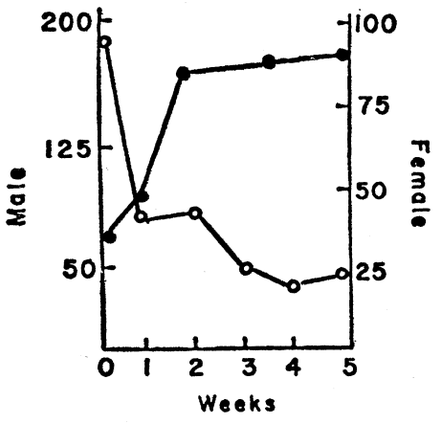

Fig. 2. The depression of serum C′ in male mice by diethylstilbestrol and the elevation of serum C′ in female mice by testosterone. ○——○, Males injected (sc) with a weekly dose of 1.5 mg diethylstilbestrol in two or three injections per week. Average of seven mice determined each point. ●——●, Females 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