

Untreated normal rabbits varied in their response to injections of distilled water. One rabbit died immediately following a single injection of 20 cc. while another withstood the injection of 250 cc. on two consecutive days without a reaction but died on the third day immediately after an attempt to introduce 300 cc. into the circulation. Hemoglobinuria occurred in all the rabbits which received large amounts of distilled water.

There were no striking changes found in the organs except those incidental to increased blood destruction. Large numbers of pigment laden phagocytes were found in the sinuses of the spleen and lymph nodes. When very large doses of distilled water were injected, the sinuses of the spleen and liver were distended with the shadows of erythrocytes; there was also evidence of pulmonary edema. The bone marrow showed no significant changes.

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Some limitations of the action of the so-called follicular hormone in birds.

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During the past twenty-five years the work of many investigators has shown that the ovaries exercise control over the rapid temporary growth and hyperemia of the uterus which is characteristic of "heat" in mammals. Various extracts made from ovaries, and from other distinct tissues, have also long been known to bring about these particular uterine changes when injected into mammals (Marshall and Jolly, 1905; Lane-Claypon and Starling, 1906; Sonnenberg, 1907; Adler, 1911). The separation from the ovary of a lipoid fraction capable of inducing these uterine changes is also well established (Iscovesco, 1912; Hermann, 1913; Fellner, 1913, 1921; Seitz, Wintz and Fingerhut, 1914; Frank and Rosenbloom, 1915); and recently both the separation and the testing of this hormone have been

more satisfactorily accomplished (Allen and Doisy, 1923; Frank and Gustavson, 1925). Still other hormones have probably been separated from the ovary (Iscovesco, 1912; Seitz, Wintz and Fingerhut, 1914; Papanicolaou, 1924), but the one considered here is the utero-stimulant¹ of Iscovesco, "das spezifische Ovarialekret" of Fellner, the "follicular hormone" of Allen and Doisy, and the "female sex hormone" of Frank and Gustavson.

No previous study has been made of the action of this hormone when injected into a bird—nor into any animal other than a mammal. Allen, and co-workers¹, however, obtained from fowl ovaries an extract which gave positive tests for the hormone when injected into rats. They were unable to obtain the hormone from egg-yolks. Fellner² extracted the hormone from the egg-yolks of the fowl and from the ovaries of fish, and obtained the typical uterine reactions in tests made on rabbits. In our rather limited studies we have obtained positive results on oviducal enlargement (little or moderate) and hyperemia in 3 of 7 tests with immature (3.5 to 6.5 mo.) ring doves. Oviducal weights from 13 controls for the three positive tests indicated an enlargement of the latter by from 10 to 60 per cent; the hyperemia was unquestionable. The lack of "specificity," as between birds and mammals, of this particular action of the hormone or substance is therefore now completely established.

Of equal or greater interest in our results are the apparent limitations of the action of this hormone in birds. Four of the seven immature doves (weight, about 150 gm. each) tested were quite unaffected although the dosage used probably ranged from 5 to 25 rat units. Three of the four doves which showed no response were given somewhat larger absolute amounts than that simultaneously given to two virgin rabbits aged 4 months. The weight of a rabbit was about ten times that of a dove, but the response was obtained in both rabbits and in neither of the doves. In two of the three positive results with doves a dosage of 5 rat units of purified hormone from the placenta* was given during 3 days and autopsies made one day later; in the third, a less marked response was obtained from approximately 22 rat

¹ Allen, E., Whitsett, J. W., Hardy, J. W., and Kneibert, E. L., *Proc. Soc. Exp. Biol. and Med.*, 1924, *xxi*, 500.

² Fellner, O. O., *Klin. Wochenschr.* (Berl.), 1925, *iv*, 1651.

* Supplied through the courtesy of Drs. R. T. Frank and R. G. Gustavson.

units† from liquor folliculi of sow administered during 6 days. This limited response under high dosage, together with the complete failure of relatively very heavy dosage to induce oviducal changes in some of our birds with the same preparation which (in relatively and absolutely smaller amounts) gave positive results in rabbits, probably indicates a less effective action of the hormone on the oviducts of birds than in those of mammals. This limitation, however, may be correlated with the fact that the functional ovaries of birds are relatively much larger than those of mammals. In other words, the ovary of the bird may normally supply the organism with larger quantities of the hormone than does the ovary of the mammal.

Still other limitations of the action of this hormone appear if this same hormone is considered as being capable also of inducing "puberty," of immediately reinforcing or increasing the growth of the larger follicles in the ovary, and of generating female sex behavior. From our knowledge of the rate and time of development of oöcytes in the ovary of the dove or pigeon, we know that injections and observations in these forms would have to include a period of nearly four weeks before an induced ovulation could be expected in an immature dove aged two or three months. We believe that ovulation is the only adequate criterion of puberty, and that conclusive proof of the capacity of this hormone to induce premature puberty in either birds or mammals is the most important theoretical point remaining for solution in work with this substance; but unfortunately our limited supply of follicular fluid has thus far prevented any adequate test of this matter. Bearing on this point nevertheless is the impression we get of rather wide-spread *degeneration* of the larger follicles in the ovaries of most (not all) treated birds.

The following facts also bear on the topic last-mentioned above. The capacity of the hormone to cause a continued growth and ovulation of ova in the ovary of mature birds has been insufficiently examined, but with entirely negative results. Four such ovulating females were tested with relatively fresh (and mostly otherwise tested) preparations. The first was given 22 rat units in 15 injections during a period of 6 days. The second was given the crude alcoholic extract from about 28 cc. of folli-

† The purified hormone for this injection was kindly supplied and tested by Dr. Edgar Allen.

cular fluid in 20 injections during 7 days. The third was given the purified hormone from about 16 cc. fluid in 14 injections during 5 days. The fourth was injected during the first 7 days of her incubation period. In none of these four cases was there any suggestion of an increase in the rate of maturation and release of ova. Possibly an action of this sort is not to be expected in the case of the pigeon, but it is certainly a fact that something connected with *season* does affect changes in ovulation rate in these animals. Two additional tests were made on older females, one a virgin of advanced age, and the other a bird that had stopped laying 3.5 mo. before beginning injections; results were negative in both cases.

The hormone or substance used by us (except as noted in footnote 3) was obtained from the liquor folliculi of the sow. It was kindly supplied by Dr. F. Fenger, of Armour and Co., Chicago, and reached us on the third or fourth day after collection and storage in two volumes of alcohol. A part of this alcohol filtrate was evaporated within a few hours at low temperature (less than 50° C.) and the injection of this fraction begun at once. The remainder was purified by the Allen-Doisy method within three days and injections begun immediately in order to avoid well known rapid loss of effectiveness of this substance. After preparation the hormone was dissolved in mazola (one lot in glycerin) and kept *in vacuo*. The material was injected subcutaneously (intraperitoneal in 1 series). Young male doves were also treated, but the results of that study, together with additional data for the female tests, will be described elsewhere.

SUMMARY

Brief reference is made to the more important earlier studies on the action of a utero-stimulating substance in mammals.

In a small group of tests of the utero-stimulating action of a placental extract, and of a substance prepared from the liquor folliculi of the sow by the Allen-Doisy method, we have obtained a few positive results—enlargement and hyperemia of the oviduct of virgin doves. This supplies the last necessary fact in proof of the lack of specificity, as between birds and mammals, of this substance.

Heavy dosage failed to affect a response in the virgin oviduct in some cases. Responses in the ovary and on sex behavior in virgin and mature doves have not been observed. An insufficient

number of cases was studied but our results indicate a less ready, and a less varied, response of the bird to this substance than has been found for mammals.

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Heavy alcoholization and prenatal mortality in mice.

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A previous investigation¹ has shown that treating female mice with light doses of alcohol fumes (beginning at 4 weeks, every day for 45 minutes in a pint bottle with 3 cc. alcohol) does not modify the prenatal mortality or any other phase of their reproduction. In the present study the same technique for administering the alcohol has been used, but each mouse was left in its bottle until in a state of deep anesthesia 5 days per week. This required 1 to 2 hours for mature mice. Experimental animals were mated at 4 weeks, and on the day of birth the mothers were returned from their isolation in pregnancy boxes to mating pens, without their young. The results are based on the number of corpora lutea associated with each successive litter of young born; the difference between the number of corpora lutea and the number of young is called the prenatal mortality. The technique of counting the corpora lutea has been described² and the reliability of this criterion of prenatal mortality discussed³.

Two main series of experiments have been performed. In the first series the mothers only were treated (data from 657 litters). The unit experiment was a litter of 4 to 6 females half of which were treated. All lived in the same pen, mated to the same male, an older sib previously found fertile.

In the second series of experiments the fathers only were

¹ MacDowell, E. C., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 480.

² MacDowell, E. C., *Anat. Rec.*, 1924, xxvii, 329.

³ MacDowell, E. C., and Lord, E. M., *Am. J. Anat.*, 1926, xxxvii, 127.