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Relaxin in the Ovary of the Domestic Sow (*Sus scrofa* L.).*

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The recent work of Albert, Money and Zarrow[1] indicates that a much higher concentration of relaxin is found in the whole, fresh ovary of the sow than in the defatted, dried corpora lutea.[2-4] They[1] also found that the hormone is distributed throughout the ovary with the exception of the follicular fluid, and that during pregnancy its concentration is 500 to 1500 times greater than during a normal estrous cycle. The present investigation was undertaken for the purpose of determining quantitatively the relaxin content of the ovary throughout pregnancy and also to find when relaxin first appears in the ovary both during pregnancy and the normal estrous cycle. In addition, studies were made of the blood, uterus and placenta for relaxin content.

Experimental procedure and methods. In order to avoid the possible loss of relaxin as a consequence of the number of manipulations in the extraction of the tissues, purification was carried out only to the point of furnishing a preparation suitable for assay purposes. Essentially the technic used was that described as step No. 1 by Albert, Money and Zarrow.[1] The tissues were obtained on the slaughtering floor and at the same time data were secured as to the age of the sow, condition of the ovary (follicles or corpora

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1 Albert, A., Money, W. L., and Zarrow, M. X., *Endocrinol.*, 1947, **40**, 370.

2 Fevold, H. L., Hisaw, F. L., and Meyer, R. K., *J. Am. Chem. Soc.*, 1930, **52**, 3340.

3 Abramowitz, A. A., Money, W. L., Zarrow, M. X., Talmage, R. V. N., Kleinholz, L. H., and Hisaw, F. L., *Endocrinol.*, 1944, **34**, 103.

4 Albert, A., Money, W. L., and Zarrow, M. X., *Endocrinol.*, 1946, **39**, 270.

lutea) and, if pregnant, the length of the fetus. The tissues were brought immediately to the laboratory and the preparation of the extracts for assay was started.

The tissues were first ground and extracted in the cold with 6 volumes of 3% HCl for 48 hours. At the end of the first extraction period the mixture was made up to 4% NaCl and the supernatant fluid separated from the residue by centrifugation. The residue was then re-extracted in the same manner for an additional 24 hours and discarded. The two supernatant fluids were combined, adjusted to pH 7.0 and dialyzed against running tap water. Adequate dilutions were prepared and the sample assayed in castrated guinea pigs by means of the relaxation of the symphysis pubis. In a few instances when the extract was too dilute, the preparation was concentrated by adding 5 volumes of cold acetone and dissolving the precipitate in a suitable volume of water.

In the present assay procedure the guinea pigs were injected with 1 μg of estradiol daily for 3 days instead of the usual 4 days and the material to be tested was injected on the morning of the fourth day. Six hours later the pelves of the animals were palpated according to the technic of Abramowitz, *et al.*[4] A guinea pig unit (G.P.U.) was defined as that amount of relaxin which produced relaxation of the symphysis pubis in two-thirds of a group of 12 or more guinea pigs. The assay was carried out by preparing a series of dilutions and determining the concentration of relaxin which gave a unit response.

It is important to note that several precautions were carefully observed. Only animals in good nutritional state and weighing 400 to 800 g were used. Furthermore, all guinea pigs were palpated prior to the start of the estradiol treatment and also just prior to the injection of the sample of relaxin. In both instances all guinea pigs with relaxed or questionably relaxed pelves were discarded. Finally, it is important to mention that it is possible to distinguish gradations in the response of the guinea pigs. However such distinctions may lead to considerable error as the test is in a large measure based on subjective judgment. Consequently, only an unquestionable relaxation of the symphysis was accepted as a positive response while doubtful or non-relaxed symphyses were considered negative.

Results. Ovaries were obtained from 15 pregnant sows with fetuses ranging in length from 3/8 to 10 3/8 inches and assayed for relaxin content on a total of 510 castrated guinea pigs. Prior to carrying out these assays, an extract was prepared of a single ovary from a sow in early pregnancy and a dose-response curve obtained. It may be seen in Fig. 1 that the results give a typical sigmoid curve similar in shape to the curve obtained with extracts of dried corpora lutea,[3] thus indicating that the assay reported pre-

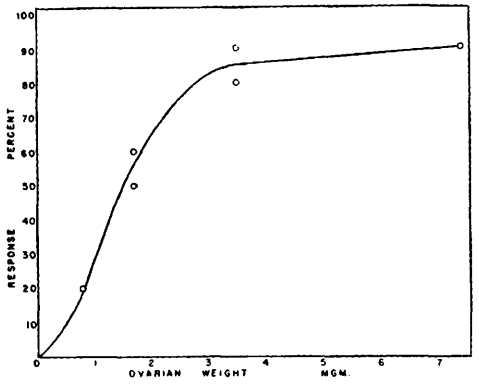

FIG. 1.

The dose-response curve of relaxin obtained from the ovary of a pregnant sow. Mg equivalent of fresh ovary are plotted against the percentage of guinea pigs showing pubic relaxation.

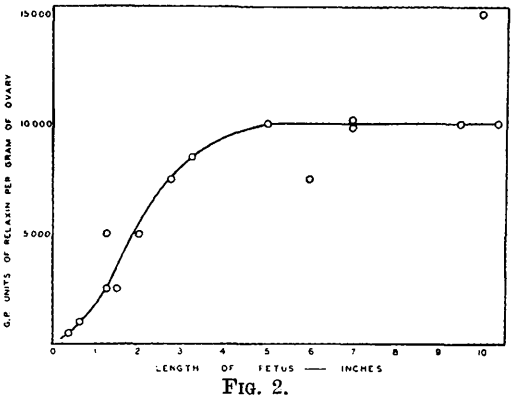

FIG. 2.

The relaxin content of the ovary of the pregnant sow plotted against the average length of the fetuses as an indication of the stage of pregnancy.

TABLE I.
Concentration of Relaxin in Blood, Placenta, Ovary, and Other Tissues of the Sow.

Age of pig	Tissue	Stage of cycle or length of fetus	No. of tests	Relaxin content G.P.U.
Gilt	Ovary	Immature	4	<1.0 per g
Mature	,,	Follicular	2	<1.0 ,, ,,
,,	,,	Luteal	3	2.5 to 5 ,, ,,
,,	Follicular fluid	Follicular	2	<1.0 ,, 2 ml
,,	Placenta	4½-inch fetus	1	0.5 ,, g
,,	,,	6 ,, ,,	2	2.5 ,, ,,
,,	,,	10 ,, ,,	2	0.5 ,, ,,
,,	Blood	5½ ,, ,,	1	2.0 ,, 1 ml
,,	,,	7 ,, ,,	2	2.0 ,, ,, ,,
,,	Uterus	Pregnant	1	<1.0 ,, g
,,	Thymus	—	1	<0.1 ,, ,,
,,	Thyroid	—	1	<0.1 ,, ,,
,,	Liver	—	1	<1.0 ,, ,,
,,	,,	—	1	<0.1 ,, ,,

viously is applicable to the present material.

Assays of the ovaries of pregnant sows (Fig. 2) indicate that the relaxin concentration rapidly increases during early pregnancy, and reaches a plateau at approximately 10,000 G.P.U. per gram of fresh ovary when the fetus is about 5 inches in length. The earliest stage of pregnancy studied was one in which the fetuses were ⅜ of an inch in length. The ovaries of this sow contained 500 G.P.U. of relaxin per gram of fresh tissue. Therefore, the relaxin content of the ovary increased some 20-fold between this early stage and that at which the fetuses attained a length of 5 inches.

An extract of the ovaries of a sow with fetuses 9½ inches long was used for making a comparison of the guinea pig and mouse assay procedures. The guinea pig assay was carried out as previously described. In the mouse assay, groups of 15 castrated female mice previously primed with estradiol were used and a unit defined as that amount of relaxin which would produce relaxation in the symphyses of two-thirds of the animals. There was complete agreement between the results of the two methods of assay as both showed a concentration of 10,000 G.P.U. of relaxin per gram of fresh ovary.

In view of the extremely high concentrations of relaxin in the ovaries of the pregnant sow it was felt desirable, for the purpose of comparison, to examine the ovaries of the gilt and of the sow during a normal estrous cycle. The tissues were collected and assayed by the guinea pig method in the same manner as described above. These assays gave negative results, at a level corresponding to 1 G.P.U. of relaxin per gram of fresh tissue, for both the ovary of the gilt and that of the sow in the follicular phase of the estrous cycle. Examination of the follicular fluid also gave negative results. However, during the luteal phase, the ovary was found to contain 2.5 to 5 G.P.U. per gram.

The relaxin content of the blood, placenta and other tissues of the pregnant sow also was determined (Table I). Several assays were carried out on the blood serum of 2 pregnant sows, one of which had fetuses of 5½ inches and the other fetuses of 7 inches. In both cases the blood was found to contain 2 G.P.U. per ml. The placentas of 3 animals having fetuses 4½, 6, and 10 inches in length were found to contain 0.5 to 2.5 G.P.U. per gram and no correlation with length of gestation was observed. Assays for relaxin in the uterus, thyroid, thymus and liver gave negative results.

Discussion. These observations indicate that relaxin is primarily a hormone of pregnancy. It is found in the ovary in small amounts (2.5 to 5.0 G.P.U. per g) during the estrous cycle, associated with the presence of a functional corpus luteum, but it begins to increase very early in pregnancy

and attains a maximal concentration of approximately 10,000 G.P.U. per g of ovarian tissue by the time the fetuses are 5 or 6 inches in length. It is rather surprising, in view of this high concentration in the ovaries, to find only 2 G.P.U. of the hormone per ml of blood serum in the pregnant animal. This is only one-fifth of the concentration found in the blood serum of pregnant rabbits.[5] However, it is 4 times the concentration found in pregnant guinea pigs[6] which indicates a wide species variation.

While the ovary must be regarded as the major source of relaxin in the pregnant sow, small amounts (0.5 to 2.5 G.P.U. per g) were also found in the placenta. Compared to the concentrations obtained in the ovary this amount is extremely small; nevertheless it is significant to note that in both the guinea pig[6] and the rabbit,[7] relaxin has been found in the placenta.

[5] Marder, S. N., and Money, W. L., *Endocrinol.*, 1944, **34**, 115.

[6] Zarrow, M. X., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 488.

[7] Zarrow, M. X., Unpublished observations.

The lack of relaxin in the ovary of the gilt and in the follicular fluid and ovary of the sow in pre-oestrus indicates that the corpora lutea are of importance in the formation of relaxative substance. The results of Albert, Money and Zarrow[1] show that relaxin is present in both the corpora lutea and the non-luteal tissue of the ovary of a pregnant sow. This may indicate that relaxin is formed in the luteal tissues and diffuses out to the surrounding area or that there is an extra-luteal site which forms relaxin under the stimulation of a corpus luteum.

Summary. No relaxin is found in the ovaries of the gilt nor of the sow during the follicular phase of the estrous cycle. There is, however, 2.5 to 5 G.P.U. of relaxin per gram of ovarian tissue present during the luteal phase of the cycle. The amount of relaxin in the ovaries increases rapidly during pregnancy and reaches a maximal concentration of approximately 10,000 G.P.U. per gram of tissue by the time the fetuses attain a length of 5 or 6 inches. The blood at mid-pregnancy contains 2 G.P.U. per ml while the placenta has 0.5 to 2.5 G.P.U. per gram.

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Electrophoretic Changes in Plasma Proteins in Patients with Pneumococcic Infections.

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Alterations of the plasma proteins in relation to successive stages of pneumococcic infections (either pneumonia or meningitis), and to important antecedent factors influencing protein metabolism, are reported in this paper. Blix,[1] who studied the plasma of pneumonia patients electrophoretically, found that the alpha-globulin concentration was more than twice normal, and gamma globulin was reduced from 76 to 64% of the total globulin concentration. Longsworth[2] confirmed these findings concerning alpha-globulin, and subsequent observers have shown that alpha-globulin generally increases in febrile reactions associated with tissue destruction, such as infectious diseases, coronary thrombosis, frac-

[1] Blix, G., *Z. f. die gesamte Exp. Med.*, 1939, **105**, 595.

[2] Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., *J. Exp. Med.*, 1939, **70**, 399.