adjacent to the carbonyl group, each of these is a "tropolone"—OH. Therefore, each of these hydroxyl groups may be involved in the tropolone function. On the other hand, if either of these groups were in positions 3, 4, or 5, these would be ring hydroxyls—pseudo-aromatic hydroxyls having perhaps a different function than the hydroxyls present in positions 2 and 7, as in α-thujaplicinol. Further, it is of interest to note that, while γ-thujaplicin and pygmaein are intermediate in effectiveness toward *Poria monticola*, α-thujaplicinol, in particular, was very potent. This, again, reflects differences in tolerance of the fungi to different chemicals (*6*).

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Neural Timing of Ovulation in Immature Rats Treated with Gonadotrophin

Abstract. Thirty-two-day-old female rats, injected two days previously with gonadotrophin from pregnant mares' serum, were given barbiturate at different times during the afternoon to determine whether the ovulation that would occur the next day could be blocked. The results indicate that ovulation depends upon release of pituitary ovulating hormone during a "critical period" between 2 and 4 P.M.

Everett *et al.* demonstrated a neural activation of the release of pituitary ovulating hormone (*1*) occurring within a 2-hour period during the afternoon preceding ovulation in adult female rats (*2*). The release of hormone was concluded to be coextensive with activation of the pituitary (*3*). The release of ovulating hormone could be prevented if nervous system blocking agents, including barbiturates, were administered prior to the onset of this "critical period" (*2*, *4*).

Cole induced ovulation consistently in immature female rats with a single injection of gonadotrophin from pregnant mares' serum (*5*). For several years we have been producing ovulation in more than 90 percent of 33-day-old female rats with a single injection of 0.4 Cartland-Nelson unit of this serum gonadotrophin. Nontreated rats seldom ovulate by this age. The treated animals mate, conceive, parturate, lactate, and raise a litter of young in an essentially normal manner.

It was of interest to know whether the ovulation in our rats depended on the release of endogenous pituitary gonadotrophin. Hypophysectomy at various times prior to ovulation indicated that the pituitary was essential up to, but not after, the late afternoon before ovulation. Experiments were then performed with barbital sodium (sodium 5,5-diethylbarbiturate) as a long-acting neural blocking agent.

Immature female albino rats, 26 days old, weighing 65 to 70 g, were obtained from the Holtzman Company. They were caged six to seven per cage with 14 hours of light and 10 hours of darkness at a room temperature of 26±1°C. When the animals were 30 days old they were given, between 9 and 10 A.M. (*6*), a single subcutaneous injection of 0.4 Cartland-Nelson unit of gonadotrophin from pregnant mares' serum (Gonadogen) in 1.0 ml of saline (*7*). Approximately 3 to 5 percent of the rats had open vaginas at 6 to 7 P.M. of the following day and they were discarded.

When the rats were 32 days old and weighed 90 to 110 g, some were injected intraperitoneally with 30 mg of barbital sodium in 0.5 ml of water at 1:55 P.M. ± 10 minutes, and others were given the same treatment at 4, 5, or 6 P.M. The controls received no barbital. Unconsciousness usually occurred within 20 to 30 minutes, and a supplemental intraperitoneal injection of no more than 1.2 mg of sodium pentobarbital (Veterinary Nembutal sodium, Abbott) was given to any individuals in which sedation was slow to develop. After 2 hours or more of unconsciousness, some rats required artificial respiration or aspiration of the pharynx, or both. The animals were autopsied during the afternoon and evening of the next day, and ovulation was determined by counting ovulated follicles and oviducal eggs.

Table 1. Blockage of ovulation by barbital treatment 2 days after administration of gonadotrophin from pregnant mares' serum. Standard errors are given in column 5.

Time of barbital injection (P.M.)	No. of rats	Rats ovulating		No. of eggs per ovulating rat*
		No.	%	
Control	15	15	100	9.60±0.43
2	19	0	0	
4	11	9	82	8.11±0.73
5	7	7	100	9.57±0.72
6	15	12	80	8.33±0.36

* Control vs. 6 P.M.: $p < .05$ (t-test). Other groups: $p > .05$.

The results are presented in Table 1. Ovulation occurred in all of the controls. Barbital, when given at 2 P.M., blocked ovulation, but when it was given at 4 P.M. or later, ovulation occurred in at least 80 percent of the animals. The number of eggs per ovulating rat was slightly smaller than that in the controls; the difference is statistically significant only for the group treated with barbital at 6 P.M.

We interpret our results to mean that there is a release of ovulating hormone from the pituitary during the period between 2 and 4 P.M. This interpretation is supported by experiments in which ovulation was prevented by hypophysectomy just prior to 2 P.M. on the 32nd day of age but was not prevented by hypophysectomy at 5 P.M. or later. The time of the critical period in our immature rats is the same as that found by Everett *et al.* in adult rats (*2*).

The partial reduction in percentage of rats ovulating in the groups receiving barbital at 4 P.M. or later may have been due to the prolonged depression produced by the high dose of barbital. A lower dose of barbital (20 mg per rat), when given at 2 P.M. to a smaller series of animals, produced consistent blockage. Nembutal (3.6 mg per rat) was also effective in blocking ovulation when injected at 2 P.M. if the individual rats remained unconscious for 2 to 2.5 hours; when given at 4 P.M. the percentage of animals ovulating was similar to that of the controls.

Recently McCormack and Meyer in our laboratory have confirmed, by the use of barbital blockage and hypophysectomy, that a similar critical period exists in serum-gonadotrophin-treated rats 8 days younger than those reported here (*8*).

The mechanism by which gonadotrophin from pregnant mares' serum brings about the release of ovulating hormone is not understood but pre-

sumably involves higher centers acting via the hypothalamus. A biological clock seems to control ovulation both in the immature rat induced to precocious puberty and in the adult. When the clock first appears in the immature rat it is already "set" to the same time as it is in the adult.

Preliminary experiments have revealed other aspects of this timing mechanism. Ovulation occurs in those animals blocked with barbital, but is a day later than in the controls. If a second injection of barbital is given 24 hours after the first, ovulation is again blocked and does not occur until 2 days after ovulation in the controls. Thus, there is a 24-hour periodicity in the release of ovulating hormone similar to that described by Everett and Sawyer in the adult rat (*4*).

It might be expected that the time of hormone release would follow the injection of the serum gonadotrophin by a constant time interval. However, when the serum gonadotrophin was given at various times within an 18-hour period the time of the critical period was the same. The setting of the timing mechanism may be determined by the lighting; when the beginning of the light period was shifted earlier or later, without change in length, the critical period shifted correspondingly.

The data in this report provide the basis for further study of the role of the hypothalamus and pituitary in controlling ovulation and for developing methods to test substances which either prevent or cause the release of pituitary ovulating hormone (*9*).

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References and Notes

1. "Ovulating hormone" is used to indicate whatever pituitary hormones are necessary for ovulation.
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6. Times are stated throughout as "colony time," using the convention of Everett and Sawyer wherein midnight, colony time, is the midpoint of the dark period. In our laboratory midnight, colony time, occurs at 1 A.M. Central Standard Time.
7. "Gonadogen" was supplied through the courtesy of the Upjohn Company. According to the Upjohn Company, "one Cartland-Nelson unit is equivalent to approximately 20 International Units."
8. C. E. McCormack and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.* **110**, 343 (1962).
9. This work was supported by grant A-804(C13) from the U.S. Public Health Service and by funds from the Wisconsin Alumni Research Foundation.

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Meiotic Drive

Matsunaga and Hiraizumi (*1*) have presented evidence suggesting that either meiotic drive or sperm competition may be responsible for an excess production of blood group O offspring from fathers heterozygous for the A and O or for the B and O alleles. As co-originator, with L. M. Sandler, of the phrase "meiotic drive," I am naturally favorably disposed toward any analysis which helps promote its usage; in this case, however, I feel that the approach is open to one criticism which may be of sufficient merit to warrant a more detailed analysis of the data.

In the method of Matsunaga and Hiraizumi it is necessary to calculate the probability that an A (or B) father will produce an O sperm. Since both homozygous AA and heterozygous AO genotypes are grouped as phenotypically of blood group A, the proportions of the two types of males are estimated by applying the Hardy-Weinberg formulation to the estimates of gene frequencies derived from observations on the Japanese population. After this is done, there appears to be an excess of progeny carrying the O alleles from that fraction of the males assumed to be heterozygous.

The pitfall in this procedure is similar to one pointed out earlier (*2*) in connection with an analysis of data from *Drosophila* populations. If there is some disturbance in a mating system such that the distribution of the genotypes in the progeny does not follow the Hardy-Weinberg expectations, then, if that disturbance is consistent, it should also have affected the frequencies of the genotypes in the preceding generation—that is, in the parents. It is not possible, therefore, to make a simple direct estimate of gene frequencies from the parental phenotypic frequencies because the Hardy-Weinberg distribution is being altered, by definition. Furthermore, if it is true, as Matsunaga and Hiraizumi point out, that postzygotic selection in the ABO blood groups also affects the genotypes differentially, then calculation of gene frequencies from the raw population data by the simple Hardy-Weinberg arithmetic and subsequent estimation of the genotypic frequencies from the gene frequencies becomes a somewhat hazardous enterprise.

Such complications can be approached by the use of appropriate programs for high-speed computers. In collaboration with Ray D. Owen we have in this way analyzed several independently collected (and self-inconsistent) sets of data on mother-child ABO blood group combinations. A test for meiotic drive of the type described was a constant feature of the program, but a drive of this type was not found to be a consistent factor in the data. Unfortunately, the data of Matsunaga and Hiraizumi (*1*) did not include either the phenotypes of the fathers (which had therefore to be assumed to be in the same distribution as those of the mothers) or specific information on family size. The possibility remains that the more detailed data that were available to us still provide evidence for some phenomenon such as meiotic drive when analyzed in a more sophisticated fashion.

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We agree that the frequencies of alleles and of genotypes, as well as of other coefficients, are best estimated by the maximum-likelihood method, but unfortunately a high-speed computer is not available in our Institute. For computing our "family size equivalent" values, the frequencies of alleles and genotypes were estimated by applying the simple Hardy-Weinberg formulation. Usually the observed phenotypic frequencies of the four blood types agree quite well with those expected from the Hardy-Weinberg law. But, of course, this agreement does not always imply that the distribution of each genotype takes place according to the Hardy-Weinberg expectation. For example, an excess in the frequency of AO but a deficiency in the frequency of AA may still give a good agreement of the observed frequency of blood type A with the Hardy-Weinberg expectation, and in that case ♀ O × ♂ A matings will give more O children than expected. However, this complication disappears if we compare the family size equivalent value for O children of ♀ A × ♂ O with the value for O children of ♀ O × ♂ A, and we can check this by taking the respective equivalent values presented in Table 1 of our earlier report (*1*).

This matter, however, requires more