with a family history of vascular disease. Subject L was a paretic 50 years of age, while subjects CH and CHg were tabetics about 60 years of age. Subject G was a 57-year-old male with a history of cardiovascular disease and alcoholism. From this small group however, conclusions cannot be drawn concerning the relationship between degree of formation of clearing factor and the disease process known to be present.

Discussion. These experiments have confirmed the work of Anderson and Fawcett(2) in which they demonstrated the presence *in vitro* of an antichylomicronemic substance in post-heparin plasma samples. However, in our experiments, incubation of the sera at 37°C appeared to be necessary to initiate clearing activity *in vitro*. It is to be noted that Anderson and Fawcett used much larger doses of heparin (50 mg) than those used in our experiments, or by Block *et al.*(4).

Several problems related to the size of the single injected dose of heparin need to be answered. For example, the larger dose of heparin used by Anderson and Fawcett(2) may make incubation of sera unnecessary to initiate "clearing activity" *in vitro*. Furthermore, large doses of heparin which not only cause greater clearing of sera *in vivo*, and perhaps for a longer period of time, may also initiate greater clearing *in vitro* after incubation with the formation of more potent "clearing factor." These problems are under investigation.

Since the work of Block and his associates (4) has indicated that the *in vivo* response to a single dose of heparin may be altered in vascular disease, further studies in patients with cardiovascular disease may also demonstrate differences in the degree of formation of "clearing factor" when compared with control subjects in the same age groups.

Summary. 1. By the use of a well-standardized fat tolerance test, it has been possible to demonstrate the clearing effect of small doses of intravenously administered heparin *in vivo*. Furthermore by use of these small doses of heparin, we have demonstrated that individuals vary in their ability to clear their own sera *in vitro* after incubation. It has also been shown that an individual who clears well *in vivo* will probably clear well *in vitro*, as in the experiments described. Apparently the potent "clearing factor" formed, as for example in subject L, was very potent for the sera of other subjects who formed less "potent clearing" factor under similar conditions.

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Premature Ovulation in the Domestic Fowl following Administration of Certain Barbiturates. (20056)

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Everett(1) and Markee(2) have recently reviewed evidence indicating that neurogenic activation of the hypophysis is essential for release of ovulation-inducing gonadotrophin not only in the rabbit, which normally ovulates following copulation, but also in the rat, a "spontaneously" ovulating species. Evidence of a 24-hour rhythm in the neural mechanism was adduced in the 5-day cyclic rat when ovulation was advanced 24 hours by progesterone(3), an action of the steroid which could be blocked by atropine or dibenamine (4). This 24-hour periodicity in the neural

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mechanism effecting release of hypophysial gonadotrophin was confirmed when Everett and Sawyer(5,6) showed that barbiturate sedation during critical hours of proestrus—a period of about 2 hours for the colony, even less for the individual rat(7)—delayed release of pituitary gonadotrophin by almost exactly 24 hours. If sedation were repeated over the same critical hours on the second day, release of the hypophysial ovulatory hormone was postponed by an additional 24 hours(6). Like atropine or dibenamine, Nembutal also blocked the release of ovulation-inducing hormones by progesterone in the rat(8).

In hens under usual conditions of maintenance and lighting, the release of ovulation-inducing hormone (OIH) for the first or C_1 ovulation of a sequence occurs at a fairly definite time of day(9,10). A number of considerations, some of which have been noted previously(10), indicate the possible participation of factors outside the usually postulated ovarian-hypophysial relationships in timing of OIH release for C_1 ovulation. In the light of findings in the rat, it appeared not improbable that the regulatory factors in question might be of neural origin in the hen also. During the course of experiments on this problem an unexpected advancement in time of C_1 ovulation was found to occur following the administration of certain barbiturates. This effect in the fowl, which does not appear to have been described previously in any species, is reported on in the present paper. Some preliminary observations indicative of a complementary action between progesterone and those barbiturates favoring prematurity of ovulation are also noted briefly.

Materials and methods. The hens included White Leghorns, Rhode Island Reds and crosses between these breeds, all in first or second years of production. The birds were battery caged, with feed and water always available. Artificial lighting was maintained from 6:00 a.m. through 8:00 p.m. Time of lay of eggs was recorded on the hour from 8:00 a.m. through 4:00 p.m. for at least 2 weeks—usually much longer—before any hen was selected either for test or control purposes. *All tests* were carried out for effect on the C_1 follicle. The hens were laying sequences of 2 or more eggs, with but a single day lapse anticipated between sequences. Such hens, as a rule, lay the terminal egg of a sequence between 1:00 and 4:00 p.m. Release of OIH for C_1 ovulation of the next sequence takes place later than 10:00 p.m. of the same day (9), and C_1 ovulation occurs between 4:00 and 7:00 o'clock of the following morning (9,11), the mean time falling not far from 6:00 a.m.(11). Barbiturates were administered in single doses between 2:30 and 4:30 p.m., thus some 12 to 16 hours before the time of normal C_1 ovulation, or at least 6 hours before the normal release of OIH. *Prematurity of ovulation* was established, in most tests, by digital palpation *via* the cloaca at appropriate intervals until the egg consequent upon ovulation had attained either the fully plumped condition or the early stages of shell formation. A total of more than 150 uninjected and carrier injected control hens were subjected, from time to time, to exactly the routine practiced on test birds. The reliability of results based on palpation was further assured by autopsy of both control and barbiturate-treated hens. The *barbiturates* used and preparation for injection follow. Barbital sodium, 200 mg/ml distilled water. Phenobarbital sodium, 100 mg/ml distilled water. Diallylbarbituric acid (Dial),† 100 mg/ml propylene glycol; also "Dial with urethane," Dial 200 mg, urethane 800 mg and monoethylurea 800 mg/2 ml ampul solution. Calcium ethylisopropylbarbiturate (Ipral),† 20 mg/ml distilled water. Pentobarbital sodium (Nembutal), 60 mg/ml distilled water; also used as prepared solution, 60 mg/ml containing 20% propylene glycol and 10% alcohol. *Dial* was administered intramuscularly and subcutaneously, with little difference in result; the remaining barbiturates were injected subcutaneously. Where a range of dosage levels is given in Table I, the great majority of tests was run at the highest level noted. In terms of *duration of depression,* phenobarbital and barbital have been classified as long, Dial and Ipral as intermediate,

† Dial and progesterone were supplied generously by the Research Department of Ciba Pharmaceutical Products, Summit, N. J., and Ipral by E. R. Squibb and Sons, New Brunswick, N. J.

TABLE I. Incidence of Premature C_1 Ovulations Following Administration of Barbiturates.

Barbiturate	Dosage, mg/kg	No. hens inj.	Premature ovulations No.	%
Barbital sodium	200	84	2	2
Phenobarbital sodium	50–150	38	0	—
Dial	80–100	57	16	28
Ipral	50–100	52	15	27
Nembutal	60	15	2	13

and Nembutal as short acting(12). Phenobarbital and barbital exhibit lengthy induction periods(12). Probably the same order of effects holds in the hen, but observations are limited. The action of barbital in fowl is to be noted, however. This drug was shown by Koppanyi, Murphy and Gray(13) to cause (at 225 mg/kg) a profound narcosis from which the birds do not recover, though they may survive for days under forced feeding. The drug is not metabolized and is very sparingly eliminated. The *induction of ovulation* with progesterone has been described elsewhere(14). In the tests described below, crystalline progesterone† was dissolved in corn oil (Mazola) and injected subcutaneously in breast regions, always distant from the site of barbiturate injection when used in conjunction with the drugs.

Results. The incidence of premature ovulations following the administration of barbiturates is recorded in Table I. There can be no question concerning the result with Dial and Ipral, since more than a fourth of the hens included in the totals were autopsied before ovulation could have occurred normally, as was confirmed by simultaneous autopsy of control birds. Nembutal probably belongs with Dial and Ipral as an effective agent in view of its action with progesterone (noted below). The exceptional number of hens treated with barbital to yield only 2 premature ovulations calls for a word of explanation. In preliminary tests to ascertain appropriate dosage levels, 1 of 4 hens receiving 200 mg/kg ovulated prematurely. The 16th hen injected thereafter did the same. In view of the lasting narcosis induced in fowl by barbital, tests were continued; none of the additional 64 hens ovulated prematurely. The results with phenobarbital were entirely negative.

Both autopsy and palpation records indicated that the interval from administration of barbiturates to premature ovulation was somewhat longer and more variable than is the same interval following the injection of luteinizing preparations at optimal dosages. If ovulation follows OIH release under barbiturate treatment by about the same interval that it follows the intravenous injection of luteinizing preparations, some 2 to 4 hours must elapse between injection of the drugs and release of OIH.

The failure to obtain a greater incidence of premature ovulations than is recorded in Table I cannot be attributed in most instances to disruption of the normal processes of OIH discharge or of follicular rupture. Of those hens which did not ovulate prematurely in response to treatment with barbital, phenobarbital, Ipral or Nembutal, 85 to 90% ovulated at the normally expected hour. Only a little better than 60% of the Dial-injected hens not ovulating prematurely did so at the normal hour. The early appearance of follicular regression in some Dial-treated hens subjected to autopsy after failing to ovulate either prematurely or at the normally expected time was suggestive of OIH discharge at subovulatory levels(15), but on this point the evidence is inconclusive. The results, taken altogether, confirm and extend the findings of Bastian and Zarrow(16) on the failure of Nembutal to block ovulation in the hen.

When it became clear that some barbiturates favored prematurity of ovulation, it seemed reasonable to expect a complementary or additive action between such barbiturates and progesterone. To test this supposition, progesterone was given at a nearly minimal ovulation-inducing level 30 to 40 minutes following the administration of Dial in one experiment, Nembutal in a second. The results, given in Table II, indicate a definite mutuality of action between each of the barbiturates and progesterone. Preliminary experiments have given some evidence, however, that the ovulation-inducing capacity of progesterone is at least partially reduced by phenobarbital; it will be recalled that no ovulations were recorded following the ad-

TABLE II. Enhancing Action of Progesterone with Dial and with Nembutal.

Treatment	No. hens inj.	Premature ovulations No.	%
Dial, 100 mg/kg	16	3	19
P,* .1 mg/hen	14	0	—
Combined	21	12	57
Nembutal, 60 mg/kg	15	2	13
P, .1 mg/hen	9	1	11
Combined	20	6	30

* P = Progesterone inj. 30-40 min. following administration of barbiturate.

ministration of this drug. The investigation of these varied relations is now in progress.

Discussion. The main objective of this report has been to establish the fact that C_1 ovulation may occur in a significant proportion of hens following the administration of certain barbiturates. The complementary action of such barbiturates (*i.e.*, those facilitating C_1 ovulation) and progesterone at near-minimal ovulatory levels strongly reinforces the direct evidence. In view of these findings, it is not surprising that the same barbiturates fail to block either normal or progesterone-induced ovulation, as Bastian and Zarrow(16) have previously reported of Nembutal.

There are no *a priori* grounds for supposing barbiturates or their metabolic derivatives can effect ovulation prematurely by direct action either on the ovarian follicle or on the anterior pituitary body. Without summarily dismissing these possibilities, the more probable supposition may be that the anterior pituitary releases OIH in response to neurogenic factors operating over a humoral pathway, such as Harris(17) has described in other connections. Drager(18) has described the required architecture, noting the absence of nerve fibers into the pars distalis. It is not likely that depression *per se* constitutes the immediate neurogenic condition for adenohypophysial activation, for the proportion of premature ovulations should then have been higher with at least some of the drugs employed. Neural excitation following a characteristic duration or intensity of depression, preceding depression (barbital?), or originating out of more complex conditions might of course be postulated.

The variable interval from injection of the drugs to ovulation becomes understandable in these terms, as does also the failure to obtain premature ovulations with phenobarbital, in appreciable proportions with barbital, or in uniformly high proportions with even the more effective barbiturates. The mutual action expressed between progesterone and effective barbiturates and the apparent absence of this effect with phenobarbital are likewise consistent with the postulated secondary appearance of neural excitation.

Although the findings presented in this paper can thus be rationalized in the suggestive, if somewhat general, terms of neural excitation, proof of such an hypothesis obviously will require data of a different order than are now at hand.

The complementary action of ovulation-facilitating barbiturates and progesterone—even if only a summation effect—is of more than incidental significance in view of the reported occurrence of progesterone in the plasma of the hen(19). Variation in the blood level of endogenous progesterone might thus become an important factor in the variability of reaction, and possibly also in timing of response to a given barbiturate. The relative small quantity of progesterone employed emphasizes this point: if all the injected steroid were taken into the blood stream without loss the blood concentration of progesterone would be increased by no more than 0.75 μg/ml. Inasmuch as the steroid was given subcutaneously, in oil, and the rate of disappearance from the blood stream is known to be high(20), the actual change in blood level was probably considerably less than 0.75 μg/ml.

Selye(21) has shown that a number of steroid compounds, among them progesterone, desoxycorticosterone acetate and testosterone, are effective anesthetic agents in the rat; progesterone, at least, acts similarly in the immature fowl(11). Like progesterone, desoxycorticosterone acetate and testosterone induce ovulation prematurely in the hen(11). The results reported on here demonstrate that this rather remarkable association of anesthetic and ovulation-inducing effects in fowl is encountered also with certain barbiturates.

It may be noted in this connection that Hertz, Allen, Tullner and Westfall(22) have recently remarked upon the association of anesthetic and endocrine properties in the non-steroid compound Amphenone "B".

Summary. Ovulatory reactions of the first follicle of the hen's ovulation sequence to barbiturates, and to barbiturates with progesterone, are described. Ovulation was found to occur prematurely in a significant proportion of hens following the administration of some barbiturates (Dial, Ipral and Nembutal), rarely with barbital, and not at all with phenobarbital. Near subovulatory dosages of progesterone acted synergistically with two of the barbiturates (Dial and Nembutal) which themselves effect ovulation prematurely, an effect which was not found when progesterone was similarly administered with phenobarbital (preliminary observations). These several observations are consonant with the supposition that prematurity of ovulation following the administration of effective barbiturates results from neural excitation secondary to depression, excitation effecting over a humoral pathway the release of ovulation-inducing gonadotrophin from the anterior pituitary body.

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New Hemophilia-like Disease Caused by Deficiency of a Third Plasma Thromboplastin Factor.* (20057)

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(Introduced by W. Antopol.)

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Hemophilia has been generally recognized as a hereditary disease characterized by the deficiency of a plasma factor which reacts with platelets to form thromboplastin(1-3). This factor, designated as anti-hemophilic globulin (AHG), is essential for the normal conversion of prothrombin to thrombin(4,5). Recently, evidence for a second plasma thromboplastin factor has been presented on the basis of 2 reports of male patients with hemorrhagic disease and coagulation findings similar to hemophilia except that the blood of these

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