indicative of gonotrophic dissociation; in other cases, follicles were measured and ovaries with the largest follicle measuring less than 300 μ in length were considered to be undeveloped. In no case would the difference in criteria used have resulted in a difference of classification of an ovary.

The results of the experiments with incandescent illumination are shown in Fig. 1 for *C. pipiens* and in Fig. 2 for *C. quinquefasciatus*. The responses of the two species under fluorescent illumination differed in minor details only, and are not presented. Ovarian development was suppressed in *C. pipiens* in response to both low temperature and short photoperiod, whereas the percentage of fed *C. quinquefasciatus* females developing ovaries was high under all conditions.

In experiments to determine the effect of the postfeeding incubation temperature, groups of *C. pipiens*, which had taken blood after having been subjected to conditions of 10°C and 12 hours photoperiod, were divided into two groups. One group was incubated at 15°C and the other at 25°C. In the females incubated at 15°C, ovarian development was suspended in about half the individuals as before, but in the group incubated at 25°C, ovarian development occurred in over 90 percent of the females. Gonotrophic dissociation, then, is dependent not only on conditioning of mosquitoes by a combination of low temperature and short photoperiod, but also on a low temperature of incubation after the feeding. Failure of some workers to demonstrate gonotrophic dissociation in *C. pipiens* in the laboratory (*7, 8*) may have been due to incubation at high temperatures after feeding.

The comparative absence of gonotrophic dissociation observed in *C. quinquefasciatus* is interesting, as it contributes to other evidence that this species cannot hibernate in areas where winters are severe. Additional evidence to support this contention is furnished by the results of the blood-feeding trials (*6*). There has been a long search for factors that limit the northward expansion of the range of *C. quinquefasciatus* into the range of *C. pipiens*, and inability to hibernate often has been cited as a possible limitation.

The demonstration of gonotrophic dissociation in *C. pipiens* in the laboratory adds little to our knowledge of its possible occurrence in this and other species of *Culex* in nature, but it certainly indicates that the question of survival of virus over a winter period is not closed, and that the possibility of hibernating females of vector species acting as reservoirs needs further study.

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Rh Factor: Prevention of Isoimmunization and Clinical Trial on Mothers

Abstract. *The results on the use of γG-immunoglobulin to Rh factor for the prevention of active immunization of Rh-negative mothers at risk appear most promising. One hundred and seven mothers in the clinical trial have been followed for periods of about 6 months to 1½ years after delivery. Of these, 48 were treated mothers who received 5 ml γG-immunoglobulin to Rh, and 59 were untreated mothers. Of the 48 treated mothers none are actively immunized; seven of the 59 control mothers have become actively immunized to Rh.*

The observation by Theobald Smith in 1909 (*1*), that in the presence of passive antibody the corresponding antigen will not immunize, has been confirmed and studied by others (*2*). From these many reports there emerged the new immunological principle that passive immunity strongly suppresses active immunity. But apparently a specific practical use for it as a means of suppressing the immune response has not been considered before.

Levine (*3*) has established that if the mother has an existing circulating antibody directed against the baby's red cells—for example, antibody to A that would be present in group O, Rh-negative mothers with a group A, Rh-positive baby—then immunization to Rh by pregnancy is uncommon. It is extremely difficult to immunize Rh-negative volunteers to Rh with injection of ABO-incompatible Rh-positive cells or with ABO-compatible cells which have been coated in vitro with an excess of antibody to Rh_0 (*4*).

In 1960 we put this principle to use and initiated a program to determine whether initial immunization of Rh-negative mothers could be prevented by the passive administration of Rh antibody immediately after childbirth (*5*). At the same time, quite independently and by another approach, Finn *et al.* (*6*) began experimental work, guided by this identical concept.

We first procured a sterile preparation of γG-globulin containing very large amounts of antibody to Rh. Starting with pooled serum with high titer from a small number of donors, γG-immunoglobulin to Rh factor was prepared, filtered, and packaged sterile in 5-ml vials as a 16.5 percent solution suitable for intramuscular injection. This material was pure γG, free from γM-globulin (19*S*). The method of processing has the effect of increasing the original antibody titer (to Rh) about 100-fold even though 75 percent of the original antibody activity present in fractions I, III, and IV is excluded in the process. Intramuscular injection of Rh-negative individuals with 5 ml of this material has produced artificial titers of antibody up to 1:128, 1 ml up to 1:32, and 0.1 ml up to 1:2.

First used in 1961, approximately 200 doses of our material have now been given to more than 120 Rh-negative individuals, with no resulting side effects. Apart from the possible accidental use in an Rh-positive individual, this material should be as safe as the commercial γ-globulin currently used for the prevention of rubella, hepatitis, and other such diseases. The experience with millions of doses of fraction

II γ-globulin has been that it does not transfer serum hepatitis.

In our first study, at Sing Sing Prison (*5*), there were nine Rh-negative male volunteers; four were treated with γG to Rh before each red cell stimulus and five acted as controls. All received an injection of 2 ml of Rh-positive blood each month for 5 months, and their blood was examined for a year. None of the treated group were immunized, but four out of the five controls became highly immunized to the Rh factor after this intensive stimulus. The second trial was then begun with 27 Rh-negative men (14 in the treated group, and 13 in the control group). On day 1 of the study all 27 men received intravenous injections of 10 ml of Rh-positive blood. Then 3 days after this red cell stimulus, the treated group (14 men chosen randomly) received intramuscular injections of 5 ml of γG to Rh. All were observed for 6 months; at 6 months none of the treated men were immunized and 6 of the 13 controls were immunized. After this, 11 of each group were given a second stimulus of 5 ml of Rh-positive blood, and the treated groups were again given 5 ml of the γG, this time 2 days after the red cell stimulus. Six months later (18 months after the start of the experiment) none of the treated men were immunized, and two more of the control group—for a total of 8 of the 13 original controls—were now immunized to the Rh factor.

Ten months after the second injection the lack of immunity in the γG-protected group was tested in nine of these men by a third antigenic stimulus of 1 ml of Rh-positive blood without the γG cover. If any of these men had attained even an extremely low level of immunity from their two earlier antigenic stimuli, they would be expected to make an accelerated or secondary immune response to this third Rh-provoking stimulus. All failed to show antibodies to Rh in their serum 24 weeks later (*5*). This meant that the suppression of antibody formation was complete, and that the men had not been left in a primed state or sensitized by their two previous (γG-covered) antigenic stimuli with Rh-positive red blood cells. Subsequently 8 of the original 14 treated volunteers received a fourth and fifth antigenic stimulus of 10 ml of Rh-positive blood without the γG-globulin cover and two of the eight are now actively immunized to Rh. Thus it is not a question that they could not be immunized at all to Rh, but rather that they were indeed protected on the previous occasions by the γG cover.

Table 1. Summary of results (numbers) of trials of γG-immunoglobulin to Rh in male volunteers.

Subjects	1st trial 1962–63	2nd trial 1963–65
Test groups		
Actively immunized	0	0
Volunteer	4	14
Control groups		
Actively immunized	4	8
Volunteer	5	13

These results showed, first of all, that our γG to Rh was quite safe. There were no side effects whatsoever. It provided complete suppression of immunization to the Rh factor in subjects heavily stimulated with Rh-positive cells (that is, with circulating antigen). It could be given up to 72 hours after the red cells and still provide a complete effect. It also did not support the suggestion of Cohen and Allton that under certain conditions passive antibody might enhance rather than suppress immunity. Such enhancement would be disastrous if it were inadvertently caused in Rh-negative mothers. We have not yet seen any sign of enhancement, and our own studies have since been extended to lower doses (that is, as little as 0.0001 ml of γG-globulin intramuscularly).

Because of the favorable findings of the male volunteer study, a trial in Rh-negative mothers was begun in April 1964. In this trial, which is still continuing, 4.5 ml of γG to the Rh_0 factor is injected intramuscularly into non-immunized Rh-negative mothers within 72 hours of delivery of an ABO-compatible Rh-positive baby. These mothers and noninjected controls are being examined at intervals with antibody screening (Table 2). In this study, the results of Kleihauer testing for fetal cells do not have any influence on whether or not a mother is admitted to the study, all mothers at risk being included.

Table 2. Clinical trial on prevention of immunization of Rh-negative mothers at risk. Results are numbers of individuals between about 6 months and 1½ years after delivery; $P = .05$.

Patients*	Immune antibodies present	No immune antibodies present
Protected†	0	48
Control	7	52
Total	7	100

* From the Rh Antepartum Clinic of the Columbia-Presbyterian Medical Center, New York City.
† In each of these protected patients the passively administered antibody had completely disappeared by about 6 months after delivery, at which time (and afterwards) no antibody could be detected by the saline, enzyme, or the "antiglobulin" methods; presumably these mothers have been protected. Two of these mothers have now been delivered of a subsequent Rh-positive, ABO-compatible, unaffected infant and have received their second injection of γG-globulin to Rh factor.

Of 174 mothers (84 protected and 90 controls) admitted to date to the clinical trial, 107 have been followed for periods of approximately 6 months to 1½ years after delivery. That no protected mother had become actively immunized was proved in every case: no antibody at all (either passive or active) could be demonstrated in each mother's serum when tested 6 months or more after delivery by both the indirect "antiglobulin" and saline methods and also by the enzyme technique. Because the presence of passive antibody might obscure early active antibody formation we did not consider any results of our clinical trial valid until the "protected" mothers had been followed until all passive antibody had disappeared. In another study (*8*), lack of active immunity was presumed in some mothers with passive antibody still present at 3 months after delivery, if no antibody could be demonstrated by the "saline" method. The absence of "saline" antibody is not a strictly valid criterion for nonimmunity because in our studies on male volunteers, over the past 3½ years, it was not at all uncommon for active immunization to the Rh factor to occur without the appearance of "saline" antibodies.

The positive trend of our results is now being confirmed by the results of others (*8, 9*). However, the final proof of the complete efficacy of this preparation will come only when the results from a number of subsequent Rh-positive pregnancies are known. In any event, the outlook is fairly promising that γG-immunoglobulin to the Rh factor will soon become a practical public health measure for the prevention of Rh hemolytic disease of the newborn.

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Visual-Cliff Preference by Infant Rats: Effects of Rearing and Test Conditions

Abstract. *In monocular tests, normally reared infant rats, aged 21 to 22 days, fail to show a side preference on the visual-cliff apparatus. Rats of the same age, but reared in an "enriched" environment, prefer the shallow side of the apparatus. In binocular tests, even normally reared animals prefer the shallow side, although experimentally reared animals show a stronger preference. The results appear to reopen the question of what cues are employed on the visual cliff.*

When placed in the visual-cliff apparatus, young animals of many species prefer to descend to the glass plate covering the shallow side rather than the deep side (*1*). Full understanding of this phenomenon may go far toward clarifying the genesis of depth perception. To this end we compared binocular and monocular performance on the visual cliff of infant rats having different early environmental histories. Both these factors have been investigated before, but never jointly (*2, 3*). Our results indicate that, in monocular tests, normally reared infant rats show no preference on the visual cliff, whereas rats reared in an "enriched" environment prefer the shallow side. In binocular tests, even normally reared animals prefer the shallow side.

The subjects in this experiment were 246 male and female hooded rats, aged 21 to 22 days, bred and reared in the University of Pennsylvania colony. They were housed until 10 days of age in normal maternity cages (38 by 23 by 23 cm). Then before the infants' eyes had opened, the litters and mothers were transferred to one of two rearing environments: for the control rats, the transfer was to a clean maternity cage; for the experimental rats, to a larger cage (63 by 23 by 23 cm) containing three hardware-cloth climbing platforms and a feeder suspended on one side, which could be climbed upon. The climbing platforms and suspended feeder were intended to provide the experimental animals with greater opportunities to experience cues as to depth and distance. In both types of cage the floor was covered with wood shavings.

On the test day the members of each litter were assigned to the various testing conditions. Half the rats were tested monocularly; half, binocularly. The animals were rendered monocular by application of a few drops of flexible collodion in 50-percent solution with equal parts of ether and ethyl alcohol, a technique used previously on chicks (*4*) but not on rats. The collodion was applied with a long cotton swab to the hair immediately above the eye, which closed as the swab approached. The collodion-covered hair was spread over the closed eye and made to adhere to the hair below the eye. Another drop or two was then applied, which soaked in and secured the eyelid shut. The entire procedure took no more than 1 minute; its only observable side effects were some squealing at the time of first application and some scratching for a few minutes thereafter. To control for these side effects, as well as for the effects of handling, we applied collodion solvent to one (closed) eye of each binocularly tested rat, and collodion solution directly above and below that eye. In every subgroup approximately equal numbers of animals had the left or right eye treated. Before being placed on the cliff, every rat was held and prevented from scratching for 100 seconds, and was then kept in an adaptation cage for 5 minutes. While the rat became accustomed to the collodion, the glass surfaces of the cliff were cleaned and interchanged to equate any olfactory cues from previous animals. After each animal was removed from the cliff, its eyes were examined to check that they looked the same as when the rat was placed on the cliff.

We performed a control experiment to ascertain the efficacy of the occlusion procedure. Thirty-one infant rats from the two rearing conditions were prepared as in the monocular testing condition but with both eyes covered. Of the 20 that descended from the start-board, 11 went to the deep side and 9 to the shallow side. These results, taken with the observation of solidly covered eyes, indicated that the collodion preparation successfully occluded vision.

The visual cliff used in this experiment, patterned after Walk and Gibson's model II cliff (*1*), consisted of two 38- by 48-cm glass plates that were separated by a 48- by 10-cm start-board located 4.5 cm above the glass surface. On the shallow side the pattern was placed immediately below the glass plate. On the deep side the pat-

Table 1. Number of rats, by groups, that chose the shallow side or the deep side, or made no choice within 10 minutes.

Conditions		Cliff					
		Normal			Equated texture density		
Rearing	Viewing	Shallow	Deep	No choice	Shallow	Deep	No choice
Normal	Monocular	15	10	6	11	14	13
Normal	Binocular	18	7	3	21	4	8
Experimental	Monocular	17	8	8	19	6	8
Experimental	Binocular	22	3	3	22	3	3