702 did not inhibit tumor promotion significantly. Neither SD-170 nor SD-702 influenced the body weight of animals during the experiment.

SD-170 strongly inhibits histidine decarboxylase ($ID_{50}$, 0.007 μg/ml) and slightly inhibits TPA-induced arachidonic acid release (40% inhibition at 20 μg/ml), while SD-702 slightly inhibits histidine decarboxylase ($ID_{50}$, 2.5 μg/ml) and strongly inhibits arachidonic acid release ($ID_{50}$, 0.35 μg/ml)[3]. Both SD-170 and SD-702 inhibit prostaglandin synthetase about 60% at 10 μg/ml[3]. Thus our results suggest that induction of histidine decarboxylase may be more important for tumor promotion than arachidonic acid release. The action of SD-170 was almost certainly biological, because neither SD-170 nor SD-702 influenced the stability of TPA when incubated with it in acetone for 3 h at 37 °C.

Retinoids[6], anti-inflammatory steroids[7] and dibromoacetophenone[8], a phospholipase $A_2$ inhibitor, are known to inhibit tumor promotion in mouse skin. SD-170 has very low toxicity, its $LD_{50}$ being 3500 mg/kg in mice and 2000 mg/kg in rats on i.p. injection[9]. Thus this lecanoric acid analogue is a new inhibitor of tumor promotion with low toxicity.

Effect of lecanoric acid analogues on mouse skin tumor promotion

| Week | Treatment | No. of tumors Size of tumors (mm diameter) 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Control (26)* | 1 | 1 | 2 | 1 | 2 | 3 | 9 | 24 | 109 | 152 |
| | SD-170 (26) | –** | – | – | – | – | 1 | 2 | 9 | 64 | 76 |
| | SD-702 (26) | 3 | – | 3 | – | 9 | 1 | 2 | 18 | 90 | 126 |
| 18 | Control (26) | – | 1 | – | 1 | 2 | 8 | 11 | 37 | 157 | 217 |
| | SD-170 (26) | – | – | – | – | 1 | – | 4 | 13 | 133 | 151 |
| | SD-702 (25) | 1 | – | – | 2 | 2 | 4 | 12 | 29 | 140 | 190 |

*Number of mice; **none

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## Prenatal stress and postnatal androgen: Effects on reproduction in female rats

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*Summary.* Heat-restraint stress applied to pregnant rats during the last trimester disrupts oestrus cycles in female offspring and alters adrenal, ovarian and uterine weights at autopsy. Sexual receptivity is left intact. Prenatal stress may operate by increasing exposure of fetal females to androgens in utero.

Stress during gestation impairs reproductive capabilities of offspring in adulthood. Prenatally stressed males exhibit a syndrome marked by diminished copulatory performance and increased lordotic potential[1-8]. Female offspring reportedly experience oestrous cycle disorders, reduced fertility, spontaneous abortions, vaginal hemorrhaging, stillbirths, neonatal mortality and low birthweight young[9-11]. Although the mechanism of prenatal stress is not clear, the abnormal actions may involve alterations in exposure to androgen. It has long been known that under normal conditions the expression of adult patterns of sexual behavior and gonadotropin secretion depend upon perinatal androgen[12-14]. Removal of androgen by perinatal castration produced genetic males who exhibit cyclic gonadotropin release and lordosis behavior characteristics of females. Conversely, early administration of androgen to genetic females produces anovulation, sterility and a reduction of sexual receptivity. Recently prenatally stressed fetal males have been shown to undergo a premature surge in plasma testosterone compared to nonstressed controls[15], and isolated plasma samples from prenatally stressed fetal females taken at about the same time as the males contain extraordinarily high levels of testosterone[16]. The question arises whether androgen may be implicated in prenatal stress by other means. If androgen plays a role in the prenatal stress syndrome, then environmental stress effects on reproduction should mimic to some extent those of exogenously-administered hormone. The present experiment compares and contrasts the effects of prenatal stress and early androgen on some reproductive functions in females.

*Materials and methods.* 24 Sprague-Dawley rats weighing 200-250 g were time-mated at Zivic-Miller (Allison Park, Pennsylvania) and sent to our laboratory. Upon arrival, females were housed individually in 24 × 32 × 16 cm fiberglas observation cages with San-i-cel bedding under a standard 12 h light/dark cycle beginning at 10.00 h, maintained on Purina chow and water ad libitum. On days 14-22 of gestation, 12 randomly selected females were subjected to stress of restraint, heat and bright light. Stress was applied by placing each female in an 18 × 8 cm semicircular Plexiglas restraining cage under 4 incandescent lights providing a surface illumination of 4280 lm/m$^2$

Effects of prenatal stress and postnatal androgen on reproduction in female offspring

| Treatment | Vaginal openings | | | Reproductive function[e] | | Sexual receptivity | | | Organ weights at autopsy (g, $\bar{X} \pm SE$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Percentage with openings | Age at openings (days, $\bar{X} \pm SE$) | N | Percentage with prolonged[c], persistent[d] oestrus, dioestrus | N | Percentage exhibiting lordosis | Lordosis quotient ($\bar{X} \pm SE$) | Adrenals | Ovaries | Uterus |
| Prenatal stress + oil[a] | 9 | 100 | 39.0 ± 0.8 | 9 | 55, 44[c] | 9 | 67 | 48.3 ± 12.9 | 0.09 ± 0.00 | 0.10 ± 0.00 | 0.61 ± 0.04 |
| Nonstress + oil[a] | 8 | 100 | 39.0 ± 1.1 | 8 | 0, 0 | 8 | 88 | 45.0 ± 11.8 | 0.07 ± 0.01 | 0.09 ± 0.00 | 0.83 ± 0.03 |
| Prenatal stress + postnatal androgen[b] | 12 | 42 | 37.2 ± 0.3 | 5 | 100, 0[d] | 12 | 8 | 1.6 ± 1.5 | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.65 ± 0.01 |
| Nonstress + postnatal androgen[b] | 15 | 27 | 40.0 ± 1.2 | 4 | 100, 0[d] | 13 | 31 | 5.06 ± 2.4 | 0.09 ± 0.00 | 0.12 ± 0.01 | 0.61 ± 0.03 |

[a] 0.05 ml sesame oil. [b] 250 µg testosterone; 0.05 ml sesame oil. [c] Prolonged: 5–9 consecutive days. [d] Persistent: Virtually throughout the experiment. [e] Reproductive status was based on vaginal smears.

and surface temperature of 34 °C. Three 45-min stress periods beginning at 10.00 h daily were alternated with rest periods of equal duration in the home cage. Females readily adapted to placement in the plexiglas cage, offering little or no resistance. After a few trials, they rapidly crawled inside and sat passively within it. 12 control females remained unhandled in their home cages. Pregnant females were observed daily for day of delivery. On the day of birth, 6 prenatally stressed and 6-non-stressed litters were randomly selected to comprise postnatal androgen groups. Female offspring were injected on day 1 with a single s.c. dose of testosterone propionate (250 µg/0.05 ml sesame oil). Control offspring were injected with 0.05 ml sesame oil. On day 21 offspring were weighed, segregated by sex and housed 2 per cage. Beginning on day 25, females were examined daily for perforation of the vaginal membrane. Vaginal smears were taken daily. Beginning at 90 days of age, females were observed for 2 oestrus cycles or for 9 consecutive days. Approaching the 2nd oestrus (or on the 9th day as the case applies), the females were observed for response to mounting by a vigorous male stud with ligated ducts. On the basis of 10 mounts by the male, a lordosis quotient was calculated for each female (LQ = No. lordosis/10 mounts × 100). The mean LQ was determined for each group and included zero scores for non-responders. The quality of the lordotic responses was rated on a three point scale ranging from: no lordosis when mounted plus resistance and aggression toward the male ('0'), mild arching of the back with some resistance to a persistent male ('1'), to deep lordotic arching of the back held after the male dismount ('2'). At approximately 130 days of age, females were sacrificed via rapid decapitation. Gonads, uteri and adrenal glands were removed and weighed.

*Results and discussion.* Although the percentage of females with vaginal openings significantly differed ($\chi^2 = 18.03$, $p < 0.001$, $df = 3$, table), only the androgen groups were significantly reduced compared to their respective oil groups (Fisher's Exact Test, p's < 0.02): prenatal stress groups did not differ from nonstress groups (p's > 0.05). Of those females with patent vaginas, age at opening did not differ significantly (p > 0.05). Oestrous cycles however were markedly different. All untreated control females had normal 4–5 -day cycles, whereas all androgenized females were in persistent vaginal oestrus. Compared to the untreated control group, the percentage of androgenized females with persistent oestrus was highly significant (Fisher's Exact Test, p's < 0.001). Also, the percentages of prenatally stressed females exhibiting prolonged oestrus or dioestrus were significantly greater than those in the untreated nonstress group (Fisher's Exact Test, $p < 0.05$). The percentages of females exhibiting lordosis were also significant ($\chi^2 = 9.82$, $p = 0.05$, $df = 3$). Both androgen groups had significantly fewer females that lordosed compared to the oil groups (Fisher's Exact Test, p's < 0.02), but prenatal stress groups did not differ from nonstress groups (p's > 0.05).

Two-way analyses of variance revealed significant differences in lordosis quotients and organ weights at autopsy (F's = 2.86, p's < 0.05, df = 3.38). Dunnett's t-test applied posthoc revealed that each androgen group exhibited a marked reduction in sexual receptivity compared to its respective oil group (t's = 5.31, p's < 0.001, df's = 15–18), but prenatally stressed groups did not differ significantly from nonstress groups (p's > 0.05). Whereas the prenatally stress group and the androgen groups did not differ significantly from each other with respect to organ weights (p's > 0.05), significant differences were found in these groups compared with the untreated nonstress controls (t's = 6.6, p's < 0.001, df's = 15–18). Moreover, adrenal and ovarian weights were significantly larger in the prenatal stress group than in the nonstress control (t's = 6.6, p's < 0.001, df = 15), whereas uterine weights were significantly lower ($t = 16.0$, $p < 0.001$, $df = 15$).

The most potent findings were that prenatal stress altered oestrous cycles and organ weights at autopsy, but not sexual behavior. The results therefore confirm Herrenkohl and Politch[11] that prenatal stress disrupts oestrous cycles as well as Beckhardt and Ward[17] that sexual receptivity remains intact. Herrenkohl[9] has described the prenatal stress syndrome in female offspring as being characterized by diminished reproductive capabilities (oestrus cycle disorders, spontaneous abortions and vaginal hemorrhaging during pregnancy, stillbirths and neonatal mortality, low birthweight young). On the other hand Beckhardt and Ward[17] report that prenatal stress does not alter reproductive functioning in female offspring. Mothers were exposed to the same heat-restraint stress known to demasculinize and feminize the sexual behavior of male offspring. Female offspring showed normal cyclicity, sexual behavior, pregnancy, parturition, pup survival and maternal behavior. While the reasons for differences in findings between the two laboratories is not clear, methodological differences need to be considered. Herrenkohl's subjects[9] for example were exposed to approximately twice the illuminance of Beckhardt and Ward[17]. If Herrenkohl's procedures are more stressful than those of Ward, there may be a sex difference in the amount of prenatal stress needed to disrupt adult reproduction. More intensive stressors may be required to alter physiology of females than to influence the behavior of males.

If prenatal stress operates by increasing exposure to androgens in utero, the sources presently unknown could include a stress response of the fetal adrenals[18], maternal adrenals[18] or fetal testes (by proximity to fetal males)[19,20]. Prenatal stress altered adrenal, ovarian and uterine weights at autop-

sy in directions similar to that of androgen (table). Such findings implicate a gonadal-adrenal interaction. Unlike androgenization, however, prenatal stress did not alter vaginal opening (table). Gorski[12-14] describes anovulatory syndromes in androgenized females where full sterility is produced by large amounts of exogenously-administered androgens at critical periods, and partial sterility by ligher amounts at less critical times. Prenatal stress may operate similarly, producing different effects on different reproductive indices in either sex, depending upon maturation and patterns of hormone release.


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## Free thyroxine in myocardial infarction

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High values for the free thyroxine fraction were found in the serum of 24 patients during the early phase of myocardial infarction. A strong correlation between the free thyroxine fraction and free fatty acids suggests that they compete for protein binding sites. The increase in free thyroxine may have undesirable effects on myocardial oxygen demand during acute myocardial infarction.

Studies on lipid metabolism after a myocardial infarction (MI) reveal several characteristic patterns. Levels of cholesterol, phospholipids, and beta-lipoproteins in serum tend to fall a few days after the onset[2]. The fatty acid composition of the serum lipids also changes with an increase of arachidonate and palmitate percentages and a decrease of linoleate[3]. Thyroxine induces similar changes[4,5]. We have therefore measured the serum thyroxine ($T_4$) and free thyroxine ($FT_4$) fractions in the early course of MI.

*Material and methods.* $T_4$, $FT_4$ fraction, and free fatty acids (FFA) were assayed in 24 men, aged 43–68 years, with MI. They were accepted for the study if the onset of symptoms had occurred less than 6 h before the admission. The diagnosis was based on clear clinical and electrocardiographic evidence in addition to increased aspartate aminotransferase values. All patients were observed in the coronary care unit with continuous electrocardiographic monitoring. Venous blood samples were taken at the time of admission, then 4 times at fixed hours during the first 24 h, and at 08.00 h on the following 3 days. Patients were fasting at 08.00 h. The control group consisted of 37 men in the same age group without obvious disease. $FT_4$ fraction was determined by the tracer equilibrium dialysis of Vaerenberg et al.[6], and FFA by the method of Trout et al.[7]. A nonparametric test, the Median test, was used for the statistical evaluation together with Kendall's correlation coefficient.

*Results.* $T_4$ concentrations in the samples drawn less than 6 h after the onset of symptoms are significantly higher than in the control group (table). In the period from 6 to 19 h the mean value is still slightly higher than in the control group, but the difference is not statistically significant. Later values do not differ from those of the controls. The $FT_4$ fraction shows high values on admission, with a mean of 0.095% against 0.047% in the control group ($p < 0.001$). Later values are lower, but remain significantly elevated during the entire period studied.

Admission values of FFA were often very high with a mean value more than 3 times the mean value of controls. A strong correlation was found between FFA and $FT_4$ frac-

Thyroxine ($T_4$), free thyroxine ($FT_4$) fraction, and free fatty acids (FFA) in patients with myocardial infarction and in controls. (Mean values with range in parentheses)

| Hours after onset of symptoms | $T_4$ (nmol/l) | $FT_4$ (%) | FFA (μmol/l) |
|---|---|---|---|
| < 6 | 184** (84–322) | 0.095* (0.051–0.195) | 1655* (563–4120) |
| 6–19 | 154 (77–368) | 0.077** (0.052–0.138) | 873** (419–1635) |
| 20–39 | 145 (89–243) | 0.075** (0.045–0.310) | 723 (187–1283) |
| 40–59 | 117 (45–254) | 0.078** (0.050–0.145) | 608 (230–1250) |
| 60–79 | 138 (59–248) | 0.076** (0.043–0.131) | 561 (168–1250) |
| Controls | 131 (53–202) | 0.047 (0.032–0.109) | 513 (184–1478) |

* $p < 0.001$; ** $p < 0.01$.