EFFECT OF POLYDIETHYLSTILBOESTROL PHOSPHATE ON THE PITUITARY CONTENT OF FSH AND ICSH IN MALE RATS

By

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ABSTRACT

The effect of a single dose of polydiethylstilboestrol phosphate (PSP) on the pituitary content of follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) was studied in intact, adult male rats after 7, 28, 103 and 161 days, respectively. The body weight and different organ weights were also recorded. The results were compared with those obtained in untreated controls and in rats treated with daily doses of diethylstilboestrol (DES) for 7 days.

The pituitary FSH and ICSH content in the control rats decreased roughly to about half during a period of approximately 5 months, i.e. from the 2nd to the 7th month of life. PSP (400 μg/100 g body weight) and DES (320 μg/100 g body weight) reduced the pituitary content of FSH and ICSH to approximately 35 and 50 % of the control values, respectively, measured on the 7th day of treatment. The gonadotrophin inhibiting effect of PSP lasted for more than 103 days. On the other hand, on Day 28 the pituitary content of ICSH was normal and that of FSH significantly elevated in the DES-treated rats. On Day 161 both PSP- and DES-treated animals had a significantly increased ICSH content, while the FSH content was normal.

Body weights, as well as the weights of the testes and accessory glands were reduced both by DES and PSP as early as on the 7th day after the commencement of treatment. Complete restoration of these parameters in DES-treated animals was found on Day 103, while PSP exerted its effect for more than 161 days. Daily injections of 3 IU of FSH and 1.5 IU of ICSH administered in the form of human menopausal gonadotrophin (HMG) to PSP-treated rats from the day of PSP injection for 14 days counteracted the weight loss of the testes and accessory glands, as well as the loss of body weight and the reduced food intake. The hypertrophy of the adrenals and the sterility produced by the administration of PSP were completely prevented by HMG injections.
Polydiethylstilboestrol phosphate (PSP) is a water soluble high molecular weight polyester of phosphoric acid and diethylstilboestrol (DES), which exerts a prolonged oestrogenic effect, when administered to spayed mice (Diczfalusy et al. 1959). The long duration of action is dependent on a slow degradation of the polymer primarily in organs containing reticuloendothelial cells (Bengtsson et al. 1963; Perklev 1964; Perklev et al. 1967).

In the present communication data will be presented on the prolonged effect of a single injection of PSP in intact, adult male rats on the pituitary content of follicle stimulating hormone (FSH), interstitial cell stimulating hormone (ICSH), body weight and weights of testes, accessory sex organs, adrenals and pituitary glands. The data are compared with those obtained in untreated control rats and rats treated for 7 days with DES. In addition, the effect of daily injections of human menopausal gonadotrophin (HMG) to PSP-treated male rats will be described.

MATERIALS AND METHODS

Preparations
The synthesis of PSP has been described previously (Diczfalusy et al. 1959). A standard preparation, Leo 116 j, was used.
DES (m. p. 169–170°C) was obtained from E. Merck, Darmstadt, Germany.
The HMG preparation (Homogonal® 69:13:CP) was prepared by AB Leo, Helsingborg, Sweden, and contained FSH and ICSH in a ratio of 2:1.
The laboratory standard of HMG was prepared from bulk material of the same origin. This was assayed for FSH and ICSH against the Second International Reference Preparation of Human Menopausal Gonadotrophin (2nd I. R. P. – HMG, 40 IU FSH and 40 IU ICSH per vial), which was kindly supplied by Dr. D. R. Bangham, National Institute for Medical Research, London.

Animals
All animals were of the Sprague-Dawley strain and supplied by AB Anticimex, Stockholm. The rats were housed in a constant temperature room providing alternating periods of 14 h of light and 10 h of darkness and maintained on tap water and a standard laboratory diet ad lib.

Bioassays
FSH assays were performed using the rat ovarian weight augmentation procedure of Steelman & Pohley (1953) with the following modifications: Injections were given once daily for 3 days and the total dose of human chorionic gonadotrophin administered was 50 IU. The animals were sacrificed 72 h following the first injection. All bioassays were carried out using a 2 + 2 design and a log dose ratio of 0.301. Usually 8 rats per dose were injected; however, when the pituitary FSH content was very low, 5 animals per dose were used.
ICSH activity was assayed by the ovarian ascorbic acid depletion method (Parlow 1961). A 2 + 2 point design was used and 8 rats were injected with each dose. The
log dose ratio was 0.477. The interval between injection and removal of the ovary for ascorbic acid determination was 4 hours.

Standard statistical methods (Bliss 1956) were used to compute the potencies of the unknown and the 95% confidence limits of error.

Comparison of the effect of PSP with that of DES

Group 1: Controls – no treatment.
Group 2: DES – 320 μg per 100 g body weight.
Group 3: PSP – 400 μg per 100 g body weight.

Each group consisted of 50 animals with an average weight of 240 g (range 230–250 g). PSP dissolved in 0.9% saline was administered s.c. as a single injection on Day 0. DES in arachis oil was injected daily by the same route on Days 0–6. The total dose (320 μg) was equivalent to the DES contained in 400 μg PSP. Twelve animals from each group were sacrificed 7, 28, 103 and 161 days following the commencement of treatment. Prostates and seminal vesicles were fixed in Bouin’s solution. The pituitary glands from each group of rats were pooled and stored in acetone at −20°C. A few days later the glands were dried in the cold room, weighed, homogenized in saline and assayed for FSH and ICSH.

Effect of PSP with and without the administration of HMG

Group 1: Controls – daily injections of saline for 14 days.
Group 2: PSP – 200 μg per 100 g body weight on Day 0 + daily injections of saline for 14 days.
Group 3: PSP – 200 μg per 100 g body weight on Day 0 + HMG – daily injections of 3 IU FSH and 1.5 IU ICSH for 14 days.

Each group was made up of 10 animals (body weight 260–280 g), which were caged separately. The body weights and the food consumption were recorded daily for each animal during the first 7 days of the experiment. On Day 7, two female rats of known fertility were placed with each male. Seven days later (Day 14) all male rats were sacrificed and various organ weights recorded. Fourteen days later the number of pregnancies was recorded.

RESULTS

Effect of DES and PSP on pituitary FSH and ICSH (Table 1)

The data demonstrate that in normal rats the pituitary concentration of FSH and ICSH decreases with increasing age of the animal. Treatment with DES and PSP caused a significant suppression of the pituitary FSH concentration in the animals sacrificed on Day 7. On Day 28, compared with the controls, the pituitary content of FSH was higher in the DES-group and lower in the PSP-group. Bioassays of the pituitary glands collected on Days 103 and 161 showed significantly decreased FSH-activity in the glands of PSP-treated rats sacrificed on Day 103.

The ICSH concentration in the pituitary glands of PSP-treated animals was significantly depressed on Days 7, 28 and 103, whereas such a decrease was found only on Day 7 but not later in the DES-treated groups. Both PSP- and

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Table 1.
Effect of diethylstilboestrol (DES) and polydiethylstilboestrol phosphate (PSP) on pituitary weight and pituitary content of gonadotrophins in male rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after first inj.</th>
<th>No. of rats</th>
<th>Pituitary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>12</td>
<td>8.37 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>12</td>
<td>9.91 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>103</td>
<td>12</td>
<td>11.22 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>12</td>
<td>11.87 ± 0.21</td>
</tr>
<tr>
<td>DES</td>
<td>7</td>
<td>12</td>
<td>9.39 ± 0.20c</td>
</tr>
<tr>
<td>7 injections s. c.</td>
<td>28</td>
<td>12</td>
<td>10.79 ± 0.20b</td>
</tr>
<tr>
<td>Total dose</td>
<td>103</td>
<td>12</td>
<td>12.60 ± 0.29c</td>
</tr>
<tr>
<td>320 μg/100 g b. w.</td>
<td>161</td>
<td>10</td>
<td>11.50 ± 0.33</td>
</tr>
<tr>
<td>PSP</td>
<td>7</td>
<td>12</td>
<td>9.88 ± 0.17c</td>
</tr>
<tr>
<td>1 injection s. c.</td>
<td>28</td>
<td>11</td>
<td>10.54 ± 0.25a</td>
</tr>
<tr>
<td>Total dose</td>
<td>103</td>
<td>12</td>
<td>12.92 ± 0.31c</td>
</tr>
<tr>
<td>400 μg/100 g b. w.</td>
<td>161</td>
<td>12</td>
<td>11.70 ± 0.18</td>
</tr>
</tbody>
</table>

* Mean ± Standard error.
** Mean and 95% confidence limits.
*** Index of precision.

a $P < 0.05$; b $P < 0.01$; c $P < 0.001$ when compared with controls.
DES-treated animals sacrificed on Day 161 had a higher pituitary ICSH content than the controls.

**Effect of DES and PSP on body and organ weights (Table 2)**

A single injection of PSP and 7 daily injections of DES in equivalent doses produced the same decrease in body weight during the first week of the experiment. During the next 3 weeks' period the growth rate of the rats in the DES-treated group and in the control group was almost the same, while the PSP-treated rats showed a diminished growth rate. On Day 103 and subsequently, there was no longer any significant difference in body weights between the DES-treated and untreated groups, whereas the body weight of PSP-treated rats was significantly depressed even 161 days after injection.

The administration of PSP and DES caused a marked decrease in the weights of the testes and of the accessory glands of rats sacrificed on Day 7. The effect was most marked on the seminal vesicles and prostate, but even the weights of the testis and epididymis were highly significantly depressed. A single injection of PSP seemed to be more active in depressing the weight of the seminal vesicles and prostate than 7 daily injections of DES. During the next 3 weeks' period (Days 7–28) the organ weights of the DES-treated rats increased almost as much as those of the controls, while the organ weights of the PSP-treated rats were further depressed. On Day 103 and subsequently there were no longer any differences in organ weights between the control and DES-treated animals, while the weights of the accessory glands of the PSP-treated rats were still significantly reduced 161 days after the injection.

The DES and PSP injections resulted in enlargement of the adrenals and pituitary glands. The adrenals responded rapidly to the DES treatment with a marked increase in weight. This effect was very pronounced in animals sacrificed on Days 7 and 28. On Day 103 the adrenal weights were not different from those of the controls. In the PSP-treated rats the adrenal hypertrophy developed more gradually. The maximum weights were recorded on Day 28, nevertheless 161 days after the PSP injection, the adrenal weights were still significantly higher than those of the controls. The weights of the pituitary glands of rats treated with DES and PSP were higher than those of the untreated controls on Days 7, 28 and 103. On Day 161 no significant differences were found.

**Effect of PSP with and without HMG on body weight and food consumption**

The results are illustrated in Fig. 1. Daily injections of HMG (3 IU FSH and 1.5 IU ICSH) partially counteracted the loss of body weight produced by the single PSP injection. However, the HMG treatment did not completely restore the normal growth rate.

The food consumption was also influenced by the different treatments. A
Table 2.

Effect of diethylstilboestrol (DES) and polydiethylstilboestrol phosphate (PSP) on body and organ weights of male rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after first inj.</th>
<th>No. of rats</th>
<th>Body weight g</th>
<th>Organ weights mg*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Seminal vesicles</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>12</td>
<td>266 ± 2</td>
<td>610 ± 23</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>12</td>
<td>349 ± 5</td>
<td>1079 ± 29</td>
</tr>
<tr>
<td></td>
<td>103</td>
<td>12</td>
<td>437 ± 11</td>
<td>1436 ± 25</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>12</td>
<td>445 ± 10</td>
<td>1431 ± 74</td>
</tr>
<tr>
<td>DES</td>
<td>7</td>
<td>12</td>
<td>214 ± 3c</td>
<td>168 ± 17c</td>
</tr>
<tr>
<td>7 injections s. c.</td>
<td>28</td>
<td>12</td>
<td>306 ± 5c</td>
<td>651 ± 24c</td>
</tr>
<tr>
<td>Total dose</td>
<td>103</td>
<td>12</td>
<td>429 ± 6</td>
<td>1505 ± 48</td>
</tr>
<tr>
<td>320 µg/100 g b. w.</td>
<td>161</td>
<td>10</td>
<td>440 ± 8</td>
<td>1234 ± 56</td>
</tr>
<tr>
<td>PSP</td>
<td>7</td>
<td>12</td>
<td>218 ± 5c</td>
<td>129 ± 7c</td>
</tr>
<tr>
<td>1 injection s. c.</td>
<td>28</td>
<td>11</td>
<td>247 ± 3c</td>
<td>62 ± 3c</td>
</tr>
<tr>
<td>Total dose</td>
<td>103</td>
<td>12</td>
<td>321 ± 8c</td>
<td>343 ± 53c</td>
</tr>
<tr>
<td>400 µg/100 g b. w.</td>
<td>161</td>
<td>12</td>
<td>366 ± 9c</td>
<td>792 ± 51c</td>
</tr>
</tbody>
</table>

* Mean ± Standard error.
a $P<0.05$; b $P<0.01$; c $P<0.001$
when compared with controls.
Effect of polydiethylstilboestrol phosphate (PSP) with and without the administration of human menopausal gonadotrophin (HMG) on food consumption and body weight of male rats. Each column and point indicates the mean of 10 animals ± S. E.

PSP injection caused a marked decrease in food intake as compared to that of the control rats. The HMG injections increased the food consumption of the PSP-treated rats, though the food intake did not reach that of the untreated controls.

**Effect of PSP with and without HMG on organ weights** (Table 3)

Daily administration of HMG (3 IU FSH and 1.5 IU ICSH) for 14 days to PSP-treated rats not only counteracted the decrease in weights of the accessory glands caused by the PSP injection, but even increased the weights of these organs as compared with those of the controls. The testicular weights of the control and PSP + HMG-injected animals were not significantly different, whereas those of the rats treated with PSP only were significantly depressed.

The weights of the pituitary glands of rats treated with PSP as well as with PSP + HMG were increased in relation to those of the controls. The weights of the adrenals were also significantly increased following the PSP injection. However, when PSP was combined with HMG, the weights of the adrenal glands decreased even below those of the controls.

**Effect of PSP with and without HMG on fertility of male rats** (Table 3)

A single injection of 200 μg PSP per 100 g body weight to male rats produced infertility 7 days after the injection, in all of the 10 treated animals. Eight of the 10 control rats were found to be fertile under the same experi-
Table 3.
Effect of polydiethylstilboestrol phosphate (PSP) with and without the administration of human menopausal gonadotrophin (HMG) on organ weights and fertility of male rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after first inj.</th>
<th>No. of rats</th>
<th>Organ weights mg*</th>
<th>No. of fertile males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Seminal vesicles</td>
<td>Prostate</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 injections s. c. saline</td>
<td>14</td>
<td>10</td>
<td>562 ± 23</td>
<td>807 ± 29</td>
</tr>
<tr>
<td>PSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 injection s. c. 200 µg/100 g b. w.</td>
<td>14</td>
<td>10</td>
<td>125 ± 18c</td>
<td>132 ± 11c</td>
</tr>
<tr>
<td>14 injections s. c. saline</td>
<td>14</td>
<td>10</td>
<td>1030 ± 49c</td>
<td>1163 ± 36c</td>
</tr>
<tr>
<td>PSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 injection s. c. 200 µg/100 g b. w.</td>
<td>14</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 injections s. c. HMG</td>
<td>14</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± Standard error.

a P < 0.05;  b P < 0.01;  c P < 0.001

when compared with controls.
mental conditions. When 10 PSP-treated rats received daily injections of HMG, all the animals remained fertile.

DISCUSSION

It has been known for a long time that the administration of DES to male rats results in impaired body growth, atrophy of the testes and accessory glands and enlargement of the adrenals and pituitary glands (Noble 1939). The data presented in this communication as to the effects of DES and PSP on the body and organ weights are in agreement with these observations.

A single injection of PSP influenced the body weight and the weight of the accessory organs for as long as 161 days after the injection. This is consistent with previous observations indicating a very slow clearance of PSP after parenteral administration. Thus, labelled material was found to be accumulated in the tissues and excreted in the urine of a steer 125 days after a single injection of radioactive PSP (Perklev et al. 1967).

The effect of long term oral DES treatment of male rats has been reported by Snair et al. (1954). These investigators administered different doses of DES 6 days a week for a period of 180 days to rats weighing 270 g and recorded the resultant loss in body and accessory organ weights. The results obtained with a daily oral dose of 7.5 μg DES, i.e. a total dose of about 1170 μg DES, are very close to those recorded by us 161 days after a single injection of about 1000 μg PSP. This indicates the practical possibility of substituting the daily oral intake of DES over a long period with a single injection of PSP.

The bioassay of the pituitary FSH and ICSH content of untreated control rats demonstrated a gradual decrease in gonadotrophin concentration with increasing age of the rats. Between approximately the 2nd and the 7th month of age the pituitary content of FSH and ICSH declined roughly to half (Table 1). Comparable results have recently been reported by Schiavi (1968), who determined the pituitary gonadotrophins in male rats of 60 and 180 days of age. The pituitary FSH and ICSH concentration of the 180-day-old rats was 63 and 35 %, respectively, of that found in the 60-day-old animals.

Daily injections of DES for 7 days caused a marked decrease in pituitary gonadotrophin content (Table 1). After discontinuation of the DES administration, the pituitary content of FSH and ICSH increased rapidly. The FSH concentration rose to a significantly higher level than that in the controls, which indicates a »rebound« effect. The pituitary gonadotrophin content of these rats remained higher than that of the control rats throughout the whole investigation period; however, the differences were statistically significant only with regard to the ICSH content of glands collected on Day 161. These data indicate that a very marked inhibition of gonadotrophin production in adult male rats
by large doses of DES does not impair the subsequent capacity of the pituitary gland to synthetize FSH and ICSH.

A single injection of PSP significantly decreased the pituitary gonadotrophin content as compared to that of the control rats for more than 100 days (Table 1). This result is consistent with the observations of a prolonged action of PSP. However, the pituitary glands collected 161 days after the PSP injection contained significantly more ICSH than those of the controls. This is in accordance with the results obtained with the DES-treated rats and also with the observations recently reported by Schiavi (1968). This investigator treated male rats at the age of 5 days with a single injection of 100 μg oestradiol benzoate. When the animals were 4 and 6 months old, the pituitary gonadotrophin content was determined. A significantly increased ICSH concentration was noted in the 6 months old animals, while no difference was recorded at the age of 4 months. The pituitary FSH concentration was not different from that of the controls at either age. These data suggest that the decrease in pituitary ICSH content with increasing age of the animals is slower in male rats previously exposed to oestrogens.

One purpose of the present study was to evaluate to what extent PSP exerts its different effects via the pituitary gland. To this end, an HMG preparation was administered to the PSP-treated rats to compensate for the inhibited secretion of endogenous gonadotrophins. Preliminary experiments indicated that daily injections of 3 IU FSH and 1.5 IU ICSH could compensate for the effect of PSP (200 μg/100 g body weight) on the testes. The results of the final experiment showed that the dosage of HMG or perhaps more likely the ratio of FSH to ICSH was not quite properly chosen (Table 3). The weight of the testes was fully normalized by the daily gonadotrophin injections to the PSP-treated animals, whereas the accessory glands were overstimulated by the treatment.

Most interesting was the effect of HMG on the weight of the adrenals of the PSP-treated rats. The hypertrophy caused by the PSP treatment is in accordance with many reports on the effect of DES on the adrenal gland (e.g. Noble 1939; Kitay 1963). Most investigators claim that the adrenal hypertrophy is caused by an increased corticotrophin (ACTH) production due to an inhibitory action of oestrogens on corticosterone secretion (e.g. Fonzo et al. 1967). The present findings, that the hypertrophy is fully counteracted by gonadotrophin administration, indicate that the inhibition of FSH and/or ICSH production might play an essential role in the oestrogen induced hypertrophy of the adrenals. The present results have been confirmed in unpublished experiments with different doses of PSP and HMG. A detailed study of this problem will be the subject of a forthcoming communication.

A decrease in the body weight of rats treated with DES has been reported by many workers (e.g. Noble 1939; Snair et al. 1954) and it has been demonstrated that most of this decrease could be accounted for by a reduced food
intake (Meites 1949). The mechanism by which DES acts is not known. The present findings that injections of HMG increased the food intake and counteracted the weight loss caused by the PSP treatment suggest that the gonadotrophins might be involved. However, since it has been shown that oestrogens are capable of reducing growth rate and food intake in hypophysectomized rats (Snair 1963; Josimovich et al. 1967) a non-pituitary mechanism must also be involved.

PSP treatment of male rats induced sterility one week after injection. Snair et al. (1954) reported the same effect on male rats receiving a daily oral dose of 10 µg of DES. Whether a suppression of mating behaviour or an inhibited spermatogenesis of the PSP-treated males was the factor responsible for the prevention of pregnancy remains to be established. Administration of exogenous gonadotrophins to the PSP-treated male rats completely prevented the development of sterility. This indicates that PSP does not exert its inhibitory action on the reproductive organs but rather on the gonadotrophins of the pituitary gland.

The data obtained in the present study would seem to justify the following conclusions. A single injection of PSP to male rats exerts an inhibitory action of long duration on the synthesis of the pituitary gonadotrophins. The administration of a moderate dose of HMG partially counteracts most of the effects on body and organ weights, food consumption and fertility caused by the PSP injection. This indicates that PSP works primarily via the pituitary gland, which does not, however, rule out the possibility of a simultaneous direct action of PSP or of its metabolites on different target organs.

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