STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION

PART XVI. FURTHER EVIDENCE OF THE ENHANCEMENT OF ANDROGENIC ACTION OF TESTOSTERONE PROPIONATE BY PROGESTERONE

by AMIYA B. KAR *and* N. N. DE, *Central Drug Research Institute, Lucknow.*

(Communicated by B. Mukerji, F.N.I.)

(*Received September 3; approved for reading on October 5, 1956*)

It seems satisfactorily established that progesterone possesses appreciable androgenic activity as tested by histophysiologic and metabolic responses of the male accessory genital organs to this hormone (for earlier references see Kar, 1949; Kar and De, 1953; and Price *et al.*, 1955). The possibility that progesterone can add to the androgenic action of testosterone was, however, originally suggested in the experiments of Morgan (1946) who showed that progesterone synergized the restoring effect of testosterone on the accessory genital organs of spayed female opossums. Subsequently, Kar and De (1953) were able to demonstrate that progesterone enhanced the androgenic effect of testosterone propionate when injected simultaneously in castrated male rats. The recent findings of Price *et al.* (1955) also tend to subscribe towards a similar physiological relationship between progesterone and androgens.

In the present paper, an attempt has been made to provide further evidence of the enhancement of androgenic effect of testosterone propionate by progesterone.

The literature on metabolic activities of the male accessory genital organs is replete with references on a positive correlation between androgenic activity and the concentration of alkaline phosphatase (Roberts and Szego, 1953). Because of the consistency of such a correlation, it has even been suggested that fluctuations in alkaline phosphatase activity in the male accessory genital organs can be used as a sensitive chemical assay method for small amounts of androgen (McCullagh and Schaffenburg, 1954). In view of these, in the present study, the alterations in alkaline phosphatase concentration in the seminal vesicles of castrated rats have been utilized as the criterion for the evaluation of androgenic effects of progesterone whether given alone or in combination with testosterone propionate.

EXPERIMENTAL PROCEDURE

Thirty adult albino rats of the Institute colony, weighing on an average 170 gm., were used in this study, out of which twenty-four were castrated. The unoperated rats and a group of six castrates served as the controls. The castrates were assigned in three groups of six animals each for receiving hormone treatments. The latter was initiated on the 31st day following castration and before the commencement of the injections, the seminal vesicles of all the castrates were examined by laparotomy in order to ensure that these had adequately regressed. All of the animals were maintained under uniform laboratory conditions throughout the experimental period.

Progesterone and testosterone propionate were injected by the intramuscular route in daily doses of 2·5 mg. (in 0·1 ml. of sterile olive oil) of each hormone for

10 days. This dosage was used for the groups which received the two hormones separately. For the group which was subjected to combined treatment 2·5 mg. of each hormone per day was injected simultaneously so that a total of 25 mg. of progesterone plus an equal amount of testosterone propionate were given per animal. The control castrate and the unoperated rats (Table I) received 0·1 ml. of sterile olive oil alone daily for the same period. The dosage used in the present study was essentially similar to that used previously (Kar and De, 1953).

The animals were sacrificed 24 hours after the final treatments. The seminal vesicles were carefully dissected out and fixed immediately in chilled 80 per cent ethyl alcohol. The serial paraffine sections of the organ (6 micra thick) were processed according to the technique of Gomori (1941) as laid down by Glick (1949) for the demonstration of alkaline phosphatase.

Results

Controls.—The pattern of distribution and concentration of alkaline phosphatase in the seminal vesicles of normal rats agreed with those of the previous workers (Bern, 1949; Melampy and Cavazos, 1953). The epithelium gave an entirely negative reaction for the enzyme and the lamina propria was only faintly positive. The latter was true for both the connective tissue and the vascular elements of the lamina propria. The muscularis gave a patchy and diffuse reaction (Pl. XVIII, Fig. 1).

Table I

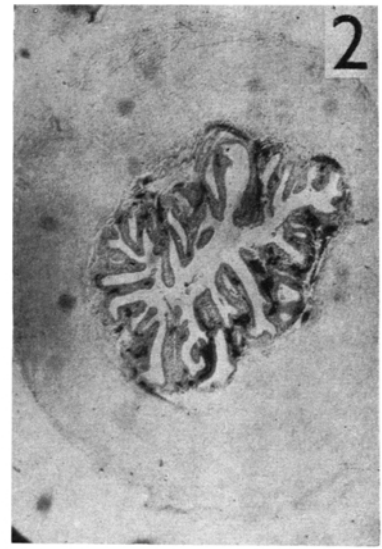
The distribution and concentration of alkaline phosphatase in the seminal vesicles of normal and experimental rats

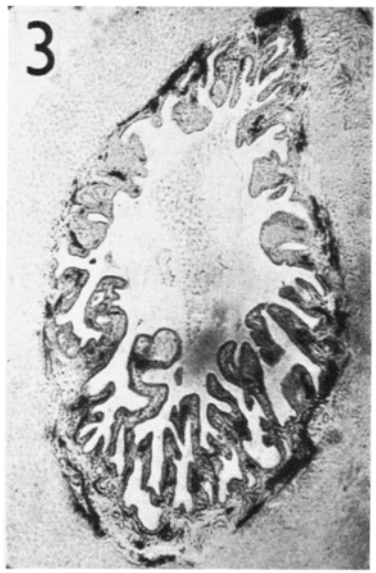
	Controls	Castrates	Castrates +progesterone	Castrates +testosterone propionate	Castrate +progesterone +testosterone propionate
Epithelium					
Nucleus	0	0	0	0	0
Cytoplasm (including secretion granules)	0	0	0	0	0
Lamina propia					
Connective tissue	+p	+p	++p	+++	++++
Blood vessels	+	0	+	++	+++
Muscularis					
Smooth muscle	+p	0	0	0	+++
Blood vessels	0	0	0	+++	+++

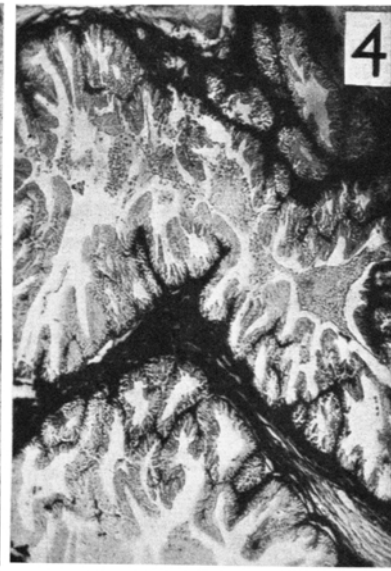
Evaluation.—

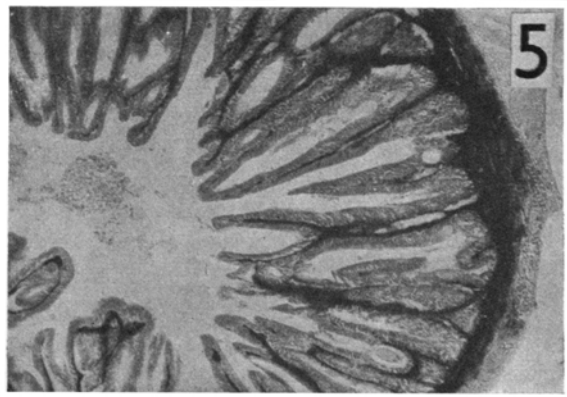
++++ = Intense phosphatase activity.
+++ = Strong phosphatase activity.
++ = Weak activity.
+ = Faint reactions.
+p = Faint and patchy reactions.
0 = Negative reaction.


1


2


3


4


5

Castrates.—The epithelium was negative for the phosphatase as in the normal controls. The other components of the organ were also devoid of phosphatase activity except for a faint reaction in the lamina propria (Pl. XVIII, Fig. 2 and Table I).

Castrates + progesterone.—The epithelium continued to give a negative reaction for phosphatase activity but the connective tissue of lamina propria was weakly positive (Pl. XVIII, Fig. 3). The blood vessels of the lamina propria stained faintly for the enzyme. The overall picture, however, was slight stimulation of phosphatase activity as compared to that of the untreated castrates.

Castrates + testosterone propionate.—No reaction for phosphatase activity was seen in the epithelium. The connective tissue of the lamina propria showed strong reactions but the vascular elements were only weakly positive. In the muscularis only the endothelium of the blood vessels gave strong reactions for the enzyme (Pl. XVIII, Fig. 4). The overall picture was considerable stimulation of phosphatase activity in sharp contrast to the untreated castrates and the normal controls (Pl. XVIII, Fig. 4).

Castrates + progesterone + testosterone propionate.—The epithelium was negative for phosphatase activity. The connective tissue of the lamina propria gave intense reactions for the enzyme and the blood vessels were also strongly positive (Pl. XVIII, Fig. 5). In the muscularis, both the fibres and the blood vessels showed strong concentrations of the enzyme. The extent of overall increase in alkaline phosphatase activity was greater than that of the androgen recipients.

Discussion

The results of the present study tend to bear out and extend the findings of Kar and De (1953) regarding the enhancement of androgenic action of testosterone propionate by progesterone. It is interesting that, at the dosage level used in this study, the weight and gross histological responses of the seminal vesicles of castrates to progesterone are negligible (Kar and De, 1953). This is to be expected as according to the recent estimation of Price *et al.* (1955) a massive dose of progesterone, several times greater than that used by us (*loc. cit.*), is necessary to reveal its androgenicity in the castrates when given alone. Nevertheless, a careful perusal of the phosphatase picture of the seminal vesicles of rats treated with progesterone (Pl. XVIII, Fig. 3) will indicate a slight but definite stimulation of phosphatase activity even at this otherwise low dosage. This not only emphasizes the sensitivity of the enzymic response in the detection of trace amounts of androgenicity but also leads to the possibility that in course of recovery of an accessory male genital tissue from the effects of chronic testicular insufficiency some metabolic features of the tissue is restored earlier than its gross morphology. The significant histochemical findings of Melampy and Cavazos (1953) can be brought to bear upon such a viewpoint. These workers reported that the activity of alkaline phosphatase in the seminal vesicles of castrated rats is restored to supernormalcy on the fifth day of androgen therapy but thereafter a relative decline in enzyme activity from the supernormal level is clearly noticeable. According to the present hypothesis, this may be due to a sequential restoration of normal phosphatase activity at a very early stage of androgen therapy, the subsequent attainment of a supernormal level with time, and final interference with the kinetics of the enzyme by sustained hormonal stimulation. To what extent such a picture parallels the physiology of the tissue concerned will be an interesting topic for research.

A final consideration of the above facts and possibilities, therefore, leads to the inference that progesterone can enhance the androgenic effect of testosterone propionate. This is indicated by changes in weight and gross histology of the seminal vesicles of castrated rats as reported earlier (*loc. cit.*) as also by the responses of a specific enzyme, the alkaline phosphatase, which is known to have a positive correlation with androgenicity.

Summary

1. Castration causes disappearance of alkaline phosphatase activity from the seminal vesicles of the rat.

2. Progesterone slightly stimulates phosphatase activity in the seminal vesicles of similar animals but testosterone causes a strong mobilization of the enzyme in this tissue.

3. Combined therapy with progesterone and testosterone propionate is associated with a greater stimulation of phosphatase activity than is noticed with the androgen alone.

4. Previous findings on the enhancement of androgenic effect of testosterone propionate by progesterone is confirmed.

Acknowledgements

The authors wish to express their gratitude to Dr. B. Mukerji for his interest in this study. Thanks are due to Sri S. Banerji for the photomicrographs.

References

Bern, H. A. (1949). The distribution of alkaline phosphatase in the genital tract of male mammals. *Anat. Rec.*, **104**, 361–370.

Glick, D. (1949). *Techniques of Histo- and Cytochemistry.* Interscience Publishers, New York.

Gomori, G. (1941). The distribution of alkaline phosphatase in normal tissues and organs. *J. Cell and Comp. Physiol,* **17**, 71–83.

Kar, A. B. (1949). Testicular changes in the juvenile pigeon due to progesterone treatment. *Endocrinol.*, **45**, 346–348.

Kar, A. B., and De, N. N. (1953). Die Wirkung Kombinierter progesteron und testosteron-propionat therapie auf die samenblasen reifer kastrierter ratten. *Endokrionol.*, **30**, 322–329.

McCullagh, E. P., and Schaffenburg, C. A. (1954). *The Testis in Glandular Physiology and Therapy.* J. B. Lippincott Co., London.

Melampy, R. M., and Cavazos, L. F. (1953). Effects of testosterone propionate on histochemical reactions of rat seminal vesicles. *Endocrinol.*, **52**, 173–187.

Morgan, C. (1946). *Assoc. Study Int. Sec. Ann. Meeting.* (Quoted by Nieburgs, H. E. (1949). *Hormones in Clinical Practice.* Cassell & Co., London.)

Price, D., Mann, T., and Lutwak-Mann, C. (1955). The stimulating effect of female hormones on the metabolic activity and histological structure of male rat accessory reproductive organs. *Anat. Rec.*, **122**, 363–380.

Roberts, S., and Szego, C. M. (1953). Steroid interactions in the metabolism of target reproductive organs. *Physiol. Rev.*, **53**, 593–629.

Explanation of Plate XVIII

(All figures are photomicrographs and are of equal magnification)

Fig. 1. Transverse section through the seminal vesicles of a control rat. Note the patchy distribution of alkaline phosphatase in the lamina propria.

„ 2. Transverse section through the seminal vesicles of a castrated rat. Note the patchy and diffuse reactions for the enzyme in the lamina propria.

„ 3. Transverse section through the seminal vesicles of a castrated rat treated with progesterone. Note weakly positive reactions in the lamina propria.

„ 4. Transverse section through the seminal vesicles of a castrated rat treated with testosterone propionate. Note the hypertrophy of the organ and stimulation of phosphatase activity.

„ 5. Transverse section through the seminal vesicles of a castrated rat treated with testosterone propionate + progesterone. Note the conspicuous foldings of the mucosa and pronounced stimulation of phosphatase activity.

Issued May 27, 1957.