Increased Concentration of Water in Uterus of the Rat Following Treatment with Relaxin.* (23351)

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It has been known for some time that relaxin induces an increase in the water content of the pubic symphysis of the guinea pig(1) and the reproductive tract of the sow(2); and that estradiol increases the water content of the uterus of the immature rat(3). Since a similarity has been shown in the end effect produced by estrogen and relaxin and since relaxin has been shown to act on the uterus of the rat(4) the following investigation was undertaken to determine whether relaxin also influenced water content of the uterus of the rat.

Material and methods. Approximately 470 female rats of the Harvard-Wistar strain were used. The animals were 24 days old at the time of relaxin administration and had been deprived of food for 72 hours prior to treatment with the hormone. All animals were maintained on 10% glucose in drinking water *ad libitum* throughout the 3-day period of food deprivation. This was necessary to insure a consistent uterine water content of approximately 80% in the untreated animals. Relaxin‡ was dissolved in water and injected subcutaneously in volume of 0.1 ml. In the time study, the rats were killed at definite intervals from 0 to 48 hours following administration of the hormone. In the study on effect of dosage of relaxin on uterine water concentration, all animals were killed 6 hours following administration of the hormone. Immediately after killing by ether, the uterus was quickly removed from the peritoneal cavity and dissected free of adhering fat and mesentery. A longitudinal incision was made in each horn and the uterus gently rolled on bibulous paper to eliminate lumenal water. The uterus was then weighed, dried in vacuum oven at 50°C for 24 hours, reweighed and the water content and dry weight determined.

Results. Approximately 95 animals were used to obtain the curve illustrating the effect of relaxin on the uterus of the rat as a function of time. Each point represents 10 or more determinations and values were secured at zero time and at 3, 6, 9, 12, 18, 24, 30 and 48 hours following a single subcutaneous injection of 50 guinea pig units (G.P.U.) of relaxin. The data reveal a marked rise in total uterine weight of 25 mg to a weight of 35.5 mg at 6 hours after administration of the hormone (Fig. 1). Thereafter the weight of the uterus showed a steady decline to a low of 22 mg at 18 hours and a return to 25.2 mg at 48 hours. The initial and highly significant increase in uterine weight at 6 hours can be accounted for entirely, on the basis of increased hydration. The concentration of uterine tissue water increased from a pre-injection value of 79.8% to 85.3% at 6 hours after injection of relaxin. No significant change in dry weight of the uterus can be noted at this time. The uterine water dropped to 82.2% at 12 hours and 81.5% at 24 hours. At 30 hours after administration of relaxin, the water content of uterine tissue was back to the pre-injection value of 79.8%. A slight drop in the dry weight of the uterus may be observed at 18 to 30 hours, however this was not significant when analyzed by the Student "T" test. Hence, one can conclude that no significant changes occurred in the dry weight of the uterus after treatment with relaxin.

A second experiment was carried out to determine whether a relationship exists between the dosage of relaxin and the concentration of water in the uterine tissue. Again immature rats at 24 days of age were used and treated as previously indicated. The hormone was administered subcutaneously in a single injection and the dosage ranged from 0.05 G.P.U. to 100 G.P.U. All animals were

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‡ Obtained in the form of a dry powder or water solution (Releasin^{Rx}) through the courtesy of Dr. R. L. Kroc, Warner-Chilcott Laboratories.

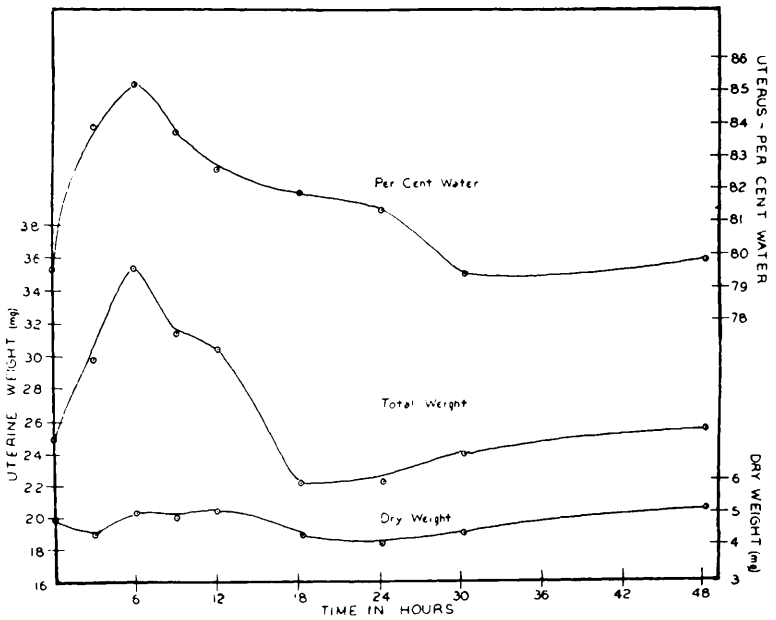

FIG. 1. Changes in % uterine water, dry wt and total wt of uterus following a single inj. of 50 G.P.U. of relaxin. Observations were made at various intervals up to 48 hr.

killed 6 hours following injection of the hormone. No statistically significant changes in water content were noted at dosages of 0.05 to 0.5 G.P.U. of relaxin. The minimum effective dose of relaxin that increased the per cent uterine water of the castrated rat was 1 G.P.U. This caused the uterine water to increase from a control value of 79.8 to 81.4% ($P=0.01$). A maximum response of 84.2% was obtained with 10 G.P.U. (Table I) and further increase in dosage of relaxin to 50 and 100 G.P.U. failed to elicit a significantly greater rise in uterine water content. The data gave a straight line relationship when plotted on a semilogarithmic basis between the dosages of 0.5 and 10 G.P.U. of relaxin.

TABLE I. Effect of Relaxin on Uterine Water Content of the Immature Rat.

No. of rats	Dose relaxin (G.P.U.)	Uterine water (% ± S.E.)*
91	No Rx	79.8 ± .24
32	.05	80.2 ± .20
32	.1	80.9 ± .20
32	.2	80.4 ± .30
32	.5	80.8 ± .26
32	1	81.4 ± .34
32	2	82.5 ± .32
32	10	84.2 ± .34
32	50	84.4 ± .38
32	100	84.5 ± .28

* S.E. = Stand. error of mean.

Discussion. These data present further evidence for a similarity in the effect produced by relaxin and estrogen and also suggest a significant dissimilarity in the action of the 2 hormones. The present report demonstrates that relaxin can act independently of estrogen in causing an increase in water content of the uterine tissue of the rat, and maximum hydration of the uterus is obtained at 6 hours following treatment with relaxin. This is identical with that seen after estrogen treatment(3). However, the uterine water returned to its pre-injection level within 24-42 hours following injection of relaxin and no subsequent response was noted during this period in either per cent water or dry weight of the uterus. In contrast to this, estradiol will induce a second rise in uterine weight at 30 hours due to an increase in dry weight and represents a true increase in protoplasmic mass. These findings suggest a marked dissimilarity between the 2 hormones and indicate that they may act via different mechanisms. Talbot *et al.*(5) have demonstrated an increase in sodium and a slight decrease in potassium of the uterine tissue following estradiol administration. This would tend to

indicate that the water shift following estradiol may be due to the action of the hormone on the electrolytes. Present experimentation is now being concluded on the action of relaxin on the electrolytes of the uterus.

It is of further interest that this effect of relaxin on uterine water content is produced without previous sensitization by estrogen. In all actions which have been previously demonstrated, it has been necessary to pretreat the animal with estrogen to obtain an effect. This necessity for estrogen priming is observed in the separation of the guinea pig pubic symphysis, the dilatation of the sow uterine cervix, and inhibition of uterine contractions in the rat.

Conclusions. Relaxin causes an increased concentration of water in the uterine tissue of the immature rat. Maximum hydration was obtained at 6 hours following administration of the hormone. The minimum effective dose of relaxin is 1 G.P.U. and the maximum response occurs following 10 G.P.U. relaxin. Contrary to the effect seen following estradiol administration, no secondary rise in uterine water content and no increase in dry weight is observed. Pretreatment with estrogen is not necessary for this action of relaxin on the water content of the uterine tissue.

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Role of Liver on Consequences of Lipid Mobilization.* (23352)

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We have reported the isolation of a lipid mobilizer (LM) from the plasma of animals administered cortisone(1-4) and from the posterior pituitary of hogs(5). The greatest sensitivity to the hyperlipemic action of LM occurred in animals which had been inadvertently exposed to minute amounts of potential hepatotoxic agents. This suggested that the liver may play an important role in determining lipid concentrations in the peripheral blood following mobilization of fat from the depots.

Materials and methods. Male and female Wistar strain rats weighing 125-250 g were obtained from the same source as those used in our previous studies(1-5). They were maintained from weaning on Wayne Chow Checkers supplemented with K penicillin G (4 g/ton) and 0.005% butylated p-hydroxyanisole. Desensitization to the hyperlipemic action of LM was accomplished by changing the diet to Warner Dog Chow which did not contain added antibiotics or antioxidants. Sensitivity was reestablished by restoring the Wayne diet. LM was either from the same batch used previously(1-4) or from a new batch. Bleeding and chemical analysis of the blood samples were by the methods previously described(1-4). Determination of liver cholesterol and fatty acids was by a modification of the Baruch and Chaikoff method(6) as furnished by Kaplan and Cohn. Lipid P in the liver was determined by the method of Zilverschmidt *et al.*(7). Liver glycogen was determined by the method of Seifter *et al.*(8). Hepatectomy was performed in one stage under sodium thiopental anesthesia (20 mg/kg). The hilus of the liver was exposed by a midline incision, ligated with No. 1 braided

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