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Effect of Theelin on Immature Guinea Pigs.

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It was previously pointed out that the vaginal smears of rabbits were not reliable as an index of hormonal activity, confirming the work of Kunde and Proud.[1] A more careful search of the literature reveals that Courrier[2] had previously observed that there was no histological change in the vaginal mucosa of several rabbits either during heat or after the administration of an ovarian extract.

We also reported an observation in 3 immature guinea pigs of the opening of the vulval orifice 48 hours after injection with the urine of a pregnant woman. To Courrier[3] again belongs the credit of having made the same observation in a single guinea pig that he injected with an ovarian extract. Laqueur[4] in studying the uterine hypertrophy of rodents after injection with an ovarian hormone, also noted the vulval opening in 2 guinea pigs but observed that one of his controls also had a wide open vagina at the end of the experimental period, namely a week. In trying to repeat our original phenomenon we found that it is not constant, appearing in but 2 of 23 animals injected with theelin-bearing fluids, while in 2 out of 34 guinea pigs the opening of the vulva, without, however, typical oestrus smears, appeared spontaneously.

Our attention was shifted, therefore, from the vulva to the effect of theelin on the uterus. Hypertrophy of the genital tract in immature animals in general is described as characteristic of the follicle hormone. Mice, rats, rabbits, and dogs, have been extensively investigated. But other than the two authors referred to above and Frank,[5] little seems to have been published. Jares[6] showed that guinea pigs are incapable of giving the Aschheim-Zondek reaction, although Papanicolaou[7] has recently demonstrated microscopic changes in the follicle following the injection into immature guinea pigs of the urine of pregnant cows.

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[1] King, E. L., and King, A. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 469.

[2] Courrier, R., *Comptes rendus Soc. de Biol.*, 1926, **94**, 280.

[3] Courrier, R., *Comptes rendus Soc. de Biol.*, 1924, **90**, 453.

[4] Laqueur, E., *Deutsch. med. Wchschr.*, 1926, **52**, 1247.

[5] Frank, R. T., Female Sex Hormone, 1929, Thomas Co., Baltimore.

[6] Jares, J. J., *Anat. Rec.*, 1931, **49**, 185.

[7] Papanicolaou, G. N., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 807.

Most authors have used the obvious hypertrophy of the uterus of rodents contrasted with a littermate control to demonstrate the uterine effect of the follicle hormone. Siddall[8] showed that in immature mice, animals weighing between 18 and 20 gm., the uterus (with the ovaries, tubes, and the cervix) weighed less than 1/400 of the total weight of the animal. Using the blood serum of pregnant women, he demonstrated the presence of theelin by daily injections over 5 days. At the end of that period the uterus was found to weigh more than 1/400 of the total weight, as for example, 1/300. Using the denominator as an index, a value over 400 would be negative, whereas a value under 400 would indicate uterine hypertrophy.

Although Frank states that in the immature guinea pig 4 to 6 days are necessary for hypertrophy of the uterus to take place, we have observed it regularly in 48 hours, and even 24 hours after the injection of theelin-bearing fluids. We have established a "ratio" between the weight of the uterus in mg. and the weight of the animal in gm. The normal should be 2.5 or less, corresponding, of course, to Siddall's value of 400. The advantages of this ratio are that it rises in value as the uterus increases in size whereas the Siddall number decreases in value. Again, the Siddall number implies an accuracy that is very hard to obtain, a change in the last significant figure representing 1/8 mg., while in the ratio as proposed an error of 25 to 36 mg. will just alter the last figure. Finally, the results in the immature guinea pig can be read in 48 or even 24 hours instead of 5 days.

The urine of pregnant women near term was used as a cheap and available supply of theelin, and 15 immature guinea pigs were injected from 7 different samples. The injections were so grouped that certain variables could be studied, although of course the number of cases is small. It was found that the intraperitoneal route gave slightly better results than the subcutaneous; that storage over night on ice did not affect the result; that the number of doses, distribution, and the amount injected had no effect on the outcome. This corresponds to the observations of others that the various reactions of the sex hormones are of a threshold type. On the other hand, one animal receiving 4.0 cc. in one dose died of peritonitis, and 3 others died of causes undetermined in 24 to 48 hours, without, however, interfering with the uterine hypertrophy. The weight of the animals seemed unimportant, although those guinea pigs in the neighborhood of 250 gm. gave the best results. An interesting

[8] Siddall, A. E., *J. Am. Med. Assn.*, 1928, **91**, 779.

observation was that 2 animals at least 6 months old but still sexually immature and weighing 350 gm., which had been injected with pregnancy urine some 3 months previously, gave typical expected reactions. In no case was any ovarian change visible to the naked eye observable. Finally the length of time between the injections and the sacrifice of the animal did not seem to play any great part in the degree of hypertrophy. The best results were obtained at the end of 6 days with one sample, but there was no progressive correlation, and in general 48 hours was ample time for the reaction.

The results with pregnancy urine were as follows:

Urine	Guinea Pigs	Ratio	Average
A	3	5.6-8.7	7.1
B	6	2.9-3.9	3.5
C	2	3.0-3.6	3.3
D			3.6
E			3.4
F			5.2
G			4.5

Since the urine of pregnant women contains large quantities of anterior pituitary-like hormone, to prove that the uterine hypertrophy was not due to this substance, 3 animals were injected with Antuitrin-S (Parke-Davis) in doses of 20, 40, and 120 units. The ratios were respectively: 2.1, 2.5, and 2.5, that is, all negative. On the other hand when Theelin (Parke-Davis) was injected in doses of 9, 35, and 90 units, the results were: 2.6, 3.5, and 3.0. This last experiment indicates again the threshold nature of the reaction, and also, that while 9 units will produce a minimal hypertrophy, 35 will give a definite reaction but 90 will not give any greater reaction. We are not ready yet, however, to discuss the quantitative relationships involved.

Among the controls, one 214 gm. guinea pig, receiving no urine, had a ratio of 1.9. Four samples of male urine were used to control the findings. This was unfortunate inasmuch as theelin has been reported on many occasions in male urine as well as in male feces, bile, and even blood, although usually in very small amounts. The results were as follows: Urine Z, 2.3 in each of 2 animals; urine Y, from the same individual some 2 weeks later, 4.0 and 4.3; urine X, 2.7; and urine W, 2.2. While standard tests for the demonstration of theelin in the male urines giving a positive reaction should have been made, we feel that it was the presence of this substance that was responsible.

Summary. Attention is called to the immature guinea pig as a suitable animal for demonstrating the presence of theelin by uterine

hypertrophy observable within 48 hours. There is proposed a numerical evaluation of this hypertrophy in the form of the ratio of the weight of the uterus in mg. to the weight of the animal in gm. Using this ratio the presence of theelin was demonstrated in pregnancy urine and a commercial preparation of theelin, differentiating these from a commercial preparation of the anterior pituitary-like hormone.

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Attempted Induction of Labor by Injections of Theelin.

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Setting aside as irrelevant to this discussion the effects which the hormones of the anterior hypophysis are supposed to exert upon the hormones of the ovary, we may accept as reasonably well established at least 3 facts: that the endometrial changes which precede menstruation are controlled during the first half of the menstrual cycle by the follicular hormone of the ovary; that they are controlled during the second half of the cycle by the lutein hormone of the ovary; and that the actual menstrual flow is precipitated by the withdrawal of the lutein hormone, with which is associated the beginning retrogression of the lutein body. To emphasize the positive rather than the negative aspect of the theory, we may say that since biologic inactivity is unlikely at any time, menstrual bleeding is initiated by the ascendancy of the ovarian follicular hormone over the lutein factor. Arguing from this premise, it does not seem unreasonable to assume that the onset of labor in a normal pregnancy which has reached term may be the result of a similar sequence of events, the ascendancy of the same follicular hormone over the same lutein hormone, which is known to be present throughout pregnancy.

To test this theory and in the hope of precipitating the onset of labor, a series of experiments was undertaken on 8 pregnant negro women at full term by injecting (subcutaneously) varying amounts

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