The action of ovary-stimulating substance of human urine of pregnancy on uterine motility in the unanesthetized rabbit

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Two to five hours before the occurrence of ovulation in the unanesthetized rabbit, the motility of the uterus profoundly diminishes from a marked type which is characteristic of oestrus, to a state approximating complete quiescence. This happens whether ovulation results from the normal stimulus of coitus or from the intravenous injection of whole human urine of pregnancy (Reynolds and Friedman, 1930a; 1930b). In view of the recent work of Friedman (1932) which shows that either growth of the Graafian follicle or ovulation and subsequent luteinization may be produced in favorable rabbits by grading the dosage of ovary-stimulating substance of pregnancy-urine, it became of interest in the present experiments to determine the changes which take place in uterine motility as a consequence of the administration of such graded, liminal amounts of the urine-substance. In addition, an attempt has been made to determine whether or not a change in motility so induced is dependent upon the presence of the ovaries or if it can occur in the absence of the tissue of the Graafian follicles and their associated structures. These experiments fall logically, then, into three groups: the uterine motility associated with 1, doses of the urine-substance that are sub-liminal for ovulation; 2, doses that are adequate to produce ovulation, and 3, the administration of the urine-substance in suitable castrated rabbits, and hence in the absence of the ovary.

Experimental procedures. The present experiments were performed with the concentrate of human urine of pregnancy in the form of the "luteinizing hormone" of Parke, Davis & Co., to which Company we are indebted for a generous supply. As will be seen below, effects other than luteinizing were obtained with this material.

In the three groups of experiments described here, each doe was one which had recently dropped a litter. Thus one was assured of a certain degree of uniformity of the sexual state in the various rabbits. One day to a week post partum uterine fistulae were prepared in the several does and

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these animals were kept segregated from all other rabbits, as well as from each other, until they were sacrificed at the termination of the various experiments. Several days were allowed to elapse between the time of operation and the commencement of the experiments. The operative procedure for preparing the uterine fistula and the method of recording therefrom have been described elsewhere (Reynolds, 1930; Reynolds and Friedman, 1930a).

Only does showing good initial motility were used, since whole urine of pregnancy inhibits the spontaneous motility. In these experiments no second or third injection of the urine-substance was made except in the presence of a background of good motility against which an inhibiting effect might be demonstrated. In the first two groups of experiments, single intravenous injections of the "luteinizing hormone" were made after an initial control record of uterine motility was obtained, and subsequent records were made at suitable intervals after the injection. Exploratory laparotomies were performed as was deemed necessary in the individual experiments. Upon recovery, later records were obtained and injections made as noted. At the end of each experiment careful examination of the uterus and ovaries or sites of ovarioectomy was made. None of the animals included in this study showed uterine infection, adhesions, or inflammation.

Results. a. Doses sub-liminal for ovulation. This series is comprised of sixteen observations of the uterine response in eight rabbits. One to three series of observations were obtained in the several rabbits with the amounts of urine-substance employed varying from 0.05-0.15 cc. in the initial injection to 0.05-0.40 cc. in the second injections and 1.0 cc. in a third injection in three does.

The essential feature of the uterine response was found to be a diminution of motility in the intervals of three to four and six to eight hours after the injection. This decrease in motility occurred in all but one instance, yet it varied, from animal to animal, from a slight decrease in the amplitude of the contractions in some instances to nearly complete quiescence in others. The more profound response was seen in most of the cases. In the one rabbit which showed no decrease in motility, the extremely marked pre-injection motility nearly doubled in the frequency of the contractions by six to eight hours after the injection of the urine-substance. Others showed this only temporarily, prior to a condition of relative inactivity. This might seem paradoxical to the general inhibitory effect described above were it not for the inexplicable observation that an increase in frequency of the contractions appears to precede a cessation of orderly, rhythmic contractions. This in turn appears to be a forerunner of feeble motility whether it be faintly rhythmic, undulatory, or arhythmical. Although this observation has been described before (Reynolds, 1931a), nevertheless, by virtue of the fact that it has been a most common observation in these
experiments, the data of the present paper have made this phenomenon seem to be a more certain indication of impending uterine inactivity than before. Its possible bearing on the uterine motility described for the rat has been recently discussed elsewhere (Reynolds, 1932).

Between the injections, laparotomies were performed and the condition of the ovaries determined. Chief of the findings disclosed by laparotomy at these times was that in not one instance was ovulation seen to have occurred, nor was there any luteinization grossly discernible. The follicles attained a diameter, in some instances, of two to three millimeters, as measured by a caliper. It is worthy of note, moreover, that in no instance in which repetitive injections were made, did ovulation of the experimentally enlarged, cystic follicles take place, even in those instances in which 0.40 cc. or more of the urine-substance was employed. It was found that such an amount of this particular batch of material was capable of producing ovulation in another doe when a single first injection was made. Whenever ovulation or grossly discernible luteinization took place as a result of a second or third injection, the response was confined to new small follicles. These findings confirm the more extensive observations of Friedman (1932).

b. Doses adequate for ovulation and luteinization. Due to the fact that whole urine of pregnancy produced 1, profound uterine quiescence which continued for twenty-four to forty-eight hours, and 2, ovulation in each instance (Reynolds and Friedman, 1930b), this series of experiments has not been repeated at length in this work other than to confirm those findings for the somewhat purified urine-substance. The earlier results were amply substantiated throughout. Only one doe was an exception, in that at the end of twenty-four hours after the injection her uterus showed pre-injection motility, but this effect wore off spontaneously by the second day.

A brief generalization, which only approximately describes the results outlined in the two sections above, may be stated in the following manner:

<table>
<thead>
<tr>
<th>Ovarian response</th>
<th>Uterine response</th>
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<tbody>
<tr>
<td>1. Abnormal follicular growth (cystic follicles) or no grossly discernible follicle growth</td>
<td>1. Transitory decrease in motility in 4-8 hours following injection; permanent return to pre-injection type in 24 hours</td>
</tr>
<tr>
<td>2. Ovulation and luteinization grossly discernible; possibly corpora hemorrhagica alone</td>
<td>2. Decrease in motility in 4-8 hours following injection with no return of motility in 24-72 hours</td>
</tr>
<tr>
<td>3. Ovulation of several follicles plus many cystic follicles from previous injections</td>
<td>3. Decrease in motility in 4-8 hours following injection, with a temporary return of motility in 24 hours followed by spontaneous occurrence of inactivity in 48 hours (only indicated in these experiments)</td>
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</table>
c. The action of the urine-substance on the oestrin-activated uterus of the castrated rabbit. Castration experiments were performed on eight animals with the injections being repeated in two animals. Thus ten observations were made in this series. Since uterine quiescence rapidly supervenes after castration, and since oestrin is the only substance that has yet induced rhythmic motility in uterine fistulae (Reynolds, 1931a), it was necessary to resort to oestrin (Theelin) injections to provide a background of spontaneous motility against which a motility-inhibiting effect of the urine-substance might be shown. Because of this it may not be said that these experiments were performed in the absence of ovarian influence, but only that they were done in the absence of ovarian tissue which might conceivably contribute to the effect.

The use of Theelin led to several complicating factors, but fortunately they were ones with which it was easy to reckon. First, the dosage was considered to be a factor of prime importance, especially in the light of recent work which showed that 5 rat units of oestrin per kilogram of body-weight overcomes the initial inhibition of motility that normally follows coitus in the rabbit. Further, since slightly less than 2 rat units per kilogram of body-weight is known to be about liminal for a good Theelin response in recently castrated rabbits, it was apparent that the Theelin dosage must be in this order of magnitude (Reynolds, 1931a). A second consideration concerned the time during the Theelin response that an inhibition of motility could be demonstrated with certainty. It is known that the time at which maximal activity occurs is about twenty to twenty-four hours after intravenous administration of Theelin to rabbits, and that such motility continues until thirty or more often forty hours after the first of four Theelin injections (Reynolds, 1931a). Accordingly, it was decided that the amount of Theelin to be used in the present experiments should be about 2 rat units per kilogram of body-weight, and that the urine-substance should be given as before, in a single intravenous injection, between the twenty-second and twenty-fourth hours after the first of four injections of Theelin.

The results obtained were most satisfactory considering the difficulties likely to be encountered. In six of the ten experiments a profound and nearly complete diminution of motility was seen; two others showed a distinct change as regards the frequency, and only two showed no change at all. Because of the fact that the decrease in motility was only transitory, and motility of the pre-injection type returned by ten and persisted for twenty-four hours or more after the injection of the urine-substance (forty-eight hours or more after the Theelin), it is obvious that this effect is a decrease which resembles that which takes place in the presence of the ovaries, yet which here occurred in the absence of ovarian tissue and so was not dependent upon it.
Particular attention should be called to the two exceptions in this series of experiments. The first of these had received 3.6 rat units of Theelin per kg.

11/20 Castrated and fistula prepared.
11/22 1.5 r.u. Theelin per kg (3.9 r.u. total, or 0.0075 cc. in four divided injections of 0.0025 cc. each in seven hours; Theelin stated to contain 50 r.u. per cc.)

A 11/23 Twenty-four hours after Theelin (time of maximum response). Normal Theelin motility. Received 1.0 cc. of urine-substance, 10:30 a.m.
B 1:30 p.m.; 3 hours after urine-substance (see text for discussion of significance of the increase in rhythm).
C 5:30 p.m.; 7 hours after urine substance.
D 9:30 p.m.; 11 hours after urine-substance. Compare with pre-injection motility in A above.
E 11/24 Twenty-four hours after urine-substance (48 hours after Theelin).
F 11/25 Forty-eight hours after urine substance (72 hours after Theelin).

A typical waning of the Theelin effect as described elsewhere (Reynolds 1931a).

Autopsy: No sign of uterine infection or adhesions. Sites of ovariecotmy clean. Time, 10 seconds. M, mechanical response to indicate the potency of the system. One-fifth original size.
kilogram of body-weight. This amount is almost certainly near the threshold for a Theelin response during the first day or so of normal pseudo-pregnancy (Reynolds, 1931b). Since this doe later showed a profound uterine response resulting from an injection of urine-substance after only 2.0 rat units of Theelin per kilogram of body-weight, it is reasonably certain that this exception may be explained on the basis of too high an initial dosage of Theelin. The second exception can hardly be included in this series of experiments for it died on the sixth day following the Theelin injections from a diffuse peritonitis. Since she had diarrhea on the third day, it is quite certain that the infection was a result of the operation at which time a brown mass of necrotic fetal membranes was removed from the vagina as it was transected in the course of preparing the fistula. Inasmuch as the condition of the doe was not suspected until the experiment was well under way, however, data from her have been included in this series, yet it seems but fair to point out that she is the only doe rendered unfavorable on account of infection, and that not of the uterus.

DISCUSSION. Theelin-activated uteri in castrated rabbits. A word should be said regarding the relatively large amounts (1.0-2.5 cc.) of urine-substance employed in these castration experiments. No attempt has been made to determine the minimal effective dose, but only to demonstrate whether or not the effect may be obtained without the follicular and associated apparatus. It would seem that until one first knows the quantitative amounts of oestrin normally present in a post partum doe, as used in these experiments, and of the various other hormones concerned in the post coital uterine quiescence, such quantitative studies would yield little to our understanding of the underlying cause of this transitory type of uterine quiescence.

Graded doses and the ovary response. The data of the above experiments completely bear out the findings of Friedman; namely, that in suitable dosage either excessive follicular growth or luteinization may occur, the latter condition being associated with ovulation or intrafollicular hemorrhage and retained ovum. They emphasize a fact pointed out by Friedman, that not only repeated injections of subliminal amounts of the urine-substance fail to elicit ovulation in the cystic follicles so produced, but that doses ordinarily adequate for ovulation are without effect on these experimentally produced cystic follicles. Chief characteristic then of the ovary-response is that the effects are regular and predictable throughout.

Graded doses and the uterine response. In striking contrast to the regularity of the ovarian response to graded doses of the urine-substance is the irregularity of the uterine response not merely to graded doses, but to the identical graded doses that effected the ovarian response. The latency of the uterine response is not shortened by increasing the dosage, nor is the intensity of the response increased. Indeed it may vary rather widely...
with the same dosage from animal to animal, or even in the same animal upon repetition of the injection. The lack of uniformity must end here however, for in one respect there appears to be a definitely predictable phase to the uterine response. It is associated with the absence or occurrence of ovulation. If marked motility of the uterine fistula results twenty-four to forty-eight hours or longer after the urine-substance injection, it is certain that ovulation has not taken place, whereas if feeble motility or none at all is seen, it is equally certain that ovulation has taken place.

Effect of the ovary on the uterine response. By the token, that the ovarian response may be predicted from the uterine response, it is therefore clear that the uterine response is influenced by and in part is dependent upon the changes that take place within the ovary, as a consequence of the injection of the gonad-stimulating agent of human urine of pregnancy. Moreover, the high degree of sensitivity to such small doses as 0.05 cc. of the urine-substance would make this seem all the more probable. Despite the fact, therefore, that the uterine response has been demonstrated in the oestrin-injected castrated rabbit it is impossible to exclude the ovary as a contributing factor when it is present.

The bearing of these findings to the physiology of the decrease in uterine motility that normally occurs in the rabbit after coitus is difficult to appraise at the present time. Especially is this so in view of the increasing doubt that has of late been cast upon the identity of the ovary-stimulating substance of human urine of pregnancy (see Wallen-Lawrence and van Dyke, 1931). The knowledge, however, that the uterine response is inseparable from the ovarian response after coitus and that it also occurs under the conditions here described leaves the question still open as to its physiological meaning. Investigations of the uterine motility in the hypophysectomized rabbit with appropriate substitution therapy will tend to show how correct the assumption is that the urine-substance effect on uterine motility simulates in this respect the behavior of the anterior pituitary extracts. Such investigations are now in progress in this laboratory.

SUMMARY

As little as 0.05 cc. of the ovary-stimulating substance of human urine of pregnancy induces a transitory decrease in the motility of the uterus of the unanesthetized rabbit, in six to eight hours. When the ovary is present only excessive follicular growth results from such a small dosage of the urine-substance. Larger amounts (0.1–1.0 cc.) in the presence of the ovary induce lasting uterine quiescence and at the same time, ovulation. With such amounts, a similar response has been demonstrated in suitable castrated rabbits. One may conclude that the effect of the urine-substance is independent of the ovary, yet when this organ is present it probably contributes to the effect.
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