not appear in the copper, mercury or anion injected controls, but appeared in equal numbers in the leaded chicks. It would seem that lead has a specific effect in the production of this particular lesion in the occasional embryo. We do not know if the defect occurs spontaneously. In the human the lesion has been considered to be of hereditary origin since it has appeared in families of the involved infants or embryos(14).

It is quite probable that meningoceles in chick embryos are a late development of the "open nervous system" described by Catizone and Gray(4). They found the lesion most frequently when lead was injected into the subgerminal cavity at 18 hours incubation. Obviously the ion injection in the albumen would be subject to greater variation in its quantitative relationship to the embryo. It is possible that a larger number of meningoceles could have been produced by injection at a different stage of incubation or by exposing the embryo more directly through subgerminal cavity injection. That the lesion could be produced even relatively late in embryonic development is evident from the observation of meningoceles in 3 of 29 embryos which developed in eggs injected with 0.75-1 mg of lead on the 5th day of incubation.

Conclusion. (1) Injection of lead into the albumen of fertile eggs may result in the production of meningoceles in chick embryos. (2) Copper and mercury ions were noted to be as toxic as similar quantities of the lead ion, but meningoceles were not seen in the embryos surviving 13 days incubation. (3) Sodium salts of anion used in the metal experiments failed to produce meningoceles.

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Failure of Nembutal to Block Ovulation in the Hen. (19341)

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Data have recently been accumulated indicating that the nervous system participates in the release of tropic hormones from the pituitary gland(1,2). Specifically it has been reported that the hypothalamus is involved in the release of the luteinizing hormone (LH), thyrotropic hormone (TSH) and adrenocorticotropic hormone (ACTH) from anterior pituitary gland(3-5). Experiments involving electrical stimulation in the rabbit have shown that ovulation occurs much more readily upon stimulation of the hypothalamus than upon direct stimulation of the hypophysis(6,7). Brooks, Beadenkopf, and Bojar(8) reported ovulation in the rabbit following injection of picrotoxin, a central nervous system stimulant. Pre-treatment with phenobarbital prevented the ovulatory-inducing action of the picrotoxin(9) and since barbiturates are known to have a selective action on the hypothalamus this would further tend to involve the diencephalon in the release of LH. Everett and Sawyer(10) have shown in the rat that Nembutal anesthesia between 2 and 4 p.m. on the

day of proestrous will prevent ovulation otherwise expected about 11 hours later. Nembutal is also effective in preventing progesterone-induced ovulation in rats(11).

The present study was undertaken to determine the role of the nervous system in ovulation in the domestic hen. In view of the definite, temporal relationship between LH release, ovulation and oviposition it is possible to calculate the time of LH release from the hypophysis after the time of lay has been determined(12-14). This should make the hen ideal for these studies in that the time of lay of the egg can be recorded without operative procedures. In addition the hen shows an asynchronous rhythm in the time of egg laying in that the first egg is laid in the morning and each succeeding egg of the clutch is laid progressively later in the day until the clutch is ended. The hen then skips a day and starts the next clutch in the morning again. This might indicate that the mechanism is different in this form from other species studied thus far. The present investigation is concerned with the action of Nembutal on normal and progesterone-induced ovulation.

Material and methods. Adult laying hens of the White Leghorn strain were used. The birds were kept in a laying battery under constant conditions of temperature and humidity and supplied with food and water *ad lib.* Artificial lighting was maintained from 8 a.m. to 9 p.m. daily. Records of the time of oviposition were kept for 4 weeks prior to the experiment in order to determine the characteristic, clutch length for the individual hens. Calculations made from these data indicated that LH release was confined to the interval between 10 p.m. and 10 a.m. This was determined on the basis that oviposition in our flock occurred between the hours of 10 a.m. and 4 p.m. and since ovulation precedes oviposition by about 26 hours(12), ovulation must have taken place between 8 a.m. and 2 p.m. Since LH is discharged from 4 to 10 hours prior to ovulation(13,14), the earliest time for LH release would be 10 hours prior to 8 a.m., and the latest time would be 4 hours prior to 2 p.m. Only those hens were used that were expected to release LH at the time of the Nembutal anesthesia. Anesthesia was induced with an initial dose of 70 mg of Nembutal given intraperitoneally. This treatment caused a deep anesthesia in 5 minutes with a duration of one-half to one hour. Prolonged anesthesia was obtained by supplementing the initial treatment with additional injections of 20 to 30 mg of Nembutal every 20 to 30 minutes. The progesterone* was dissolved in sesame oil and injected intramuscularly.

TABLE I. Inability of Nembutal to Block Natural Release of LH in 13 Hens. Anesthesia was maintained for 12 hr (10 P.M. to 10 A.M.).

No. of hens	Time of lay in clutch	No. of hens that laid next egg	% of hens showing apparent release of LH during anesthesia
4	Start	4	100
8	Middle	7	88
1	End (?)	0	0

Results. In a preliminary experiment Nembutal anesthesia was induced in a number of hens at the time calculated for LH release. The anesthesia was maintained for only 2 hours. No interference with ovulation was observed in any of the hens. To remove any doubt as to the failure of Nembutal to prevent ovulation, a second experiment was designed in which the hens were maintained under deep Nembutal anesthesia for 12 hours. A total of 13 hens were used during different stages of their clutch. Anesthesia was started at 10 p.m. and maintained until 10 a.m., the next morning. The occurrence of ovulation was judged by digital palpation through the cloaca and by the time of subsequent lay. Eleven of the 13 treated hens or 85% ovulated indicating a failure of the Nembutal anesthesia to block the normal release of LH (Table I). In one instance the bird may have been at the end of the clutch so that no ovulation could be expected. If this hen is eliminated from the group, then ovulation occurred in 92% of the treated birds.

A third experiment was designed to study the effect of Nembutal anesthesia on pro-

* Progesterone was obtained through the courtesy of Dr. Henderson, Schering Corp., Bloomfield, N. J.

TABLE II. Inability of Nembutal to Block Progesterone Induced Ovulation.

Group	Anesthesia, hr	No. of hens	% ovulating	Mean interval inj. to lay, hr
I	—	12	83	35.1 ± .9*
II	1.5	14	100	35.4 ± 1.3
III	3	5	80	34.4 ± 1.5
IV	6	13	77	35.6 ± 1

* Stand. dev.

gesterone-induced ovulation(15). In all instances 1 mg of progesterone was injected intramuscularly at 11 a.m. on the last day of the clutch. Table II shows the results involving a total of 44 hens, divided into 4 groups. The first group consisted of the control birds and received only progesterone. Groups II, III and IV received progesterone and in addition were kept under anesthesia with successive intraperitoneal injections of Nembutal for 1.5, 3, and 6 hours duration, respectively. The initial injection of Nembutal was made at 10:50 a.m., so that the birds were in deep anesthesia by the time the progesterone injections were made.

Eighty-three per cent of the control group of 12 hens, treated at the end of the clutch, ovulated after the intramuscular injection of 1 mg of progesterone (Table II). However, Nembutal anesthesia for 1.5 to 6 hours failed to block ovulation in the progesterone treated birds. Group II under anesthesia for 1.5 hours showed 100% ovulation, Group III under anesthesia for 3 hours showed 80% ovulation, and Group IV under anesthesia for 6 hours showed 77% ovulation. It is also of interest to note that the mean interval from the time of injection of the progesterone to the time of lay was not affected by the anesthesia (Table II).

Discussion. It has been known for some time that the induction of pseudopregnancy can be inhibited by gaseous anesthetics(16) and barbiturates(17), and it has been suggested that the block occurred in the hypothalamus preventing the release of LTH(17). Recently, the investigations by Everett and his collaborators(10,11) indicating that Nembutal can block ovulation in the rat, led them also to conclude that the hypothalamus was involved in the release of LH. Similar conclusions were arrived at from work on the rabbit(6).

The present results would tend to indicate that the central nervous system may not always be involved in the release of LH in the hen. The failure of Nembutal to inhibit ovulation when anesthesia was maintained for a period of 12 hours should rule out the possibility of miscalculating the time of LH release. In addition, the failure of Nembutal to inhibit progesterone induced ovulation, where the time of LH release is more exact, constitutes added evidence that a stimulus (progesterone) may bring about the release of LH by direct action on the pituitary gland. That the nervous system may impinge on the phenomenon of ovulation is obvious from the influence of light on the time of oviposition but it would appear that in the hens the mechanism is different from that seen in the mammal.

Summary. (1) Nembutal anesthesia was induced for periods of 2 and 12 hours in laying hens in an attempt to inhibit the normal release of LH. Ovulation occurred in more than 80% of the birds despite the length of anesthesia, indicating a release of the luteinizing hormone during the time of narcosis. (2) In a second experiment hens were anesthetized with Nembutal 1.5 to 6 hours and an attempt made to induce ovulation by progesterone. Ovulation was obtained in 100% of the birds anesthetized for 1.5 hours, in 80% of the birds anesthetized for 3 hours, and in 77% of the birds anesthetized for 6 hours. This compares favorably with the 83% ovulation noted in the control hens treated only with progesterone. It may be concluded that Nembutal-induced anesthesia does not interfere with the release of LH in the hen.

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Cytologic Changes in Rat Adenohypophysis Following Administration of Adrenocorticotrophin or Cortisone. (19342)

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An increased pituitary basophile count has been found at post mortem examination of patients dying after receiving pituitary adrenocorticotrophic hormone (ACTH)(1) or cortisone(2). In addition, the hyaline changes described by Crooke(3) were seen in many of the basophile cells after the administration of both hormones(1,4). These changes have usually been considered to be characteristic of Cushing's syndrome. The appearance of these findings following the administration of adrenal cortical hormones strongly supports the theory that the pituitary alterations in Cushing's syndrome are a secondary manifestation of increased adrenal cortical activity. An attempt was therefore made to reproduce these lesions in experimental animals and to define the circumstances under which the changes occur.

Experimental. Male Sprague-Dawley rats were treated with ACTH* or with a microcrystalline suspension of cortisone (Cortone, Merck) according to the schedule in Table I. The daily dose of ACTH was administered subcutaneously in 3 portions—one-fourth of the total at 8:00 A.M. and at noon, and one-half at 5:00 P.M. The phenol control solution was given in a similar manner, in the same volume as the ACTH solution (.025 ml/100 g at 8:00 A.M. and noon; .05 ml/100 g at 5:00 P.M.). A group of normal rats was examined without having been subjected to any trauma. The effects of "stress" were also studied in animals fasted for 15 hours in individual cages in a cold-room at 4°C. At the end of the experimental period, the animals were anesthetized with intraperitoneal pentobarbital. The adrenals were removed, cleaned and weighed on a Roller-Smith torsion balance to the nearest milligram.

The pituitary glands were removed by the dorsal approach. They were fixed in 10% solution of USP formaldehyde, embedded in paraffin and sectioned serially at 5 μ. Multiple sections were stained for differential counting by Mallory's 1% acid fuchsin, orange G, aniline blue stain(5). Selected sections were also stained by a modification of the periodic acid Schiff technic(6). A minimum of 6000 cells of the pars distalis were counted from each gland by the method of Rasmussen(7). In all but two instances, 3 or 4 sections from different areas were included in the count. Two sections from each gland were evaluated for Crooke's hyaline change of the basophile cells. The identity of the slides was unknown at the time of these observations which were repeated on 3 separate occasions.

* Wilson's Corticotrophin, kindly supplied by Dr. David Klein of the Wilson Co.