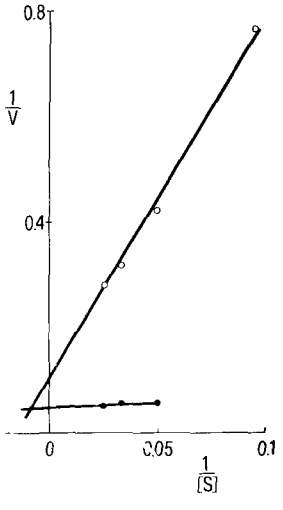
citrate dehydrogenase (E.C.1.1.1.42), 0.72 units (Sigma); NADP, 0.55 μmoles (Sigma); steroid substrate and *Tris*-HCl, 50 m*M*, pH 7.45 (containing 3 m*M* $MgCl_2$) to a final volume of 1.5 ml. Tritiated tracers of steroid substrates were used as required to quantitate the hydroxylation of the steroid being studied. Other details of the assay procedure were as previously described[10]. Controls showed that, in the absence of NADPH generator, conversion of 17α-hydroxyprogesterone(17α-OHP, 40 μ*M*) to 11-deoxycortisol was about 0.8% under the conditions of the assay procedure. Controls using tritiated progesterone (40 μ*M*) as the sole steroid inclusion showed production of 17α-OHP (2.46%) and 11-deoxycortisol (0.95%) under similar reaction conditions. These control data were taken into account in the calculation of relevant substrate and product values. The cytochrome P450 content was 0.17 nmol/mg protein.

The Figure shows the 21-hydroxylation of 17α-OHP to 11-deoxycortisol in the presence of increasing quantities of substrate. Under the conditions studied the rate of the reaction was limited by the concentration of the substrate. The *Km* for 17α-OHP was 4.3 μ*M* and the V_{max} for the reaction, 2.6 nmol/min/mg protein. It was observed that the addition of 40 μ*M* progesterone (unlabelled) causes a marked decrease in the rate of 21-hydroxylation of 17α-OHP. The double-reciprocal plot demonstrates that the inhibition by progesterone is of the 'mixed type'[11]. Further data on the possibility of 17α-OHP influencing the 21-hydroxylation of progesterone could not be undertaken at the present time due to the presence of the very active 17-hydroxylation of progesterone by the human adrenal microsomal preparations.



21-Hydroxylation of 17α-hydroxyprogesterone by human adrenocortical microsomes in the presence and absence of progesterone. V, nmoles 11-deoxycortisol produced in 5 min incubation; [S], concentration of 17α-OHP, μ*M*; •, only 17α-OHP present; ⊙, 17α-OHP plus progesterone (40 μ*M*) present. Incubation products separated by thin-layer chromatography on silica-gel IB-F (Baker) in benzene:acetone 3:1.

It is concluded from these studies that progesterone does have an inhibitory action, with possible regulatory implications, on the 21-hydroxylation of 17α-OHP by human adrenocortical microsomes. It is not possible to determine unequivocally at the present time as to whether the 21-hydroxylation of these two steroid substrates are effected at the same or at separate active sites. It may well be that the inhibitory effect of progesterone observed results from multiple actions on the multiprotein[5] enzyme system and complex hydroxylation mechanism concerned[12].

Summary. Progesterone inhibits the 21-hydroxylation of 17α-hydroxyprogesterone by human adrenal cortex microsomes. The possible light this finding may shed on the genetic condition, the 'adrenogenital syndrome' is discussed. *Km* and V_{max} data for the above hydroxylation reaction are given.

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Effect of Cyproheptadine Hydrochloride on Spermatogenesis[1]

While investigating some endocrine effects of cyproheptadine hydrochloride in rats, we observed that chronic administration of the drug to young male rats caused a slight but consistent stimulation of spermatogenesis. But this was not statistically significant as revealed by spermatodynamic study of testicular sections (unpublished data). Since the spermatogenesis in young adult rats is optimal and a further stimulation is difficult to obtain, the effects of a potential stimulant of spermatogenesis may not be very obvious. Hence, we investigated the potentiality of cyproheptadine hydrochloride as a stimulant of spermatogenesis in old rats where the process is normally retarded. The results of these experiments, which prompted us to investigate the effect of this drug on artificial cryptorchid testis, along with some interesting observations in the latter experiment, are presented here.

Materials and methods. A group of 6 male rats aged $2^1/_2$ to 3 years were treated orally with cyproheptadine hydrochloride (5 mg/kg/day) for 48 days. A similar untreated group served as control. In the second experiment, 2 groups of 6- to 8-month-old young male rats, were made unilaterally cryptorchid. One group was treated with cyproheptadine hydrochloride (5 mg/kg/day) for 15 days from the day of operation. At the end of the treatment, all the rats were killed and weights of testis of old rats,

[1] This gives a part of the work done by K. V. Jogi for Ph. D. thesis.

abdominal testis of cryptorchid rats, along with weights of accessory sex organs, were noted. The testes were processed for histology and stained with PAS and haemotoxylin.

Result. Testis sections from untreated old control rats were characterized by absence of secondary spermatocytes and spermatids. Only pyknotic spermatogonia, resting spermatocytes, and empty spaces interspersed with abundant hyperplastic Sertoli cells could be seen. Leydig cells were mostly degenerated (Figure 1). Sections from cyproheptadine-treated old rats showed active spermatogenesis. Early stages of gametogenic elements could be seen in almost all the tubules. However, advanced stages of spermatogenesis and fully mature spermatozoa could be seen only in a few tubules depicting normal spermatogenesis. Leydig cells appeared to be healthy (Figure 2). Sections of cryptorchid testis showed depletion of all gametogenic elements except a few spermatogonia and a row of resting spermatocytes along with limiting membrane. Leydig cells appeared to be hypertrophic. In cryptorchid testis of cyproheptadine hydrochloride-treated rats, spermatogenesis progressed up to the secondary spermatocyte stage. In some tubules, the secondary spermatocytes were in large numbers, filling the lumen. Cyproheptadine treatment did not produce significant change in the weights of testis and accessory sex organs in either of the groups.

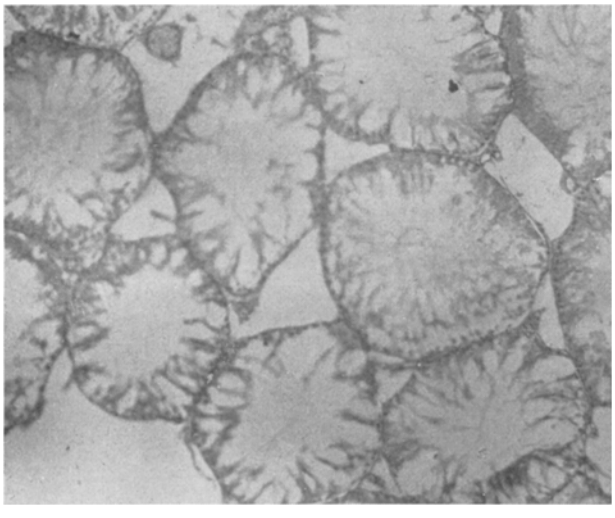

Fig. 1. Section of testis from old rat. × 100.

Fig. 2. Section of testis from old rat treated with cyproheptadine hydrochloride. × 100.

Discussion. The results indicate that spermatogenesis is stimulated in old rats treated with cyproheptadine for 48 days. A 48-day treatment was chosen in order to cover a complete spermatogenic cycle[2]. In cryptorchid rats, maximum testicular damage was reported after 15 days' retention[3]. Cyproheptadine-treated rats were therefore sacrificed on the 16th day to observe the effect of treatment at a time when damage is maximal.

Since, both in old rats and in cryptorchid rats, a definite stimulation of spermatogenesis was obtained, it is probable that a similar mechanism is involved in both cases. PENG et al.[4] reported that, in old male rats, both pituitary FSH and LH were lesser than in young adult rats. However, in cryptorchid rats both FSH and LH levels were higher than in intact rats, but in such rats, associated stimulation of ventral prostate weight was not obtained, suggesting injury to Leydig cell due to cryptorchidism[5]. Although the mechanism of action of cyproheptadine on spermatogenesis is not clear, it is possible that it exerts its effect directly on the testis. Further, it is to be elucidated whether it is primarily acting on gametogenic elements, or whether its primary action is stimulation of Leydig cell. Nelson reported HCG induced stimulation of androgen production in cryptorchid testis failed to stimulate spermatogenesis[6]. Hence it is likely that stimulation of spermatogenesis is a direct effect of cyproheptadine both in cryptorchid and senile testis[7].

Summary. Administration of cyproheptadine hydrochloride (5 mg/kg/day) to old rats for 48 days stimulated spermatogenesis. It also initiated spermatogenesis in the abdominal testis of unilaterally cryptorchid rats after a 15-day treatment.

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