nique (*4*) or the tanned erythrocyte procedure (*9*), it is suggested that these discrepancies may be attributable to the fact that different substances are being measured by the different indicator systems. Thus, one is drawn to the conclusion that either there are several different properdins, or that properdin is a family of cross-reacting antibodies capable of combining with zymosan. The inability to correlate properdin levels determined by the various procedures might then be due to the fact that antigens such as tanned erythrocytes, T2, T6, and T7 phage are measuring a specific portion of the properdin or normal antibody pool which may vary both in quantity and quality from serum to serum.

Before the conclusion is drawn that properdin is normal antibody, however, one distinguishing feature of the properdin system should be stressed. This is the demonstration by Pillemer *et al.* (*10*) that C′ is required not only for the manifestation of the viricidal, bactericidal, and hemolytic reactions attributed to the properdin system but also for the combination of properdin with zymosan and other polysaccharides reactive in the system. While C′ may be essential for bactericidal and hemolytic reactions by immune antibody, it is clear that it is not required for the formation of antigen-antibody complexes. Thus, if properdin is to be considered normal antibody, it will be necessary to revise our concepts concerning the nature of normal antibody-antigen reactions in order to incorporate C′ as an essential cofactor. While it may be premature to postulate a role for C′ in such reactions, the multivalent or cross-linking concept of C′ as presented by Weigle and Maurer (*11*) might be considered with respect to this problem. It is possible to envisage C′ as a stabilizer of readily dissociable immune complexes by virtue of its cross-linking activity. Thus, it might both play a role in the formation of certain low-avidity antibody-antigen complexes and serve as an essential component for the manifestation of such unions where bactericidal or hemolytic reactions are involved. It is hoped that experiments being undertaken may shed further light on the role of C′ in properdin or normal antibody reactions, or both (*12*).

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Infection of Human Volunteers with Type 2 Hemadsorption Virus

The hemadsorption viruses, types 1 and 2, are new members of the myxovirus group which have recently been recovered from infants and children with respiratory illness (*1*). Subsequent studies have provided evidence that a considerable proportion of respiratory illness in children during the winter of 1957–1958 was associated with the hemadsorption viruses (*2*). A study to determine whether type 2 virus could produce infection and illness in adults was performed with male volunteers (*3*). Although placebo controls were not employed in this pilot study, valuable information was acquired because illness was correlated with the occurrence of infection.

The virus used for the inoculum was derived from an infant with acute laryngotracheobronchitis. The original throat swab specimen from this infant was inoculated into a monkey kidney tissue-culture flask maintained with Eagle's basal medium without serum. After 7 days' incubation at 36°C the tissue culture fluid was filtered through a Selas 0.03 filter and stored at −50°C. Safety tests were carried out in monkey kidney and HeLa cultures and in suckling and adult mice, guinea pigs, rabbits, and various bacteriologic media. No bacteria or contaminating viruses were detected.

Thirty-two healthy male volunteers, ranging in age from 21 to 46 years (mean, 28 years) were selected from among the inmates at the Patuxent Institution at Jessup, Md. Seven of the men did not have neutralizing antibody for type 2 virus. The remaining 25 volunteers had antibody levels of 1:4 to 1:128 (mean of 1:21). The volunteers were isolated in separate cells and had an independent medical examination each day by two physicians. The laboratory techniques for virus isolation, virus identification, and serologic testing by hemagglutination-inhibition, complement-fixation, and neutralization have been described (*1*).

Each volunteer was given a total of 1 ml of undiluted tissue culture fluid by swabbing the posterior oropharynx and the lower palpebral conjunctivae and by spraying and instillation into the nose and oropharynx. Each volunteer received 80 TCD_{50} as determined by a simultaneous titration.

Throat swabs taken from all the volunteers on the 2nd, 3rd, 6th, and 7th days were tested simultaneously for virus in monkey kidney tissue culture by the hemadsorption technique (*4*). Type 2 virus was recovered from 24 of the men. Virus was not detectable on the 2nd day after inoculation but was isolated from 10 of the volunteers on the 3rd day. Twenty-two were positive on the 6th day, with 12 of the isolations coming from individuals with a negative test on the 3rd day. Nineteen volunteers had virus on the 7th day, with two of these individuals shedding virus for the first time.

A total of 25 men developed a rise in antibody for type 2 virus. Each of the volunteers from whom virus was recovered had a serologic response. Twenty-two men developed a rise in neutralizing antibody, 18 in hemagglutination-inhibition antibody, and 14 in complement-fixing antibody. The one individual with a positive serologic response from whom virus was not recovered developed antibody demonstrable by neutralization, hemagglutination-inhibition, and complement-fixation. None of the volunteers developed a rise in complement-fixing antibody for adenovirus or Asian influenza.

When clinical illness failed to develop by the 5th day, the men were released from isolation. On the 6th day, however, six of the men reported to the infirmary complaining of respiratory illness and immediately all volunteers were returned to isolation. Examination of the other volunteers revealed that additional individuals had onsets of respiratory illness on this day. The onset of these illnesses coincided with the time when the greatest number of volunteers had virus demonstrable in their upper respiratory tracts. For the most part the illness was mild with prompt and uneventful recov-

ery occurring within 2 to 3 days. The volunteers were released from isolation on the 11th day.

The most common symptoms of this "cold-like" illness were nasal obstruction and discharge, coughing, and sneezing. Less frequent were sore throat, headache, ocular complaints, vague chest pain after coughing, and chilliness. Aches, vomiting, anorexia, and hoarseness were present in an occasional individual. Physical examination revealed a reddened edematous pharynx at the onset of clinical symptoms. A rhinorrhea that was serous and later mucoid, and inflamed nasal mucosa and tonsils, were the next most common findings. Four individuals had lacrimation and a mild conjunctivitis. Lung fields were clear by auscultation, percussion, and x-ray. Four men, one of whom showed no evidence of infection, had a low-grade fever (100 to 101°F). White blood counts were within normal limits.

Eighteen of the volunteers developed clinical illness. In this group, 17 were shown by laboratory tests to have been infected with type 2 virus; this agent was isolated from 16 of the men, and the remaining volunteer developed an antibody rise. In the group of 14 who did not become ill, only eight were infected with type 2 virus. A significant correlation between clinical illness and infection is suggested by the data (P equals 0.035 by the chi-square test with Yates correction for small numbers). The physicians who examined the volunteers were in good accord about what constituted clinical illness.

Eight days after the premature release of the volunteers from isolation a small outbreak of respiratory illness occurred in the general prison population. Eighteen individuals who were not in the study became ill with a mild "cold-like" illness without fever that lasted 2 to 3 days. Type 2 virus was recovered from eight of the individuals in this group.

The results of this study suggest that type 2 virus can cause respiratory illness in adults. This conclusion is consistent with the findings of previous epidemiologic investigations which indicated an association of this virus with minor as well as severe respiratory illness in small children (*2*).

It is of interest that of the 25 men who were infected with as small a quantity of virus as 80 TCD_{50}, 18 had neutralizing antibody levels of 1:4 to 1:64 (mean of 1:13) prior to challenge. Previous antigenic experience either with type 2 virus or a related agent may be one of the factors responsible for the mildness of the observed illness. The prolonged incubation period seen with type 2 virus is not unlike that observed with a nasal secretion (NS 111) employed by Jackson *et al.* in their volunteer studies (*5*). In the present study the apparent long incubation interval could reflect either a property of the virus or the small size of the challenge inoculum.

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Induction of Tolerance of Skin Isografts from Male Donors in Female Mice

In certain inbred strains of mice, Eichwald and Silmser (*1*) and subsequently other workers (*2*) have observed that whereas skin *isografts*—that is, grafts transplanted between members of the same inbred strain—are permanently accepted when transplanted between animals of the same sex or from females to males, females usually reject grafts from male donors after an initial period of well-being of highly variable duration. Similar results have been obtained with isografts of thymic and of lymph node tissue (*3*).

Table 1. Fate of skin isografts transplanted to mice of the C57BL/6 strain.

Sex		No. grafted	Distribution of survival times of grafts (days)		
Donor	Recipient		< 30	> 30 < 60	> 60
M	M	16			16
F	F	23			23
F	M	29			29
M	F	29	9	16	4*
M	F†	20			20

* Breakdown of three of these grafts was complete by 100 days.
† Injected at birth with male spleen cells.

The most obvious and acceptable explanation of this rejection of tissues of male origin by the females is that originally postulated by Eichwald and Silmser (*1*). Like the destruction of skin *homografts* transplanted between mice of *different* strains, it is the result of an immunological response or sensitization on the part of the host against a "foreign" transplantation antigen (or antigens) in the graft, determined, in the present instance, by a histocompatibility gene (or genes) located on the Y chromosome and therefore not present in females. The strongest evidence in support of this immunogenetic interpretation is the fact that a female which has already rejected a male graft will reject a subsequent graft from a male donor much more rapidly (*1*)—that is, there is a "second-stage phenomenon" (*4*), as with skin homografts. Attempts to discover an endocrinological factor in the rejection of these male isografts have so far been unsuccessful (*5*).

In an attempt to obtain further evidence that the rejection of male tissues by females is a straightforward transplantation immunity phenomenon, we have attempted to induce a state of immunological tolerance (*6*) in female mice with respect to the putatively antigenic tissues from male donors. For this work mice of the C57BL/6 strain were employed, since the females were known to reject male grafts with almost complete uniformity and more rapidly than females of the other strains so far investigated. In our control series (Table 1), 28 of 29 females rejected grafts from male donors within 100 days, the median survival time of these grafts, with its confidence limits, being 33.8 (28 to 41 days).

Forty-seven newborn mice, belonging to seven litters, were each injected intravenously with a suspension containing 5 to 10 million living spleen cells prepared from adult male donors. When the animals had grown up, each of the 20 females present was challenged with a graft of isologous skin from a male donor. These grafts have now been under careful observation for 60 days. The fact that all of them are still in perfect condition, with excellent hair crops, demonstrates that a very high degree of tolerance must have been conferred upon the females as a consequence of their exposure to the "male" antigen (or antigens) at birth.

Besides giving strong support to the straightforward immunogenetic explanation of the rejection of male isografts by females, the present findings show that the induction of a very high degree of tolerance is possible, even in response to an exceedingly feeble antigen. Attention is also drawn to the fact that an inbred strain in which practically all the