Oviductal Glycogen Content of Laying and Nonlaying Hens and of Estradiol Stimulated Pullets* (33829)

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A tremendous increase in oviduct weight of the hen occurs coincident with sexual maturity, while a large decrease occurs when egg production stops (1). Estrogen secretion maintains the reproductive tract in a functional state and controls the reproductive processes of the female fowl. The immature chick oviduct also responds dramatically to estrogen and several biological assays have been developed utilizing oviduct weight as a measure of estrogenic activity (2–5). Since estrogen causes hypertrophy and hyperplasia of the chicken oviduct, energy metabolism is probably accelerated to meet the needs of the growing tract. Alterations in the oviducal level of glycogen, the storage form of carbohydrate energy, might be expected to reflect alterations in metabolism during these periods of estrogen-induced growth. The present investigations studied the changes in oviducal glycogen in the immature chick after a single injection of 17β-estradiol and the differences in the oviducal glycogen of the laying and nonlaying hen.

Materials and Methods. The female white leghorn chickens were raised in batteries on standard broiler ration. Mature white leghorn hens were maintained in individual cages, fed a standard ration *ad libitum* and were exposed to artificial lights from 6 a.m. to 8 p.m. When sacrificed the chickens were decapitated. In the 5-week-old chicks (body wt, 314 $\pm$ 3 g) the entire oviduct was taken as a single sample and used for chemical analysis. In the 12-week-old birds (body wt 945 $\pm$ 26 g), and laying hens, the oviduct was divided into its component anatomical parts, the infundibulum, magnum, isthmus, and uterovagina and each section was analyzed separately. Glycogen determinations were carried out by the anthrone method of Seifter *et al.* (6) and glucose was analyzed by the glucose oxidase method (7). Statistical comparisons were made using Student's t test with corrections for unequal group size.

Results and Discussion. A maximal 6-hr response in oviducal glycogen was elicited by 25 μg of estradiol in a single injection ($p < 0.001$, Table I), and the total glycogen per oviduct remained approximately constant for doses ranging from 25 to 1000 μg of estradiol. Although 25 μg of estradiol induced maximal stimulation of glycogen synthesis at 6 hr, this glycogen level was not maintained for 24 hr (Table II). Fifty μg of estradiol was required to increase oviducal glycogen for 24 hr and a maximal response was obtained with 100 μg. The growth of the oviduct with the larger doses of estradiol was reflected in a greater glycogen concentration per oviduct at 24 hr than at 6 hr.

A time study was undertaken at 6, 18, 24, 36, and 48 hr to more completely describe the course of these biochemical events. The larger doses of estradiol maintained the increased oviducal glycogen for a period of 24 hr. Between 24 and 48 hr glycogen content decreased and returned to the control level. The results suggest that with lower amounts of estradiol, the estrogen was not available for the entire 24-hr period and the oviducal responses elicited initially were not stimulated further. The large doses apparently provided continual estrogenic stimulation as the hormone was adsorbed from the oil solution at the subcutaneous injection site.

The glycogenic effects of estradiol on the

* The material presented herein is taken from a thesis submitted to The University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific paper number A1489, Contribution No. 4132 of the Maryland Agricultural Experiment Station (Department of Poultry Science).

TABLE I. The 6-hr Oviducal Responses of the 5-Week-Old Chick to a Single Injection of 17β-Estradiol.[a]

Estradiol (μg)	Oviduct wet wt (mg)	Glycogen (μg/100 mg of wet wt)	Glycogen (μg/100 mg of dry wt)	Glycogen (μg/oviduct)
0	33.9 ± 0.8	39 ± 2	221 ± 12	13 ± 1
10	36.8 ± 1.4	43 ± 3	240 ± 19	16 ± 1
25	43.1 ± 1.8	61 ± 4	369 ± 25	28 ± 2
50	45.1 ± 2.1	67 ± 4	400 ± 32	31 ± 4
100	37.7 ± 3.6	67 ± 4	393 ± 27	26 ± 3
500	43.6 ± 4.1	61 ± 4	418 ± 26	21 ± 3
1000	49.9 ± 2.1	42 ± 3	296 ± 26	24 ± 3

[a] Each value is the mean ± SE (standard error of the mean) of 5–15 samples.

chick oviduct agree qualitatively with the changes that occur in the estrogen stimulated rat, rabbit, or sheep uterus (8, 9). Although the mammalian uterus has a higher control glycogen concentration (400 μg/100 mg of dry wt) than the chicken oviduct, both had a 2–3-fold increase in glycogen 6 hr after an estradiol injection. Larger doses of estradiol were necessary to elicit a glycogen increase in the chicken oviduct than in the uterus of an ovariectomized rat of comparable body size. In the rat, 0.2 μg of 17 β-estradiol caused an increase in uterine glycogen, while in the chick 25 μg were required.

No changes in chick oviducal glucose were observed. The glucose concentrations were 74 μg/100 mg of wet tissue, 427 μg/100 mg of dry weight and the total glucose content of the oviduct was 33 μg. In the rat and sheep, the uterine increase in glycogen after estradiol was accompanied by an increase in uterine glucose (8). In the rabbit, estrogen did not evoke an increase in uterine glucose, although uterine glycogen was elevated to the same extent as in the rat or sheep. Consequently, it appears that the pathways of glycogen synthesis in the chicken oviduct and the rabbit uterus are not dependent upon increases in free uterine glucose.

Although the oviduct of the chicken is a discrete entity, it can be divided into five distinct anatomical parts—the infundibulum, magnum, isthmus, uterus, and vagina. All portions of the immature oviduct had the same glycogen response after estradiol administration (Table III). The total glycogen content of each section of the control oviduct was as follows (μg): infundibulum, 4.3; magnum, 7.0; isthmus, 4.5, and uterovagina, 21.6. Six hr after treatment with 300 μg of 17β-estradiol, the content of these sections was 11.8, 17.4, 12.2, and 40.0 μg of glycogen, respectively, representing increases of 173, 148, 169, and 85%, respectively.

TABLE II. The 24-hr Oviducal Responses of the 5-Week-Old Chick to a Single Injection of 17β-Estradiol.[a]

Estradiol (μg)	Oviduct wet wt (mg)	Glycogen (μg/100 mg of wet wt)	Glycogen (μg/100 mg of dry wt)	Glycogen (μg/oviduct)
0	33.9 ± 0.8	39 ± 2	221 ± 12	13 ± 1
10	36.9 ± 1.3	35 ± 1	185 ± 8	13 ± 1
25	43.8 ± 2.2	37 ± 2	216 ± 10	18 ± 2
50	41.9 ± 1.9	53 ± 3	288 ± 17	22 ± 2
100	42.8 ± 2.6	58 ± 6	327 ± 31	25 ± 2
500	64.6 ± 3.7	66 ± 6	421 ± 35	42 ± 2
1000	83.0 ± 5.0	51 ± 4	342 ± 26	45 ± 4

[a] Each value is the mean ± SE (standard error of the mean) of 5–15 samples.

TABLE III. The Glycogen Response of the Various Anatomical Portions of the 12-Week-Old Chick Oviduct to 300 mg of Estradiol.

Oviduct portion	Glycogen (μg/100 mg of dry wt $\pm$ SE)		% of control
	Control	6 hr estradiol	
Infundibulum	133 $\pm$ 12	356 $\pm$ 37	268
Magnum	114 $\pm$ 9	254 $\pm$ 22	223
Isthmus	114 $\pm$ 7	322 $\pm$ 22	282
Uterovagina	129 $\pm$ 8	244 $\pm$ 15	189

The upper oviduct (infundibulum and magnum) of the laying hen had a lower glycogen concentration than corresponding segments of the oviduct of the nonlaying hen (Table IV). The reverse was true in the lower oviduct (isthmus and uterus) that is, the lower oviduct of the laying hen had a higher glycogen concentration than the lower oviducal sections of the nonlaying hen. The glucose concentration was lower in all oviducal sections of the laying hen than the oviducal sections from the nonlaying hen, while cloacal glucose was higher in the laying hen than in the nonlaying hen.

The comparison of biochemical constituents on a concentration basis may give a false picture of the real nature of biochemical alterations when large differences occur in organ weights or in the number of cells per unit weight. Consequently, consideration should also be given to the total oviducal content of a particular constituent and/or to an evaluation of changes in that constituent in each oviducal cell. The chemical composition of a tissue may be expressed in terms of its DNA content. If the tissue constituents are expressed in terms of amounts per unit of DNA, and if the unit of DNA chosen is the amount per nucleus, it is possible to obtain an approximate estimate of the average amount of each biochemical constituent per cell. Table V shows the oviducal glycogen content per 100 μg of DNA in the various portions of the voiduct of 12-week-old chicks, immature chicks, treated with estradiol, laying hens, and nonlaying hens. Two- to threefold increases in oviducal glycogen were observed in both the immature treated with estradiol and the laying hen. The isthmus of the laying hen showed an exceptional increase of 7$\times$ that of the nonlaying hen. When the oviduct of the laying hen became nonfunctional, its glycogen content decreased to a level comparable to the oviduct of the immature chick. Since the oviducal glycogen increase is a typical estrogen response, the increase in oviducal glycogen that occurred when the nonfunctional oviduct becomes functional (nonlayer to laying bird) can probably be attributed to the elaboration of estrogen by the ovary.

Summary. Administration of 17β-estradiol to immature chicks elicited a 2–3-fold increase in oviducal glycogen. The laying hen had an oviducal glycogen content (μg/unit of DNA) similar to the immature bird treated with estrogen, while the oviducal glycogen concentration of the nonlayers was similar to the immature controls. The elevated oviducal glycogen of the laying hen is probably a result of estrogen secretion.

TABLE IV. Glycogen and Glucose Concentration of the Various Portions of Oviducts from Laying and Nonlaying Hens.

Tissue	Glycogen (μg/100 mg of dry wt)		Glucose (μg/100 mg of dry wt)	
	NL[a]	L[b]	NL[a]	L[b]
Infundibulum	218 $\pm$ 24	187 $\pm$ 16	651 $\pm$ 81	635 $\pm$ 33
Magnum	145 $\pm$ 37	63 $\pm$ 9[c]	379 $\pm$ 30	150 $\pm$ 19[c]
Isthmus	198 $\pm$ 37	321 $\pm$ 40	491 $\pm$ 53	386 $\pm$ 44
Uterus	134 $\pm$ 21	277 $\pm$ 39	469 $\pm$ 44	352 $\pm$ 30
Cloaca	423 $\pm$ 96	386 $\pm$ 59	387 $\pm$ 62	500 $\pm$ 42

[a] NL = nonlaying hen; mean $\pm$ SE for 4 birds.
[b] L = laying hen; mean $\pm$ SE for 17 birds.
[c] NL vs L, $p < 0.005$.

TABLE V. Oviducal Glycogen Content per Unit of DNA as Influenced by Estrogen Stimulation.

Oviduct section	Glycogen (μg/100 μg of DNA)			
	Immature[a]	Immature + estradiol[b]	Laying hen	Nonlaying hen
Infundibulum	3.0	11.2	10.3	6.2
Magnum	2.7	6.3	6.2	3.7
Isthmus	2.8	8.6	29.2	4.1
Uterus	4.0	8.7	13.4	4.1

[a] Twelve-week-old chick.

[b] Twelve-week-old chick injected with 300 μg of 17β-estradiol 6 hr before killing.

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Received Dec. 18, 1968. P.S.E.B.M., 1969, Vol. 131.

Quantitative Analysis of Prolactin by Disc Electrophoresis and Its Relation to Biological Activity (33830)

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Up to the present time the pigeon crop sac response has been the most extensively used method for the assay of prolactin, and many modifications of this method have been reported (1, 2). Recently, disc electrophoresis was employed for semiquantitative analysis of prolactin in the anterior pituitary of several species (3–6). However, a quantitative method of analysis of prolactin by this method and its relation to the values obtained by the pigeon bioassay have not been investigated. The present experiments were carried out to study this problem. The possibility of using this method to measure prolactin in medium after incubation of anterior pituitary tissue was also examined.

Methods and Results. Disc electrophoresis on polyacrylamide gel was carried out by the method of Davis (7). The concentration of acrylamide was 7.5% and a current of 4 mA/column was applyed at pH 9.5. After electrophoresis, the column was stained in 1% amido schwarz solution for 1 hr. The optical density of prolactin band was measured with a microdensitometer (Canalco, model E).

Relation between dose of standard prolactin and optical density of the band obtained by disc electrophoresis. A standard bovine prolactin preparation (NIH-P-B2, 20 IU/mg)[1] was dissolved in distilled water, and electrophoresis was performed on 10–150 μg. Figure 1 shows the relationship between the dose of prolactin and the optical density of the bands obtained. The regression equa-

[1] The standard prolactin preparation was kindly provided by the Endocrinology Study Section, NIH.