*Summary.* 1. Liver slices of rat, mouse, dog, guinea pig, rabbit and pigeon were incubated with reserpine-$C^{14}$. 2. Demethylation of the 4-methoxy position of trimethoxybenzoic acid moiety of reserpine was observed to occur, with rat liver slices most active in this respect. 3. Hydrolysis of reserpine was observed to occur with liberation of free trimethoxybenzoic acid. Guinea pig liver slices were most active in this respect. 4. Esteratic activity resides chiefly in the microsomes of guinea pig liver. 5. Demethylation or hydrolysis or both by liver slices is not correlated with dose requirement for typical tranquilizing response. 6. Rate of excretion of $C^{14}$ by the guinea pig was studied *in vivo* after intravenous injection of reserpine-$C^{14}$.

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## Delaying Action of Gonadotrophins on Ovulation in the Hen.* (22059)

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The ovulation-inducing hormone (OIH) in the hen is probably the luteinizing fraction (LH)(1-3), although this action of LH independently of the follicle stimulating hormone (FSH) has not been demonstrated in either birds or mammals(4). As in other spontaneously ovulating animals such as the rat(5) and cow(6), OIH release in the hen is apparently effected over nervous pathways (7-12). The interval from OIH release (or from neural excitation for OIH release) to ovulation is estimated to be not more than about 8 hours(11-15), although it may be greater in some hens(10). A number of pharmacological agents which block LH release in the rat(5,16) were shown recently to suppress ovulation of the first (or $C_1$) follicle of the hen's cycle following a single injection 38 hours before expected ovulation(17). The agents included anesthetic [diallylbarbituric acid (Dial), pentobarbital Na (Nembutal), and Phenobarbital Na], anticholinergic (atropine sulfate) and antiadrenergic (SKF-501 and Dibenamine) drugs. It seemed highly improbable that some, at least, of these drugs (*e.g.,* atropine or Nembutal) could directly block the OIH release mechanism 30 hours following their injection. Suppression of ovulation was attributed instead to disruption of nervous controls over pituitary gonadotrophin secretions required for maintenance and maturation of the ovarian follicle. The prevalence of follicular atresia in hens injected 38 hours before expected $C_1$ ovulation and sacrificed shortly after the hour of normally expected

* These results were presented at the 44th Annual Meeting of the Poultry Science Assn., East Lansing, Mich., Aug. 9-12, 1955.

ovulation(18) added weight to this conclusion. Ovulation of the second or $C_2$ follicle was also suppressed by the agents enumerated above, and again in certain instances, under conditions which pointed to disruption of gonadotrophin secretion for maintenance or maturation of the follicle rather than to simple "blockade" of OIH release(18 and unpublished observations).

One obvious approach to the problem thus posed was to administer gonadotrophins in the expectation that these might counteract the drug action. An anterior pituitary (AP) preparation was accordingly given intramuscularly shortly before injection of representative "blocking" agents. Contrary to expectations, the AP preparation appeared to enhance the suppressing or delaying action of the drugs. These observations led to the suspicion that the AP preparation itself might delay ovulation. This proved to be so. Conceivably, any of several hormones presumably carried by the whole AP preparation might have accounted for its delaying action. Not exhausting all these possibilities, it was nevertheless found that appropriate combinations of LH and FSH could duplicate the effects of the whole AP preparation. In a word, pituitary gonadotrophins may serve in the hen as highly effective "blocking" agents.

*Materials and methods.* Hens of White Leghorn and crossbred (White Leghorn x Rhode Island Red) extraction served in all but a few test groups; some Rhode Island Red hens were used in these latter groups. The battery caged birds were under artificial illumination from 6:00 a.m. through 8:00 p.m. and had access to feed and water *ad libitum*. Practically all hens were ovulating in 3-day "closed" cycles, ovulating, that is, consecutively on each of 2 days, failing to ovulate on the third day to complete one cycle, and then ovulating on the next day to initiate another cycle(11,12). Typically, the first or $C_1$ ovulation in the 3-day cycle occurs at about 6:00 a.m., the second or $C_2$ at around 10:30 a.m. of the following day. The interval between $C_1$ and $C_2$ ovulations is thus approximately 28.5 hours. Since ovulation fails to occur on the third day of the cycle, but does so at 6:00 a.m. of the day thereafter, the interval between the $C_2$ ovulation of one cycle and the $C_1$ ovulation of the succeeding cycle is some 43.5 hours. All injections were timed for effect on $C_2$ ovulations. Two measures of response are recognized. The first is simply the incidence of suppressed ovulations, regardless of the fate of the follicle whose ovulation was prevented. The second is the incidence of $C_2$ ovulations which were delayed only from the normally expected hour of one day (ca. 10:30 a.m.) until early morning hours (ca. 6:00 a.m.) of the following day. In this type of delay the follicle whose ovulation was prevented subsequently behaves like the $C_1$ follicle of the naturally occurring closed cycle. Such delayed ovulations are therefore designated "$C_1$ lapses", or merely "lapses". The induced $C_1$ lapse is identical with the "1-day delay" in ovulation noted elsewhere(11), and is a special case of the "$C_1$" type" of delayed ovulation first recognized by Rothchild and Fraps(19). Theoretically, the $C_1$ lapse can be induced only if the specific follicle whose ovulation was prevented is also the follicle subsequently to ovulate. Autopsy findings confirmed this important expectation. The appearance of the $C_1$ lapse may therefore be accepted as evidence that normally expected OIH release was prevented or failed to occur despite the presence of an ovulable follicle. The occurrence of normal ovulation, of the $C_1$ lapse, or of longer delays in ovulation was established by palpation procedures described earlier(11,20). Percentages of suppressed ovulations or of $C_1$ lapses appearing in the Tables are based on the total number of injected hens in each group. Despite care in selection of the birds, an occasional hen will fail to ovulate as expected. A very low incidence of suppressed ovulations or of $C_1$ lapses ($<10$ or 20%) is therefore of little or no significance. The "L/S ratio," expressed in percent, gives the proportion of $C_1$ lapses included in the total of suppressed ovulations. Since follicular atresia is ruled out in the $C_1$ lapse, but not necessarily in the case of ovulations delayed for greater intervals (2 days or more), the L/S ratio serves as an index of relative follicular immunity to indicated experimental procedures. The "blocking" agents were injected into the muscles of the

TABLE I. Effect of Male Chicken Anterior Pituitary (AP) on Incidence of Delayed $C_2$ Ovulations following Administration (4:00-5:00 P.M.) of Several "Blocking" Agents.

| Injected agents | No. hens inj. | Suppressed ovulations No. | % | $C_1$ lapses No. | % | L/S ratio (as %) |
|---|---|---|---|---|---|---|
| Atropine | 12 | 10 | 83 | 5 | 42 | 50 |
| AP + atropine | 11 | 10 | 91 | 8 | 73 | 80 |
| Nembutal | 15 | 8 | 53 | 5 | 33 | 63 |
| AP + nembutal | 9 | 8 | 89 | 8 | 89 | 100 |
| Phenobarbital | 11 | 10 | 91 | 1 | 9 | 10 |
| AP + phenobarbital | 11 | 11 | 100 | 2 | 18 | 18 |

leg at the following dosages: atropine sulfate, 80 mg/kg body weight, Nembutal, 60 mg/kg, and phenobarbital sodium, 100 mg/kg. The drugs were taken up in distilled water immediately before use, one ml water carrying the mg/kg equivalent. Some deaths resulted from the intramuscular injection of Nembutal at the 60 mg/kg level, although none had been encountered when the same dosage was given subcutaneously in other experiments(17).

The AP preparation consisted of anterior pituitary glands from adult Rhode Island Red male chickens. Upon removal, the glands were crushed between glass plates, air dried and subsequently powdered. The powder was suspended in distilled water, 2 or 4 mg/ml, usually shortly before injection into the pectoral muscles; a few suspensions were carried (under refrigeration) over several days. When used with "blocking" agents, the AP preparation was injected some 30-40 minutes before administration of the drugs. The LH and FSH fractions were prepared from anterior pituitary glands of sexually mature male chickens by procedures previously described in the fractionation of sheep pituitary tissue (21). Shortly before injection the fractions were dissolved, separately or together, in distilled water in concentrations which required the administration of not over 1 ml/hen. The solutions were injected into the pectoral muscles. Progesterone was dissolved in corn oil (Mazola), 5 mg/ml, and was injected into the pectoral muscles, 1 mg/hen.

*Results. i. Effects of AP on drug action.* Examples of drug-induced suppression of $C_2$ ovulation and AP effects on the drugs are given in Table I. Atropine alone or phenobarbital alone suppressed a high proportion of expected ovulations, yet AP appeared to intensify—however slightly—the suppressing action of the drugs. Nembutal alone suppressed only 53% of expected ovulations, leaving a wider margin for display of any enhancing action by AP. Significantly, AP with the drug increased the incidence of suppressed ovulations from 53 to 89%. The incidence of $C_1$ lapses increased appreciably when AP was used with atropine (from 42 to 73%) or with Nembutal (from 33 to 89%). With phenobarbital the AP effect was negligible, though in the direction of increased incidence. A high proportion of ovulations suppressed by AP + atropine subsequently exhibited $C_1$ lapses (L/S = 80%), and all ovulations suppressed by AP + Nembutal did so.

*ii. The effects of AP alone.* Preliminary tests with AP at several dosage levels indicated that 2 mg/hen, injected at about 4:00 p.m., yielded a fairly high incidence of sup-

TABLE II. Incidence of Suppressed Ovulations and $C_1$ Lapses following Intramuscular Injection of AP, 2 mg/Hen, at Differing Times before Expected $C_2$ Ovulation.

| Time of inj. (p.m.) | Inj. to ovulation (hr) | No. hens inj. | Suppressed ovulations No. | % | $C_1$ lapses No. | % | L/S ratio (as %) |
|---|---|---|---|---|---|---|---|
| 1:30 | 21 | 7 | 5 | 71 | 4 | 57 | 80 |
| 4:30 | 18 | 22 | 20 | 91 | 18 | 82 | 90 |
| 6:30 | 16 | 12 | 8 | 67 | 4 | 33 | 50 |
| 11:30 | 11 | 7 | 0 | — | — | — | — |

TABLE III. Incidence of Delayed $C_2$ Ovulations following Intramuscular Injection (ca. 5:00 P.M.) of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Combinations of the Fractions.

| Quantities | | No. hens | Suppressed ovulations | | $C_1$ lapses | | L/S ratio |
|---|---|---|---|---|---|---|---|
| LH, mg | FSH, mg | inj. | No. | % | No. | % | (as %) |
| .005 | — | 11 | 2 | 18 | 2 | 18 | 100 |
| .01 | — | 11 | 4 | 36 | 2 | 18 | 50 |
| .02 | — | 12 | 7 | 58 | 4 | 33 | 57 |
| .04 | — | 14 | 6 | 43 | 5 | 36 | 83 |
| — | 1.0 | 9 | 1 | 11 | 0 | — | — |
| — | 2.0 | 21 | 7 | 33 | 6 | 29 | 86 |
| .02 | .5 | 14 | 8 | 57 | 7 | 50 | 88 |
| .02 | 1.0 | 27 | 21 | 78 | 16 | 59 | 76 |
| .02 | 2.0 | 14 | 13 | 93 | 13 | 93 | 100 |

pressed ovulations. This level was used in all experiments summarized in Table II, which in part were carried out to ascertain variation of effect with hour of injection. Despite the relatively small number of hens injected at 1:30 p. m., the results leave little doubt that AP effectively suppressed ovulation when administered as much as 21 hours before the expected event. Four of the 7 treated hens (57%) exhibited $C_1$ lapses, or 80% of the 5 in which ovulation was suppressed. Following 4:30 p. m. injections, ovulation was suppressed in 20 out of 22 hens. Eighteen of the 22 injected hens (82%), or 90% of the 20 in which ovulation was suppressed, exhibited $C_1$ lapses. Ovulation was suppressed in 67% of 12 hens injected at 6:30 p. m., but $C_1$ lapses occurred in only 4 or 33%. As was anticipated from earlier experience, injection of AP at 11:30 p.m. failed to delay ovulation in any of 7 hens.

*iii. Effects of LH, FSH and LH + FSH.* As can be seen in Table III, LH suppressed few ovulations at the 0.005 mg level, and no large proportion at any of the dosages administered. The incidence of $C_1$ lapses was relatively low at the 0.02 mg level (33%), which suppressed ovulation in 58% of 12 injected hens. At the 0.04 mg level the proportion of suppressed ovulations dropped somewhat, although the incidence of $C_1$ lapses increased slightly; the L/S ratio increased from 57 to 83%. Administration of FSH alone at the 1.0 mg level was without appreciable effect in suppressing ovulation. The moderate incidence of suppressed ovulations following injection of FSH at the 2 mg level might possibly be attributed to contaminating LH, and is discussed later. In combining LH and FSH, LH at the 0.02 mg level was injected with increasing levels of FSH (Table III). The incidence both of suppressed ovulations and of $C_1$ lapses is seen to increase with increasing FSH levels, though at the 1 mg level of FSH the proportion of $C_1$ lapses did not keep pace with that of suppressed ovulations. This was reflected in the L/S ratio, which came to 76%. The combination of 0.02 mg LH + 2 mg FSH suppressed ovulation in 13 of 14 injected hens, and all of these exhibited the $C_1$ lapse.

*iv. Progesterone effects.* Twenty hens were injected with AP (2 mg/hen) at about 4:30 p. m. for delay of $C_2$ ovulation. The same hens received progesterone (1 mg/hen, intramuscularly) at about 2:30 a. m. of the following morning, *i.e.*, at about the hour of normally expected OIH release. Fifteen of the 20 hens (75%) ovulated either normally or in response to progesterone. This result is to be compared with the effect of AP alone (4:30 p. m. injections, Table II); here only 2 of 22 hens (9%) ovulated at the normally expected hour. It is to be noted that the incidence of progesterone-induced ovulations in AP treated hens is of the same order as was the incidence of $C_1$ lapses following the administration of AP alone.

*Discussion.* When injected intravenously, 0.001 mg/hen, 14 hours before expected $C_1$ ovulation, the same LH fraction used in the present experiments induced ovulation prematurely in some 50% of treated birds(3). The

FSH fraction was about equally effective at the 0.5 mg/hen level. This quantity of FSH (500 times the LH dose) presumably carried sufficient LH as a contaminant to account for the induced ovulations. The moderate effectiveness of FSH in delaying $C_2$ ovulation (Table III, 2 mg/hen) probably represents, in part at least, the action of LH. If this is so, we may conclude that FSH in itself has little or no capacity to delay $C_2$ ovulation, but that it substantially increases the potency of LH in this respect. The increased effectiveness of LH in combination with FSH observed here is apparently another instance of the augmentation effect so often encountered when the two fractions are combined(22,23).

Incomplete or partial stimulation of the ovulable follicle by subovulatory doses of LH may result in atresia(24) and thus "suppress" its normally expected ovulation. Van Tienhoven(25) has shown recently that interruption of progesterone-induced OIH release may likewise cause atresia of the ovulable follicle. Interruption of normal OIH release would presumably have the same effect. In the present experiments, atresia of the ovulable follicle might have occurred (though not necessarily), in any hen which failed to exhibit the $C_1$ lapse. Under appropriate conditions, however, gonadotrophins are capable of inducing the $C_1$ lapse in practically all injected birds. It is accordingly this characteristic effect which calls for examination and, if possible, explanation.

A first question concerns the immediate source of the suppressed ovulation appearing in the $C_1$ lapse. The fact that the suppressed $C_2$ ovulation is typically delayed only until the approximate hour of $C_1$ ovulation suggests failure of nervous stimulation for normally expected OIH release, such stimulation occurring at the outset of the next diurnally recurrent period of high neural sensitivity(11, 12). The response of gonadotrophin treated hens to progesterone, which causes OIH release by stimulation of the neural component of the release mechanism(7-10,12,25), confirms this inference. We may then conclude that the gonadotrophin-induced $C_1$ lapse makes its appearance in consequence of a transient "blockade" of the nervous activity required for OIH release.

There are no grounds for supposing that gonadotrophins might act directly as "blocking" agents (*e.g.*, like central depressants). On the contrary, we should expect them to act directly and perhaps exclusively upon the ovary and its secretory functions(4). The causes of gonadotrophin-induced lapses must then be sought in modified concentrations of circulating hormones of ovarian origin.

In appropriate combinations, LH and FSH would be expected to favor estrogen production in the hen, as they certainly do in the rat(26,27). Although estrogen may cause ovulation in the rat and other polyestrous mammals(4,28 for references), it delays ovulation in both the ring dove(29) and the hen (11). The proportion of $C_2$ ovulations suppressed by estradiol benzoate was only moderate in the hen (about 50%), but some 90% of these subsequently exhibited the $C_1$ lapse. Moreover, estrogen apparently induced $C_1$ lapses by increasing threshold requirements in the neural component of the OIH release mechanism(11). In all important respects, then, estrogen and gonadotrophin effects are similar. The gonadotrophin-induced lapse may thus have its origin, in part at least, in elevated levels of circulating estrogen.

Despite the similarity of qualitative actions, gonadotrophins appear to be more effective than either estradiol benzoate or diethylstilbestrol in delaying $C_2$ ovulation. This may mean that the endogenous estrogen is more "potent" than are the injected substances, or that it is secreted in a fashion not reproduced by injection. There remains, however, another possible aspect of endogenous estrogen production which may not be encountered when estrogen is injected. The gonadotrophin enforced elaboration of estrogen may be associated with delayed or decreased production by the ovary or the maturing follicle of the hormone—possibly a progestin(11,12)—believed to stimulate the neural component of the OIH release mechanism. In this event, gonadotrophin-induced lapses may stem in part from subthreshold levels of the postulated circulating excitation hormone.

If exogenous gonadotrophins result in an altered balance of ovarian hormones (estrogen

and progestin), it is not impossible that the naturally occurring OIH release—whether this consist of FSH and LH or LH alone—may do the same. Such a recurrent periodicity in ovarian hormone levels might well play an important part in the determination of lag relationships, length of cycle, and time of onset of the period of lapse(11,12). There is little point in carrying these speculations further at this writing, but they may serve to underscore the very real need for identification of the sex steroids of the ovulating hen and determination of varying concentrations with reference to critical events in the cycle.

The experiments described in this paper do not tell us much about the manner in which the usual nervous "blocking" agents delay ovulation when the delay (or suppression) cannot be attributed to direct blockade of the neural component of the OIH release mechanism(17,18). The increased proportions of $C_1$ lapses effected by the AP preparations in Nembutal or atropine treated hens may well signify, however, some amelioration of drug action by gonadotrophins or a gonadotrophin. Experiments looking toward a clarification of these relationships are in progress.

*Summary.* Antiadrenergic, anticholinergic or central depressant drugs suppress or delay, in varying proportions, ovulation of the second or $C_2$ follicle of the hen's sequence following their administration some 18-20 hours before normally expected ovulation. In an attempt to counteract these effects, a whole anterior pituitary (AP) preparation from cocks was injected intramuscularly shortly before injection of several of the drugs. The incidence of suppressed ovulations was increased by the AP preparation, and increased proportions of suppressed ovulations exhibited $C_1$ lapses, *i.e.*, the normally expected $C_2$ ovulation was delayed only until the hour of typical $C_1$ ovulation on the day following its suppression. Intramuscularly injected 18-19 hours before expected $C_2$ ovulation, the AP preparation alone suppressed ovulation in most hens, with a high proportion of $C_1$ lapses. Under similar conditions, luteinizing hormone (LH) or follicle stimulating hormone (FSH) from cock pituitaries suppressed ovulation in some hens. Administration of 0.02 mg LH + 2 mg FSH/hen suppressed ovulation in 93% of injected birds, and all of these exhibited the $C_1$ lapse. Progesterone injected at about the hour of normally expected release of ovulation-inducing hormone (OIH) effected ovulation in most hens previously treated with the AP preparation, indicating that gonadotrophin-induced lapses resulted from a transient "blockade" of the nervous mechanism controlling OIH release. Since estrogens similarly "block" $C_2$ ovulation in the hen, the gonadotrophin-induced delay may be attributed to increased levels of circulating estrogen. Enforced estrogen production may however be associated also with reduced or delayed output of the hormone believed to excite the nervous component of the OIH release mechanism.

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## Effect of Temperature and Humidity on Nasal Flora of Mice. (22060)

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The possibility that atmospheric conditions are reflected in changes in physiological status of the nose and nasopharynx which are related to susceptibility to upper respiratory infections and poliomyelitis have been suggested. Thus, Armstrong(1) observed a correlation between absolute humidity and incidence of poliomyelitis in certain localities and suggested that atmospheric humidity influences susceptibility by its action on the nasopharynx.

In the present investigation an attempt was made to demonstrate local alterations in normal microflora of nasal passages of mice after exposure to different atmospheres of temperature and humidity, preliminary to a study of association of these changes with local resistance.

*Material and methods.* White Swiss male mice (9-12 g) were used. Atmospheres of low and high humidity were prepared in metal chambers with glass covers in which cages containing the mice were placed. A low relative humidity in range of 25-30% was maintained by introducing dry air into an enclosed chamber and measured directly by means of hydrodial supplied by the Bendix Co. of Baltimore, Md. A high relative humidity was maintained by introducing air saturated with water into the enclosed chamber. Three sets of the experimental chambers were maintained at 21°C, to study the effect of humidity and normal temperatures. One set was maintained at approximately 31°C to study the effect of increased temperature and humidity. Mice were kept at 21°C with a relative humidity of 45-50% for at least 2 to 3 weeks before they were subjected to experimental conditions. They were fed a balanced diet, and every care taken to avoid infection, exposure, or overcrowding. Bacterial flora of the nasal tract was determined by plating nasal washings on blood agar plates. Nasal washings were obtained as follows: Mice were killed by ether. The skin covering anterior part of head was removed after being moistened with ethyl alcohol. The nasal bone covering the nasal passage was lifted using aseptic technic, thus exposing the nasal cavity. Using a capillary pipette, .05 ml of sterile buffered saline was introduced and after drawing it up and down several times, the washing was spread on a blood agar plate using sterile bent glass rod. The plates were incubated at 37°C for 48 hours and the number and type of normal flora were determined. Nasal washings from 20 mice which had been exposed to low humidity were tested for antibacterial action on isolated gram positive and gram negative bacteria.

*Results.* The effect of temperature and humidity on normal flora of nasal passage was studied by exposing 4 groups of mice to conditions mentioned previously for 3 weeks. The results summarized in Table I indicated

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