

## STUDIES ON CYTOCHEMISTRY OF HORMONE ACTION.

### PART X.—THE HORMONAL MODIFICATION OF ALKALINE PHOSPHATASE ACTIVITY IN THE TESTIS AND IN SOME MALE GENITAL ACCESSORIES OF THE GUINEAPIG.

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#### THE PROBLEM.

The present paper embodies the extent to which the alkaline phosphatase activity in the testis and in some male genital accessories of the guineapig can be influenced by various hormones.

#### REVIEW OF THE LITERATURE.

Kabat and Furth (1941) observed that only slight alkaline phosphatase activity is present in the spermatogenic cells of the adult human testis but the basement membrane of the tubules exhibits more prominent reactions for the enzyme. Recently, Bern (1949) made a comparative study of the distribution of alkaline phosphatase in the male genital system of several species of mammals including the species under report. In an earlier paper Gomori (1941) reported the occurrence of this enzyme in the testis of the rabbit and the guineapig and the details of enzymatic distribution in the two species, as given by Bern (1949), agree with those presented by the former author. Bourne (1943) also observed the presence of alkaline phosphatase activity in the guineapig's testis. Dempsey *et al.* (1949) noted that in the rat this enzyme is demonstrable both in the tubules and in the intertubular tissue. After hypophysectomy the phosphatase disappears from the testicular components but replacement therapy with whole pituitary powder causes a return of the enzyme to its original distribution and intensity in all the elements. Wislocki (1949) reported the presence of alkaline phosphatase in the interstitial cells and in the tubular elements of the testis of two species of deer, *Odocoileus virginianus borealis* and *Cervus nippon*.

Kabat and Furth (1941) observed that alkaline phosphatase activity is entirely absent from the epithelium of the adult human prostate and seminal vesicles. The endothelium of the capillaries of the latter, however, gives intense reactions for the enzyme whereas the muscular stroma of both the organs contains only a trace of the phosphatase. Gomori (1941) noted that marked enzyme activity is present in the bladder epithelium of the rabbit and the guineapig. Bourne (1943) obtained negative Gomori reactions for alkaline phosphatase activity in the vas deferens and in the seminal vesicles of the guineapig. Zorzoli and Stowell (1947) noted the presence of strong alkaline phosphatase activity in the muscular stroma of the bladder of the rat, mouse, and the guineapig. They also remarked on the pronounced enzyme activity in the nuclei of the epithelium of guineapig's vas deferens and the subepithelial connective tissue of rat's epididymis. Atkinson (1948) reported that marked phosphatase activity is present in the stromal elements of the seminal vesicle of the adult mouse. Occasional traces of the enzyme are

also demonstrable in the nuclei of the mucosal epithelium. Castration causes a diminution of phosphatase activity which, however, is restored on replacement therapy with androgen. Soullairac and Thibault (1948) demonstrated that intense enzyme activity is visible in the vas deferens, seminal vesicles, prostate, and Cowper's glands of the rat. Castration causes a suppression of phosphatase activity which can be re-established by androgen therapy. Desoxycorticosterone acetate is only partially effective in restoring the enzyme activity but estrogen has no effect in this respect. Dempsey *et al.* (1949) observed pronounced phosphatase activity in the stroma and in the capillaries immediately underneath the epithelium of the rat's seminal vesicle. After hypophysectomy or gonadectomy, the enzyme activity considerably declined or disappeared. The restoration of the normal enzymatic complement is accomplished by the injection of pituitary powder or testosterone into the operated rats. Wislocki (1949) noted the occurrence of alkaline phosphatase in the seminal vesicles and in the ductus epididymidis of the two species of deer referred to in the previous paragraph. Bern (1949) studied the distribution of this enzyme in the male genital accessories of several species of mammals including the guineapig.

In the testis of juvenile sparrows the enzyme is present in the seminiferous tubules and in the endothelium of the interstitial blood vessels (Kar, 1951*a*). Desoxycorticosterone acetate treatment causes a pronounced loss of phosphatase activity from practically all the testicular components. The distribution of the enzyme in the testis of the adult pigeon exhibits a pattern which is more or less similar to that in the sparrow (Kar, 1951*b*). However, in the pigeon the Leydig and the fibroblast cells of the interstitium also show pronounced phosphatase activity. Treatment with sexual hormones is associated with a marked inhibition of enzymatic activities in the testicular components. There is, unfortunately, no report of the presence of alkaline phosphatase in the ductus deferens of birds.

A careful perusal of the works cited in the foregoing paragraphs makes it evident that considerable efforts have been made to study the distribution of alkaline phosphatase in the male genital system of different species of animals, but attempts to modify its activities by hormonal treatments have been rather meagre. We would like to emphasise in this connection that the component organs of the male genitalia are nicely suited for studies on hormone-enzyme relationships and extensive researches on this system are sure to adduce many valuable evidences to the current thesis that the hormonal actions are mediated through the modifications of enzymatic activities.

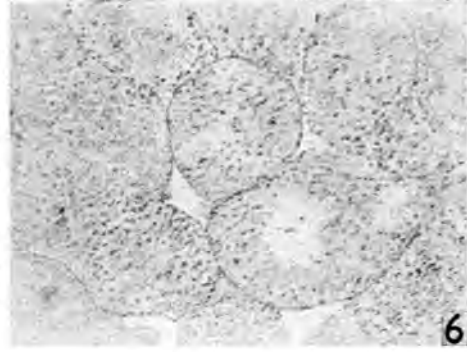
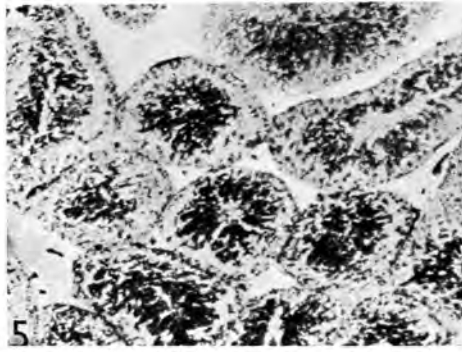
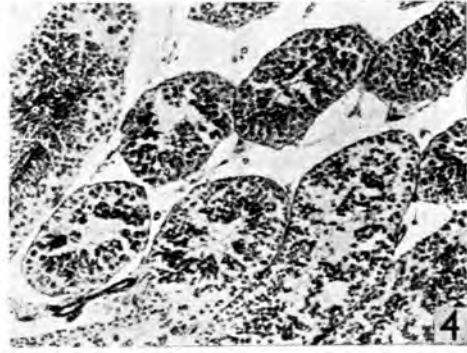
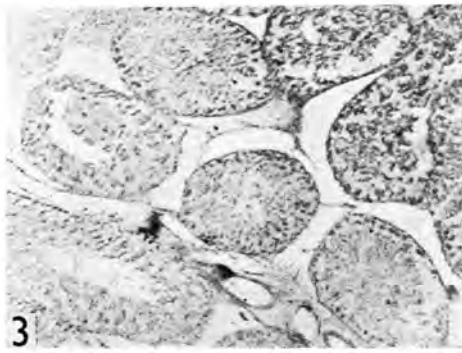
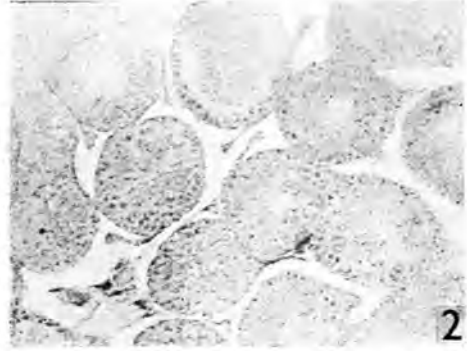
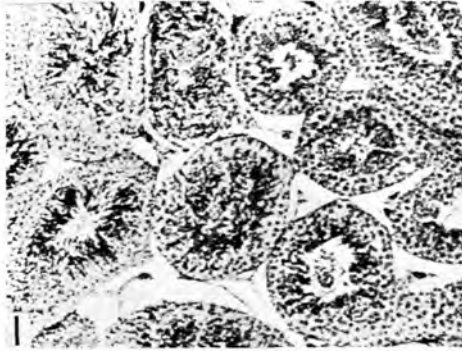
#### EXPERIMENTAL.

The details regarding the allotment of the animals for receiving treatments and the dosage used for different hormones have already been presented in a previous paper of this series (Kar and Ghosh, 1952). The male genital organs of normal and hormone-treated guineapigs were carefully dissected out on autopsy and were processed according to the technique of Gomori (1941) for the demonstration of alkaline phosphatase. The tissue sections were incubated in the substrate mixture for three hours and were finally mounted without any counterstaining.

#### RESULTS.

##### A. Testis.

*Controls:* The basement membrane of the tubules stains positively for the phosphatase. The enzyme activity is present in all the tubular elements. But in the spermatogonia and in the spermatocytes the phosphatase activity is more intense in the nucleus than in the cytoplasm. The Sertoli cells alone show uniform enzyme activity in the nucleus as well as in the cytoplasm (Pl. VII, fig. 1).



Phosphatase is also present in the chromosomes of the dividing tubular elements. Among the intertubular elements the endothelium of the blood vessels and the nucleus of the Leydig cells show intense phosphatase activity. The cytoplasm of the latter gives only a faint reaction for the enzyme.

*Testosterone propionate treatment:* There is a total loss of phosphatase activity from practically all the testicular components (Pl. VII, fig. 2).

*Desoxycorticosterone acetate treatment:* A pronounced loss of phosphatase activity is evident in the testis. However, small amounts of the enzyme are retained in the spermatogonia, the spermatocytes and in the endothelium of the intertubular blood vessels (Pl. VII, fig. 3).

*Progesterone treatment:* An overall reduction in phosphatase activity is evident in the organ. The basement membrane of the tubules stains faintly but the cellular elements show positive reactions for the enzyme. However, the reactions here are much less intense than in the tubular elements of the control animals (Pl. VII, fig. 4). In the interstitium the marked phosphatase activity is retained in the endothelium of the blood vessels but a definite loss is evident in the Leydig cells.

TABLE I.

*The distribution of alkaline phosphatase in the male genital accessories of normal and hormone-treated guineapigs.*

	Controls.	Androgen treated.	DCA-treated.	Progesterone treated.	Estradiol treated.	Stilbestrol treated.	Gonadotrophic hormone treated.
<i>TESTIS</i>							
Basement Membrane ..	++	-	-	+(F)	-	+(F)	+(F)
Seminiferous epithelium ..	++	-	+(F)	+	-	+	+(F)
Interstitium ..	++	-	+(F)	+	-	+	+(F)
<i>SEMINAL VESICLE</i>							
Epithelium ..	++	+	+	+	+	+(F)	++
Fibro-muscular stroma ..	-	-	-	-	-	+(F)	-
<i>PROSTATE</i>							
Epithelium ..	+(F)	-	-	-	++	-	+(F)
Basement membrane ..	-	-	-	-	-	-	-
<i>EPIDIDYMIS</i>							
Epithelium ..	++ <sup>n</sup>	++ <sup>n</sup>	++ <sup>n</sup>	++ <sup>n</sup>	++ <sup>n</sup>	++ <sup>n</sup>	++ <sup>n</sup>
Basement membrane ..	++	+	++	++	++	+	++
<i>VAS DEFERENS</i>							
Epithelium ..	+(F)	-	+(F)	+(F)	+ <sup>n</sup> (F)	++ <sup>n</sup>	+ <sup>n</sup> (F)
Fibro-muscular stroma ..	-	-	-	-	++	++	-

Legend:— + = Positive reaction.  
 ++ = Very strong positive reaction.  
 - = Negative reaction.  
 +(F) = Faint reaction.  
<sup>n</sup> = Only nuclear phosphatase activity.

*Estradiol dipropionate treatment:* Typical castration effects are seen in the testis of the treated animals. Total loss of phosphatase activity is clearly visible in the testicular components.

*Diethylstilbestrol treatment:* The enzymatic responses to this hormone treatment strikingly resemble those elicited by the luteoid. The basement membrane of the tubules gives only a faint reaction for the enzyme (Pl. VII, fig. 5). Moderate nuclear phosphatase activity is retained in the tubular elements. Similar reactions are also seen in the Leydig cells but the endothelium of the interstitial blood capillaries exhibits marked phosphatase activity.

*Gonadotrophic hormone treatment:* Stimulation of some components of the testis is discernible upon histological examination. But a pronounced loss of enzymatic activity is visible in our preparations (Pl. VII, fig. 6; also see Table I).

### B. Seminal vesicle.

*Controls:* Seminal vesicle has an external muscular layer of longitudinal fibres and an internal circular muscle layer. The mucosa is considerably pleated in appearance and its epithelium is of the columnar type. The muscle layers are entirely negative for the phosphatase but spectacular enzyme activity is visible in the mucosa (Pl. VIII, fig. 7). The reactions are so intense that the contour of the epithelial cells is totally obscured by the granular deposits of cobalt sulfide.

*Testosterone propionate treatment:* The muscle layers stain negatively for the enzyme. There is a definite reduction of phosphatase activity from the epithelial cells of the mucosa. This enzymatic loss appears to be more pronounced in the cytoplasm than in the nucleus (Pl. VIII, fig. 8). Marked phosphatase activity, however, is retained in the endothelium of the blood capillaries located in the axial portion of the mucosal pleats.

*Desoxycorticosterone acetate treatment:* Muscle layers give negative reactions for the enzyme. There is only a slight loss of phosphatase activity from the mucosa. This is evident from the fact that the cellular contours are clearly distinguishable in our preparations (Pl. VIII, fig. 9) and not obscured by cobalt sulfide deposits as in the control animals (Table I and text-fig. 1).

*Progesterone treatment:* Muscle layers stain negatively for the phosphatase. The enzymatic responses in the mucosa appear to be the same as in the DCA-treated animals.

*Estradiol dipropionate treatment:* No phosphatase activity is visible in the muscle layers. There is a slight enzymatic loss from the mucosa and the reactions appear to be similar as in the DCA-treated animals.

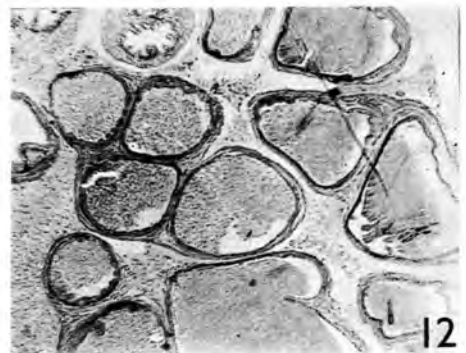
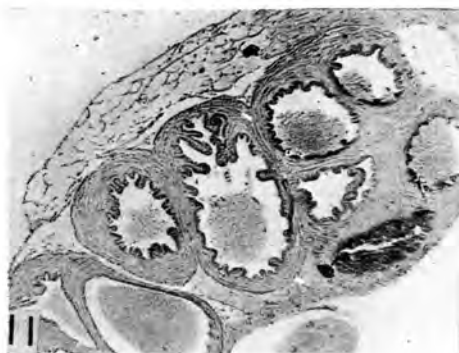
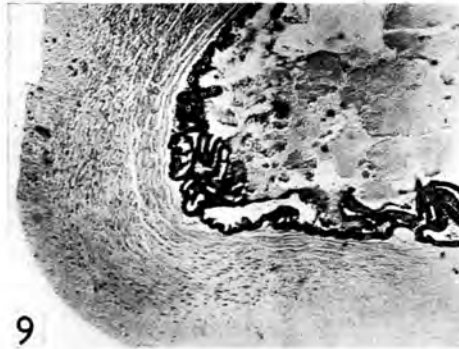
*Diethylstilbestrol treatment:* The organ presents an infantile appearance. The longitudinal muscle layer is negative for the phosphatase but slight reactions are given by the nuclei of the circular muscle fibres. The sub-mucus region shows maximum phosphatase activity. This is evident in the nucleus of the cells in this region as well as in the endothelium of the blood vessels (Pl. VIII, fig. 10). Marked enzyme activity is also seen in the connective tissue extending into the axial portion of the slightly pleated mucosal folds. The epithelial cells of the mucosa, however, give only a faint reaction in the nucleus.

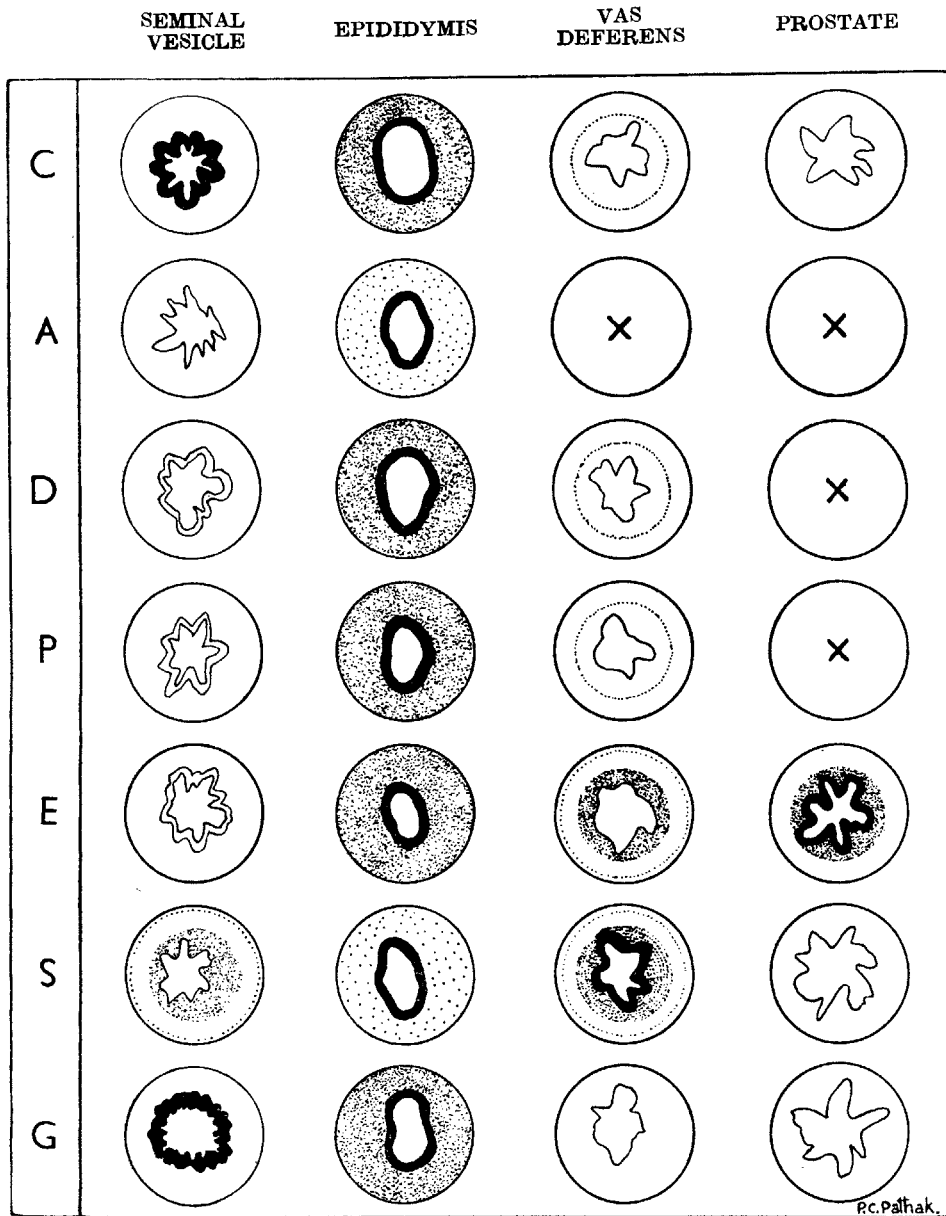
*Gonadotrophic hormone treatment:* Muscle layers stain negatively for the phosphatase. In the mucosa the intensity of reaction is comparable to that in the controls.

### C. Prostate.

*Controls:* No phosphatase activity is seen in either the basement membrane surrounding an alveolus or in the fibro-muscular septum separating two alveoli. Only negligible amounts of the enzyme are present in the columnar epithelial cells of the mucosa (Pl. VIII, fig. 11; Table I and text-fig. 1).

*Testosterone propionate treatment:* Practically no enzyme activity is seen in the prostatic components.





TEXT-FIG. 1. Diagram to show changes in the distribution of alkaline phosphatase in the male genital accessories of normal and hormone-treated guineapigs. Heavy dark line indicates very pronounced phosphatase activity in the mucosa, double thin lines less intense activity, and single thin line mere positive reaction in the same component. Stippled areas indicate locations of stromal phosphatase activity. Closely stippled areas correspond to strong enzyme activity zones and sparsely stippled areas indicate locations where the reactions are much less intense. C, controls; A, testosterone propionate treated; D, desoxycorticosterone acetate treated; P, progesterone treated; E, estradiol dipropionate treated; S, diethylstilbestrol treated; G, gonadotrophic hormone treated; and X, negative reaction.

*Desoxycorticosterone acetate treatment:* The enzymatic response to the corticoid treatment is entirely negative.

*Progesterone treatment:* The phosphatase does not show any response to the luteoid treatment.

*Estradiol dipropionate treatment:* The basement membrane and the inter-alveolar septum stain negatively for the enzyme. Positive reactions for the phosphatase, however, are given by the connective tissue extending into the axial portion of the slightly pleated mucosa (Pl. VIII, fig. 12). The epithelial cells of the mucosa also exhibit prominent phosphatase activity.

*Diethylstilbestrol treatment:* Only moderate reactions for the phosphatase are visible in the nucleus of the epithelial cells of the mucosa (Pl. IX, fig. 13). In other components the enzyme activity is practically absent.

*Gonadotrophic hormone treatment:* The nuclei of the mucosal epithelial cells stain faintly. In other components the phosphatase activity is nil.

#### D. Epididymis.

*Controls:* The basement membrane of the tube stains intensely for the phosphatase (Pl. IX, fig. 14). The nucleus of the epithelial cells shows marked enzyme activity but responses are very slight in the cytoplasm.

*Testosterone propionate treatment:* There is a reduction in phosphatase activity in the basement membrane of the tube but the reactions in the epithelial cells are more or less similar to that in the controls (Pl. IX, fig. 15).

*Desoxycorticosterone acetate treatment:* The basement membrane of the tube and the nucleus of the epithelial cells stain intensely as in the control animals but there is a total loss of phosphatase activity from the cytoplasm of the latter elements.

*Progesterone treatment:* The reactions are more or less similar to those observed in the DCA-treated animals.

*Estradiol dipropionate treatment:* The responses appear to be the same as in the control animals.

*Diethylstilbestrol treatment:* There is a definite loss of phosphatase activity from the basement membrane of the tube as well as from the cytoplasm of the epithelial cells but the nuclear enzyme activity is retained to the control level (Pl. IX, fig. 16; Table I and text-fig. 1).

*Gonadotrophic hormone treatment:* The enzymatic responses are more or less similar to that in the control animals.

#### E. Vas deferens.

*Controls:* The muscle layers are negative for the phosphatase. The endothelium of the blood vessels in the sub-mucosal region and the mucosal epithelial cells show only a faint reaction for the enzyme (Pl. IX, fig. 17).

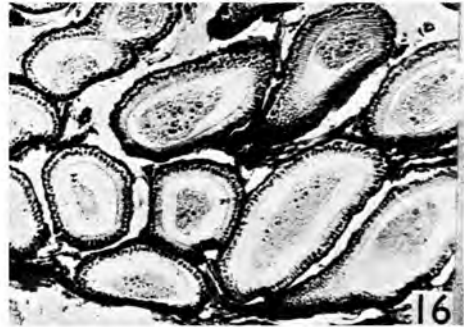
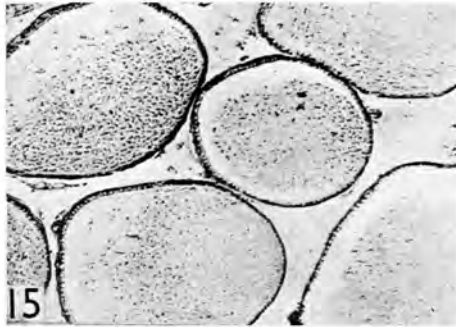
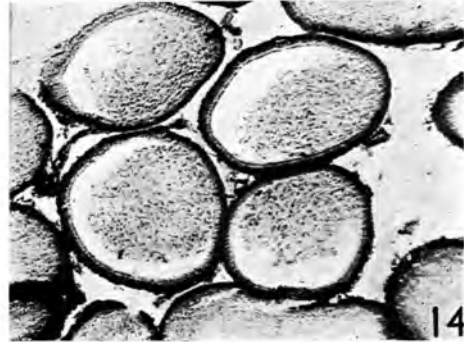
*Testosterone propionate treatment:* The components of the vas deferens are entirely negative for the phosphatase.

*Desoxycorticosterone acetate treatment:* The reactions are more or less same as in the control animals.

*Progesterone treatment:* The phosphatase responses are comparable to those encountered in the vas deferens of the control animals.

*Estradiol dipropionate treatment:* The muscle layers give faint positive reactions for the phosphatase but the sub-mucosal connective tissues and the endothelium of the blood vessels in this region show intense enzyme activity (Pl. IX, fig. 18; Table I and text-fig. 1). Only negligible quantities of the phosphatase are present in the nucleus of the epithelial cells of the mucosa but the cytoplasm is practically negative for the enzyme.





*Diethylstilbestrol treatment:* The nucleus of the epithelial cells shows intense phosphatase activity but in other components the reactions are more or less similar as in the estradiol dipropionate-treated animals.

*Gonadotrophic hormone treatment:* The epithelial cells alone show very faint reactions for the enzyme but the other components are entirely negative for the phosphatase.

#### DISCUSSION.

Our results on the distribution of alkaline phosphatase in the testis and the genital accessories of the guineapig are found to differ in several respects from those reported by the previous authors. Thus, Bern (1949) and Gomori (1941) obtained negative reactions for the enzyme in the interstitial stroma of the testis. We, however, observed phosphatase activity in the nucleus of the Leydig cells and in the endothelium of the interstitial capillaries. According to Bourne (1943) the seminal vesicles are totally devoid of enzyme activity. Bern also noted very faint reactions only in the fibro-muscular wall of the organ. In contrast, we observed intense phosphatase activity in the fibro-muscular wall of the seminal vesicles of this species. The last-named author also reported the epididymal epithelium to be negative for the enzyme but we have unmistakable evidence of strong nuclear phosphatase activity in the epithelium of the same organ. Our findings on the distribution of the enzyme in the vas deferens are practically in agreement with those of Bern and Bourne but differ markedly from those of Zorzoli and Stowell (1947) who demonstrated pronounced phosphatase activity in the nuclei of the mucosal epithelium. It appears probable that these differences in the location and distribution of the enzyme are due to the different periods of incubation employed by the various authors. It is, however, interesting to note in this connection that the picture of phosphatase distribution seen in the guineapig's testis is comparable in every way with that in the adult pigeon (Kar, 1951b).

The hormonal treatments caused considerable loss of testicular phosphatase activity in the guineapig, but among the different hormones used in this study the effects of progesterone and diethylstilbestrol were less severe in this respect than the rest, since appreciable amounts of the enzyme were clearly discernible in some components of the testis. The other hormones in our list inhibited phosphatase activity to such an extent that practically no trace of the enzyme was visible in our preparations. It has recently been reported that DCA inactivates phosphatase activity in the testis of sparrows (Kar, 1951a) and likewise responses are also elicited in the pigeon's testis by the gonadal hormones (Kar, 1951b). If we reckon these findings against those made in this study, we find that the action of these hormones in the two species of vertebrates studied by us are strikingly similar.

The enzymatic response in the genital accessories of the guineapig to steroid hormones brings to light some interesting facts. Thus, testosterone propionate caused a reduction in phosphatase activity in all the accessory organs but DCA exerted no influence whatsoever on epididymis and vas deferens, although the enzyme activity in the seminal vesicles and the prostate was inhibited to a marked extent. Progesterone resembled the corticoid in modifying the phosphatase activity in all the organs but estradiol dipropionate augmented enzyme activity in the prostate and in the vas deferens. However, the ovarian hormone considerably depressed phosphatase activity in the seminal vesicles and did not alter the enzymatic picture of the epididymis. The action of diethylstilbestrol was practically in line with that of the other estrogen, whereas gonadotrophic hormone differed from the steroid hormones in that it failed to evoke any enzymatic response in the accessory genitalia of our material.

It will be evident from the above that of all the hormones used in this study the androgen alone had uniform effects on phosphatase activity of the genital accessories. Now, viewing the situation from a slightly different angle we find

that the actions of DCA and progesterone on enzyme activity in the accessories were remarkably alike and the same was also the case with the estrogens. On two previous occasions we observed that the cortical hormone and progesterone exerted identical influences on the adreno-cortical phosphatase activities in the guineapig and the pigeon (Kar and Ghosh, 1952; Kar, 1951a). We had further noticed that estradiol dipropionate and diethylstilbestrol consistently inactivated alkaline phosphatase in the guineapig's adrenal cortex (Kar and Ghosh, 1952). It is now well known that the chemical structure of the corticoid and the luteoid are great deal alike (Burrows, 1949). The two hormones also have some similarities in their physiological effects (Turner, 1948). On the other hand, the estrogens used by us have lesser chemical affinities but more of physiological likeness (Grollman, 1942). Our studies, therefore, clearly indicate that these hormones with close chemical or physiological similarities (or both) are also cytochemically alike.

#### SUMMARY.

The cytochemical demonstration of alkaline phosphatase activity has been made in the male genital system of the guineapig. Steroid hormones like testosterone propionate, DCA, estradiol dipropionate and the non-steroid gonadotrophic hormone considerably inhibited testicular phosphatase activity, but the effects of progesterone and diethylstilbestrol were less striking in this respect. The phosphatase in the accessory genital organs responded uniformly to androgen but the effects of other steroid hormones were somewhat variable. The gonadotrophic hormone, however, failed to evoke any enzymatic response in the accessory genitalia. The remarkable cytochemical likeness between some hormones is pointed out.

#### ACKNOWLEDGMENTS.

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#### EXPLANATION OF PLATE VII.

*(All figures are photomicrographs and are magnified  $\times 30$ .)*

1. Section through the testis of a control guineapig showing alkaline phosphatase activity.
2. Section through the testis of an androgen-treated guineapig. Note the disappearance of the enzyme.
3. Section through the testis of DCA-treated guineapig. The loss of phosphatase activity is comparatively less than in fig. 2.
4. Section through the testis of a progesterone-treated guineapig. Compare with figs. 2 and 3.
5. Section through the testis of a diethylstilbestrol-treated guineapig. Note the continuation of enzyme activity in some tubular components.
6. Section through the testis of a gonadotrophic hormone treated guineapig. Note the absence of phosphatase activity.

#### EXPLANATION OF PLATE VIII.

*(All figures are photomicrographs and are magnified  $\times 30$ .)*

7. Section through the seminal vesicle of a control guineapig. Note pronounced phosphatase activity in the mucosal pleats.
8. Section through the seminal vesicle of an androgen-treated guineapig. Note the reduction of enzyme activity from the mucosa.
9. Section through the seminal vesicle of a DCA-treated guineapig.
10. Section through the seminal vesicle of a diethylstilbestrol-treated guineapig. Note the presence of phosphatase activity in the fibro-muscular stroma and in the sub-epithelial region.
11. Section through the prostate of a control guineapig. The phosphatase activity is negligible.
12. Section through the prostate of an estradiol dipropionate-treated guineapig. Note the presence of the enzyme in the mucosa.

#### EXPLANATION OF PLATE IX.

*(All figures are photomicrographs and are magnified  $\times 30$ .)*

13. Section through the prostate of a diethylstilbestrol-treated guineapig.
14. Section through the epididymis of a control guineapig. Note the marked phosphatase activity in the basement membrane of the tube.
15. Section through the epididymis of an androgen-treated guineapig. Note the loss of phosphatase activity.
16. Section through the epididymis of diethylstilbestrol-treated guineapig.
17. Section through the vas deferens of a control guineapig.
18. Section through the vas deferens of an estradiol-treated guineapig. Note the presence of pronounced enzyme activity in the sub-epithelial stroma.