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

females reject male grafts may be regarded as a ready-made source of "isogenic resistant" (*7*) animals, and this may have some practical application for immunological studies (*8*).

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Effects of Intracerebral Injection of Anticholinesterase Drugs on Behavior in Rats

Krech, Rosenzweig, *et al.* have reported that the level of cholinesterase in localized brain areas correlates with the maze learning behavior of rats (*1*). Animals that utilized visual cues (visual hypothesis) to run an insoluble maze had a lower average cholinesterase activity in visual and somesthetic areas than those that followed spatial cues (spatial hypothesis). These workers proposed (i) that the cortical cholinesterase level is directly proportional to the rate of cortical acetylcholine metabolism; (ii) that a high level of acetylcholine metabolism implies a readier synaptic transmission of impulses, which is responsible for the more adaptive, probabilistic, spatial hypothesis; and (iii) that a low rate of acetylcholine metabolism results in a more stimulus-bound, visual hypothesis. An indirect test of these propositions was the finding that rats displayed increased visual hypotheses in maze running after intraperitoneal injection of small doses of pentobarbital sodium. This drug inhibits acetylcholine synthesis (*2*).

In the study reported here (*3*), attempts were made to test whether the cortical level of acetylcholine influences the maze running behavior of rats. Two anticholinesterase drugs, physostigmine (eserine) and diisopropyl fluorophosphate (DFP), were injected either directly into the visual or somesthetic cortex, or directly into one of the lateral ventricles. Such injections were expected to inhibit cerebral cholinesterase, leading to an increase in cortical acetylcholine. A subsequent shift from visual to spatial hypotheses in rats so treated would support the propositions set forth above.

Thirty-four naive male hooded rats, 3 to 4 months old, were used (*4*): 13 of the Tryon Maze-Bright strain S1 (spatial hypothesis), 13 of the Tryon Maze-Dull strain S3 (visual hypothesis), and 8 of a crossed strain between these two (S13). The experimental period for each rat ranged from 25 to 115 days. We used a four-unit Y-maze (*5*), and followed the training method and schedule of reinforcement used by Krech *et al.*

All animals were operated upon under ether anesthesia. Four 1/8-in. holes were made in the skull, bilaterally above the visual and somatic areas, for intracortical injections. Such injections were made, without use of anesthetic, by inserting a 26-gauge needle, 2.5 mm long, through the skin and into the holes. Sometimes 2 percent procaine hydrochloride was put on the scalp before injection. For intraventricular injection, a ventricular tube was implanted unilaterally with stereotaxic apparatus (*6*). To make such injections, a 27-gauge needle, long enough to reach the lateral ventricle, was inserted through the tube.

After appropriate taming and food deprivation, preliminary training consisted of 6 days in which the rats were given either a random-reward or a progressive-reward schedule. Drug experiments were run with a random-reward schedule. Twelve trials (48 choices) were given each day. There were 2 days of testing with drug injection, 2 days of testing with saline injection, and then 2 days of testing without injection. This sequence was repeated on the same rats up to five times. Seven micrograms of eserine or DFP, in 0.10 ml of saline, were injected into both visual cortical areas or both somatic areas of each animal on each day of intracortical injection. This dosage was found to be just subconvulsive. For intraventricular injection, three different concentrations of eserine were used (2, 4, and 7 μg in 0.05 ml of saline). In control experiments, the appropriate volume of normal saline was used. Since some fluid may back out from the needle track after injection, the actual amount and distribution of drug in the brain was uncertain (*7*).

No overt behavioral effects were observed in about 30 percent of the drug injections. In the remaining 70 percent of the cases (see Table 1) general behavioral disturbances of various sorts were evident. These ranged from excessive washing, grooming, and chewing to tremor, ataxia, incoordinated walking, and, after intraventricular injection, circling to the side opposite the injection. Finally, grand mal convulsions occurred in 16 cases after drug injection and in six cases after saline injection. The rats usually started to run the maze immediately after injection. When there were severe motor disturbances or convulsions, they remained immovable for periods of from 5 to 15 minutes before running.

The results of maze testing are summarized in Table 1. The number of choices of each rat for each day was calculated, according to the criterion of Krech *et al.*, as visual hypothesis, spatial hypothesis, or no hypothesis (a minimum of 33 out of 48 choices of light or dark was scored as visual hypothesis, of

Table 1. Total number of days on which rats of each strain displayed visual, spatial, or no hypothesis behavior, and general behavioral disturbances under each of the experimental conditions. The number of days of testing of each rat varied from 4 to 30. Every rat of each strain was not run under all the conditions. S, spatial hypothesis; V, visual hypothesis; No, no hypothesis; Beh, general behavioral disturbances; Occ., occipital area; Som., somatic area.

Injection	S_1 (spatial) $N = 13$				S_3 (visual) $N = 13$				S_{13} (mixed) $N = 8$			
	S	V	No	Beh	S	V	No	Beh	S	V	No	Beh
Intracortical												
Eserine, Occ.	7	0	3	5	1	7	4	10	4	2	4	2
Eserine, Som.	7	3	3	10	6	9	5	12	7	0	3	4
DFP, Occ.	6	0	3	6	2	6	1	6	—	—	—	—
DFP, Som.	5	0	2	7	0	7	1	6	—	—	—	—
Saline, Occ.	8	3	3	0	5	9	2	2	7	2	2	0
Saline, Som.	13	0	3	3	4	12	2	1	3	0	3	0
No injection	20	2	2	0	11	27	8	0	3	1	5	0
Intraventricular												
Eserine (2 μg)	7	0	1	1	1	5	2	1	2	4	0	0
Eserine (4 μg)	6	0	2	4	0	8	2	6	2	3	1	2
Eserine (7 μg)	11	3	5	13	2	6	0	5	5	6	4	5
Saline	14	2	4	3	3	15	4	3	4	7	5	1
No injection	25	3	9	0	2	21	6	0	3	12	6	0