the egg-laying monotremes, such as the duckbill platypus and the Australian ant-eater, would require a factor comparable to that present in birds to stimulate the act of incubation and thus provide for the hatching of the young.[12] It would seem reasonable that this same factor would play a role in the initiation of lactation at the close of the incubation period in many ways comparable to the relation of this factor in stimulating the gradual proliferation of a crop secretion to be utilized by the newly hatched squab.

The male pigeon takes part in the incubation of the eggs and in the feeding of the squabs. It is strange, therefore, that the inactive male pigeon pituitary contains definitely less lactogenic hormone than the female. In this respect it is quite comparable to the sex difference in the lactogenic content of mammals.[13]

The mechanism by which the pigeon pituitary is activated to the production of lactogen and the incubation of the eggs is not known. In the male, Patel[11] has indicated an association with the incubating female and the act of sitting on the eggs to be necessary for the formation of crop milk. Further, castration in one incubation cycle did not influence crop milk production by the male in that cycle but prevented "milk" production in succeeding periods.

The mechanism by which the lactogenic hormone in birds is stimulated to increased production in order to produce broodiness and crop milk formation and in the primitive mammals, the egg-laying monotremes, broodiness and milk secretion may be a sensory nervous stimulation comparable to the influence of light upon the gonadotropins of the pituitary. Whether the estrogens and androgens play roles in mediating the effect requires further investigation.

Summary. The lactogen content of the anterior pituitary of two types of pigeons, the common and the White King, were compared in both sexes. As in mammals, the pituitaries of the male pigeons contained only ½ to ⅓ as much lactogen as the female by all methods of comparison even though the male pigeon aids in the incubation of the eggs and in feeding the squabs.

The common female pigeon, although considerably lighter in body weight than the White King, contains as much or more lactogen per pituitary. Per 100 g body weight, the pituitary of the White King contains only ½ to ⅔ as much lactogen as the common pigeon.

[11] Patel, M. D., *Physiol. Zool.*, 1936, **9**, 129.

[12] Turner, C. W., *The Comparative Anatomy of the Mammary Glands*, Pub. Univ. Coop. Store, Columbia, Mo., 1939.

[13] Hurst, V., and Turner, C. W., *Endocrinology*, 1943, **31**, 334.

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Inactivation of Estrone in Normal Male Rabbits.

GERSON R. BISKIND AND MORTON A. MEYER.

From the Department of Pathology, Mount Zion Hospital, and the Division of Pathology, University of California Medical School, San Francisco.

The metabolism of estrogens in rabbits has been the subject of several conflicting reports. Smith and Smith[1] recovered less than 10% of the injected estrogens in the urine of these animals; Frank and his collaborators[2] found practically none in the blood after 30 minutes. The original observations were confirmed by Pincus and Zahl[3,4] and their findings implicated the uterus as an agent in the conversion of estrone to estriol, and the ovaries

[1] Smith, G. S., and Smith, O. W., *Am. J. Physiol.*, 1931, **98**, 578.

[2] Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 1229.

[3] Pincus, G., *Cold Spring Harbor Symposia on Quant. Biol.*, 1937, **5**, 44.

[4] Pincus, G., and Zahl, R. A., *J. Gen. Physiol.*, 1937, **20**, 879.

in the conversion of estradiol to estrone. Subsequent experiments showed that the ovaries and uterus are not necessary for the conversion of *a*-estradiol to estrone and *b*-estradiol.[5,6] Using the *in vitro* technic Heller[7] and Heller and Heller[8] reported that rabbit liver can inactivate estrogens in a manner similar to rat liver and that the function is not altered by the pregnant state.

Numerous observations indicate that the liver is a specific site of inactivation of estrogens in rats, dogs, and human beings; these investigations have been summarized elsewhere.[9,10] As the liver exerts so profound an effect on the metabolism of estrogens in these species, it is important that this function be considered in any study on the inactivation or interconversion of estrogens *in vivo*. Moreover, the hepatic estrogen-inactivating function is readily affected by nutritional deficiency,[10] even of moderate degree. Thus the experiments to be described were undertaken to ascertain whether or not the liver of the intact rabbit, like that of the other species mentioned, also inactivates estrogen. The method previously used in rats[11,12] has been applied to the rabbit and confirms that in this species too the liver alone is capable of inactivating estrone. Male animals were employed to eliminate any possible interfering activity by the uterus or ovaries.

Methods. The young male rabbits used in this experiment varied in weight between 1200 and 2500 g, and were between 3 and 4 months old. In each of 3 of these animals 2 weighed pellets of estrone were implanted subcutaneously. In each of a second group of 3 animals 2 similar pellets were implanted in the spleen. A third group of 3 animals served as controls. The diet consisted of a standard brand of rabbit pellets.* The weight of each animal was recorded weekly and all showed a continuous gain. The experiment was terminated 91 days after the pellets were inserted. After nembutal anesthesia and exsanguination the testicles were dissected, cleaned of all adherent structures, and weighed immediately on a damped balance. The testicles were fixed in Bouin's solution together with the seminal vesicles, prostate and epididymis. The usual methods were employed for dehydrating, embedding in paraffin, sectioning and staining selected blocks of these tissues.

The experimental data are presented in Table I. It is evident that the weights of the testicles of the animals with the pellets in the spleen are of the same order of magnitude as the controls, while the testicles of the group implanted subcutaneously weigh only about one-sixth as much. Variations in the weights of the animals and in the amount of estrone absorbed were not sufficient to influence the above result.

The microscopic sections of the testicles of the animals with the pellets in the spleens show no significant histologic changes from the normal controls. There is pronounced alteration of the histologic pattern of the testicles of the group implanted subcutaneously; the spermatic tubules are shrunken and are lined by several layers of large vacuolated cells that have replaced entirely the normally active spermatogenic cells. The lamina propria is fibrous and thickened. This histologic pattern is very similar to that found after experimental cryptorchidism in the rabbit,[13] and in normal male rats implanted with estrone pellets in the subcutaneous tissues for 42 days.[12]

The microscopic sections of the prostate and seminal vesicles of the group with the pellets in the spleen are almost indistinguishable from the normal controls. A slight interstitial edema accompanied by a scattering of

5 Fish, W. R., and Dorfman, R. I., *J. Biol. Chem.*, 1941, **140**, xl; 1942, **143**, 15.

6 Heard, R. D. H., Bauld, W. S., and Hoffman, M. M., *J. Biol. Chem.*, 1941, **141**, 709.

7 Heller, C. G., *Endocrinology*, 1940, **26**, 619.

8 Heller, C. G., and Heller, E. J., *Endocrinology*, 1943, **32**, 64.

9 Doisy, E. A., Thayer, S. A., and Van Bruggen, J. T., *Federation Proceedings*, 1942, **1**, 202.

10 Biskind, M. S., and Biskind, G. R., *Endocrinology*, 1942, **31**, 109.

11 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

12 Biskind, G. R., *Med. Surg. Tributes to Harold Brunn*, Univ. Calif. Press, 1942, p. 41.

* Globe A-1 Wonder Rabbit Pellets, Pillsbury Flour Mills Co., Los Angeles.

13 Biskind, G. R., and Glick, David, *Arch. Path.*, 1937, **23**, 363.

TABLE I.
Estrone Pellets Implanted for 91 Days in the Spleen or in the Subcutaneous Tissues of Male Rabbits: Effect on Testicles; Comparison with Normal Controls.

Animal No.	Location of pellet	Body wt		Wt of testes		Amt estrone absorbed, mg
		Beginning, g	End of exp., g	Right, g	Left, g	
6	—	1850	3370	2.84	2.63	—
7	—	1200	2925	2.35	2.12	—
8	—	2400	3600	2.85	2.75	—
	Avg	1817	3278	2.68	2.50	
9	Subcu.	2500	3750	0.45	0.47	1 pellet lost
10	"	1850	3045	0.43	0.47	4.1
11	"	1700	3075	0.44	0.44	3.0
	Avg	2017	3290	0.44	0.46	3.6
13	Spleen	1800	3000	2.38	2.65	4.0
15	"	2450	3300	2.66	2.70	4.9
16	"	2300	3600	2.62	2.71	4.3
	Avg	2217	3300	2.55	2.69	4.4

lymphocytes and phagocytic mononuclear cells is the only histologic deviation from the normal. The epithelial elements of these organs are similar in these two groups. The atrophic condition of the prostate and seminal vesicles of the animals implanted subcutaneously is readily distinguished from the above. Accompanying the shrunken epithelial elements of these organs is a diffuse scattering of inflammatory cells in the acini and interstitial tissues, with edema and fibrosis of the latter.

Discussion. In previous experiments of this type, performed on rats, a control group of animals was employed to exclude the possibility that the spleen may alter in some manner the absorbed estrogen before it is inactivated by the liver. In this type of control, the pellet was placed in the spleen; then that organ was transplanted into the overlying abdominal wall. After an interval to permit the development of vascular adhesions, the splenic vessels were ligated, thus excluding the spleen from the portal system and forcing the absorbed hormone into the systemic circulation. The hormone under investigation always exerted its specific action under these conditions. The uniformity of results in the previous experiments indicated that the spleen played a passive part as the site of absorption; therefore that control group was omitted in this experiment.

A possible explanation of the slight inflammatory reaction in the prostate and seminal vesicles of the group with the pellets in the spleen is that for a short period immediately after implantation of the pellet some of the hormone escapes inactivation in the liver, as happens in female rats.[11] During this time the organs may have been altered by the hormone with the production of an inflammatory reaction that has persisted long after the epithelial elements have returned to normal.

The absence of any specific action of the absorbed estrone in the animals with pellets in the spleen indicates that the compound is completely inactivated in the liver. Even though estrogens are excreted with bile and may enter into an entero-hepatic circulation, as shown by Cantarow *et al.*,[14] there was still no opportunity in our experiment for the absorbed hormone to be influenced by the genital organs before it reached the liver. Hence, one may conclude that the liver is capable of inactivating estrone without the intermediation of other organs, and that this function takes place in the rabbit as well as in other species previously mentioned.

Summary. The liver of normal male rabbits has the ability to inactivate estrone as shown by the presence of normal testicles and genital apparatus in a group of three animals examined 91 days after the implantation of estrone pellets in the spleens. Alterations in the structure of the testicles in rabbits with subcutaneously implanted pellets corresponded to

[14] Cantarow, A., Rakoff, A. E., Paschkis, K. E., Hansen, L. P., and Walking, A. A., *Endocrinology*, 1943, **32**, 368.

the changes found in similarly treated male rats, and to the changes produced by experimental cryptorchidism in rabbits.

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Lactation Activity, Chemical Composition, and *in vitro* Metabolism of Rat Mammary Tissue.

MAX KLEIBER, ARTHUR H. SMITH, AND PHILLIP LEVY.
From the College of Agriculture, University of California, Davis.

During work on the metabolic effect of lactogenic hormone one of us observed that mammary tissue from a rat in full lactation consumed *in vitro* 2.7 ± 0.3 cmm of oxygen per mg of dry matter per hour. Mammary tissue from a rat that had suckled only one young, weaned 8 days before the test, consumed *in vitro* only 0.7 ± 0.2 cubic millimeter of oxygen per mg of dry matter per hour.

We have repeated and extended these *in vitro* measurements of the metabolic rate of mammary tissue because the work offered an opportunity to connect metabolic rate with "activity," a term often used rather vaguely.[1] The relation of metabolic rate to the amount of "active" tissue has a sound meaning only when the activity is defined and measured independently of the metabolic rate itself.* Lactation provides such an independent definition af activity. During the trials described here we noted that both lactation activity and metabolic rate are related to the nitrogen and water content of the mammary tissue.

Method. The rats used were bred in our colony from the Long-Evans strain and kept on our ordinary colony diet. On the day of the respiration trial they were killed by a blow on the head. Immediately the thorax was opened; the mammary gland was placed in oxygenated balanced ion glucose solution containing phosphate buffer;[2] and parts of the gland, excised with a forceps, were placed in buffered balanced-ion glucose solution and subjected to Warburg's standard procedure of micro respiration trial in a water-bath kept at 37°C. The time between killing the rat and installing the manometers in the waterbath averaged to 18 ± 1 minute and did not exceed 27 minutes. The amount of tissue used in each respiration chamber was measured by determining the nitrogen in the sample after the trial by the micro Kjeldahl method. In extra samples of the same tissue, the nitrogen : dry matter ratio and the water content were determined.

Results. Table I summarizes the results of these measurements with mammary tissue taken on the 20th day of pregnancy and tissue taken on the 21st day of lactation. The number of fetuses per pregnant rat ranged from 9 to 11. Each of the 8 lactating rats included in the means suckled 6 young. This is the size to which each larger litter in our colony is reduced at birth.

The lactating mammary tissue contained less than half as much dry matter as the mammary tissue from the pregnant rats. The mammary tissue of a rat that had suckled only 3 young contained, on the 21st day of lactation, 26% of dry matter—a figure higher than that for any rat with 6 young. The mammary dry-matter content of a rat with only 2 young was as high as 35%. Lactation, therefore, increases the water content of the mammary gland. The nitrogen content of the dry mammary tissue had the same trend as the water content of the fresh gland. The dry matter of the lactating mammary glands averaged over 7 times as much nitrogen per

[1] Benedict, F. G., *Vital Energetics*, 1938, 199.

* Otherwise one relates just two different expressions for only one variable.

[2] Kleiber, M., and Cole, H. H., *Am. J. Physiol.*, 1939, **125**, 747.