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Antagonistic effect of progesterone towards estradiol dipropionate-induced changes in glycogen content in uterus and vagina of P-mice

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Summary. Estradiol dipropionate induces an increase (3-fold) in the uterine glycogen content and a decrease (4-fold) in the vaginal glycogen content of Parkes (P) mice. Progesterone antagonizes this estradiol dipropionate-induced response in both the uterine and vaginal tissue. The degree of this antagonism is more pronounced in the uterus than in the vagina.
Key words. Mice; uterus; vagina; sex steroids; glycogen.

The effect of estrogen on uterine and vaginal glycogen content is well studied in a number of mammalian species. Further attempts were made to find out the effect of progesterone on estrogen-mediated response of uterine and vaginal glycogen levels in the rat and as a result contradictory findings have been reported. For instance, the combined administration of estrogen and progesterone did not produce any change in the uterine glycogen content as compared to the estrogen-treated animals[2]. In contrast, the conjoint administration of estrogen and progesterone caused a decrease in the uterine glycogen content in comparison to the condition when estrogen was given alone[3,4]. Moreover, the conjoint treatment with estrogen and progesterone produced a decrease in uterine glycogen content and an increase in vaginal glycogen content as compared to the rats which received estrogen alone[5]. This indicated the organ-specific differences in the glycogen content of uterus and vagina in response to a combined treatment with estrogen and progesterone. Therefore, the problem of estrogen-progesterone interaction seemed to be of remarkable importance. Similarly, many other investigators have also examined the glycogen level as a biochemical correlate of the process of vaginal keratinization[6,7]. These observations led us to investigate the effect of progesterone on the estrogen-induced response of uterine and vaginal glycogen contents of albino mice (P-strain).

Material and methods. There are several inbred albino strains of the common house mouse, *Mus musculus* Linn. Various strains differ in the duration and phases of the estrous cycle and in the response to exogenous female sex hormones[8]. Adult virgin female mice of the Parkes (P) strain (60–100 days old) were used in the present investigation. They are polyestrous rodents having an average 4–5 days of estrous cycle. The parental stock of P-mice was obtained from the Central Drug Research Institute (CDRI), Lucknow (India); and they were bred in the animal house of the Department of Zoology, Banaras Hindu University. All female mice were maintained with food and water ad libitum under laboratory conditions at 25 ± 3 °C and natural photoperiod (13L:11D) for 14 days prior to experimentation. They were bilaterally ovariectomized and 7 days after ovariectomy were divided into the following four groups for the treatment:

Group I (control): 0.05 ml olive oil.
Group II: 20 µg estradiol dipropionate (E-DIP).
Group III: 2 mg progesterone.
Group IV: 20 µg E-DIP+2 mg P (1:100).

Each individual received daily the above mentioned physiological doses by s.c. injection continuously for 7 days. Ampoules of hormonal injection (estradiol dipropionate and progesterone) were purchased from Ciba-Geigy Ltd., Bombay, and olive oil was used as a carrier vehicle. Animals were killed by cervical dislocation 24 h after the last injection. Uteri and vaginae were collected immediately after sacrifice from experimental and control groups for biochemical estimation. Glycogen was determined by Montgomery's[9] method, and the content determined (µg/mg wet wt tissue) using a linear standard curve for glycogen. Student's t-test was employed for statistical analysis.

Results. It is evident from the table that the administration of estradiol dipropionate to ovariectomized mice produced a 3-fold increase in uterine glycogen content while progesterone reduced the glycogen content in comparison to the control. The combined treatment with estradiol dipropionate and progesterone at a dose ratio of 1:100 resulted in a decrease in uterine glycogen content as compared to the control value. In the vagina, estradiol dipropionate caused a 4-fold decrease in the glycogen content while progesterone showed an insignificant decrease. However, the conjoint treatment with estradiol dipropionate and progesterone at 1:100 dose ratio revealed a statistically insignificant difference in the vaginal glycogen content as compared to the control value.

Moreover, the significant differences were noticed in uterine as well as vaginal glycogen content when the values of group IV

Effects of exogenous female sex steroids on the glycogen contents of uterus and vagina of P-mice

Group	Treatment	Uterine glycogen (µg/mg wet wt)	Vaginal glycogen (µg/mg wet wt)
I	Control	0.808 ± 0.056	2.213 ± 0.141
II	Estradiol dipropionate (E-DIP)	2.469 ± 0.033 ($p < 0.001$)	0.565 ± 0.008 ($p < 0.001$)
III	Progesterone (P)	0.677 ± 0.053 ($p < 0.05$)	2.060 ± 0.091 ($p > 0.10$)
IV	*E-DIP + P (1:100)	0.168 ± 0.040 ($p < 0.001$)	2.214 ± 0.050 ($p > 0.20$)

The results are expressed as mean ± SEM of 3–4 individual determinations. P values are given in parenthesis in comparison to their control values. *$p < 0.001$ vs group II.

were compared with the values given in group II (see table). As a result, the uterine glycogen content showed a decrease in response to the conjoint treatment with estradiol dipropionate and progesterone, while an increase in the vaginal glycogen content was observed.

Discussion. It is well established that the glycogen metabolism in normal mammalian tissues is regulated by the changes in glycogen synthase and glycogen phosphorylase activities due to alteration in the phosphorylation state of these two enzymes. Estradiol brings about the conversion of glycogen synthase b to a, so that the synthase a/b ratio is elevated and concomitantly glycogenesis is stimulated[10]. It has been reported that the accumulation of glycogen proceeds at a greater pace than its consumption in uterine tissue[11]. Thus the estrogen-induced glycogen deposition in the uterus is a result of both the effects. However, the estrogen-induced decline in vaginal glycogen may perhaps be due to high glycogen consumption to meet the physiological demand of heavy vaginal keratinization[12]. Progesterone alone caused a decrease in uterine glycogen; this is in agreement with the previous report in the rat[4]. On the other hand, progesterone produced only an insignificant decrease in vaginal glycogen content when compared with the control value. This indirectly strengthens the fact that the content of vaginal glycogen largely depends on its consumption for cellular proliferation in vagina. It is shown in the table, that during combined administration of the two female sex-hormones (estradiol dipropionate and progesterone), progesterone abolished the estrogenic-effect on uterine (increasing-effect) and vaginal (reducing-effect) glycogen content in P-mice. This is similar to a previous observation in albino rats[5]. Ultimately, due to progesterone antagonism to an estradiol ester a reduced level of uterine glycogen, well below the control value, was noticed. The vaginal glycogen content in group IV approached a value equivalent to the control. So, unlike the conditions reported in the albino rat[5], it appears that the antagonizing effect of progesterone is more pronounced in the uterus than in the vagina of P-mice.

Antagonistic effects of estradiol dipropionate and progesterone on uterine and vaginal histology of the mouse have been documented[13]. Similarly, in the present investigation, the progesterone antagonism to an estradiol ester is demonstrated in relation to uterine and vaginal glycogen content of P-mice. Various workers have reported the modulation of estradiol activity by progesterone[14–16]. On the question of estrogen-progesterone interactions in relation to glycogen content, it is more reliable to speculate that the antagonistic action of progesterone on an estradiol receptor[17,18] evokes progesterone antagonism to estradiol dipropionate in uterus and vagina. But the dissimilarity in the degree of antagonism in both the two organs (uterus and vagina) could be attributed to the differences in the rate of absorption and/or retention of sex hormones in the target organs[19].

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Granulocyte adhesion to nephritic glomeruli through recognition of activated C4 and C3 in immune deposits

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Summary. Sections of rat kidney with bovine serum albumin nephritis were incubated either with a single component of complement or with several components in sequence and then reacted with granulocytes. The average number of granulocytes bound to a nephritic glomerulus was elevated in sections incubated with C4 or C3 and increases were most significant when C14, C142 or C1423 were incubated.

Key words. Granulocyte; complement; immune adherence; immune deposit; glomerulonephritis.

Most types of glomerulonephritis in humans are considered to be induced by immune complexes accumulated in glomeruli. Although leukocytic accumulation is also characteristic of glomerulonephritis, the precise mechanism is not clear. Results derived from experiments with the Arthus reaction in vivo and related systems[2] have led to speculation that chemotactic factors play an important role in the accumulation of leukocytes within glomeruli. However, any extrapolation of evidence derived from studies of the Arthus reaction must include awareness of differences between inflammation in glomeruli and skin. The sites of leukocyte accumulation in glomeruli are essentially the capillary lumen and the mesangium, whereas leukocytes infiltrate into the extravascular space of the skin in the Arthus reaction. Moreover, glomerular function is to filter low molecular substances from plasma into the urinary space. This should make it difficult for chemotactic factors to maintain the concentration gradient essential for their activity, because they are usually small in molecular size and are filtered easily through the glomerular basement membrane. However, activation of the complement system on immune complexes in glomeruli can generate these chemotactic factors. We report here the essential role of immune adherence through complement receptors on granulocytes (Gr) in the adhesion of Gr to glomerular immune complexes, according to a Gr binding assay in vitro[3]. The purpose of this study was