the color reaction, the liquid was treated with Folin's tungstic acid reagent and the tests carried out on the filtrate.

The analytical *results* were:

	Sample I	Sample II
Chloral hydrate	60 μg/cc	50 μg/cc
Trichloroacetic acid	22.5	15
Urochloralic acid (calculated as chloral hydrate)	44	35

Urine: Chloral hydrate and trichloroacetic acid negative; urochloralic acid: 560 μg/cc.

Blood: Trichloroethanol, 25 μg; trichloroacetic acid, 32.5 μg; chloral hydrate, 32.5 μg/cc.

In view of the small quantities of amniotic fluid collectable in the third rabbit, the potential error in the figures given is considered to be large, and the results should be evaluated rather as qualitative than quantitative.

It seems that at the time of gestation of the third rabbit, kidney function has set in and the transudative portion, containing the compounds circulating in the blood, is already mixed with urochloralic acid of urinary origin. At a later stage the urinary liquid replaces the reabsorbed transudate leading to a more watery fluid containing urochloralic acid only.

Summary. The results indicate that in the early stage of pregnancy chloral hydrate and its metabolites pass from the blood into the amniotic fluid. However, urochloralic acid, which is the form by which chloral hydrate is excreted by the kidneys, was found also at a stage when the gestation had progressed to two-fifths. In later pregnancy only urochloralic acid was found, which indicates that in the later stages the amniotic fluid contains essentially the products of foetal renal excretion.

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Received October 20, 1953. P.S.E.B.M., 1953, v84.

Blockade of Ovulation in the Hen with Adrenolytic and Parasympatholytic Drugs.* (20676)

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The involvement of the nervous system in the release of the ovulatory hormone (LH) has been demonstrated in several mammals. Electrical stimulation of the hypothalamus of the rabbit causes ovulation at levels that are ineffective when applied directly to the pituitary gland(1,2). Similarly other types of experiments on the rat have also indicated a neural component in ovulation(3). Recently Markee, Everett & Sawyer(4) have reviewed the data indicating the presence of a neural and neurohumoral link in the release of LH not only in the rabbit which ovulates following copulation but also in the rat, a non-spontaneous ovulator. The presence of a 24-hour rhythm in the neural link was indicated by the ability of progesterone to advance ovulation by 24 hours in the rat(5) and the ability of the barbiturates to delay ovulation by approximately 24 hours(6,7). Nembutal is also effective in preventing progesterone induced ovulation in rats(8).

The present study was undertaken to clarify the role of the nervous system in ovulation in the domestic hen. Like the rat, the hen is a cyclic, spontaneous ovulator. However, the hen differs from the rat in that the ovulatory

* Aided by a grant from the Purdue Research Foundation.

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rhythm of the former is typically asynchronous with regard to the 24-hr day. The successive follicles of a clutch are ovulated progressively later each day until the clutch is ended by a terminal ovulation occurring around noon. In view of the findings in the rat it was deemed possible that a neurogenic factor is also present in ovulation in the hen. Recently we noted a failure on the part of Nembutal to block normal or progesterone-induced ovulation(9) indicating the possibility of a different mechanism in the bird. However, Fraps and Case (10) while confirming the above observations of Bastian and Zarrow(9) also reported premature ovulation with certain barbiturates. These authors suggested the possibility that the ovulation was due to neural excitation following administration of the barbiturates. Since the adrenolytic and parasympatholytic drugs have been used to great advantage in establishing neural control of ovulation in the rat and rabbit, the present study is concerned with the action of such drugs in the hen.

Material and method. Adult laying hens of the White Leghorn strain were used in this study. The birds were kept in a laying battery under conditions of constant temperature and humidity and supplied with food and water *ad libitum*. Artificial lighting was maintained from 8 A.M. to 9.P.M. daily. The time of ovulation was determined by digital palpation through the cloacca and also by recording the time of oviposition for all the birds by an automatic recorder(11). Records of the time of oviposition were kept for several weeks prior to each experiment in order to determine the characteristic, clutch length for each bird. All experiments involved the C_1 or first egg of the clutch and only hens laying a sequence of 2 or more eggs separated by a single day of failure to ovulate were used. Since LH release occurs 4 to 10 hours prior to ovulation (12,13) and ovulation of the C_1 follicle occurs from 4 A.M. to 7 A.M.(14), all injections were started at 8 P.M. preceding the day of expected ovulation. The atropine was dissolved in water at a concentration of 300 mg per ml and the adrenolytic drug, SKF-501,‡ was dissolved in a 50% mixture of propylene glycol and water at a concentration of 25 mg per ml.

‡ The authors are indebted to Dr. Fellows, from Smith, Kline & French, for the SKF-501.

TABLE I. Inhibition of Normal Ovulation in the Hen by Adrenolytic and Anticholinergic Agents.

No. of hens	Treatment	No. of hens ovulating	% blocked
12	Saline control	10	17
17	Atropine sulfate	5	71
27	SKF-501	6	79

Results. In the first experiment, each of 20 hens were injected hourly from 8 P.M. to 8 A.M. with 150 mg of atropine sulfate. The drug was given intraperitoneally in a 0.5 ml volume per injection and produced such symptoms as dropping of feathers, lowering of wings and panting. In some instances the birds appeared to be near death and the hourly injection was skipped. Three of the hens died and do not appear in the results. The SKF-501 was injected intramuscularly at a dosage of 10 mg per kg body weight. Each hen received an injection at 8 P.M. and a second at 1 A.M. No deaths were recorded for this treatment. Both of the drugs blocked the expected ovulation (Table I). Atropine produced a 71% block and SKF-501 a 79% block. Compared with the control birds that received only saline hourly for 12 hours, the inhibition by the 2 drugs is statistically significant.

The second experiment was designed to study the effect of the two drugs on progesterone-induced ovulation. One mg of progesterone was injected intramuscularly at 4 P.M. on the last day of the clutch *i.e.*, on the evening of the day preceding the next expected ovulation. The first injection of atropine was made at 3:45 P.M. *i.e.*, 15 minutes before the ovulation-inducing injection of progesterone and was continued hourly thereafter for 4 hours. The SKF-501 was injected intraperitoneally in a single dose of 10 mg per kg body weight at 3:45 P.M.

While progesterone alone caused 90% of the hens to ovulate, only 20% of the birds ovulated following treatment with atropine and progesterone and 56% of the birds ovulated following SKF-501 and progesterone (Table II). This represents an 80% block by atropine and a 44% block by SKF-501. In both instances the differences, when compared to

TABLE II. Inhibition of Progesterone-Induced Ovulation in the Hen by Adrenolytic and Anticholinergic Agents.*

No. of hens	Treatment	No. of hens ovulating	% ovulating
10	Controls	9	90
10	Atropine sulfate	2	20
16	SKF-501	9	56

* All 3 groups of hens used in this experiment received 1 mg of progesterone intramuscularly.

the effect of progesterone alone, are statistically significant.

Discussion. The present results are consistent with the hypothesis established for the rat and the rabbit that a neural pathway is involved in the mechanism regulating ovulattion in the hen. Furthermore the action of atropine and SKF-501 would indicate that the neural pathway may have both an adrenergic and cholinergic link. The inhibition of both the normal and the progesterone induced ovulation by atropine and SKF-501 are identical with the results obtained in the rat(3) and the rabbit(15), and also indicate the presence of a neural factor in the mechanism that is concerned with ovulation in the hen. The present results however do not clarify the action of Nembutal with regard to its failure to prevent ovulation(9) and its ability to facilitate the process(10). Actual studies on the level of neural activity may be necessary before a definite explanation can be obtained. However, in spite of the failure of Nembutal to interfere with ovulation, these results indicate that a neural link is present in ovulation in the bird.

Summary and conclusions. Both atropine and SKF-501 block the normal occurring ovulation and the progesterone-induced ovulation in the hen. It is suggested that a neural link must be present in the mechanism regulating ovulation in the hen. This link may be similar to that postulated for the rat and the rabbit.

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Received October 22, 1953. P.S.E.B.M., 1953, v84.

Motility in a Species of Non-Flagellated Bacteria. (20677)

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Though there has been dispute concerning the role of flagella in bacterial motility, flagella have been found on most motile bacteria when examined under the electron microscope. Exceptions include members of the genera *Beggiatoa* and *Thiothrix* and of the order *Myxobacteriales,* in which creeping on a solid substrate has been described or in which flexible cell walls have been observed. In *Fusobacterium girans,* described in detail elsewhere (Macdonald)(1), motility which appears to be independent of flagella, creep-