ously reported for pooled, dried egg-whites, and for the dried oviduct of the hen.[9,10] The use of fresh materials and the more complete extraction of the samples by mechanical homogenization may account for the higher titers reported here.

The oviducts and ovaries of the 10 non-laying hens were in varying stages of atrophy. The magna of these birds were, however, uniformly negative to avidin test, indicating that avidin production is correlated closely with full ovarian and associated oviducal function.

Summary and Conclusions. Quantitative avidin assays of the various portions of the oviducts of 21 actively laying and of 10 non-laying hens indicate (1) that avidin is found at all levels of the magnum, or albumen-secreting portion of the oviduct of the laying hen, (2) that avidin does not occur in either the isthmus or uterus of the laying hen, (3) that avidin is not found in the atrophic magna of non-laying hens, and (4) that the avidin content of regions of the magna below the level of an oviducal egg is higher than in regions above the egg. Avidin production appears to be associated, either directly or indirectly, with complete reproductive function of the ovary.

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Induction of Avidin Formation in the Avian Oviduct by Stilbestrol Plus Progesterone.

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In the preceding paper we have presented observations indicating that the presence of avidin in the oviduct of the hen is functionally related to the full reproductive phase of ovarian activity.[1] Since the endocrine products of the ovary are known to control in large part many of the functions of the oviduct, we have undertaken to determine the effect of certain ovarian hormones upon the formation of avidin.

The present report describes the experimental induction of avidin formation in the oviducts of sexually immature birds and of hens which were out of egg production for some time preceding and throughout the course of the tests.

It is thus far not clear whether or not the ovary of the bird produces steroid hormones in addition to estrogens. Consequently our experimental procedure was based primarily upon hormonal mechanisms known to occur in mammalian species. In these forms it is established that the ovary secretes at least two hormones, namely, the follicular hormone which is primarily responsible for tissue proliferation in the genital tract, and the corpus luteum hormone, progesterone, which, among other effects, induces secretory activity in the endometrium.[2] Accordingly, we undertook to determine whether avidin formation might be induced in the oviduct of the bird by the synergistic action of follicular hormone and progesterone.

Material and Methods. One hundred and twenty-two immature chickens ranging from 4 to 14 weeks of age constituted 4 distinct experimental series (Table I). In all 4 series birds were treated with stilbestrol alone, and with stilbestrol plus progesterone. Three of the series included untreated or oil injected controls, and in 2 of the series groups were injected with progesterone alone. The steroids were dissolved in corn-oil and injected daily subcutaneously. The essential differences

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TABLE I.
Avidin Assays of Magna of Immature Chickens.

Breed and age of birds	Initial treatment: Substance inj.	Daily dose	Days inj. No.	Secondary treatment: Substance inj.	Daily dose, mg	Days inj., No.	Magna assayed, No.	Result: + No.	— No.	Range of titre, units
Cross Bred	Stil.	0.5 mg	18				6	2	4	0.8-1.0
(W.L. ♂ × R.I.R. ♀)	Stil.	0.5 "	12	Prog.	0.2	6	6	6	0	0.8-2.0
4 to 6 wk	Prog.	0.2 "	18				6	0	6	—
	Oil	0.2 ml	18				6	0	6	—
White Leghorn	Stil.	0.5 mg	8				15	0	15	—
6 wk	Stil.	0.5 "	6	Prog.	0.2	2	13	13	0	0.4-0.8
White Leghorn	Stil.	0.2 "	18				33	0	33	—
10 wk	None						7	0	7	—
Cross Bred	Stil.	1.0 "	18				7	1	6	2.8
(Misc.)	Stil.	1.0 "	12	Prog.	0.4	6	7	7	0	0.6-2.5
10 to 14 wk	Prog.	0.4 "	18				7	0	7	—
	None						9	0	9	—

Stil.—Stilbestrol.
Prog.—Progesterone.

between the respective series were age of birds and length of treatment with either stilbestrol or progesterone.

In addition to the immature birds, 35 hens which had terminated their laying season were likewise treated with varying combinations of stilbestrol plus progesterone or with stilbestrol alone, as is recorded in Table II. Complete confirmatory evidence that the ovaries of these hens were non-functional was afforded by the finding of marked gonadal atrophy at autopsy.

The magnum of each hen was prepared for avidin assay in accordance with the procedure outlined in the preceding paper.[1]

Results and Discussion. The data presented in Tables I and II show that injection of stilbestrol followed by progesterone proved effective in inducing avidin formation in every bird so treated, 26 immature and 6 non-laying chickens. In direct contrast, only 3 of the 61 immature birds and only 4 of the 29 non-laying hens treated with stilbestrol alone showed evidence of avidin formation. The oviducts of all control birds and of those receiving progesterone alone were negative to avidin assay.

The strikingly uniform results obtained with the combination of stilbestrol and progesterone leads one to conclude that the elaboration of avidin may be regarded as one more of the numerous reactions known to depend upon the synergistic action of follicular and luteal hormones.

It is difficult to account for the occasional positive response to stilbestrol alone. Such reactions may be due to the endogenous production of a lutein or physiologically equivalent steroid as a result of an estrogen-conditioned release of luteinizing hormone from the pituitary. Indirect support is afforded this

TABLE II.
Summary of Avidin Assays of Immature Chickens and Non-laying Hens.

Treatment	Immature ♀ ♀: Magna Assayed No.	Results: Positive No.	Negative No.	Non-laying hens: Magna Assayed No.	Results: Positive No.	Negative No.
Stilbestrol	61	3	58	29	4	25
Stil. + Prog.	26	26	0	6	6	0
Progesterone	13	0	13	—	—	—
Oil or None	22	0	22	10	0	10

hypothesis by the fact that such a release mechanism has been repeatedly demonstrated in the rat.[2,3] The variation in frequency of positive response to stilbestrol alone shown in the respective series may arise out of the specific conditions of pretreatment with stilbestrol, or endogenous hormonal or other factors may differ in birds of the several series.

The identity of the hormones secreted by the ovary of the bird is not clear. It is generally agreed that estrogenic hormone is produced by the avian ovary, but the existence of a true corpus luteum hormone is open to doubt.[4] Nevertheless, recent observations by Dunham and Riddle[5] indicate that certain steroids other than those of the estrogenic type exert distinctive effects in the bird, and Fraps and Dury[6] have found that progesterone will regularly induce premature ovulation of any follicle of the hen's normal clutch sequences. Moreover, the induction of ovulation by progesterone and testosterone in amphibia suggests that non-estrogenic steroids may normally play a role in such lower forms.[7-9]

It should be emphasized that our data do not establish the ovarian source of the second steroid involved in avidin production. In this connection the physiological similarity of gonadal and adrenal steroids in their effects on the genital tract of mammals must always be kept in mind. Nevertheless, our data do indicate that the experimental induction of avidin formation in the avian oviduct requires the action of some (unidentified) steroid in addition to estrogenic hormone.

The hormonal control of avidin production suggests further that the avidin-biotin complex may be intimately related to basic mechanisms involved in the physiology of reproduction in birds.

Summary. The production of avidin, the antibiotin factor responsible for dietary egg-white injury, has been experimentally induced in the oviducts of both immature chickens and non-laying adult hens by administering stilbestrol followed by progesterone. This finding demonstrates a specific effect of progesterone in the bird, and suggests that the avidin-biotin complex may play a role in the physiology of avian reproduction.

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