

**Effects of Intranasal Administration of Hormonal Steroids
on Serum Testosterone and Spermatogenesis in
Rhesus Monkey (*Macaca mulatta*)**

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ABSTRACT

Spraying estradiol-17 β , progesterone or norethisterone intranasally in adult male rhesus monkeys (7.5-11 kg BW) at a daily dose of 30 μ g/day for a period of 60 days resulted in a decrease of testicular size, arrest of spermatogenesis and a significant reduction in serum levels of testosterone. No changes were observed in the solvent-treated controls.

INTRODUCTION

Estrogens (Balze et al., 1954; Frick et al., 1976) or progestins (Heller et al., 1958; Johanson and Nygren, 1973) administered by parental routes are known to suppress spermatogenesis and also to reduce levels of testosterone in the circulation. The dose of steroids required to be administered by the oral or systemic routes to achieve such effects is quite large. Studies carried out in our laboratories have shown that ovarian functions can be impaired by administering extremely small quantities of progestins by the intranasal route to rhesus monkeys (Anand Kumar et al., 1977). In view of these findings it was of interest to determine if testicular functions are also impaired in adult rhesus monkeys following intranasal administration of estradiol-17 β (E), progesterone (P), or norethisterone (NET) at a daily dose of 30 μ g of each steroid given over a period of 60 days.

MATERIALS AND METHODS

Animals

Eleven adult male rhesus monkeys, each weighing

between 7.5-11.0 kg were used. The animals were individually caged and fed with pelleted diet (Hindustan Lever) supplemented with fresh fruits, vegetables and freely available water. The animals were maintained in well aerated rooms and were not subjected to control of temperature or photoperiods.

Before the start of the experiment, the animals were trained daily for 2 weeks to sit on a restraining chair for a period of 10 min at the start of the training; the period was gradually extended to 2 h at the end of the training.

Spraying of Steroids

The animals were restrained on a chair daily between 1000 and 1030 h and sprayed intranasally with the steroid or solvent (control) using a glass atomizer according to the procedure described previously (Anand Kumar et al., 1977). The steroids used were E, P or NET and the daily dose was 30 μ g. The total period of spraying was 60 days.

Histology

Testicular biopsies containing a few seminiferous tubules were obtained from all animals after sedation with Ketlar. The biopsies were taken 1 day prior to the start of the treatment (Day 0) and the day after the last administration of steroids (Day 61). The tissues were processed and sectioned using routine histological procedures and stained with hematoxylin and eosin.

*Blood Sampling and Estimations
of Testosterone and Cortisol*

Blood samples were collected by venipuncture

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according to the schedule shown in Fig. 1. Serum was separated and stored at -20°C until all samples from each animal were subjected to the estimation of testosterone by specific radioimmunoassay (RIA). The RIA procedures used were as described in the WHO Method Manual (1977). The antiserum used was supplied by the WHO and had a maximum cross reaction of 14% with dihydrotestosterone and 6.0% with androstenediol. The sensitivity of the assay was 5 pg. The inter- and intraassay variations were 4.8–7.2% and 4.9–8.3%, respectively.

Plasma cortisol was estimated by fluorimetric method (Mattingly, 1962). The inter- and intraassay variations were 3.8–6.9% and 5.2–9.3%, respectively.

Statistical Evaluation

The mean value of serum testosterone levels in samples obtained on the day prior to the start of the treatment period, 30 days after treatment and on the day following the end of the 60 days of treatment (Day 61) were compared by Cochran's modified t test (Snedecor and Cochran, 1967).

RESULTS

Before the start of the treatment, the testes were well developed and were situated in the scrotum. The testes decreased in size after 30 days of steroid administration, and the reduction in their size was more marked at the end of 60 days of treatment. In the E-treated monkeys, the testes were not only reduced in size but also had retracted into the inguinal canal at the end of 60 days of treatment.

The testes of the solvent-treated controls remained unaffected.

Histology

Testicular histology prior to the treatment as well as in the solvent-treated controls revealed the occurrence of all stages of spermatogenesis (Fig. 2a). In the E- and NET-treated monkeys, there was a marked reduction in the diameter of the seminiferous tubules, and the tubules contained only spermatogonia and Sertoli cells (Fig. 2b,c). All other stages of spermatogenesis (spermatocytes, spermatids and spermatozoa) were absent. In the P-treated monkeys (Fig. 2d), the reduction in the diameter of the seminiferous tubules was not as marked as that observed in E- or NET-treated monkeys; the tubules, however, had fewer spermatids as compared with controls.

Serum Testosterone Levels

The mean values of the serum testosterone levels (Fig. 3) in blood samples obtained at 10 min intervals (Fig. 1a) during the forenoon prior to the start of the treatment varied between animals as well as between the months during which the experiments were carried out. The variations were within the range of serum testosterone levels normally encountered in adult male rhesus monkeys in our colony during the different months of the year (unpublished observations).

A progressive decline in the mean values of serum testosterone levels was evident in all the animals treated with steroids (E, P or NET),

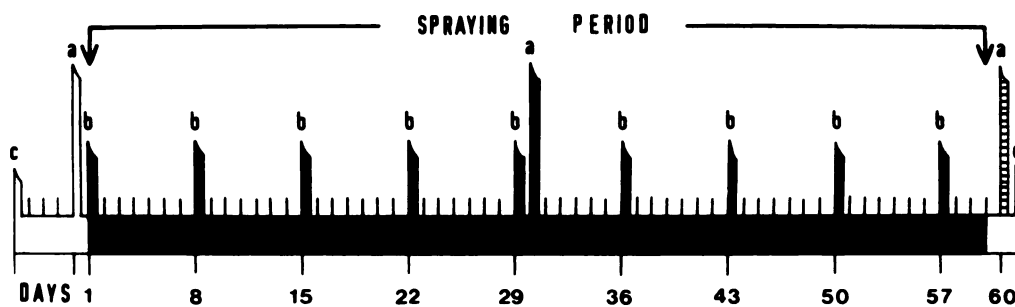


FIG. 1. Experimental design to show the period of intranasal spraying (dark bars) and collection of blood samples (a, b and c).

a) Blood samples taken at intervals of 10 min over a period of 2 h (1000–1200 h) on the day prior to the start of the spraying, 30 days after spraying and on the day following the end of the 60 days of spraying for estimating serum levels of testosterone.

b) Three blood samples taken weekly at intervals of 15 min (1000–1030 h) for estimating serum levels of testosterone. The first sample was taken just prior to the spraying and the others at 15 and 30 min after spraying.

c) Blood samples taken at 0800 h and 2000 h 4 days prior to the start of the spraying period and 2 days after the end of spraying period for estimating levels of plasma cortisol.

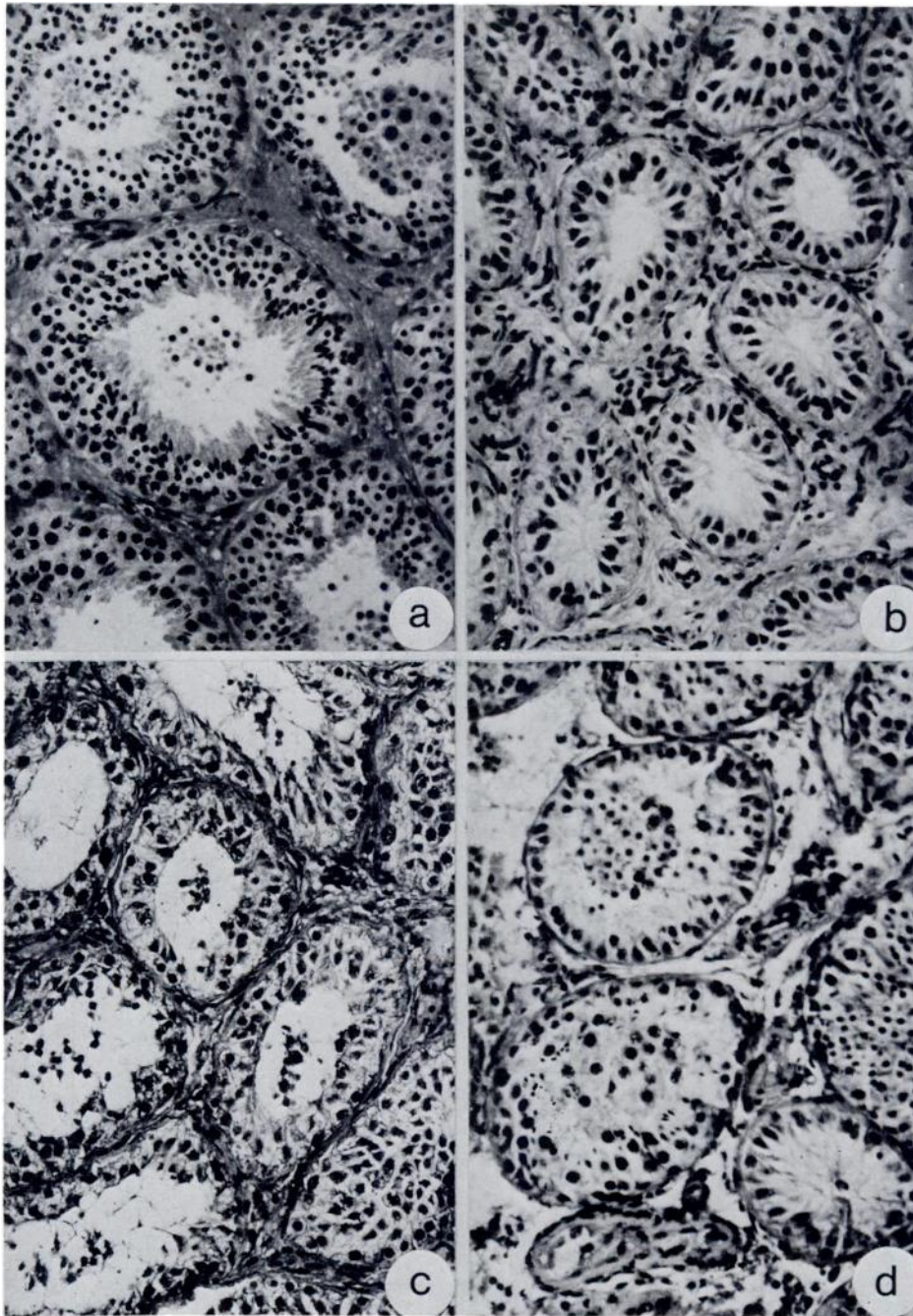


FIG. 2. Testicular histology of rhesus monkeys treated with a) solvent only, b) E, c) NET or d) P. A reduction in the diameter of the seminiferous tubules and the arrest of spermatogenesis are evident in the E- or NET-treated animals. Progesterone treatment caused least effects on testicular histology, but note that there are fewer spermatids in the tubules as compared with the controls. X240.

and at the end of the treatment period the reduction was statistically significant as compared with the mean values of serum testosterone levels observed in blood samples obtained before the start of treatment (Fig. 3). Serum testosterone levels did not show any

change in the solvent-treated controls (Fig. 3).

The pattern of serum testosterone levels observed in the three samples taken weekly according to the schedule described (Fig. 1b) is shown in Fig. 4. Values for one representative animal from each treatment group are indi-

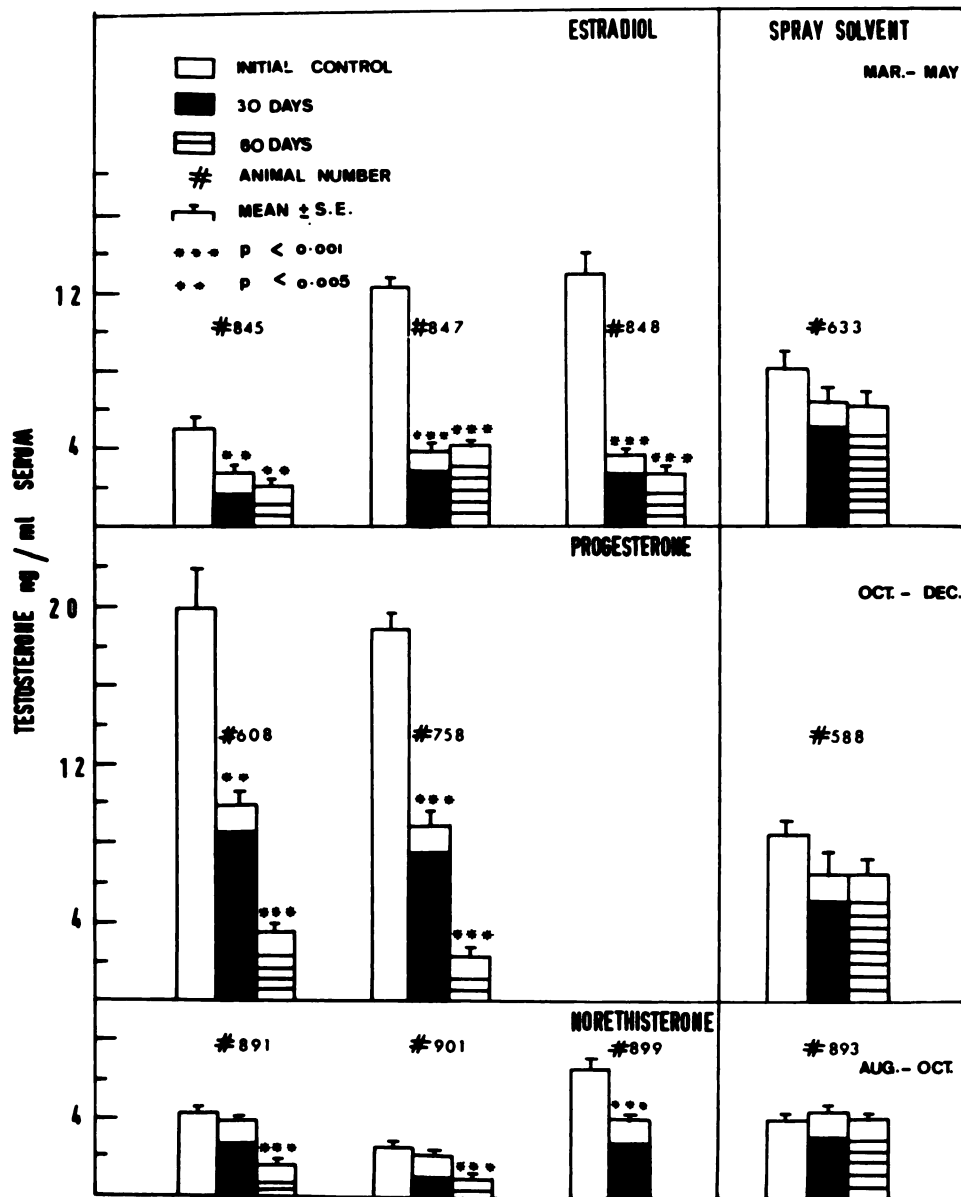


FIG. 3. Serum levels of testosterone in rhesus monkeys treated with various steroids or solvent alone. Each of the 3 bars in a set indicates testosterone values in samples taken from each animal before the treatment, after 30 days of treatment and on the day following 60 days of treatment. One of the animals treated with NET died after 40 days of treatment due to causes not related to the treatment, and studies could not be completed in this animal. Each bar represents the mean values (\pm SEM) of testosterone in blood samples collected at 10 min intervals.

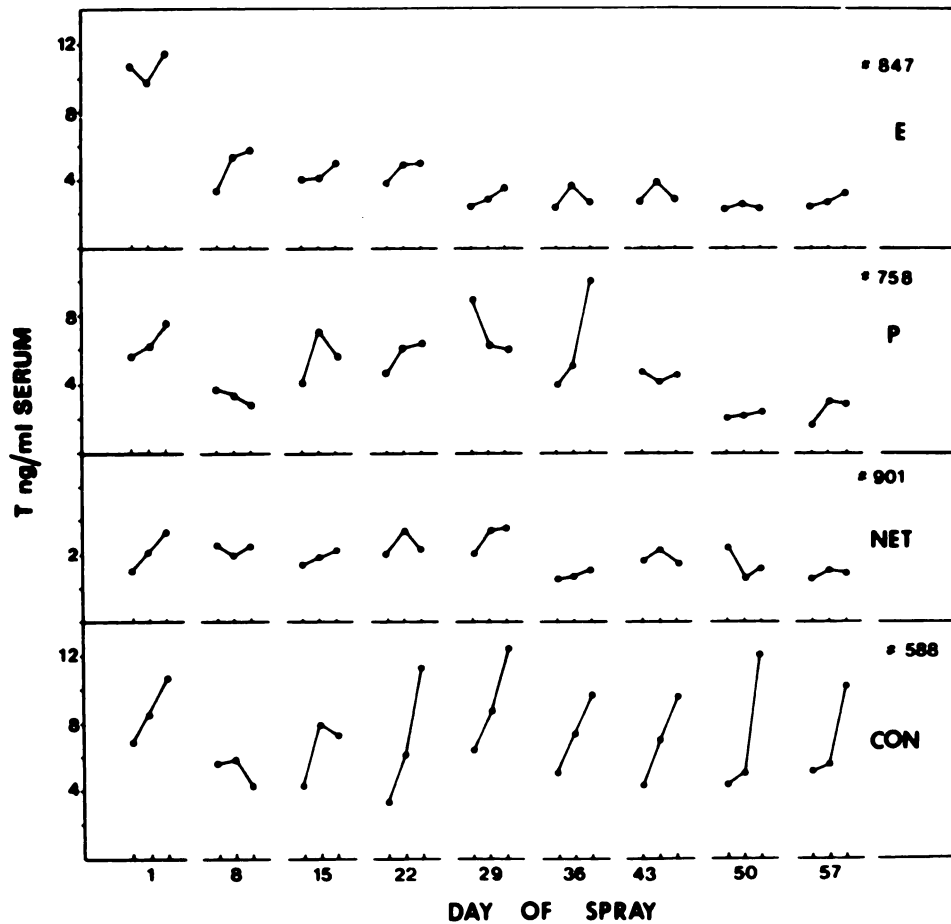


FIG. 4. Serum levels of testosterone in weekly blood samples taken at 0, 15 and 30 min from representative animals of each treatment group.

cated. In most of the animals serum testosterone showed a sharp rise in samples taken 15 min after spraying steroid or solvent alone. The reason for this is not known. However, in all the steroid-treated animals testosterone levels dropped after 30 min of spraying.

Plasma cortisol levels before, during and at the end of treatment with steroids or solvent showed no significant changes and these were within the normal range of values reported for rhesus monkeys (Michael et al., 1974).

DISCUSSION

The present studies have shown that there is an impairment of spermatogenesis and a significant reduction in levels of circulating serum testosterone following intranasal administration of E, P or NET to adult rhesus monkeys. Animals sprayed with the solvent alone did not

show such changes. Plasma cortisol levels in all the animals before and after the treatment were within the normal range for this species, and therefore, it would seem that the observed changes were due to the treatment.

The regressive changes in testicular function following intranasal administration of steroids are essentially similar to those already reported following oral or systemic administration of estrogens or progestins (Balze et al., 1954; Heller et al., 1958; Geller et al., 1965; McLeod, 1965; Johansson and Nygren, 1973; Frick et al., 1976). However, the main difference between the previously reported studies and ours lies in the dosages used to achieve comparable effects. Suppression of spermatogenesis occurs in human subjects following daily i.m. injections of as much as 50 mg of P, which is $\sim 700 \mu\text{g}/\text{kg}$ BW. Synthetic progestins (Nilvar, Nortutin and Enovid) need to be injected at a

daily dose of $\sim 400 \mu\text{g/kg}$ BW (Heller et al., 1958). In our studies similar effects on the testes were observed by administering as little as $2.72\text{--}4 \mu\text{g/kg}$ BW of the steroids by the intranasal route.

This difference in the dosages to achieve comparable effects may well be due to the way in which steroids are transferred to the target tissues by different routes. The pattern of steroid transfer into the cerebrospinal fluid (csf) has been shown to differ between the intranasal route of administration and systemic injections (Anand Kumar et al., 1978). Steroids sprayed intranasally reach a much higher concentration in the csf as compared with i.m. injections. Therefore, the concentration of steroids in the brain, where neuroendocrine mechanisms regulate the secretion of pituitary gonadotropin, would also be much higher following intranasal administration compared with systemic injection.

It is therefore possible that regressive changes in the testes occur when considerably lower dosages of the steroids are administered by the intranasal route than by the systemic routes because the steroids reach higher concentration in the brain when administered intranasally.

Thus the present studies have shown that steroid administered by the intranasal route do affect testicular function. However, a much larger series of studies needs to be carried out to determine the effects of such treatment on mating behavior and to evaluate spermatogenesis following cessation of treatment. Such studies are under way in our laboratories.

ACKNOWLEDGMENTS

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