mary-normal-amyl alcohol was found to be the most effective disinfectant when tested by the agar cup plate method for testing antiseptics and disinfectants. There was a regular decrease in germicidal action from the primary to iso- to secondary to tertiary arrangements of the carbon atom chain for any given number of carbon atoms.

14062

Relation Between Ovarian Function and Avidin Content in the Oviduct of the Hen.

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The dietary studies of Boas[1] and of Parsons[2] demonstrated that raw egg-white contains a factor capable of producing in rats and other species a specific toxic syndrome. This condition is characterized in rats by a scaly dermatitis, loss of hair, reduction in weight, diarrhea, spasticity of the hind limbs, and a general debility which ultimately leads to death. The harmful agent in egg-white has been shown by Eakin *et al.*[3] to exert its toxic effect by rendering the biotin in the diet unavailable to the animal. Because of this affinity for biotin, the egg-white injury factor has been named "avidin."[3]

Biotin, originally considered only as a growth-essential for yeast, has subsequently been identified as the dietary factor which either prevents or cures egg-white injury.[4] Biotin was first isolated from dried egg yolks by Kögl and Tonnis[5] and its chemical structure has been elucidated recently by du Vigneaud.[6] It is particularly noteworthy that the chicken is among those species shown to require biotin for growth and survival.[7,8]

Hertz and Sebrell[9] have recently presented evidence indicating that avidin arises as a secretory product of the mucosa of the oviduct of birds and amphibia. We wish to present at this time further data concerning the relationship of avidin content of the oviduct to the functional state of the ovary of the hen. In addition, we have attempted to determine more specifically the particular tissues of the oviduct which are concerned with avidin secretion.

Material and Methods. Thirty-one adult hens weighing between 2 and 3 kg were maintained in separate cages on an adequate stock diet. Of the 21 laying birds, 8 were sacrificed at known times following estimated time of ovulation in order to obtain the oviducal egg at various levels in the magnum. Eight hens were autopsied immediately following lay; the 5 remaining hens carried eggs *in utero* at autopsy. Ten non-laying hens, seasonally out of production, were sacrificed to provide oviducts in varying degrees of atrophy.

The avidin content of the various oviducal tissues was determined by the method of Eakin *et al.*[10] as modified by Hertz.[11] This method

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TABLE I.
Avidin Assays of Magna and of Albumen of Oviducal Eggs.

		Units of avidin per g of fresh material				
		In magnum†			In albumen	
Bird No.	Position of egg in tract*	above egg level	at egg level	below egg level	of oviducal egg	of last laid egg
N4084	7/18	1.0	1.0	2.5	2.5	1.7
N3828	9/18	1.0	2.5	5.0	2.5	2.5
E221	8/21	1.25	1.25	2.5	2.5	5.0
E284	10/17	1.0	3.3	5.0	2.0	
K4593	11/16	0.3	1.25	1.25	1.0	
N3833	End of magnum	2.0	3.3	—	1.0	
N4168	4/16	1.0	1.25	2.5	1.0	
K5520	4/15	2.5	1.7	5.0		

*Fractions indicate the proportion of the total length of the oviduct (inches) which had been traversed by the egg at the time of autopsy.

†Avidin titers for the mid-portion of the magna of 8 laying hens without eggs in oviducts at time of autopsy and of 5 hens with eggs *in utero* ranged from 1.0 to 3.3 units per gram of fresh tissue. Assays of the mid-portion of the magna of 10 non-laying hens were all negative.

is based upon the inhibition of growth of a given strain of yeast which multiplies at a rate proportional to the concentration of available biotin in the culture medium. A unit of avidin is the amount required to inhibit completely the yeast growth supported by one μg of biotin. Assays are considered negative when results indicated less than 0.01 unit of avidin per g of fresh tissue.

The portions of the oviduct which were separately assayed for avidin included the magnum, the isthmus, and the uterus, the several parts being identified according to the terminology of Richardson.[12] No attempt was made to assay the tissues of the infundibulum. In several instances the albumen of an oviducal egg as well as that of the last laid egg was also assayed. In 8 cases in which an egg was found in the magnum at autopsy, samples of the magnum immediately above, below, and at the level of the egg were assayed.

Tissue samples weighing 1 g were taken from specified regions or from the middle of each segment of the tract to be assayed for avidin. This tissue, in 500 cc of distilled water, was thoroughly homogenized at room temperature in a "Waring Blendor." All subsequent dilutions were made from this initial preparation.

Results and Discussion. Complete data on the 8 hens carrying eggs in the magnum, or albumen-secreting portion of the oviduct, at autopsy are summarized in Table I. Avidin was found to occur only in the magnum, the titer ranging from 1 to 5 units per g of fresh tissue. The titer of the tissue below the level of the oviducal egg was somewhat greater than was the titer of tissues from the portions of the magnum previously traversed by the egg or at the level of the egg. This differential obtained irrespective of the relative position of the egg at the time of autopsy, indicating that the portion of the magnum from which albumen had already been secreted had also discharged a part, but in no case all, of its avidin content.

Avidin contents of 1 to 3.3 units were found in the mid-region of magna of 5 hens with eggs *in utero* and of 8 hens autopsied following lay but prior to subsequent ovulation. The presence of an egg in any portion of the oviduct is thus not a necessary condition for presence of avidin in the magna of laying hens.

Assays of the uteri and isthmi of 7 laying hens were uniformly negative, thus limiting the occurrence of avidin to the albumen-secreting portion of the tract.

The albumen of oviducal eggs found at autopsy and that of previously laid eggs was found to contain from 1 unit to 5 units of avidin per g of fresh egg-white. Considerable individual variation is thus seen. The higher titers are somewhat in excess of those previ-

[12] Richardson, C. K., *Phil. Trans. Roy. Soc. London*, Series B, 1936, **255**, 149.

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.

14063

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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1 Fraps, R. M., Hertz, R., and Sebrell, W. H., PROC. SOC. EXP. BIOL. AND MED., 1943, 52, 140.