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RAT UTERUS RENIN-LIKE ACTIVITY: EFFECT OF STIMULI AND HORMONES

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1 The characteristics of renin-like activity in rat uterus were studied. The optimum temperature was 50°C and optimum pH 4.0. Potassium (100 mM) enhanced this activity but sodium or calcium had no effect.

2 Uterine renin-like activity was unchanged 24 h after bilateral nephrectomy.

3 Renin-like activity in the uterus increased slowly from birth to 4 weeks of age and faster between the 4th and 8th week.

4 Ovariectomy caused a considerable fall in uterine renin-like activity.

5 The following factors, known to affect renin secretion in the kidney, i.e., hypovolaemia, sodium loading, adrenalectomy, administration of desoxycorticosterone acetate and injection of isoprenaline, had no effect on uterine renin-like activity.

6 Stilboestrol, an oestrogen, caused a significant increase of uterine renin-like activity. Progesterone and testosterone had no significant effect on this activity but blocked the increase caused by stilboestrol.

7 During pregnancy there was a small but significant fall of renin-like activity in the uterus in the first week and a continuous increase throughout the later period of pregnancy.

8 It is concluded that uterine renin-like activity is independent of kidney renin and does not respond to stimuli affecting kidney renin. Uterine renin activity is hormone-dependent and may be governed by the ratio, oestrogen : progesterone.

Introduction

Renin-like activity has been demonstrated in several tissues: submaxillary glands of mice (Cohen, Taylor, Murakami, Michelakis & Inagami, 1972) and rats (Gutman, Levy & Shorr, 1973), brain (Ganten, Minnich, Granger, Hayduk, Brecht, Barbeau, Boucher & Genest, 1971) and in human as well as in rabbit uterus (Gross, Schaechtelin, Ziegler & Berger, 1964; Geelhoed, Vander & Carlson, 1970). During pregnancy significant changes in the plasma renin-angiotensin system have been demonstrated (Skinner, Lumbers & Symonds, 1972). However, the possible correlation of uterine renin-like activity and plasma renin has not been clearly demonstrated. Furthermore, although several types of stimuli (changes in sodium balance, mineralocorticoids, β-adrenoceptor activation, hypovolaemia) have been shown to affect the release of renin from the kidney, the possible physiological stimuli which affect uterine renin have not been established.

We have characterized the properties of a renin-like activity in rat uterus and have studied the action of various possible stimuli on this activity. The results are described in this paper.

Methods

Female albino rats of the Hebrew University strain, weighing 150–210 g were used throughout. In some experiments, specified in the results section, immature female rats of different ages, were used. The rats were killed under ether anaesthesia by stretching of the neck and the uteri were immediately removed. Homogenates were made of samples consisting of 2–3 uteri.

Operations

All operations were carried out under ether anaesthesia.

Bilateral adrenalectomy was performed through a mid-line incision in the back. Sham-operated rats underwent the same procedure but the adrenals were not removed. Three days after the operation, the uteri were removed.

Bilateral nephrectomy was performed through a midline incision of the abdomen. The same procedure was carried out in the sham-operated animals, but the kidneys were left intact. The uteri were removed 24 h after operation.

For ovariectomy both ovaries were removed through two small incisions along the ventrolateral sides of the abdomen. The same procedure was carried out in sham-operated rats, except for removal of the ovaries. The uteri were taken out on the tenth post-operative day.

Treatment

1. Stilboestrol was injected intramuscularly, 0.5–0.7 mg/kg, on the eighth day after ovariectomy.
2. Progesterone was injected intramuscularly, 5–7 mg/kg, on the eighth day after ovariectomy.
3. Testosterone was injected intramuscularly, 6.3–8.3 mg/kg, on the eighth day after ovariectomy.
4. Dexamethasone was injected subcutaneously, 2–2.7 mg/kg, on the eighth day after ovariectomy.
5. Combined treatment (a) Stilboestrol and progesterone were administered simultaneously (0.5–0.7 mg stilboestrol and 5–7 mg progesterone/kg, both i.m.) on the eighth day after ovariectomy. (b) Stilboestrol, (0.5–0.7 mg/kg, i.m.) and dexamethasone, (2–2.7 mg/kg, s.c.,) were injected simultaneously on the eighth day after ovariectomy. (c) Testosterone, (6.3–8.3 mg/kg, i.m.) and stilboestrol, (5–7) mg/kg, i.m.) were injected simultaneously on the eighth day after ovariectomy.

Control ovariectomized rats were injected with an equivalent volume of oil. Following the various treatments described, the uteri were removed on the third day after drug injection.

6. Desoxycorticosterone acetate (DOCA) 5–7 mg/kg, was injected intramuscularly for three days. Control rats were injected daily with equal volumes of oil. The uteri were removed on the fourth day.
7. Isoprenaline (in 0.9% w/v NaCl solution (saline)) was administered subcutaneously, 1 mg/kg to rats, 2 h after bilateral nephrectomy. Control rats were nephrectomized and injected with saline. The uteri were removed 2 h after the injection.
8. Hypovolemia was induced according to the method of Stricker (1966), by subcutaneous injection of 4 ml/rat of a 20% solution of Carbowax in saline. Control rats were injected with an equivalent volume of saline. The uteri were removed after 4 hours.
9. Sodium loading. The animals were given 1.7% NaCl solution to drink for five days. Control rats drank tap water. The uteri were removed on the fifth day.

Removal of uteri for assay

The uteri were carefully separated from the ovaries and cut at the junction with the vagina. The uteri were then cleaned of fat and attached blood vessels, blotted on filter paper and weighed. The uteri of pregnant rats were separated also from foeti and placentae.

Homogenization

The uteri were placed in distilled water (w:v = 1:10) and were then subjected to three consecutive cycles of freezing and thawing. They were then homogenized in a Virtis Homogenizer, model 45, speed position-30, for 3–5 minutes. The homogenate was centrifuged at 10,000 *g* for 30 min, at 4°C. The supernatant (enzyme solution) was kept at −20°C until assayed.

Assay of renin-like activity in uterus

The enzyme solution (1 ml) was incubated with 1 ml renin-substrate (prepared from plasma of nephrectomized rats, according to Boucher, Menard & Genest, (1967)), 1 ml of resin (Dowex 50WX2, 100–200 mesh, to bind and protect angiotensin formed in the reaction), 1 ml citrate-phosphate buffer (pH 4.0) and 1 ml KCl 0.5 M (final concentration of K^+ in the reaction mixture was 0.1 M). Incubation was carried out at 50°C for 2 h in a thermostatic bath with constant shaking. The reaction was stopped by immediate freezing. The reaction conditions differed from this protocol in the initial experiments, when the optimal conditions for the enzyme activity were investigated. The reaction mixture was passed through Dowex 50WX2 resin columns, washed with water and eluted with diethylamine and NH_4OH, as previously described (Gutman *et al.*, 1973). The angiotensin in the eluate was assayed in nephrectomized, male rats injected with pentolinium, as described by Boucher *et al.* (1967). The amount of angiotensin in the sample was assessed by comparison with standard solutions of angiotensin II (Hypertensin, CIBA).

Drugs

The following drugs were used: Val^5-hypertensin-asp-βamide (Angiotensin II; CIBA-GEIGY Ltd, Basel), polyethylene glycol, mol. wt. 20,000 (Carbowax; Chemorad, Tel-Aviv), dexamethasone (ampoules of 400 mg/ml in saline; Ikapharm, Ramat-Gan, Israel), desoxycorticosterone acetate (DOCA, 5 mg/ml ampoules Organon, Holland), isoprenaline sulphate a gift from Teva, Jerusalem), pentolinium (Ansolysen; May & Baker), progesterone (Luteopur, ampoules of 50 mg/ml in oil; Teva, Jerusalem), stilboestrol dipropionate (dissolved in oil; Ward, Blenkinsop & Co.) and testosterone (Homosteron, ampoules containing 25 mg/2 ml oil; Teva, Jerusalem).

Results

Characterization of renin-like activity in rat uterus

Effect of pH Figure 1 shows the effect of pH on renin-like activity in the rat uterus. Optimal activity

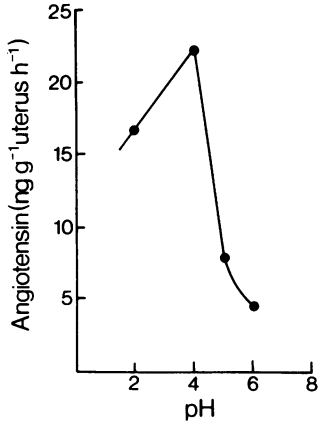

Figure 1 Effect of pH on renin-like activity of rat uterus. Incubations carried out at 37°C, for 18 hours. Each point is the mean of 4 experiments.

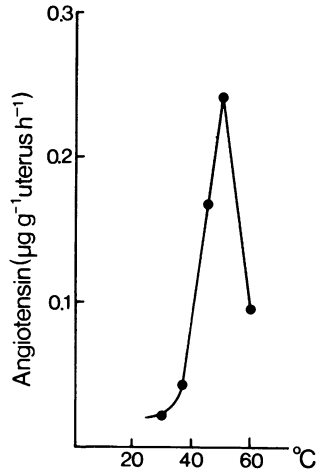

Figure 2 Effect of temperature on renin-like activity of rat uterus. Incubations carried out at pH 4, for 5 hours. Each point is the mean of 3 experiments.

was obtained at pH 4.0 with a sharp drop in activity at higher pH. Pre-incubation of the enzyme solution, without substrate, at pH ranging from 2 to 8, for 1 h, did not affect the activity of the enzyme on subsequent incubation with substrate at pH 4.0.

Effect of temperature Figure 2 shows the uterine renin-like activity at different temperatures. The optimal activity was obtained at 50°C with a sharp drop at lower and higher temperatures. Pre-incubation of the enzyme solution, without substrate, at temperatures of 4–50°C for 1 h caused no significant change of activity on subsequent incubation with substrate. Pre-incubation of the enzyme without substrate at 60°C for 1 hr caused inactivation of 65%.

The effect of various ions The effect of addition of various ions on renin-like activity in the rat uterus was next studied.

(a) Addition of Ca^{2+} (5–20 mM) or Na^{+} (150 mM) had no significant effect on uterine renin-like activity.

(b) Addition of K^{+} at final concentrations of 50–500 mM caused a substantial increase of the activity of the enzyme, with an optimum at 100 mM. At this concentration, activity was 2.25 times higher than in the absence of potassium.

Following these preliminary experiments, optimal assay conditions were set at 50°C, pH 4.0 and K^{+} 100 mM.

Changes in uterine renin-like activity with age

Uterine renin-like activity was assayed in rats aged 1, 2, 4 and 8 weeks. Figure 3 shows that the renin-like activity rose slowly up to 4 weeks of age. A sharp elevation was noted between the fourth and eighth week.

The source and function of uterine renin-like activity

Bilateral nephrectomy To ascertain whether uterine renin-like activity originated from the kidney, bilateral nephrectomy was performed in female rats. Twenty four hours after nephrectomy, when plasma renin had completely disappeared, uterine renin-like activity showed no significant change, compared to sham operated rats, as shown in Table 1. This experiment shows that uterine renin-like activity is independent of kidney renin.

Hypovolaemia This is one of the major stimuli for release of renin from the kidney. To study further a possible correlation of kidney and uterine renin, hypovolaemia was induced in female rats by subcutaneous injection of the polymer polyethylene-glycol (Carbowax). No significant change in renin-like activity in the uterus was observed, compared to saline-injected rats, as shown in Table 1.

Na^{+} loading Another stimulus which affects kidney renin, sodium loading, was given to female rats. No significant change in uterine renin-like activity was found, as seen in Table 1. Such treatment decreases kidney renin (Gross 1967, Vander 1970).

Effect of DOCA Mineralocorticoid administration causes a decrease of kidney and plasma renin (Pettinger, Marchell & Augusto, 1971). To

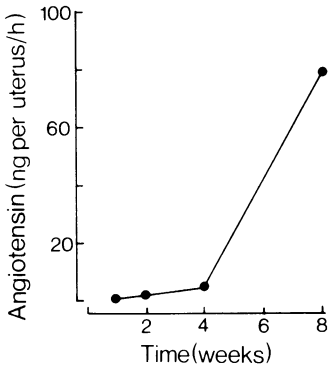

Figure 3 Effect of age on renin-like activity of rat uterus. Incubations carried out at 50°C, pH 4 for 2 hours. Each point is mean of two experiments.

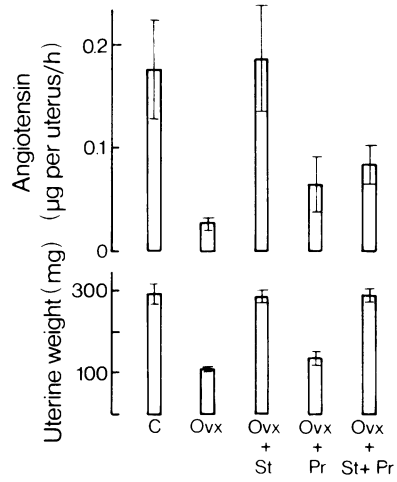

Figure 4 Effect of stilboestrol, progesterone and combined treatment on renin-like activity of rat uterus. Incubations carried out at 50°C, pH 4 for 2 hours. Vertical bars show s.e. mean. For renin-activity: C = control ($n = 8$); Ovx = ovariectomy ($n = 7$); St = stilboestrol ($n = 7$); Pr = progesterone ($n = 5$); St + Pr ($n = 8$).

characterize further uterine renin-like activity, desoxycorticosterone acetate was injected intramuscularly into female rats. Table 1 shows that no significant change in uterine renin-like activity was observed.

Bilateral adrenalectomy Adrenalectomy causes a significant increase in kidney renin (Hasegawa, Nasjletti, Rice & Masson, 1973), but as seen in Table 1, there was no significant change in uterine renin-like activity compared to sham-operated rats.

Isoprenaline Activation of β-adrenoceptors causes release of renin from the kidney. Table 1 shows that no significant change in uterine renin followed injection of isoprenaline.

Effect of stilboestrol, progesterone and testosterone Figure 4 shows the effect of stilboestrol and progesterone, given separately or combined, to

Table 1 Effect of various treatments on uterine renin-like activity

	Renin-like activity (Angiotensin, ng per uterus/h)		
	Control	*Experimental*	P
Nephrectomy	176 ± 57 (6)	186 ± 50 (8)	NS
Isoprenaline	148 ± 45 (5)	155 ± 20 (8)	NS
Adrenalectomy	104 ± 20 (8)	109 ± 12 (10)	NS
DOCA	124 ± 18 (14)	148 ± 66 (7)	NS
Na^+ loading	124 ± 18 (14)	164 ± 17 (8)	NS
Hypovolaemia	124 ± 18 (14)	115 ± 35 (9)	NS

Figures in parentheses indicate *n*. Nephrectomy—bilateral nephrectomy was performed in the experimental group and sham-operation in the control group, 24 h before removal of uteri. Isoprenaline—bilateral nephrectomy was performed 2 h before administration of saline (control) or isoprenaline, (1 mg/kg, s.c.), (experimental). Adrenalectomy—bilateral adrenalectomy was performed in the experimental group and sham-operation in the control group, 3 days prior to removal of uteri. DOCA—The mineralocorticoid was injected (1.0 mg/rat, i.m.) for three consecutive days; control rats were injected with the equivalent volume of oil. Uteri were removed on the fourth day. Na^+ loading—rats were given 1.7% NaCl to drink for 5 days, before removal of uteri; control rats were given tap water. Hypovolaemia—was induced by subcutaneous injection of 4 ml/rat of 20% Carbowax in saline, in the experimental group and of saline in the control group, 4 h before removal of uteri. Results are expressed as mean ± s.e. mean, NS—difference between control and experimental group not significant.

ovariectomized rats. Uterine renin-like activity decreased significantly after ovariectomy as compared to sham-operated rats. When ovariectomized rats were treated with stilboestrol (oestrogen-like) there was a significant rise in renin-like activity compared to that of ovariectomized rats. Renin-like activity in uteri of ovariectomized rats treated with stilboestrol was not significantly different from that of control, sham-operated rats.

Ovariectomized rats treated with progesterone showed a small but significant elevation of uterine renin-like activity compared to ovariectomized rats injected with oil (figure 4). However, the activity was considerably lower than in control, sham-operated rats.

Ovariectomized rats treated with both stilboestrol and progesterone showed a significantly lower renin-like activity in the uterus than ovariectomized rats treated with stilboestrol alone. Thus, when both hormones were administered simultaneously their effect was not additive but antagonistic (Figure 4).

The results were similar when renin activity was compared per gram uterus. Thus, in the control group the activity was 601 ng angiotensin per g uterus/h; after ovariectomy the activity diminished to 224 ng g^{-1} h^{-1}; following stilboestrol administration to the ovariectomized rats the activity increased to 799 ng g^{-1} h^{-1}, after progesterone administration to ovariectomized rats-467 ng g^{-1} h^{-1}, while combined treatment of ovariectomized rats with stilboestrol and progesterone resulted in renin activity of only 351 ng g^{-1} h^{-1}, considerably below that of stilboestrol-treated rats.

In order to find whether the action of progesterone was non-specific, i.e. a function of its corticosteroid property, the effect of the corticosteroid dexamethasone was studied. Ovariectomized rats were given either stilboestrol or dexamethasone, or a combined treatment. Dexamethasone had no effect on uterine renin-like activity in ovariectomized rats or on the elevation of uterine renin caused by stilboestrol in ovariectomized rats, calculated as renin activity per uterus or per gram uterus (Table 2).

Testosterone administration reduced uterine renin-like activity in ovariectomized rats when calculated per gram uterus and reduced the effect of stilboestrol on uterine renin (decrease of 37.5 ± 7.2%) without a significant change in uterine weight (Table 3).

Changes in uterine renin-like activity during pregnancy

Dog and rabbit uterine renin have been reported to increase during pregnancy (Johnson, Ryan & Reyes-

Table 2 Effect of dexamethasone and stilboestrol on renin-like activity of rat uterus

Treatment	*Renin-like activity (Angiotensin, ng per uterus/h)*	*(Angiotensin ng g^{-1} h^{-1})*	*Uterine weight (mg)*
Ovariectomized	5.5	65.5	84
Ovariectomized + Stilb	68.0	246.4	276
Ovariectomized + Dexa	6.4	65.3	98
Ovariectomized + Stilb + Dexa	67.0	234.3	286

Stilb = stilboestrol (0.1 mg/rat, i.m.) Dexa = dexamethasone (0.4 mg/rat, s.c.) Each value is the mean of two experiments. The incubations with renin-substrate were carried out without the addition of K^+, therefore the activities were lower than in other experiments.

Table 3 Effect of testosterone and stilboestrol on renin-like activity of uterus

Treatment	*Renin-like activity (Angiotensin, ng per uterus/h)*	*(Angiotensin ng g^{-1} h^{-1})*	*Uterine weight (mg)*
Ovariectomized	51 ± 10 (3)	526	97 ± 14 (9)
Ovariectomized + Stilb	198 ± 47 (3)	723	274 ± 8 (7)
Ovariectomized + Test	56 ± 14 (3)	357	157 ± 12 (9)
Ovariectomized + Stilb + Test	121 ± 38 (6)	465	260 ± 14 (18)

Figures in parentheses indicate *n*. Stilb = stilboestrol (0.1 mg/rat, i.m.) Test = testosterone (1.25 mg/rat, i.m.). Results given as mean ± s.e. mean

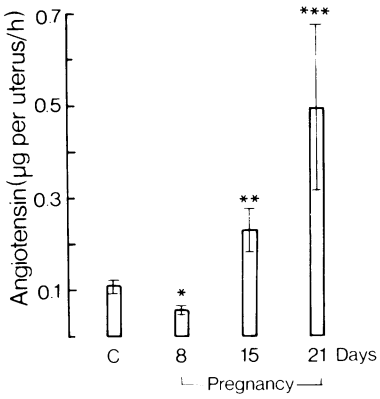

Figure 5 Effect of pregnancy on renin-like activity of rat uterus. Incubations carried out at 50°C, pH 4, for 2 hours. Vertical bars show s.e. mean. Control, $n=11$; 8 days pregnancy, $n=5$; 15 days pregnancy, $n=6$; 21 days pregnancy, $n=3$.

Rodrigues, 1971). The effect of pregnancy in the rat is shown in Figure 5: there was a significant initial decrease in uterine renin-like activity (44%) at the beginning of pregnancy, and a steady rise later in pregnancy. Renin-like activity was also assayed in the placentae. The mean content of renin-like activity per placenta increased from 21 at 15 days of pregnancy, to 53 ng angiotensin released per placenta/h, on the last (21st) day of pregnancy.

Discussion

The presence of renin-like activity in extra-renal tissues has been reported in several species. Gross *et al.* (1964) described such renin-like activity in the placenta and uterus of the rabbit. Gutman *et al.* (1973) have identified such activity in the submaxillary gland of the rat, and Ganten *et al.* (1971) in the brain of the dog.

Recently, Carretero, Bujak & Houle (1971) showed that the various extra-renal renin-like enzymes catalyze the production of the same product, angiotensin. Gross *et al.* (1964) have shown that the final product of incubation of rabbit uterus homogenate is angiotensin and Hodary, Carretero & Hodgkinson (1969) demonstrated that angiotensin is formed upon incubation of dog uterine homogenate. Geelhoed *et al.* (1970) have shown the presence of renin-like enzyme in the human uterus. However, whereas the role of kidney renin in sodium balance is well established (Brown, Lever & Robertson, 1967), no functional significance of extra-renal renin has been clearly demonstrated.

One possibility is that extra-renal renin merely reflects adsorption of circulating renin from the blood into various tissues. This possibility has been eliminated in the case of the rat submaxillary gland (Gutman *et al.*, 1973) as well as for the utero-placental complex of the dog (Carretero, Polomski, Piwonska, Afsari & Hodgkinson, 1972) and the rabbit uterus (Gordon, Ferris & Mulrow, 1967). The experiments reported in the present paper corroborate these findings in the case of the renin-like enzyme of the rat uterus. Twenty four hours after bilateral nephrectomy of the female rat there was no reduction in the uterine renin-like activity (Table 1), whereas plasma renin disappears completely within a few hours. However, these findings do not refute the possibility that uterine renin-like stores do originate from the kidney but are released very slowly.

The uterine renin-like enzyme has been shown to be similar though not identical to kidney renin in both the dog (Carretero & Houle, 1970) and rabbit (Anderson, Herbert & Mulrow, 1968). The present paper has also shown that although the rat uterus renin-like enzyme is similar to kidney renin, its characteristics differ from those of kidney renin in the pH optimum and the enhancement of the reaction by K^+ but not by Na^+.

The effects of physiological stimuli which regulate kidney renin were studied on the uterine renin-like enzyme. Hypovolaemia had no effect on the uterine enzyme, whereas it is known to stimulate the release of kidney renin (Gross 1967; Vander 1970). Na^+ loading also showed no significant effect on the uterine renin-like activity, though it inhibits the release of kidney renin (Vander, 1970). Injection of a mineralocorticoid (DOCA) had no effect on the uterine renin-like activity, but it decreases plasma renin level (Pettinger *et al.*, 1971). Bilateral adrenalectomy had no effect on the uterine enzyme, whereas it stimulates the release of kidney renin, due to the enhanced sodium loss. These findings suggest that uterine renin is not affected by the same physiological stimuli that regulate kidney renin.

Several authors have studied the renin-angiotensin system in plasma during pregnancy. Thus, Talledo (1967) found differences in renin-angiotensin during normal and toxaemic pregnancies; Skinner *et al.* (1972) analysed changes in the renin system during pregnancy. Both of these studies, as well as others, have shown elevation of plasma renin activity during pregnancy. Various reports have also shown an increase of uterine renin during pregnancy: Ferris, Gordon & Mulrow (1967), and Johnson, *et al.* (1971) found an increase of renin in rabbit uterus. However, the possible relation of plasma renin and uterine-renin during pregnancy has not been demonstrated. Furthermore, in view of the independence of uterine-renin response to stimuli which affect plasma renin, such a relationship seems rather remote. Therefore, uterine renin-like enzyme may have a local function rather than play a role in systemic renin-angiotensin.

The ontogenesis of renin-like activity in the uterus follows sexual maturation. Thus, Figure 3 shows a slow rise from birth to the age of 4 weeks, and a sharp rise between the fourth and eighth week, when the female rat reaches puberty. This course could be reversed by ovariectomy. As seen in Figure 4 ovariectomy of mature rats caused a profound fall of uterine renin-like activity. The decrease of this activity in ovariectomized rats could be prevented completely by administration of an oestrogen, stilboestrol, while progesterone induced only a small and incomplete recovery of uterine renin-like activity after ovariectomy. (Figure 4.) In view of the qualitatively similar effects of progesterone and an oestrogen, it was rather surprising that combination of these treatments in ovariectomized rats caused a smaller elevation of renin-like activity than the oestrogen alone. Progesterone, therefore, seems to antagonize the effect of oestrogen on uterine renin-like activity. The increase of uterine weight caused by the oestrogen was not antagonized by progesterone.

In a recent communication, Hsueh, Peck & Clark (1975), demonstrated reduction of the amount of oestrogen-receptor in the uterus of immature rats by treatment with progesterone. This could be a possible mechanism for the phenomenon described in the present paper. However, in our experiment, carried out in mature female rats, progesterone did not reduce the uterine weight-gain induced by stilboestrol. A similar finding, inhibition of the increase of uterine renin induced by stilboestrol, followed injection of testosterone, without an effect on uterine weight (Table 3). One could have ascribed the effect of progesterone on uterine renin to corticosteroid-like effects, but dexamethasone, a potent glucocorticoid, had no effect on the action of oestrogen and, on uterine renin (Table 2).

The effects of oestrogen and, possibly, of progesterone were reflected also in the rat uterine-renin during pregnancy. Figure 5 shows that there was a gradual increase in uterine renin during pregnancy, except for a significant fall during the first week. If progesterone antagonizes the effect of oestrogen on uterine renin, then the ratio oestrogen: progesterone will be the effective parameter. It is interesting, therefore, that from the reports of Wiest (1970) and of Yoshinaga, Hawkins & Stocker (1969), this ratio was lower during the first 10–12 days of pregnancy than during the later periods of pregnancy. It is suggested that uterine renin may depend on the ratio oestrogen: progesterone.

Y.G. is an Established Investigator of the Chief Scientist, Israel Ministry of Health. The results described in the paper were presented at the 33rd Meeting of the Israel Physiological and Pharmacological Society, Haifa, June 1975.

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