Table 1. Concentrations of catecholamines in the plasma of rats.

Sex or state; No. of samples	Concentration ± SE (μg liter^{-1})	
	Norepinephrine	Epinephrine
Male, 6	0.73 ± 0.16	1.38 ± 0.15*
Estrus, 7	0.44 ± 0.05†	2.74 ± 0.39
Diestrus, 5	1.14 ± 0.24	1.98 ± 0.13
Metestrus, 4	1.08 ± 0.25	2.17 ± 0.56
Pregnant, 8	0.26 ± 0.13‡	2.33 ± 0.10

* Statistically different from estrus, diestrus, and pregnancy, $P < .05$. † Statistically different from diestrus, $P < .05$. ‡ Statistically different from metestrus, diestrus, and male, $P < .05$.

rine to norepinephrine in the plasma could alter the relative uptake of these two amines by the uterus and thus change the relative amounts in the uterus. Our purpose was to determine whether changes in the concentrations of epinephrine and norepinephrine in the plasma of rats during the estrous cycle and pregnancy occur in a manner consistent with this hypothesis.

Adult male and female Simonsen rats, weighing 200 to 250 g, were used; all were anesthetized with pentobarbital sodium at 30 mg kg^{-1}. The state of estrus was determined by microscopic examination of vaginal washings from the nonpregnant females. Following laparotomy, 4 to 5 ml of blood was withdrawn from the abdominal aorta into a syringe previously rinsed with heparin; blood from three to four animals was pooled for each sample, and plasma catecholamines were assayed (*6*).

Concentrations of epinephrine and norepinephrine in plasma from male, estrous, diestrous, metestrous, and pregnant rats are listed in Table 1. The important differences may be summarized as follows: (i) the concentration of norepinephrine in plasma from estrous and pregnant animals was significantly lower than from diestrous animals, (ii) the concentration of epinephrine in plasma from estrous and diestrous females was statistically greater than from males, and (iii) epinephrine was of greater concentration than norepinephrine in the plasma of all groups tested.

Published data on uterine concentrations of catecholamine (*1, 3, 7*) led us to two correlations of interest: The concentration in tissue of epinephrine or norepinephrine was directly proportional to the concentration of the particular amine in the plasma; and, as the ratio of epinephrine to norepinephrine in the plasma increased, the percentage of total uterine catecholamines, represented by epinephrine, also increased. These correlations, along with the data presented, are consistent with the hypothesis that the cyclic variations in uterine catecholamines observed in the rat result from variations in the ratio of epinephrine to norepinephrine in the plasma and in the transport of these amines into the uterus.

In all groups studied the mean epinephrine concentration was higher than the corresponding norepinephrine concentration. In most species the concentration of norepinephrine exceeds that of epinephrine (*6*). It could be argued that the epinephrine values reported in Table 1 were elevated because of adrenal-gland discharge caused by the blood-sampling procedure employed. Stern and Brody (*8*) reported values for the epinephrine and norepinephrine concentrations in plasma from female rats during hexobarbital anesthesia; their blood samples came from the inferior vena cava. Their values were similar to the diestrous-plasma values shown in Table 1, although the epinephrine concentration was somewhat lower. Rubinstein (*9*) also reported epinephrine values in rat plasma; he obtained blood samples, by cardiac puncture, from males under pentobarbital anesthesia; his animals were acutely adrenalectomized just before the cardiac-puncture procedure. His average epinephrine concentration in plasma was essentially the same as that for the male group listed in Table 1. Thus the values reported from our study are in close agreement with values reported in the literature and obtained by use of the trihydroxyindole procedure. There are indications that these values were not due to excessive discharge of the adrenal gland although the extent of discharge is unknown. Much higher values than those just considered were reported by Anton and Sayre (*6*), who indicated that their value for epinephrine was possibly due to adrenal discharge. The finding that the epinephrine concentration in plasma was higher in females than in males agrees with an earlier finding in humans (*10*). Catecholamine concentrations in the circulation also were reported to vary during human pregnancy (*11*).

The finding that the epinephrine content (relative to body weight) of the adrenal glands was higher in female rats than in males (*12*) may relate to the sex difference noted in our study for the concentrations of this amine in plasma. While the variations in catecholamines in plasma appear to correlate well with their concentrations in the uterus, the findings may also have more general significance.

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Environmental Control of Ovarian Development in Mosquitoes of the Culex pipiens Complex

Abstract. *Gonotrophic dissociation, a condition in which the ovaries remain undeveloped in female mosquitoes that have taken a full blood meal, occurs in* Culex pipiens *L., when incubated at low temperature (10° to 15°C) with short photoperiod and held at low temperature after feeding. Gonotrophic dissociation occurred sporadically in* Culex quinquefasciatus *Say after conditioning by low temperature, irrespective of photoperiod. Two major considerations are posed: first, the importance of gonotrophic dissociation to hibernation potential; and, second, the potential of a hibernating female mosquito to serve as a virus reservoir.*

The term gonotrophic dissociation (*1*) is commonly applied to any situation where the ovaries remain undeveloped in female mosquitoes that have taken a full blood meal. Although gonotrophic dissociation in this sense has been reported as occurring in members of the *Culex pipiens* complex from

Japan (*2*) and Russia (*3*), it has never been reported for North American species of the genus *Culex*. Gonotrophic dissociation may have direct bearing on the ecology of the mosquito-borne viral encephalitides. A long-standing question is how the viruses survive interepidemic periods in temperate regions. Although hibernating females of vector species often have been suspected of harboring the particular virus involved, the possibility that they actually do so in nature is argued against on the basis of experiments with *C. tarsalis* Coquillett, the principal vector of western equine encephalitis. Attempts to isolate virus from hibernating females of this species were, with a single exception, unsuccessful (*4*). Furthermore, ovaries of hibernating females were always found in the nulliparous condition (*5*). The evidence for nulliparity is direct, but implicit in this observation is that the mosquitoes in question have never had a blood meal, hence have had no opportunity to become infected. Nulliparity, however, is only valid as evidence of lack of a previous blood meal as long as gonotrophic dissociation does not occur in prehibernating females of natural populations. This is a report of the occurrence of gonotrophic dissociation in a colonized strain of *C. pipiens* L. under the influence of artifically induced environmental conditions.

Mosquitoes used in this study were from a colony of *C. pipiens* established from field-collected material from West Lafayette, Indiana. For comparison, investigations were also carried out with mosquitoes from a colony of *Culex quinquefasciatus* Say established from material collected at Vero Beach, Florida. All treatments were carried out in incubators modified from household refrigerators [about 10.3 m^3 (12 ft^3)]. Larvae of both species were reared under constant conditions: 28°C and a daily photoperiod of 16 hours of light and 8 hours of darkness. Other features of the rearing procedure were standardized as closely as possible. Adults were provided with 5 percent sucrose solutions at all times during the experiments. When larval development was completed, pupae were divided at random into test groups. Each group was subjected to a different combination of temperature and photoperiod from this time until seven days after emergence of adult mosquitoes. At that time, blood-feeding response was determined, and the fed females were held for subsequent determination of ovarian development. In all, 16 combinations of temperature and photoperiod were tested (10°, 15°, 20°, and 25°C; and 10, 12, 14, and 16 hours daily photoperiod). Illumination within the incubators came from two different sources. In one series of experiments, unshielded 40-watt incandescent lamps were used, and there was a fluctuation in air temperature of 1° to 2°C between light and dark phases. In the other series of experiments, water-jacketed 20-watt daylight fluorescent lamps were used, and there was no systematic fluctuation of air temperature. The entire series of 16 combinations was run twice with each illumination source.

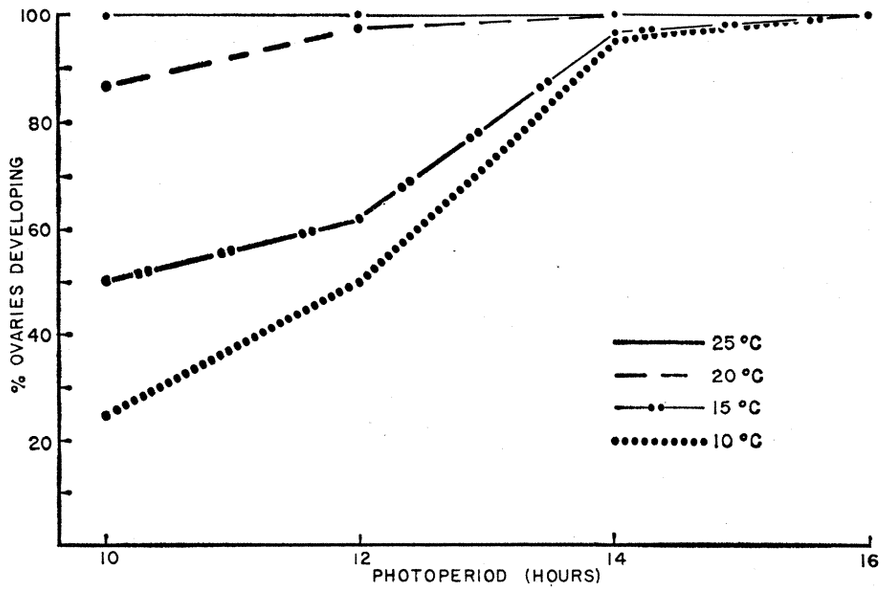

Fig. 1. Ovarian development in *Culex pipiens* under different conditions of temperature and of photoperiod regulated by incandescent illumination.

The blood-feeding trials were conducted at 25°C by placing the female mosquitoes in jars and exposing them overnight to a chicken whose leg projected into the jar through a hole in the jar lid. Each treatment consisted of 50 females divided into groups of 10 individuals in each of five jars (*6*). Blood-fed females were segregated from non-fed females the following morning and incubated at the temperatures maintained before feeding. After all the blood was digested in all females of a group, ovarian development was determined by dissection. In some cases, ovaries, with follicles in which no yolk granules were visible under magnification (× 100) of a compound microscope, were considered undeveloped and

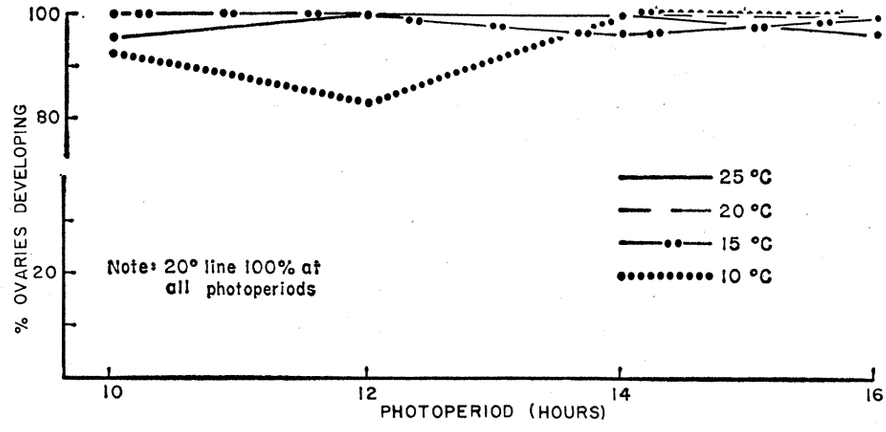

Fig. 2. Ovarian development in *Culex quinquefasciatus* under different conditions of temperature and of photoperiod regulated by incandescent illumination.

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