show a requirement for free inorganic iron by these enzymes. On the other hand, folate conjugase activity was similar in the jejunal mucosa of iron-deficient and control puppies. No histological abnormalities were noted in the mucosa of the iron-deficient animals. It is suggested that iron deficiency may cause a functional impairment in the brush border region of the growing animal before structural changes occur.

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Blockade of Ovulation in the Rabbit with Catecholamines and Sympathomimetics* (33616)

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The literature dealing with pharmacologic agents that block ovulation in various species of animals was reviewed by Everett in 1961 (1). Although a variety of drugs which influence the central or autonomic nervous systems have the ability to inhibit both "spontaneous" and reflexogenous" ovulation, they do not appear to be pharmacologically similar to one another. This fact is increasingly obvious when one considers that parasympatholytic compounds (such as atropine and Banthine) (1–4), adrenergic blocking agents (such as Dibenamine) (5–14), as well as reserpine and chlorpromazine (15, 16) all block ovulation under certain conditions.

Many pharmacologic agents which are effective in blocking ovulation can also produce marked disruption of the sympathetic nervous system. Phenoxybenzamine (Dibenzyline), a representative of the beta haloalklyamine family, blocks the uptake of exogenous norepinephrine and endogenous epinephrine (17, 18). Two hours after administration of Dibenamine, the tissue levels of catecholamines are increased (19). Both reserpine and chlorpromazine are effective in blocking ovulation. The pharmacologic properties of reserpine reside in its ability to deplete the stored catecholamines of the sympathetic nervous system (20–22). Similarly, chlorpromazine has an effect on the catecholamines: it increases both the tissue levels of catecholamines and their metabolism (19, 23). Atropine favors the expression of

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TABLE I. Effect of Epinephrine, Norepinephrine, and Isoproterenol Administered 0–1 Minute *Postcoitum* on Ovulation.[a]

Drug	No. of animals per group	Drug dosage (μg/kg)						
		0	25	50	100	200	300	400
Epinephrine	3	8.3 ± 0.3		8.5 ± 0.5	1.6 ± 1.7	3.0 ± 3.0	0	Fatal
Norepinephrine	2	8.0 ± 0		8.5 ± 1.5	6.0 ± 0.7	8.5 ± 0.5	Fatal	
Isoproterenol	2	8.5 ± 0.5	9.0 ± 1.0	8.0 ± 0	6.5 ± 0.5	Fatal		

[a] Tabular values are mean (± SE) numbers of ovulations per rabbit; means not underscored by the same line are significantly different from one another ($p < .05$) (Tukey's Multiple Range Test).

the sympathetic nervous system by blocking the action of the parasympathetic responses.

It is well established that these agents, as well as others, are effective in blocking ovulation, presumably by interfering with the release of gonadotropic hormones. How the release of gonadotropic hormone is blocked is not known. It is possible that the various pharmacologic agents known to block ovulation do so by increasing the activity of the sympathetic nervous system. Evidence in support of this has been obtained by Eskin (24) who has reported that high doses of epinephrine administered before coitus will inhibit ovulation in the rabbit. It was the purpose of the present experiments to investigate this possibility further, by administer ng drugs known to directly increase the activity of the sympathetic nervous system and studying the effects of these drugs on coitus-induced ovulation in estrous rabbits.

Materials and Methods. Virgin female Dutch belted rabbits weighing from 1.8–2.5 kg were isolated for at least 1 week. After isolation, the females were placed in the male's cage for breeding. If the female did not mate within 4 min, she was returned to isolation for another week. Females which accepted the males were injected, via the marginal ear vein, with various doses of catecholamines. All injections were accomplished within 1 min *postcoitum*. The concentration of the catecholamines was adjusted with ascorbic saline (100 μg/ml ascorbic acid and 0.9% NaCl solution) so that the injection volume was between 0.2 and 0.5 ml. Animals requiring an excessive amount of restraint were removed from the experimental design.

Since all females did not breed at the same time, randomization was accomplished by randomizing the treatments within a given experimental period. The same male was used throughout the experiment, but did not service more than four females per day or more than 10 females per week.

After treatment, all animals were returned to their respective isolation cages for 1 week. On day 7 after breeding, the animals were anesthetized with sodium pentobarbital (approximately 30–50 mg/kg) and laparotomies were performed. The corpora lutea were counted and considered to be indicative of ovulation.

The various sympathetic amines employed at 0–1 min after breeding were epinephrine, norepinephrine, isoproterenol, amphetamine, and serotonin.

The second phase of this study was designed to ascertain the response of the most effective dose of these agents on ovulation when administered at varying time periods after breeding. In these experiments epinephrine, isoproterenol, and 5-hydroxytryptamine were utilized.

The statistical methods employed consisted of analysis of variance of a randomized complete block design and Tukey's Multiple Range Test for determination of specific areas of significance (25).

Results. Effect of graded dosages of various catecholamines on coitus-induced ovulation. The effects of graded dosages of epinephrine, norepinephrine, isoproterenol, amphetamine,

TABLE II. Effect of Amphetamine and Serotonin, Administered 0–1 Minute *Postcoitum*, on Ovulation.[a]

Amphetamine[b] (mg/kg)				Serotonin[c] (mg/kg)					
0	6	7	8	0	0.5	1.0	2.0	3.0	4.0
7.5 ±.5	6.0 ±.7	6.0 ±4.0	7.5 ±.5	8.7 ±.3	6.0 ±1.0	1.7 ±1.7	3.7 ±3.7	2.0 ±1.5	0

[a] Tabular values are mean (± SE) number of ovulations per rabbit; means not underscored by the same line are significantly different from one another ($p < .05$) (Tukey's Multiple Range Test).

[b] Two animals per group.

[c] Three animals per group.

and serotonin upon ovulation, when administered i.v. 0–1 min after mating, are presented in Tables I and II. Epinephrine significantly reduced numbers of ova shed at dosages of 100 and 200 μg/kg, completely prevented ovulation at 300 μg/kg, and was fatal at 400 μg/kg.

Norepinephrine and isoproterenol, which were lethal at dosages of 300 μg/kg and 200 μg/kg, respectively, failed to influence either the percentages of does which ovulated or the numbers of ova shed at sublethal doses. Amphetamine, at dosages up to 8 mg/kg was neither fatal nor inhibited ovulation.

Serotonin was tolerated at dosages as high as 4 mg/kg, but completely prevented ovulation at this dose, and significantly reduced the number of ova shed at dosages of 1–3 mg/kg.

The effects of epinephrine, serotonin, and isoproterenol administered at various times after breeding. Epinephrine (300 μg/kg) was administered at 0–1, 5, 10, 20, and 30 min after breeding (Table III). This treatment again completely blocked ovulation at 0–1 min and effectively reduced the number of ova shed at 10 through 30 min after breeding. However, the 300-μg dose was 100% fatal when administered at 5 min postcoitum. When the 200 μg/kg dose was utilized (Table IV), at the same time interval, it was not fatal and did significantly ($p < .01$) reduce the number of ova shed.

TABLE IV. Effects of Epinephrine (200 μg/kg) Administered at 5 min *Postcoitum* on Ovulation.

	No. of animals	Average no. ovulations	SE
Control saline	5	8.60	0.22
5 min postcoitum[a]	5	1.80	1.07

[a] Significantly blocked ovulation $p < .01$.

TABLE III. Effect of Time of Administration, *Postcoitum*, on Blockade of Ovulation by Epinephrine, Isoproterenol, and Serotonin.[a]

Time of administration (minutes *postcoitum*)	No. of animals per group	Drug and dosage		
		Epinephrine (300 μg/kg)	Isoproterenol (100 μg/kg)	Serotonin (4 mg/kg)
Control[b]	3	10.7 ± 2.1	8.3 ± .3	8.7 ± 0.3
0–1	3	0[c]	6.5 ± .5	0[c]
5	3	Fatal	0.3 ± .3[c]	9.0 ± 1.0
10	3	2.0 ± 1.2[c]	1.3 ± 1.3[c]	6.0 ± 2.6
20	3	1.5 ± 1.5[c]	6.0 ± 2.0	
30	3	4.7 ± 2.4[c]		

[a] Tabular values are mean (± SE) numbers of ovulations per rabbit.

[b] No drug administered.

[c] Significantly different from untreated controls ($p < .05$, Tukey's Multiple Range Test).

Isoproterenol (100 μg/kg) reduced ($p<.05$) the number of ova shed when given 5 and 10 min after breeding but was ineffective at other time periods (Table III). When serotonin (4 mg/kg) was administered at 0–1 min *postcoitum* it completely blocked ovulation, while it was not effective at 5 and 10 min after breeding (Table III).

Discussion. Although many pharmacologic agents are known to prevent ovulation, probably by inhibition of secretion of the pituitary gonadotropins, it seems surprising that such a wide array of pharmacologic agents should be so effective. One characteristic of many pharmacologic compounds which block ovulation is their ability to affect the sympathetic nervous system. If they are acting through their ability to alter the sympathetic nervous system, then one should be able to block ovulation with drugs known to directly affect the sympathetic nervous system.

As shown in the present studies, certain sympathetic amines do block coitus-induced ovulation when given alone. Admittedly the doses of these various sympathetic amines are rather high although no greater than the doses of other pharmacologic compounds which have been used to block ovulation. The high doses that were required may be a reflection of the exceptionally rapid detoxification mechanism for catecholamines.

The same dose of epinephrine (300 μg/kg) which was effective in blocking ovulation at 0–1 and 10 min after breeding was fatal to all animals when administered at 5 min after breeding. At 5 min *postcoitum* 200 μg epinephrine per kilogram of body weight was not fatal and did block ovulation. These results imply that resistance to the effects of high doses of epinephrine has been reduced at this time. This may have resulted from the liberation of endogenous epinephrine and/or norepinephrine in response to mating. The effectiveness of the various amines used in this study suggests that agents primarily involved in pressor responses do not effectively block ovulation. The indirect sympathomimetic (amphetamine) or a direct sympathoamine (norepinephrine) did not block ovulation. Catecholamines that possess vasodilatory activity, such as serotonin, isoproterenol, and epinephrine, block ovulation at 0–1 min, 5 and 10 min, and at 0–1, 5, 10, 20, and 30 min *postcoitum,* respectively.

Whether or not disruption of the sympathetic nervous system is a common mechanism of action by which many pharmacologic agents initiate their effects on ovulation remains unknown. It is of interest to speculate upon possible sites at which the blockade may be exerted. In the early studies of Sawyer *et al.* (2, 3, 6, 7) several antiadrenergic and anticholinergic agents effectively blocked ovulation in the rabbit only if administered 0–1 min after breeding. If administration was delayed 5 min, these compounds were ineffective, suggesting that the blockade was exerted at the central nervous system level, rather than at the level of the pituitary or ovary. In the present experiments, such critical timing was necessary only with serotonin. Isoproterenol was effective only when administered later (5 or 10 min *postcoitum,* and epinephrine still possessed some blocking ability when administered as late as 30 min after breeding. These observations suggest that the latter two compounds may act at a point (or points) later in the relex arc than at the level of the central nervous system. Possible sites include the hypophyseal portal vessels (perhaps via vasomotor effects which interfere with blood flow to the pituitary at a critical period), or the adenohypophyseal cells themselves. The possibility of interference with blood flow through the ovary cannot be completely excluded as an explanation for the blockade; epinephrine has been observed to markedly reduce blood flow through the testis (26) and the ovary (Currie, G. N., and Armstrong, D. T., unpublished observations). Experiments in which intrapituitary infusions of preparations of "LH-releasing factor," (27) are compared with i.v. injections of exogenous LH, should help distinguish between these various alternative sites of the actions of catecholamines in blocking ovulation.

Summary. Elevation of sympathetic tonus by injecting various catecholamines blocks coitus-induced ovulation in rabbits. Epine-

phrine, isoproterenol, and serotonin block ovulation when given at various times after mating. A dose of epinephrine (300 μg/kg) which is well tolerated by rabbits at other times was lethal to 100% of does when administered 5 min after mating. Norepinephrine and amphetamine do not prevent ovulation when given at 0–1 min *postcoitum*.

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