Infertility Induced in Mice by a Single Injection of Testosterone Propionate.* (20989)

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The influence of androgens on the female reproductive tract has received considerable attention but comparatively little is known about the effects of these steroids on fertility. In general, reproductive performance in female rats is adversely affected by testosterone propionate. Daily administration of testosterone propionate to female rats from birth to 44 or 175 days of age induced sterility(1). Furthermore, if the androgen was given on alternate days starting at 6 days of age and continued until the rat was 38 days old, mating attempts failed as long as 10 weeks later (2). Adult female rats treated with 2 mg of testosterone propionate daily for 4 weeks failed to exhibit vaginal plugs although caged with males. A month after cessation of treatment, remating was attempted with 98% success(3). It would appear that the age at which androgen injections are started is an important factor in the establishment of a permanent or temporary sterility. To further explore this premise, the influence of single injections of testosterone propionate given to mice at definite prepuberal ages was tested for an effect on fertility.

Methods. Swiss albino mice raised in our laboratory were used in this study. The mice were maintained on Rockland complete mouse diet pellets. The diet was supplemented by a mixture of cod liver oil and Mazola oil on bread twice weekly and by lettuce once each week. Female mice of 5, 10 and 20 days of age were given a single subcutaneous injection of 1.0 mg of testosterone propionate (Oreton, Schering)‡ in oil. The single injection permitted a minimum of disturbance to the nursing animals which were kept with their mothers until weaning. The mice selected were of such number as to enable an approximately equal distribution of littermates between control and injected groups. Fertility was tested when the mice reached 90 days of age by placing the treated and control mice from one litter with a male. The time after admitting the male until littering was recorded as well as the litter size, sex distribution and the number and weight of young carried to weaning at 22 days of age. Litters were reduced to 6 animals at 3 days after birth to permit more uniform growth.

Results. The single injection of testosterone propionate to 5-day-old mice sharply curtailed fertility. These mice were kept with proven males for 100 days (now 6 months old) without obtaining litters from 14 animals. In contrast, all littermate control mice delivered litters in a month. Two mice destroyed their litters but the others carried their young to weaning. Lactation was subnormal in this control group, however, as indicated by the weaning weight of the pups (Table I).

Androgen administration at 10 days of age had less effect on fertility than when given at 5 days of age as only 4 of 12 mice failed to produce litters. Those mice which were fertile, dropped litters at essentially the same time as controls, had a normal number of pups per litter and exhibited good lactation.

Testosterone propionate injections at 20 days of age failed to diminish fertility (Table I).

Ovaries of infertile mice revealed relatively few follicles, no corpora lutea and a marked concentration of sudanophilia in the prominent stroma. Furthermore, follicular atresia was increased by androgen in 5-day-old mice examined at various times after injection but had little effect on older mice(4).

Estrogen will also induce infertility in mice

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TABLE I. Influence of Age and Testosterone Propionate (1 mg) on Reproductive Performance of Prepuberal Mice.

Treatment	No. mice	No. preg.	Days in breeding to 1st litter, avg (range)	Avg No./litter	Avg* weaning wt (g)
Control	14	14	31.6 (22-40)	6.6	5.9
T.p. at 5 days	14	0	Infertile	—	—
Control	13	13	33.3 (22-46)	6.5	10.0
T.p. at 10 days	12	8	37.0 (28-55)	7.0	9.7
Control	14	14	39.4 (22-60)	7.1	11.7
T.p. at 20 days	16	15	35.7 (22-71)	7.8	10.9

* Per individual at 22 days.

and the prepuberal ages appear to be the more sensitive periods(5,6).

Summary. Mice 5, 10 and 20 days of age were given a single 1 mg injection of testosterone propionate. Fertility was tested when these mice were 3 months old and compared with littermate controls. No litters were obtained with mice treated at 5 days of age despite 100 days in breeding. Fertility of mice treated when 20 days old, however, was not decreased whereas mice treated at 10 days of age assumed an intermediate position.

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Distribution of Succinic Dehydrogenase and Hyaluronidase in Adult Rat Testis.* (20990)

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A study of testicular hyaluronidase has revealed, in confirmation of the work of Perlman *et al.*(1) that freezing and thawing and sterile autolysis of homogenates of testicular tissue make possible the extraction of large amounts of this enzyme. These steps were therefore incorporated into a reproducible method for the measurement of the hyaluronidase content of rat testes(2). The relatively low activity found in extracts of fresh unfrozen and unautolyzed tissue suggested that hyaluronidase might be localized within a structure which was altered by freezing and autolysis.

Arnesen, Buxton, and Dulaney(3) concluded from differential centrifugation experiments with homogenates of mouse testis suspended in hypertonic sucrose solution (.88 Molar) that hyaluronidase activity was localized in the so-called "soluble" fraction. The fractions obtained by these workers were tested neither for activity of an enzyme of known localization, nor for nitrogen content. In the present investigation the determination of succinic dehydrogenase activity and of nitrogen content have been employed as biochemical indications of the effectiveness of the separation procedure(4). Optimum conditions for the measurement of the hyaluronidase content of testicular fractions are still being sought, hence the results to be reported are of a preliminary nature.

Methods. Adult albino rats were killed by means of a blow on the head, the testes were

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