EFFECTS OF PROLACTIN AND LH ON THE ACTIVITY OF $\triangle^5$-3β HYDROXY-STEROID DEHYDROGENASE, DIHYDRO-OROTIC DEHYDROGENASE, B-HYDRO-XYBUTYRATE DEHYDROGENASE AND GLUCOSE - 6 - PHOSPHATE DEHYDRO-GENASE IN THE TESTIS OF THE DWARF MICE

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Summary: The activity of $\triangle^5$-3**B** Hydroxysteroid hydro-genase, dihydro orotic dehydrogenase, B-Hydroxybutyrate dehydrogenase and glucose-6-phosphate dehydrogenase was studied in the testes of hereditary dwarf mice treated with Prolactin or LH. Two months old dw/dw dwarf mice were injected twice daily, for 14 days, either with 100 μg ovine Prolactin, or 5 μg ovine LH twice a day. Prolactin treatment increased the activity of all the enzymes assessed. Treatment with LH stimulated the activity of all the enzymes as compared to the saline treated animals but less than the Prolactin treated ones. The data bearing that the increased activity of several oxidising enzymes in the testes of hereditary dwarf mice is increased with Prolactin, is consistent with the suggested effect of this hormone on testicular steroidogenesis.

Key words: Prolactin LH $\triangle^5$-3B hydroxy steroid dehydrogenase
dihydro-orotic dehydrogenase B-hydroxy butyric dehydrogenase
glucose-6-phosphate dehydrogenase

INTRODUCTION

Dwarf mice are Prolactin deficient (8) but appear to produce normally both LH and FSH (2). Administration of Prolactin to male dwarf mice has been shown previously to increase the weight of seminal vesicles, spermatogenesis, and fertility (3,4). It has been suggested that in the mouse, Prolactin acts synergistically with LH on testicular steroidogenesis and that these effects may be due to increased esterified cholestrol (5). In the presence of LH Prolactin stimulates spermatogenesis, increases plasma testosterone levels and the formation of testosterone *in vitro* (5,11). This work was undertaken to further elucidate the effect of Prolactin on testicular function. The effect of Prolactin on the activity of some oxidising enzymes in the testis was studied.

MATERIAL AND METHODS

Heredit ry dwarf mice dw/dw of the dw/J strain were obtained from the Jackson Laboratory, Bar Harbor, Main. At the onset of the study the animals were approximately 60 days old and wei hed from 5 to 8 grams. Two groups of twelve and ten male dwarfs were respectively dministered 200 μg ovine Prolactin (NIH-P-S9) or 10 μg LH (NIH-LH-S16) in 0.05 *ml* volume subcutaneously. Half of the daily dose was given in the morning and half

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in the afternoon. The controls received the same volume of normal saline. The animals were treated for two weeks and killed with ether on the 15th day. Uninjected non dwarf litter mates and randon bred Charls River CD-I males served as normal controls. The testes, epididymes and seminal vesicles were weighed and testes were immediately frozen on dry ice. Sections were cut in the crystat at 16 μ thickness at -18°C and allowed to dry at room temperature for half an hour. The sections of the testes of Prolactin treated, LH treated and saline treated control dwarf mice were always incubated in the same jar. The following enzymes were studied : $\triangle^5$-3B Hydroxysteroid dehydrogenase (3B HSD) (15), dihyrdro-orotic dehydrogenase (OD) (9), B-Hydroxybutyrate dehydrogenase (HBD) (14) and Glucose -6-Phosphate dehydrogenase (G-6-PD) (14). Nitro blue-tetrazolium served as final hydrogen acceptor in all these reactions.

RESULTS

Normal (non dawrf mice): There was no differences in the activity and the distribution of the studied enzymes betweon the testes of normal mice and of the dw/J strain. The G-6-PD and OD were present in the Leydig cells as well as in the seminiferous tubules (Fig. 1A and

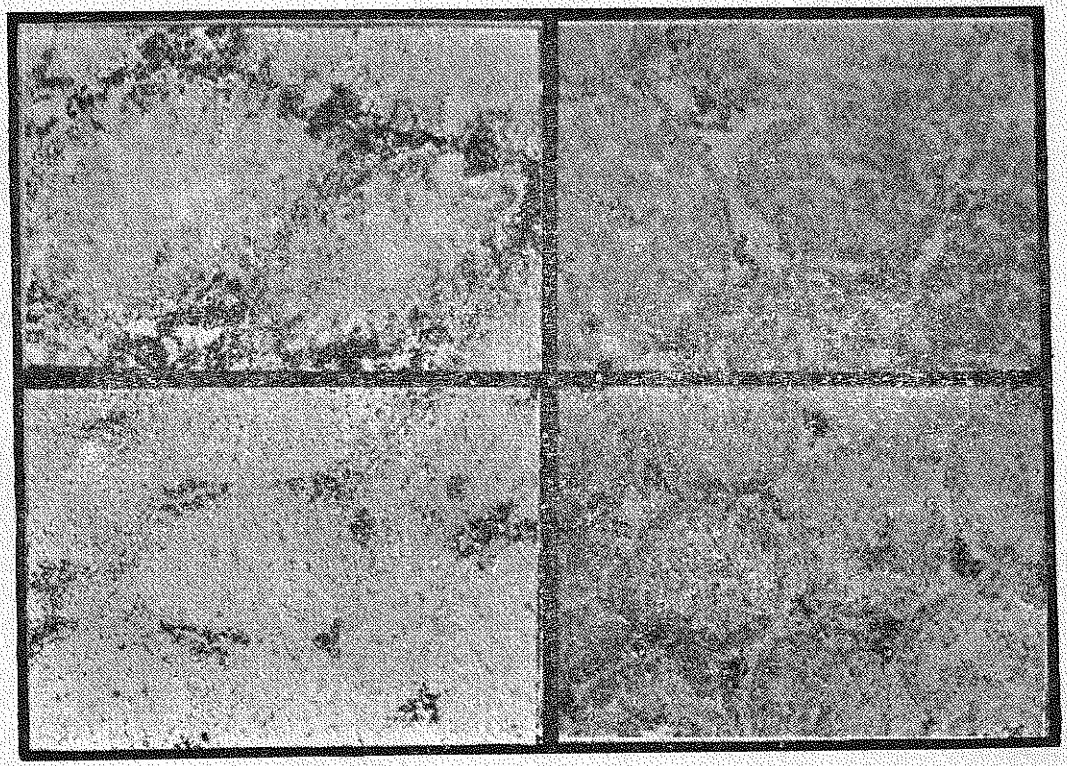

Fig 1: Transverse section of the testis, showing Glucose-6-phosphate dehydrogenase activity in the Leydig cells. A. Non dwarf litter mate, B. Saline treated dwarf mice, C. Prolactin treated dwarf mice, D. LH treated dwarf mice. Nitroblue tetrazolium (X 200).

3A) 3B HSD and HBD were present only in the intestitial cells (Fig. 2A and 4A). In the germinal epithelium the activity of the OD seemed to be limited to the most peripheral layer of cells (Fig. 3A) and that of G-6-PD was present in the Leydig cells as well as in the lumen of the tubule (Fig. 1A).

Saline treated dwarf mice: The distribution of the enzymes in the testes of dwarf animals was the same as in normal animals. However, Leydig cells were far fewer in number and consequently the enzyme activity was seen in smaller areas. In dwarf mice the tetrazolium granules were always less abundent than in the normal animals (Figs. 1B, 2B, 3B and 4B).

Prolactin treated dwarfs: The activity of G-6-PD was increased in this group as compared to the LH treated one (Fig. 1C and D). 3B HSD activity was increased by Prolactin administration; the NBT granules were darker and were present in a larger area as compared

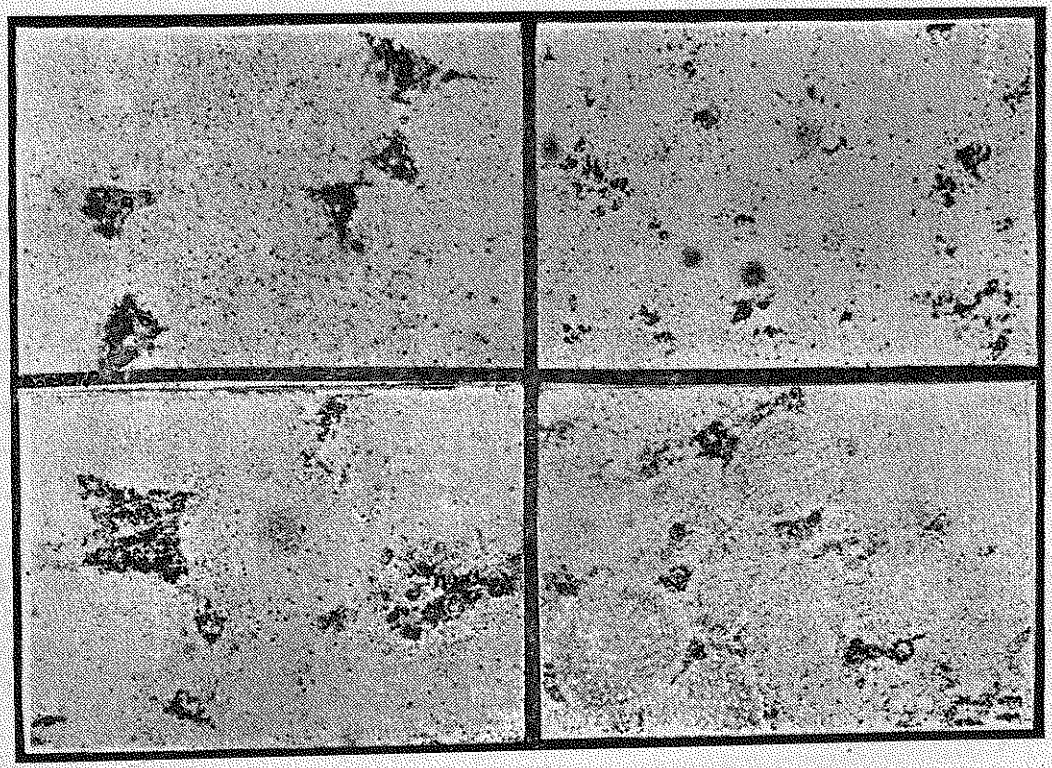

Fig. 2: Transverse section of testis, showing Δ^5-3B Hydroxy steroid dehydrogenase activity in the Leydig cells. A. Non dwarf litter mate, B. Saline treated dwarf mice, C. Prolactin treated dwarf mice, D. LH treated dwarf mice. Nitroblue tetrazolium (X 200).

to the saline treated animals (Fig. 2C). The OD was also increased in the Prolactin treated animals (Fig. 3C). There seem to be presence of this enzyme in the peripheral germinal layers only very little is present in the Leydig cells. There is a definite increase in the activity of HBD which is greater than in the LH treated animals (Fig. 4C and D).

The weights of testis, epididymus and seminal vesicles were also increased more with Prolactin treatment than with LH. (Table I).

LH treated dwarfs: The activity of all the enzymes assessed was increased in these animals as compared to the saline treated ones, but the intensity of the tetrazoluim granules and areas of deposit were not increased as much as in the Prolactin treated animals, (Table II).

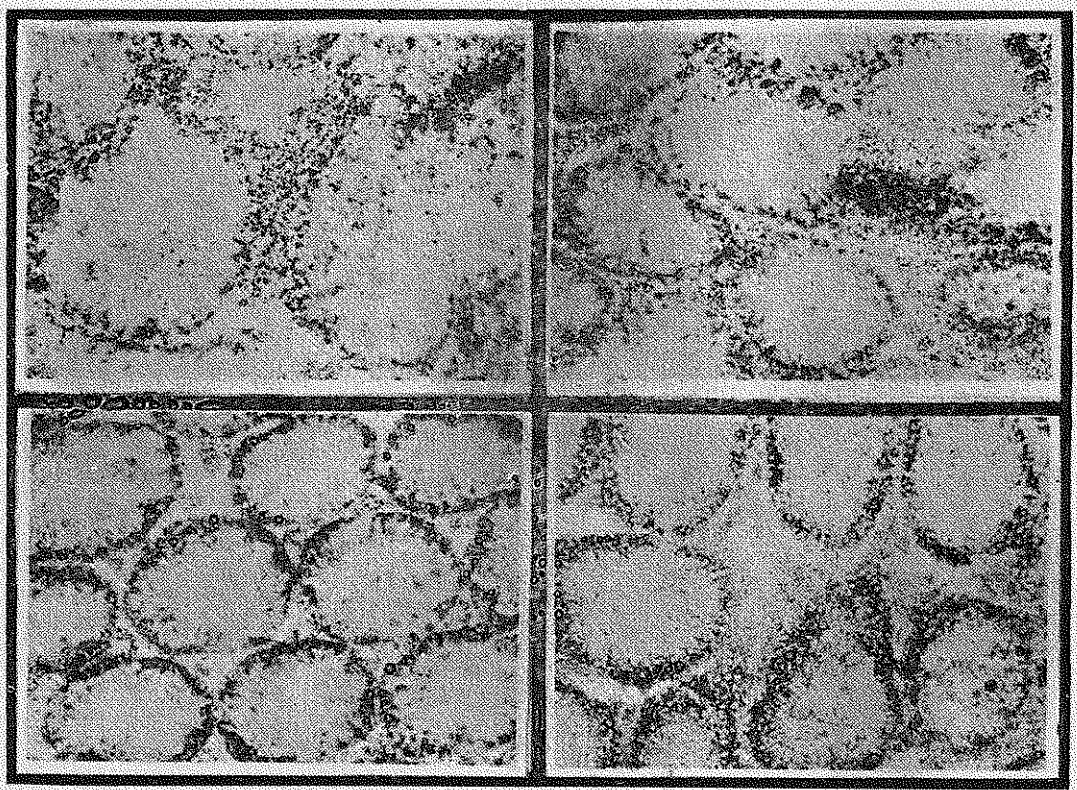

Fig. 3: Transverse section of testis, showing dihydro-orotic dehydrogenase activity in the Leyding cells and to the most peripheral layer of cell in the tubules. A. Non dwarf litter mate, B. Saline treated dwarf mice, C. Prolactin treated dwarf mice, D. LH treated dwarf mice. Nitroblue tetrazolium (X 200).

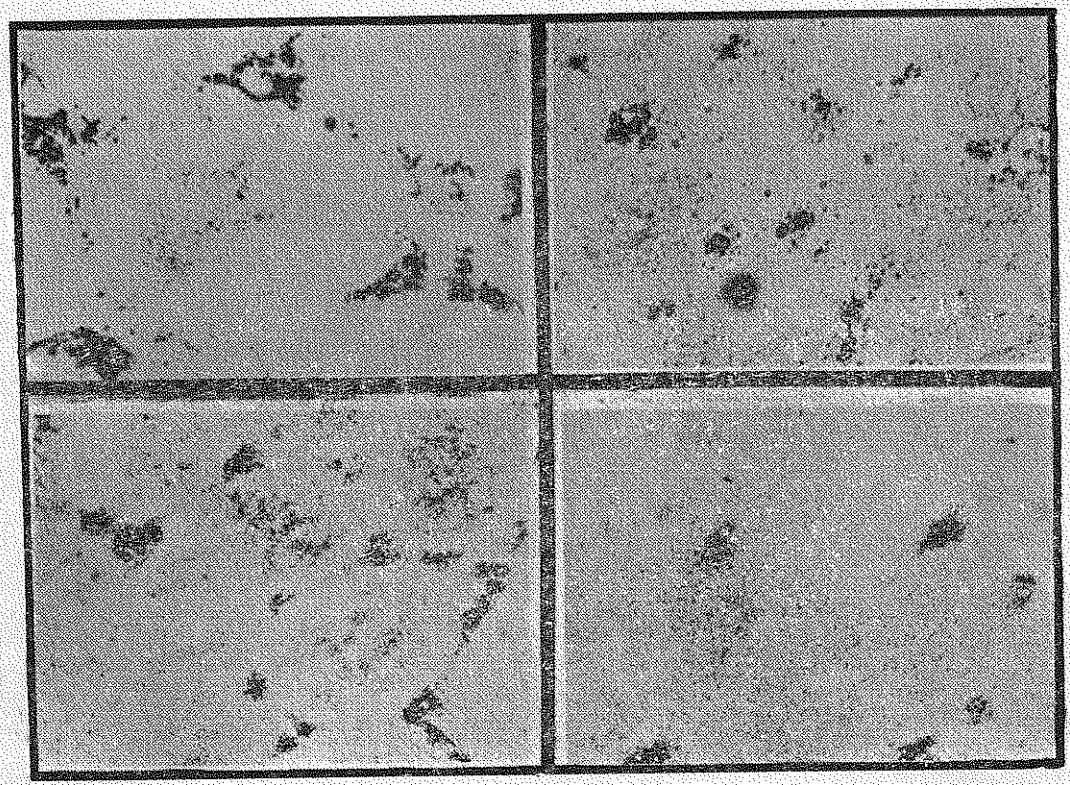

Fig. 4: Transverse section of testis, showing dihydrobutrate dehydrogenase activity in the Leydig cells and in the lumen of the seminiferous tubules in the mature sperms. A. Non dwarf litter mate, B. Saline treated dwarf, C. Prolactin treated dwarf, D. LH treated dwarf. Nitroblue tetrazolium (X 200).

Table I.

	Prolactin treated mg.	*LH treated mg.*	*Saline treated mg.*
Testes	97.7** ± 8	74.8 ± 14	69.0 ± 8
Epididymus	20.4* ± 1	13.5 ± 0.2	12.1 ± 1
Seminal vesicles	18.3* ± 1	8.3 ± 1	10.8 ± 1

**P < 0.0025
*P < 0.01

Table II

Enzymes	*Prolactin treated Leydig cells/ Tubules*	*LH treated Leydig cells/ Tubules*	*Saline treated Leydig cells/ Tubules*	*Normal litter mates Leydig cells/ Tubules*
G-6-PD	+++/++	++/+	+/+	++++/++
Δ^5-3B HSD	+++/—	++/—	+/—	+++/—
OD	+/++	+/+	+/+	++/++
HBD	++++/—	++/—	+/—	++++/—

DISCUSSION

The interstitial cells of the normal rat and mouse testis exhibit activity of all the enzymes studied (13). 3B HSD is involved in several steps of steroidogenesis including the convertion of pregnenolone to progestrone, G-6-PD is utilised in Hexose monophosphate shunt, HBD converts Ketobutyric acid to β-hydroxy butric acid and OD is involved in pyrimidine synthesis.

In the saline treated dwarf mice these enzymes were present in the Leydig cells, but their activity was definitely lower than that observed in normal males. This is consistent with the observation that these animals have atrophic accessory reproductive organs, low yield of spermatogenesis and are presumably androgen deficient (8). Cavallero *et al.* (7) observed stimulation of the activity of G-6-PD and HBD in the Prolactin and LH treated dwarf mice to the same extent. This discrepancy is probably due to the use of much older animals in the present study (60 days rather than 21 days at the onset of treatment) and to different genetic background of the dwarfs.

In the present study Prolactin appeared more effective than LH in stimulating the activity of several dehydrogenases. After these experiments were started, the stimulation by Prolactin of the activity of 3B HSD in the testes of dwarf mice was demonstrated by biochemical assay (12). This was most likely due to the fact that dwarf mice are Prolactin deficient but their pituitary releases adequate, if not normal amounts of LH (1).

The increased activity of HBD observed in the Prolactin treated dwarfs appear consistent with the suggestion that in the male mice Prolactin can make more cholesterol available for steroidogenesis (6). Field (10) reported that HBD can be coupled with coenzyme A to synthesize active acetate. Elevated activity of this enzyme could threfore contribute to the suggested increase of testosterone bio-synthesis in Prolactin treated dwarf mice. The fact that the activity of G-6-PD is not increased by LH confirms the observations of Field and shows that neither LH nor HCG affect the metabolism of glucose via HMP shunt in the testes *in vitro*. Prolactin increased the activity of G-6-PD in the Leydig cells and in the germinal epithelium (Fig. 1C). The increased activity of this enzyme would make more TPNH available which, in turn could be utilized for the synthesis of cholesterol. This would substantiate the findings of elevated cholesterol content in the testes of Prolactin treated dwarfs.

The activity of 3B HSD was increased by both Prolactin and LH indicating a potential for increased steroidogenesis. After these experiments were started, the stimulation by Prolactin of the activity of 3B HSD and 17B HSD in the testes of dwarf mice was demonstrated by biochemical assays (12). The activity of OD is also increased by Prolactin and by LH, Prolactin increases spermatogenesis and causes an increase in nucleic acid synthesis. It also

causes proliferation of the Leydig cells and an increase in androgen production. Stimulation of enzymatic activity in the interstitial tissue of dwarf mice treated with Prolactin confirms the morphologic indications of stimulation of the Leydig cells observed in the haematoxylin and eosin and PAS stained histological sections of the testes of dwarf mice chronically treated with Prolactin (Bartke and Russfield, unpublished observations).

In the present study the effects of injecting 200 μg Prolactin daily were greater or similar to the effects of injecting 10 μg LH. The LH activity of NIH-P-59 Prolactin was reported to be less than 0.4 $\mu g/mg$ and consequently the effects of Prolactin treatment can not be attributed to its LH contamination. It can be concluded that Prolactin stimulates the activity of several oxidising enzymes in the testes of hereditary dwarf mice and that this may partially explain its apparent effect on the biosynthesis of testicular androgens.

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