HISTOCHEMICAL DEMONSTRATION OF PHOSPHOMONOESTERASE SECRETION IN THE GENITAL TRACT OF THE DOMESTIC COCK

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(Received 8th August 1961)

Summary. Intense acid-phosphatase activity was found in the distal parts of the cells lining the entire length of the vas deferens of the domestic cock and there was evidence that the enzyme(s) is secreted in large amounts into the seminal plasma. Some acid phosphatase(s) was also produced in the vasa efferentia and seminiferous tubules.

Intense alkaline-phosphatase activity was found only in secretions of certain parts of the vasa efferentia, in the tunica intima of all small blood vessels and in intertubular tissue. From the evidence, it is considered that some alkaline phosphatase(s) might possibly diffuse into the seminal plasma from all regions of the genital tract, but it is likely to be small in amount. The significance of the presence of phosphomonoesterases in seminal plasma is discussed.

INTRODUCTION

The seminal plasma of mammals displays a relatively high phosphomonoesterase activity. Some species show greatest activity at an acid pH and others at an alkaline pH, depending on the extent of development of certain accessory reproductive organs. The bulk of the activity is considered to reside in secretions of either the prostate gland or seminal vesicles and is largely non-specific as shown by the wide variety of substrates that are used in its investigations (Mann, 1954).

During the course of studies on fowl semen physiology, Bell & Lake (1960, 1962) and Wilcox (1961) discovered that the seminal plasma of the domestic cock had a relatively high acid-, and some alkaline-phosphatase activity in spite of the absence in this species (Gallus domesticus) of the typical accessory reproductive organs. In view of this observation, a cytochemical study was undertaken to find the origin of the enzyme in the secretions of the male reproductive tract and the results are reported herein. A preliminary account has been given (Lake, 1960).

MATERIAL AND METHODS

Reproductively active Brown Leghorn cocks varying in age between 5 months and 2 years were killed by dislocation of the neck vertebrae and immediately
portions of the testis, epididymal region, vas deferens (upper, middle and lower regions), ejaculatory duct, internal vascular body, cloacal vascular tissue (Nishiyama, 1955; Lake, 1956) and lymph fold were dissected out and fixed in neutral formalin for 6 to 16 hr. (The 10% neutral formalin solution had the following composition: 40% formaldehyde solution 50 ml, distilled water 450 ml, NaH₂PO₄.2H₂O 2-26 g, Na₂HPO₄. 3-25 g.) Portions of the ureter were also examined. Different cocks were killed at various times after semen had been collected from them to ensure that spurious negative results would not be obtained as a result of exhausted secretory epithelia at any given time of examination.

Diazocoupling methods for the investigation of tissue phosphatase activity (Pearse, 1960) were explored. Initially, frozen sections (20 to 30 µ thick) were incubated for 15 to 66 min at 18 to 37°C (depending on tissue and degree of phosphatase activity) in appropriate substrate-dye mixtures at pH 4-9, 5-5 and 8-9. The substrate was always sodium α-naphthyl phosphate. For acid-phosphatase activity, the liberated α-naphthol was coupled with the stable diazonium salts of either 4-benzamido-2:5-dimethoxy aniline (Fast Blue RR salt, Gurr Ltd), α-dianisidine (Fast Blue, Gurr Ltd), p-nitrobenzene-azo-dimethylaniline (Fast Black salt K, Gurr Ltd) or α-amino azotoluene (Fast Garnet GBC salt, I.C.I. Ltd). For alkaline phosphatase, the coupling agents were 4-chloroanisole-2-diazonium chloride (Fast Red RC salt, Gurr Ltd) and the stable diazotate of 5-chloro-α-toluidine (Diazo red TR salt, Light & Co Ltd).

The method finally selected as the most suitable for the more extensive investigation and assessment of the degree of activity of the acid phosphatase in the genital tract was as follows: Sections were incubated at 37°C for 15 min in a substrate medium composed of 100 mg sodium α-naphthyl phosphate and 100 mg Fast Garnet GBC salt or 500 mg of Fast Blue RR salt dissolved in 100 ml 0-2 m-acetate buffer, pH 5-5 or 4-9 (Walpole, 1914). The substrate media were made up 30 min before use with thorough shaking and filtered directly onto the slides in an incubation vessel. At pH 5-5, the site of enzyme activity with the latter salt was indicated by blackish-blue granules, and with the former by brick-red granules. A solution of 0-002 m-sodium fluoride was used in control substrates to inhibit acid-phosphatase activity. Alkaline-phosphatase activity was best investigated at pH 8-9 by the following method: Sections were incubated at 37°C for 20 to 30 min in the filtrate of a solution composed of 0-1 g MgCl₂.6H₂O, 50 mg sodium α-naphthyl phosphate, 500 mg borax and 250 mg of either Diazo Red TR salt or Fast Red RC salt in 110 ml distilled water.

All sections were mounted in glycerine and examined immediately, although it was found that they could be stored at 2°C for at least 2 weeks if necessary.

RESULTS

The chief sites of alkaline- and acid-phosphatase activity in the genital tract of the domestic cock are listed in Table 1. Since it was desired to locate the origin of the phosphatase(s) found in the semen of the cock, a search for
evidence of enzyme secretion was concentrated on the various lining epithelia of the different parts of the genital tract. The immediate subepithelial tissue was also examined closely as there is always a possibility of phosphatase diffusing

**TABLE 1**

**SITES OF PHOSPHOMONOESTERASE ACTIVITY IN THE GENITAL TRACT OF THE DOMESTIC COCK**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Alkaline phosphatase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubules</td>
<td>* (S) Few granules scattered throughout germinal epithelium</td>
<td>**** (S) All located in area of late spermatocytes and spermatids</td>
</tr>
<tr>
<td>Intertubular tissue</td>
<td>***** Diffusely scattered throughout fibrillar and cellular layer surrounding tubules</td>
<td>* Few scattered granules</td>
</tr>
<tr>
<td>Tunica intima of blood vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Epididymal region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short epididymis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vas efferens</td>
<td>**** (S) Present in certain cell groups only</td>
<td>***** (S) Especially in large irregular-shaped tubules</td>
</tr>
<tr>
<td>Subepithelial tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commencement of vas deferens</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vas deferens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All regions (epithelium)</td>
<td>— Perhaps few granules. ** In sinuses and cords of blood vessels</td>
<td>****** (S)</td>
</tr>
<tr>
<td>Subepithelial tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ejaculatory duct</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External, cloacal surface epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood vessels and subepithelium sinuses</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td><strong>Cloacal tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular body (internal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urodaeal erectile vascular tissue:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Surface glandular epithelium</td>
<td></td>
<td>** Mainly in matrix of cells</td>
</tr>
<tr>
<td>(b) Subepithelial tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph folds:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Subepithelial tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other organs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ureter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteries</td>
<td>** (S) In few areas ** (S)</td>
<td></td>
</tr>
</tbody>
</table>

* to ****** = degree of activity  
— = absence of activity  
(S) = secreted into lumen

from these sites to the lumen. Thus, in Table 1 are recorded an assessment of (a) the degree of enzyme activity in these parts, and (b) the likelihood of the enzyme(s) being actively secreted into the lumen. The latter judgement was
made after examining many stained sections of the appropriate tissues and observing the structure and enzyme activity in the apical portions of the cells in the epithelia, and the presence of positive material in the lumina of the ducts. Bern (1949) has discussed the feasibility of differentiating between ‘stromal’ and ‘secretory’ enzyme material in cytological sections of tissues.

TESTIS

The bulk of the acid-phosphatase activity was in the region of the cytoplasm of the spermatocytes and spermatids (Pl. 1, Fig. 1) and from its distribution it appeared that much of it was expelled into the lumen of the seminiferous tubules together with other discarded cytoplasmic material from the germinal epithelium. Smith & Cress (1960) recently demonstrated acid phosphatase in sections of the seminiferous tubules of the domestic cock and provided some evidence of a change in distribution with advancing age.

The bulk of the alkaline phosphatase was distributed amongst the fibrillar components of the intertubular tissue (Pl. 1, Fig. 2). Judging by the type of distribution of alkaline-phosphatase activity, the organization of the basement membrane and adjacent areas of the tubules appeared to be more complex than previous histological work on the testis would lead one to suppose. In addition to large cells, there appeared to be a few interwoven layers of fibrillar material and small connective-tissue cells. It is of interest to note that Lacy & Rotblat (1960) have recently described the boundary of seminiferous tubules in the rat as being comprised of four layers, two fibrillar and non-cellular and two containing cells.

There was some granular alkaline-phosphatase reaction scattered throughout the entire cells of the germinal epithelium and some of it appeared to be expelled into the lumen of the tubule.

EPIIDYMAL REGION

The small tissue mass observed macroscopically at the concave-shaped medial side of each testis in the fowl is referred to as the epididymal region. There is no distinct long coiled epididymis divided into three portions as in the mammals. A general longitudinal section through this region and part of the testis is shown in Pl. 1, Fig. 3. The epithelium of most of the vasa efferentia tubules showed intense acid-phosphatase activity, and it was less intense in the cells of the true short epididymis (Pl. 1, Fig. 4). A large amount of the enzyme material appeared to be shed into the lumen. Subepithelial connective-tissue elements contained no demonstrable acid-phosphatase activity, but there was fairly extensive alkaline-enzyme activity in this area. Intense alkaline-phosphatase reaction was observed in certain isolated cell groups of the vasa efferentia (Pl. 1, Fig. 5), and there was evidence of secretion into the lumen. Cells in the typical short epididymis showed little or no alkaline-phosphatase activity in their cytoplasm.

VAS DEFERENS

The epithelium of all regions of the vas deferens, including the extreme distal sac-like portion, showed the most intense acid-phosphatase activity (Pl. 1,
Fig. 6) in the genital tract. Much of the enzyme material was shed into the lumen as the result of the holocrine- and apocrine-secretory activity of the cells. There was no significant alkaline-phosphatase activity in the epithelium, and there was a little general activity scattered in the form of granules in the subepithelial connective tissue and muscle (Pl. 2, Fig. 7).

**Ejaculatory Duct**

There was neither acid- nor alkaline-phosphatase activity in the internal epithelium of this region of the genital tract. However, there was a little activity in the external folded epithelium, much of which appeared to be at the base of the cells and not secreted (Pl. 2, Fig. 8). The alkaline-enzyme activity was generally sparsely distributed in the connective tissue and especially among the extensive vascular sinuses and capillaries that engorge with blood during erection for copulation.

**Internal Vascular Body, Lymph Folds and Other Erectile Vascular Tissue Surrounding the Urodaeum in the Cloaca**

The external epithelium of the lymph folds showed acid-phosphatase activity in the cells (Pl. 2, Fig. 9), but little of this material appeared to be secreted. The alkaline-phosphatase reaction was sparsely scattered in the connective tissue, chiefly in the region of the numerous tissue spaces.

The two internal vascular bodies, which are spongy red-coloured structures consisting of cell cords, spaces and small blood vessels all surrounded by a capsule, are situated adjacent to the sac-like distal portions of the vasa deferentia, and are surrounded by the internal muscular tissue of the cloaca. They regulate the flow of lymph-like fluid into the lymph folds when the latter erect as part of the complex copulatory organ of the fowl (Nishiyama, 1955). There was scattered alkaline-phosphatase activity only among the cell cords and numerous blood capillaries. The very vascular erectile tissue of the urodaeum of the cloaca, thought to have connexions with the vascular bodies (Lake, 1957), has numerous mucus-secreting cells in the external epithelial glands and acid-phosphatase activity was found mainly confined to the intracellular matrix and little appeared to be secreted (Pl. 2, Fig. 10). Alkaline-phosphatase activity was scattered chiefly in the subepithelial tissue among the numerous blood and tissue spaces.

**Discussion**

It has been possible to demonstrate intense acid-phosphatase activity in certain parts of the genital tract of the domestic cock. Previously (Lake, 1957), it was believed that there was little of this type of enzyme activity present, but this could be accounted for by the fact that the study was made on paraffin-embedded sections with Gomori's technique under conditions where much of the enzyme activity would have been destroyed (Pearse, 1950).

From the evidence obtained in the present study, it is possible, barring selective reabsorption of enzymes en route, that acid-phosphatase activity in the seminal plasma of the domestic cock (Bell & Lake, 1962) is derived from
secretions in the entire length of the genital tract, including the sac-like distal portions of the vasa deferentia. The bulk of the enzyme material is produced in the vasa deferentia and this may be significant in view of the fact that the spermatozoa of the fowl are held in these parts of the genital tract and not in the epididymides as in mammals. Allen & Slater (1957, 1958) showed alkaline- and acid-phosphatase activities in different parts of the extensive epididymal canal of the mouse. They identified several different types of phosphatases, some of which were shown to be dependent on androgen secretion.

The alkaline-phosphatase activity in the genital tract of the cock is in general confined to subepithelial tissues such as connective tissue, tunica intima of small blood vessels and smooth muscle. It is possible that some of this alkaline phosphatase(s) enters the seminal plasma by diffusion through the epithelium. However, there are certain cell groups in the epithelium of the vasa efferentia that appear definitely to secrete alkaline phosphatase directly into the lumen; these cells have previously been shown to secrete Schiff-positive and lipid material (Lake, 1957).

Rollinson (1954) discussed the cytochemical evidence for alkaline- and acid-phosphatase activity in various parts of the reproductive tract of many mammals. In the Zebu bull, acid phosphatase was found mostly in the epididymis, the ampulla and the seminal vesicle, little or none being found in the prostate gland. On the other hand, Wolf, Kabat & Newman (1943) showed that the secretory epithelium of the human prostate displayed intense acid-phosphatase activity, whilst it was less distinct in the epididymis, seminal vesicle and vasa deferens. In man, the cytochemical evidence bears out the quantitative biochemical work on seminal plasma showing the great production of acid phosphatase by the prostate gland. In spite of the cytochemical evidence for the secretion of phosphatases by parts of the male genital tract of mammals other than the seminal vesicles or prostate gland, little or no attention has been given to the separate activities of these enzymes in a consideration of the physiology of semen. In the fowl, with the absence of the seminal vesicles and prostate gland, it should be possible to gain interesting information on the importance of the vasa deferens phosphatase(s) to the viability of spermatozoa in vivo, and also in vitro during semen-storage experiments. In this respect, it is appropriate to make the following remark about the presence of the phosphatase activity in seminal plasma in view of the type of cellular activity in the epithelium of the genital tract. In the germinial epithelium, one finds the extrusion of cells (spermatozoa) and cytoplasmic material taking place, and in the cells of the vasa efferentia and vasa deferentia the holocrine and apocrine types of secretion occur, resulting in the extrusion of secretory products and some cytoplasm. Thus, one might consider that with such processes occurring there would be a release of enzymes more directly implicated in important intracellular metabolic pathways and that their presence in seminal plasma is fortuitous.

ACKNOWLEDGMENTS

I wish to express thanks to I.C.I. Ltd for a generous gift of Fast Garnet GBC salt.
REFERENCES


EXPLANATION OF PLATES

PLATE 1

Fig. 1. Distribution of acid-phosphatase activity in the seminiferous tubules. Note little activity in the basal layers of cells. Fast Blue RR salt. ×49.

Fig. 2. Distribution of alkaline-phosphatase activity in the testis, showing most of the activity confined to the basement membrane and interstitial tissue. Fast Red RC salt. ×49.

Fig. 3. Low power picture of a longitudinal section through part of the testis and the entire epididymal region of the domestic cock. Stained for acid-phosphatase activity with Fast Blue RR salt. S = seminiferous tubules; MR = mediastinum and rete testis; VE = vas efferens; VS = vas deferens. ×5.

Fig. 4. Distribution of acid-phosphatase activity in vas efferens and epididymis proper. Most intense activity in the former. VE = vas efferens. Fast Blue RR salt. ×72.

Fig. 5. Alkaline-phosphatase activity in parts of the vasa efferentia. VE = vas efferens. Note some activity also in the subepithelial tissue. Fast Red RC salt. ×65.

Fig. 6. Intense acid-phosphatase activity in the middle portion of the vas deferens. This type of reaction was also evident in the remainder of the vas deferens including the distal sac-like portion. Fast Blue RR salt. ×42.

PLATE 2

Fig. 7. Portion of the vas deferens showing only slight alkaline-phosphatase reaction in the epithelium and subepithelial tissue. Fast Red RC salt. ×98.

Fig. 8. Longitudinal section of an ejaculatory duct showing a negative acid-phosphatase reaction in the internal epithelium, but a slight reaction in the basal portions of the cells of the external epithelium. Fast Blue RR salt. ×42.

Fig. 9. A portion of the lymph fold in the cloaca of the domestic cock showing some acid-phosphatase activity chiefly in the basal portions of the epithelial cells. Fast Blue RR salt. ×75.

Fig. 10. A portion of the erectile vascular tissue surrounding the urodaeum of the cloaca showing moderate acid-phosphatase activity in the basal portions of the epithelial cells and subepithelial tissue. Fast Blue RR salt. ×75.
[Facing p. 362]