STUDIES ON HORMONAL ACTIONS OF DIHYDROXYPROGESTERONE ACETOPHENIDE, ESTRADIOL ENANTHATE AND THEIR MIXURES

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Recent global trends of population increase has turned attention to hormonal control of ovulation as an effective contraceptive. Consequently, oral contraceptives have been studied and are generally utilized in the North America and Europe. These are used in a combination of progestogens and estrogens, in either a combination or sequential method. A near perfect contraceptive result is effected, however, carelessness or misuse often results in failure (1).

It has been reported that acetophenone derivatives of 16α, 17α-dihydroxyprogestosterone show long-lasting potent progestational activity (2-4). In view of this, attempts have been made to produce a contraceptive effect via a monthly intramuscular injection of the steroid which has been dissolved in vegetable oil. In this administration a certain prolonged concentration of the steroid is maintained in the blood, subsequently, the question is raised as to endocrinological side effects of the steroid or toxicities. The objective of the present work is to determine in rats, hormonal action of a mixture of dihydroxyprogesterone acetophenide and estradiol enanthate, one of the test preparations of parental contraceptive, developed by Squibb Institute for Medical Research.

MATERIALS AND METHODS

Sixty-one female and 30 male adult Wistar strain rats were used. Steroids included dihydroxyprogesterone acetophenide (16α, 17α-dihyroxy-4-pregnene-3, 20-dione, cyclic-16, 17-acetal with acetophenone, β-methyl-α-phenyl) (hereafter referred to as DHPA), estradiol enanthate (1, 3, 5(10) estratriene-3, 17β-diol, 17-ester with heptanoic acid) (hereafter referred to as EE) and Deladroxate (hereafter referred to as DDX). All are products of Squibb Institute for Medical Research. DDX is an oily solution, containing 150 mg of DHPA and 10 mg of EE per milliliter. This was diluted 10 fold with castor oil when used in doses less than 0.1 ml. Castor oil was always used for dissolution of both DHPA and EE. Control animals were given only castor oil. The steroid was intramuscularly injected into the femur in a single dose. Details are given in Tables 1-3.

The animals were weighed daily. Vaginal smears were taken at definite times each day to determine effect of the steroid on the estrous cycle. The rats were sacrificed and
**TABLE 1. Experimental conditions.**

| Drug   | Content  | No. of animals | Body weight |
|--------|----------|----------------|-------------|
| DDX 0.01 ml | DHPA 1.5 mg | 5              |             |
|        | EE 0.1   |                |             |
|        | 0.02     | 5              | 160         |
|        | DHPA 3.0 |                |             |
|        | EE 0.2   |                |             |
|        | 0.04     | 5              |             |
|        | DHPA 6.0 |                |             |
|        | EE 0.4   |                |             |
| DHPA 0.2 | DHPA 3.0 | 5              | 175         |
|        | EE 0.2   |                |             |
| Castor oil | 0.2      | 5              |             |

**TABLE 2. Experimental conditions.**

| Drug   | Content  | No. of animals | Body weight |
|--------|----------|----------------|-------------|
| DDX 0.1 ml | DHPA 15.0 mg | 5              |             |
|        | EE 1.0   |                |             |
|        | 0.2      | 5              | 205         |
|        | DHPA 30.0|                |             |
|        | EE 2.0   |                |             |
|        | 0.4      | 5              |             |
|        | DHPA 60.0|                |             |
|        | EE 4.0   |                |             |
| DHPA 0.2 | DHPA 30.0| 6              | 215         |
|        | EE 2.0   |                |             |
| Castor oil | 0.2      | 5              |             |

**TABLE 3. Experimental conditions.**

| Drug   | Content  | No. of animals | Body weight |
|--------|----------|----------------|-------------|
| DDX 0.4 ml | DHPA 60.0 mg | 5              | Adult male rats |
|        | EE 4.0   |                |             | 280         |
| Castor oil | 0.4      | 5              | Adult male rats (10 days after castration) |
| DDX 0.04 | DHPA 6.0 | 5              |             |
|        | EE 0.4   |                |             |             |
| Castor oil | 0.4      | 5              |             |
| DDX 0.4 | DHPA 60.0 | 5              | Adult male rats |
|        | EE 4.0   |                | 400         |
| Castor oil | 0.4      | 5              |             | 8           |

Sacrificed on 20th-21st day following drug administration.
generally produced continuous cornification, that is, the typical estrogenic effect. Figure 2a represents estrous cycles of various groups according to the Koyama-Nakao's method (5) (Fig. 2b).

3. Effects on uterus

After DDX administration in doses ranging 0.01 ml-0.4 ml, the uterus showed macroscopical findings which could be divided into two types independent of the doses: One was characterized by relatively massive, medium hypertrophy with hyperemia, while the other was characterized by large uterine hydrops with an extremely thin translucent wall and transparent aqueous content. This uterine hydrops observed in the estrus of the normal rat was unexpectedly large in size. Since such hydrops were observed in all the cases given EE, but not in any cases after DHPA, it can evidently be attributed to the estrogenic action of EE. However, in cases given EE alone (0.2 mg), the hydrops was not so large as after DDX. Therefore EE and DHPA are considered to act in synergism in producing the huge
uterine hydrops (Fig. 3). Table 4 shows the presence or absence of such hydrops in each group.

After DDX, uterus weight increased, with wide variation depending on the presence or absence of hydrops. Within the range of 0.01-0.04 ml of DDX, the uterus weight tended to increase in proportion to the dose, however, in larger range of 0.1-0.4 ml, only a slight increase of the weight was observed when compared in smaller doses. After DHPA, the results were scarcely different from those in the control irrespective of the dose, but after EE, the weight gain was observed without exception (Table 5).

Histological changes were more complicated than macroscopical findings. As above
### Table 5. Weights of body and various organs in female rats.

| Conditions | Body weight at sacrifice (mg) | Hypophysis (mg) | Adrenal (mg) | Thyroid (mg) | Thymus (mg) | Spleen (g) |
|------------|-------------------------------|-----------------|-------------|-------------|-------------|-----------|
| DDX 0.01 ml | 161.0 ± 7.8**                | 9.8 ± 1.3       | 56.7 ± 2.7** | 17.8 ± 4.1  | 128.0 ± 35.0** | 0.62 ± 0.14 |
|            | 0.02 ‡                      | 160.2 ± 2.9**   | 8.8 ± 1.1   | 48.6 ± 2.5** | 16.0 ± 4.8  | 136.2 ± 56.0** | 0.63 ± 0.4* |
|            | 0.04 ‡                      | 161.6 ± 8.1**   | 8.6 ± 2.1   | 47.0 ± 2.7** | 21.0 ± 3.3  | 123.8 ± 24.7** | 0.61 ± 0.10* |
| DHPA 3.0 mg | 191.4 ± 12.1                | 8.0 ± 1.6       | 59.2 ± 4.2  | 20.8 ± 3.6  | 270.8 ± 102.4 | 0.78 ± 0.12  |
| EE 0.2 ‡   | 170.2 ± 7.0*                | 10.6 ± 1.3      | 57.0 ± 2.7  | 19.6 ± 4.5  | 158.4 ± 30.1** | 0.64 ± 0.13  |
| Castor oil 0.2 ml | 186.2 ± 11.0            | 8.6 ± 1.7       | 61.2 ± 3.5  | 21.0 ± 3.1  | 299.4 ± 53.0  | 0.75 ± 0.08  |
| DDX 0.1 ml | 224.8 ± 13.4*               | 15.4 ± 3.8      | 60.4 ± 8.1* | 16.4 ± 2.4  | 45.2 ± 16.9** | 0.52 ± 0.12  |
|            | 0.2 ‡                       | 210.2 ± 10.6    | 19.0 ± 4.6**| 53.6 ± 7.8**| 18.4 ± 5.4  | 46.4 ± 16.9** | 0.52 ± 0.06  |
|            | 0.4 ‡                       | 183.4 ± 24.1*   | 13.2 ± 3.7  | 53.4 ± 9.2**| 15.0 ± 2.2  | 50.0 ± 34.2** | 0.43 ± 0.11  |
| DHPA 30.0 mg | 231.3 ± 16.0               | 10.8 ± 1.5      | 58.8 ± 9.4* | 16.2 ± 3.9  | 173.7 ± 20.3** | 0.53 ± 0.12  |
| EE 2.0 ‡   | 194.2 ± 7.9*                | 17.6 ± 2.4*     | 60.2 ± 6.7* | 18.2 ± 2.5  | 83.8 ± 19.6** | 0.42 ± 0.48  |
| Castor oil 0.2 ml | 224.2 ± 12.8             | 11.2 ± 1.8      | 73.4 ± 7.5  | 18.2 ± 3.7  | 359.8 ± 86.6  | 0.56 ± 0.83  |

| Conditions | Liver (g) | Kidney (g) | Heart (g) | Mandibular gland (g) | Uterus (g) | Ovary (mg) |
|------------|-----------|------------|-----------|----------------------|------------|-----------|
| DDX 0.01 ml | 7.7 ± 0.7  | 1.25 ± 0.03 | 0.69 ± 0.02 | 0.33 ± 0.04          | 0.67 ± 0.53 | 28.6 ± 4.7** |
|            | 0.02 ‡   | 7.7 ± 0.6  | 1.27 ± 0.07 | 0.62 ± 0.08          | 0.37 ± 0.08 | 1.33 ± 1.69 | 29.6 ± 4.6** |
|            | 0.04 ‡   | 6.8 ± 0.8  | 1.24 ± 0.05 | 0.60 ± 0.06          | 0.35 ± 0.07 | 1.91 ± 2.01 | 30.8 ± 7.5** |
| DHPA 3.0 mg | 8.3 ± 0.9  | 1.29 ± 0.06 | 0.66 ± 0.05 | 0.41 ± 0.07          | 0.35 ± 0.05* | 90.2 ± 26.8 |
| EE 0.2 ‡   | 7.6 ± 0.8  | 1.25 ± 0.05 | 0.69 ± 0.05 | 0.39 ± 0.08          | 1.00 ± 0.28** | 27.6 ± 7.5** |
| Castor oil 0.2 ml | 7.8 ± 0.6  | 1.29 ± 0.06 | 0.71 ± 0.04 | 0.41 ± 0.10          | 0.46 ± 0.08  | 94.6 ± 26.6 |
| DDX 0.1 ml | 8.4 ± 1.1  | 1.38 ± 0.16 | 0.72 ± 0.04 | 0.36 ± 0.05*         | 2.84 ± 3.32  | 57.4 ± 18.4** |
|            | 0.2 ‡    | 9.6 ± 1.5* | 1.49 ± 0.19 | 0.76 ± 0.07          | 0.42 ± 0.02  | 1.10 ± 1.06 | 59.6 ± 20.6** |
|            | 0.4 ‡    | 7.9 ± 1.6  | 1.36 ± 0.14 | 0.66 ± 0.09          | 0.38 ± 0.08  | 1.60 ± 2.51 | 56.0 ± 21.6** |
| DHPA 30.0 mg | 9.1 ± 0.9* | 1.50 ± 0.11 | 0.79 ± 0.05 | 0.44 ± 0.05          | 0.48 ± 0.05  | 72.8 ± 20.7* |
| EE 2.0 ‡   | 8.0 ± 0.6  | 1.44 ± 0.11 | 0.74 ± 0.07 | 0.35 ± 0.03          | 2.33 ± 0.27** | 44.0 ± 14.1** |
| Castor oil 0.2 ml | 7.7 ± 0.8  | 1.43 ± 0.10 | 0.80 ± 0.10 | 0.47 ± 0.07          | 0.58 ± 0.32  | 102.6 ± 15.3 |
mentioned, EE is considered chiefly responsible for the uterine hydrops produced by DDX, and fairly large hydrops were microscopically visible after administration of EE alone, thus

Fig. 4. Adult rat uterus after i.m. injection of 0.4 ml of DDX. Showing well developed muscular layers and endometrium. hematoxylin-eosin stain, × 70.

Fig. 5. Adult rat uterus after i.m. injection of 0.4 ml of DDX. The wall is very thin and marked dropsy visible, hematoxylin-eosin stain, × 70.

Fig. 6. Adult rat uterus after i.m. injection of 3.0 mg of DHPA. Progestational change is visible in the endometrium. hematoxylin-eosin stain, × 70.

Fig. 7. Adult rat uterus i.m. injection of 3.0 mg of DHPA. Atrophy of muscular layer is observed. hematoxylin-eosin stain, × 70.

Fig. 8. Adult rat uterus after i.m. injection of 2.0 mg of EE. The wall thinned and dropsy is observed. hematoxylin-eosin stain, × 70.

Fig. 9. Adult rat uterus after i.m. injection of 0.2 ml of castor oil. hematoxylin-eosin stain × 70.
both endometrium and muscular layer were thinner after EE than in the control. In DDX
given cases in which large hydrops were observed, the uterine wall was found microscopically

Fig. 10 Adult rat ovary after i.m. injection of 0.2 ml of DDX. Complete disappearance
of lutein body is observed. hematoxylin-eosin stain, x 28.
Fig. 11 Adult rat ovary after i.m. injection of 0.4 ml of DDX. Atrophy of stroma and
great lutein bodies are observed. hematoxylin-eosin stain, x 28.
Fig. 12 Adult rat ovary after i.m. injection of 3.0 mg of DHPA. No different from con-
trol. hematoxylin-eosin stain, x 28.
Fig. 13 Adult rat ovary after i.m. injection of 2.0 mg of EE. Lutein body disappearance
and atrophy of stroma are observed. hematoxylin-eosin stain, x 28.
Fig. 14 Adult rat ovary after i.m. injection of 2.0 mg of EE. Great lutein body is visible.
hematoxylin-eosin stain, x 28.
Fig. 15 Adult rat ovary after i.m. injection of 0.2 ml of castor oil. Growing follicles and
lutein bodies are visible. hematoxylin-eosin stain, x 28.
extremely thin with flattened endometrial cells. Those cases where DDX produced massive hypertrophy of the uterus without hydrops, dilatation and vacuolization of endometrial cells, enlargement of the uterine cavity, marked development of the uterine gland and hypertrophy of the muscular layer were microscopically observed in varying grades. These changes were independent of DDX dosage. They represent progesterone effect under influence of estrogen action. In DHPA given cases, some exhibited gestagen effects such as dilatation of endometrial cells and accelerated development of the uterine gland, however, the majority were poorly developed. Rather there was a tendency to atrophy of the muscular layer, which supports the aforementioned uterine weight loss found after DHPA (Figs. 4-9).

4. Effects on ovary

Ovary weight was significantly decreased in DDX administration, however, rate of the decrease was not always proportionate to the dose of DDX, and while marked decrease was elicited by such small doses as 0.01-0.04 ml, the decrease was not so great after administration of relatively larger doses of 0.1-0.4 ml. In a dose of 3.0 mg, DHPA produced no loss in the ovary weight, and even 30.0 mg resulted in very little weight loss. There was a great ovary weight loss with EE, but as in DDX greater effect were seen in smaller doses rather than in larger ones (Table 5).

Histologically, smaller doses of DDX produced marked atrophy of the stroma and nearly total disappearance of the lutein body. After administration of larger doses of DDX, nearly half the cases showed marked atrophy of stroma with total disappearance of the lutein body. The other half exhibited atrophy of stroma concurrently with the presence of numerous large lutein bodies, which macroscopically presented a mulberry-like appearance. Relationships between the presence of these large lutein bodies, change in estrous cycle and development of uterine hydrops have not been clarified. As for DHPA effect, atrophy of

| Conditions | Cases |
|------------|-------|
| DDX 0.01 ml |       |
| 0.01       |       |
| 0.02       |       |
| 0.04       |       |
| DHPA 3.0 mg |       |
| EE 0.2     |       |
| Castor oil 0.2 ml | |
| DDX 0.1 ml |       |
| 0.1        |       |
| 0.2        |       |
| 0.4        |       |
| DHPA 3.0 mg |       |
| EE 2.0     |       |
| Castor oil 0.2 ml | |

- Atrophy without lutein body
- Atrophy with large lutein body
- Normal
stroma accompanied by disappearance of lutein body was occasionally observed after administration of 30.0 mg, but in the majority histological findings after DHPA scarcely

Fig. 16. Mammary gland after i.m. injection of 0.4 ml of DDN. Well developed alveolus is visible as well as mild in enlarged duct; hematoxylin-eosin stain, 70.

Fig. 17. Mammary gland after i.m. injection of 0.2 ml of castor oil. Mammary gland is developed, hematoxylin-eosin stain, 70.

Fig. 18. Testis in adult rat after i.m. injection of 0.4 ml of DDN. Depressed spermatogenesis and polyhedral giant cell are visible; hematoxylin-eosin stain, 70.

Fig. 19. Testis in adult rat after i.m. injection of 0.2 ml of castor oil. Various stages of spermatogenesis are observed; hematoxylin-eosin stain, 70.

Fig. 20. Thyroid in adult female rat after administration of 0.2 ml of DDN. Distinction between cortical area and medullary portion is not clear, marked acute involution is visible; hematoxylin-eosin stain, 70.

Fig. 21. Thyroid in adult female rat after administration of 0.2 ml of castor oil. hematoxylin-eosin stain, 70.
Table 7. Weights of body and various organs in male rats.

| Conditions          | Body weight at sacrifice (g) | Hypophysis (mg) | Adrenal (mg) | Thyroid (mg) | Thymus (mg) | Spleen (g) | Liver (g) |
|---------------------|-----------------------------|-----------------|--------------|--------------|-------------|------------|-----------|
| DDX 0.4 ml          | 265.2 ± 37.9 **             | 12.6 ± 2.3      | 49.6 ± 6.6   | 19.0 ± 2.4   | 54.4 ± 10.3 ** | 0.70 ± 0.27 | 9.8 ± 0.9 |
| Castor oil 0.2 ml   | 340.4 ± 49.4                | 13.2 ± 2.7      | 52.0 ± 5.1   | 16.0 ± 3.2   | 230.2 ± 59.7 | 0.61 ± 0.17 | 11.4 ± 1.7 |
| Castration + DDX 0.04 ml | 362.6 ± 57.9             | 20.8 ± 3.4 **   | 64.8 ± 10.9  | 27.8 ± 4.8   | 97.4 ± 27.9 ** | 0.85 ± 0.20 | 12.2 ± 2.1 |
| Castration + Castor oil 0.2 ml | 371.6 ± 43.8       | 14.6 ± 0.9      | 68.0 ± 7.5   | 29.4 ± 7.8   | 272.2 ± 59.2 | 0.97 ± 0.15 | 11.3 ± 1.8 |
| Castration + DDX 0.4 ml | 295.8 ± 57.9             | 15.4 ± 1.5      | 52.0 ± 17.8  | 21.4 ± 3.6   | 74.8 ± 31.4 ** | 1.03 ± 0.28 | 12.1 ± 1.8 |
| Castration + Castor oil 0.2 ml | 352.0 ± 43.3           | 13.0 ± 3.8      | 54.8 ± 11.0  | 17.0 ± 3.4   | 452.6 ± 86.4 | 1.03 ± 0.37 | 11.8 ± 2.0 |

| Conditions          | Kidney (g) | Heart (g) | Mandibular gland (g) | Testis (g) | Epididymis (g) | Seminal vesicle (mg) | Prostate (mg) | M. levator ani (mg) |
|---------------------|------------|-----------|----------------------|------------|----------------|-----------------------|---------------|---------------------|
| DDX 0.4 ml          | 2.02 ± 0.23 | 0.87 ± 0.07 ** | 0.35 ± 0.11 ** | 2.06 ± 0.44 * | 0.51 ± 0.69 ** | 234.0 ± 108.6 ** | 295.0 ± 106.2 ** | 148.6 ± 44.1 |
| Castor oil 0.2 ml   | 2.36 ± 0.36 | 1.18 ± 0.11 | 0.54 ± 0.05         | 3.08 ± 0.62 | 1.14 ± 0.17 | 1122.0 ± 290.9        | 670.4 ± 212.1 | 233.8 ± 79.4 |
| Castration + DDX 0.04 ml | 2.59 ± 0.46 | 1.20 ± 0.30 | 0.59 ± 0.12        |           |               |                       |               | 165.4 ± 14.4 | 171.0 ± 20.5 | 157.0 ± 42.4 |
| Castration + Castor oil 0.2 ml | 2.48 ± 0.26 | 1.19 ± 0.12 | 0.58 ± 0.05        |           |               |                       |               | 164.0 ± 37.5 | 133.0 ± 17.2 | 166.0 ± 40.8 |
| Castration + DDX 0.4 ml | 2.00 ± 0.51 | 1.06 ± 0.15 | 0.48 ± 0.19        |           |               |                       |               | 164.2 ± 68.4 | 129.8 ± 77.6 | 143.2 ± 31.1 |
| Castration + Castor oil 0.2 ml | 2.24 ± 0.31 | 1.18 ± 0.23 | 0.43 ± 0.16        |           |               |                       |               | 121.6 ± 56.1 | 77.8 ± 27.5 | 108.8 ± 39.3 |

# : Standard deviation
* : P<0.05
* * : P<0.01
differed from those of the control group. In EE-given cases, various microscopical changes as seen after DDX were observed, and the atrophy of stroma with disappearance of lutein body was especially remarkable (Figs. 10–15). Table 6 shows histological findings in the ovary under various experimental conditions as classified by the presence or absence of lutein body.

5. Effects on mammary gland

DDX elicited marked changes in the mammary gland, in proportion to the dose. With a relatively large dose of DDX (0.1 ml), development of yellow-whitish, thickened, glandular tissue was macroscopically seen in all the cases. Also histologically, the mammary gland was well developed after DDX, and in many cases, enlarged milk duct filling milk was observed. DHPA and EE, when given in relatively large doses, induced evident development of mammary gland tissue, which could be seen either macroscopically or histologically, however, the stage of development was clearly lower than after DDX, which indicates the presence of synergism between DHPA and EE (Figs. 16 and 17).

6. Effects on male gonad

1) Adult intact rats

Ten days after the administration of 0.4 ml of DDX, the testis, epididymis, seminal vesicle, prostate, and M. levator ani clearly weighed less than those of the control (Table 7). Histologically, the epithelium of the seminiferous tubule was atrophied, and spermatogenesis was either suppressed or absent. Particularly in one case, characteristic polinuclear giant cells appeared in association with absence of spermatogenesis. In the epididymis, atrophy of the epithelium and deficiency in sperms were visible, and the seminal vesicle and prostate exhibited considerable atrophy of the epithelium (Figs. 18 and 19).

2) In adult castrated rats

When 0.4 ml of DDX was given to rats 10 days following castration, the seminal vesicle, prostate and M. levator ani tended to increase in weight, when compared to castrated controls (Table 7).

Also histological evidence indicated a recovery tendency in the seminal vesicle and prostate in which intense atrophy of epithelium had been produced by castration when 0.4 ml DDX had been administered.

7. Effects on adrenals

Significant reduction of adrenal weight in female rats was evident after DDX administration. Thirty mg of DHPA and 2.0 mg of EE likewise diminished adrenal weight. In male rats, the adrenal weight decreased with DDX, but not to the extent of the females. Reasons why differences in reduction of the adrenal weight exist between male and female, is still open for discussion (Tables 5 and 7).

In accordance with reduction of the adrenal weight, atrophy of the zona fasciculata and zona reticularis, and tendency of enlargement of the zona glomerulosa were histologically observed in the adrenal cortex.

8. Effects on thymus

Administration of DDX, DHPA or EE all induced remarkable weight loss of the thymus,
however weight loss with DHPA was far less than that with EE. For this reason, great weight loss after DDX is considered to be ascribed to synergism between DHPA and EE (Tables 5 and 7).

Histologically, DDX, DHPA and EE produced the picture of acute involution of the thymus. Effects of DDX and EE were particularly prominent. Changes were similar to those elicited by glucocorticoid, the cortico-medullary border became indistinct, lymphocytes reduced in number, and pyknosis and occasionally karyorrhexis were observed (Figs. 20 and 21).

9. **Effects on liver**

When given in relatively large doses, DDX, DHPA and EE tended to increase the liver weight. Thus both DHPA and EE had weight increasing effects (Tables 5 and 7).

Histologically, no marked change was visible in female rats’ livers even after liver weight markedly increased. With administration of large doses of DDX, there was swelling of the hepatic cells in male castrated and uncastrated rats. There was also fatty degeneration shown peripherally in the lobule, and was occasionally centrally observed. After 0.04 ml of DDX, however, no such changes were visible.

**DISCUSSION**

In 1960 and 1962, it was reported that remarkable body weight loss and histological atrophy of the ovary were observed in rats after successive administration of glucocorticoids. It was considered that absolute organ weight expresses “true action” of drugs better than relative weight per 100 g body weight, when the drug results in a remarkable body weight loss action (6–8). Since DDX has potent body weight loss action, organ weights have been presented herein as of absolute weights.

Inhibitory effects of DDX on body weight gain was considerably evident, and there was no recovery tendency even 3 weeks after a single injection. It was clear that the component EE was responsible for this inhibitory effect. The estrous cycle, which indicates the function of the ovary, became irregular after DDX injection, and recovery from the irregular cycle was still not observed after 3 weeks. Effects could depend on slow absorption of the steroid.

It is noteworthy that even a single injection of a small dose of DDX strongly inhibited body weight gain. After a large dose, marked inhibition of body weight gain was accompanied by depilation and emaciation. Fatty degeneration of the liver in male rats should also be considered.

After injection of EE, estrus II continued, and after DDX, there was shown a picture of estrus I or diestrus in many cases. This apparently indicates antiestrogenic actions of the progestogen in DDX.

In the uterus, DDX resulted in huge hydrops in some cases. Such uterine hydrops was observed in no cases after DHPA and in all cases after EE, it is evident that uterine hydrops produced by DDX can be attributed to EE. With administration of EE alone the hydrops was not so large as in cases with DDX. It is probable that synergism between
EE and DHPA contributed to the formation of these huge hydrops, Lerner et al. (9) reported that DHPA did not inhibit ovum transportation nor nidation in the reproductive tract, however, the wall of the uterus in the picture of large hydrops induced by DDX administration was very thin and with no recognizable development of the uterine gland. Therefore, it might be said that the state of this uterus is unfavorable for nidation.

There are many reports on the contraceptive action of steroids, especially gestagen (9–16). It has been reported that progesterone inhibited increase of LH content in the blood of castrated female rats (17), and when in women were given a combination of ethynodiol diacetate and mestranol, the peak of LH release in the midcycle was abolished (18). However, LH content in the hypophysis of the female rat increased after progesterone administration (19) and it is known that progesterone does not affect LH production but only inhibits release of same (20). Since ovulation is induced by action of LH on mature follicles under influence of FSH, ovulation can be inhibited simply by suppression of LH release. For this purpose administration of progestogen is adequate. The present experiments show that EE exerted a strong inhibitory action on the ovary while DHPA had only a weak inhibitory effect. Consequently it appears that EE plays a principal role in the inhibition of ovulation by DDX. This means that EE would induce a kind of negative feedback, which would bring suppression of FSH secretion, which in turn would block growth of follicles, thus inhibiting ovulation. Progestogen appears to play only a supplementary role by blocking ovulation through inhibition of LH secretion in cases where maturity is seen despite suppression of FSH release.

From the data shown in Table 7, after administration of DDX and EE in a small doses, most of the cases revealed a strongly atrophied ovary with disappearance of the lutein body, however, in groups treated with large doses of DDX, a very large lutein body was detected in the atrophied ovary in half the cases. It has been reported by Rothchild (21) that LTH release is braked by the central nervous system, and in cases where there is considerable progesterone the brake is released and LTH secretion begins. It could be considered that the already existing lutein body at the time of a single injection of a large dose of DDX had increased considerably in size. LTH release in cases treated in large doses of DDX could be attributed to the fact that well-developed mammary glands filled with milk as is generally shown in all DDX treated groups. It is therefore presumed that progesterone induces a negative feedback for LH release and positive feedback for LTH release (21).

After DDX injection, the gonads, seminal vesicles and prostate were atrophied and spermatogenesis was inhibited. This can be ascribed to inhibition of FSH and LH release. On the other hand, DDX also exerted androgenic and anabolic actions on male rats, even though these were very weak.

Furthermore, besides inhibition of body weight gain, DDX produced histologically, suppression of zona fasciculata and zona reticularis of the adrenal cortex and acute involution of the thymus. As DHPA has no glucocorticoid-like activity (2, 3), these changes observed after DDX administration can be attributed to the component EE.

As to side effects of oral contraceptives, various reports have been published (1, 16, 22).
HORMONAL CONTRACEPTIVE

It is said that none are serious except for thrombosis and that most of the side effects disappear after continuous use. After DDX, however, body weight, general condition and endocrine glands were considerably affected as a result of the strong toxicity of the component EE. Inhibition of ovulation is realised more by the combined use of progestogen and estrogen than by progestogen alone (23). Since estrogen produced stronger inhibitory effects than progestogen as described herein, elimination of estrogen is not justified. Further research is being done on the estrogenic component of the estrogen-progestogen combination, to determine the proportion of estrogenic component for contraceptive application thereby making available a less toxic estrogenic steroid.

SUMMARY

Rats were given an i.m. single injection of various doses of Deladroxate (DDX) and of its components, dihydroxyprogesterone acetophenide (DHPA) and estradiol enanthate (EE). After administration of DDX or EE, except for large dose of DDX, atrophy of the ovary was observed with disappearance of lutein body and inhibition of follicular development. With a large dose of DDX, a very large lutein body was often detected in the atrophied ovary.

After DDX administration, the estrous cycle became irregular, and recovery failed for as late as 3 weeks.

After DDX administration, male rat gonads were markedly suppressed both macroscopically and histologically. Mammary gland of rats after DDX administration was intensely hypertrophied to the milk filling stage.

After DDX administration, one rat uterus was relatively massive, rather hypertrophied with hyperemia and the other has very large hydrops.

Body weight, weight of thymus and adrenal gland decreased after DDX administration with the latter two organs histologically involuted.

Liver weight was often increased and fatty degeneration was seen in the lobules.

Most of the changes described above, that is, inhibition of ovulation and toxicity to other organs, can be explained by the action of EE. The effect of DHPA was not remarkable, with exception of the uterine gland development. This compound revealed no toxicity.

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