Measurement of pH in the rat epididymis *in vivo*

N. Levine and Helen Kelly

Department of Physiology, New York Medical College, Valhalla, New York 10595, U.S.A.

Summary. Antimony microelectrodes were used to measure pH in rat seminiferous tubules and epididymides *in vivo*. Acidification of fluid leaving the testis occurs primarily in the initial segment and to a lesser extent in the intermediate zone.

Introduction

The pH of the fluid in the seminiferous tubules (Levine & Marsh, 1971) and rete testis of rats (Tuck, Setchell, Waites & Young, 1970) is about 7.4. However, between the testis and the caput epididymidis the pH falls to about 6.5 (Levine & Marsh, 1971). Cohen, Hoffer & Rosen (1976) have demonstrated substantial carbonic anhydrase activity in many of the cells of the epididymis as well as in the efferent ductules. Carbonic anhydrase has been associated with acidification or bicarbonate ion reabsorption in many tissues, although it is apparently not necessary for acidification in all tissues in all circumstances (Giebisch, Malnic, De Mello & De Mello Aires, 1977; Maren, 1977). No actual measurements have been made of the pH of epididymal fluid in the regions of the epididymis before the caput proper (initial segment, intermediate zone) to determine the precise site(s) of acidification. All previous measurements of epididymal fluid pH have been made *in vitro* on fluid collected from the epididymis. In the early regions of the epididymis, where acidification presumably occurs, fluid collection by micropuncture is difficult. In this paper we describe the results of a method for measuring epididymal fluid pH that enabled us to make pH measurements *in vivo* and in hitherto inaccessible regions of the epididymis.

Materials and Methods

Male Sprague-Dawley rats (300-350 g) were anaesthetized with 100 mg Inactin (Byk Gulden)/kg body weight, and the seminiferous tubules or epididymis were prepared for micropuncture and electrical measurements as previously described (Levine & Marsh, 1971). The pH measurements were made *in vivo* in the following regions: seminiferous tubules, beginning of the initial segment just distal to the isthmus, the end of the initial segment, the middle portion of the intermediate zone and the caput distal to the epididymal flexure. The initial segment was considered to be confined to the relatively vascular region just distal to the isthmus (Hamilton, 1975). The pH electrodes used were antimony electrodes with sharply bevelled 20 μ m tips.

An antimony/antimony oxide electrode undergoes the following reaction: $2\text{Sb(s)} + 3\text{H}_2\text{O} \rightleftharpoons \text{Sb}_2\text{O}_3 + 6\text{H}^+ + 6\text{e}^-$ and can, therefore, be used to measure pH (Vieira & Malnic, 1968; Solomon & Albert, 1969; Karlmark, 1973). The electrodes were made by filling borosilicate glass tubing (1.0 mm diameter, 0.2 mm wall thickness) with a molten antimony column a few cm long. After the antimony had solidified, the antimony section of the tube was pulled out in a microburner to about 0.5 mm diameter. An electrode with a tip of 0.5 μ m was then made in a microforge from the narrow antimony-containing tube. Standard micropipette pullers could not be used because the antimony column tended to break. The 0.5 μ m antimony tip was ground to a 20 μ m bevel on an Arkansas stone. After the pipette had been bevelled a copper wire was heat-sealed into the back of the antimony column. The electrode was calibrated by inserting it and a reference glass electrode, filled with 3 M-KCl and connected by a 3 M-KCl-agar bridge to a calomel half-cell, into small droplets of a series of buffers about 0.15 pH units apart. The potential differences were recorded on a Keithley 600B electrometer. The slope of our electrodes

was 50.0 ± 0.1 mV/pH unit at 27°C . In some electrodes the slope changed at pH 7.00 to about 40 mV/pH unit. Similar results were obtained by Vieira & Malnic (1968). Care was always taken to calibrate the electrode in the range of the expected pH measurement.

For pH measurements *in vivo* a correction had to be made for the spontaneous transmural electrical potential difference (see Levine & Marsh, 1971, for discussion). This was accomplished by inserting a 2–5 μm tipped glass microelectrode, filled with 3 M-KCl-agar connected via a 3 M-KCl-agar bridge to a calomel reference electrode, into the lumen with the antimony electrode. The difference in electrical potential between the two electrodes represents the antimony pH potential. It was found that the regions between the seminiferous tubules and the beginning of the caput proper are isopotential and have a small transmural electrical potential of about 5 mV (lumen negative) which is similar to our previous electrical measurements (Levine & Marsh, 1971). The recorded pH potentials were stable for at least 20 min and only those potentials that were stable for at least 2 min were used. To check the accuracy of this type of pH measurement *in vivo* further, a third micropipette filled with a pH 6 phosphate buffer was inserted into the epididymal lumen in one group of rats after the pH measurements had been made. This third micropipette was positioned a few centimeters away from the measuring electrodes and the buffer rapidly perfused around them. The pH of the perfused buffer in 10 such experiments was 6.05 ± 0.04 (s.e.m.) and was not significantly different from the actual buffer pH. All estimates of statistical significance were evaluated by using Student's *t* test.

Results

The results of the pH measurements *in vivo* in the different regions of the epididymis and in the seminiferous tubules are presented in Table 1. Most of the acidification in the epididymis occurred in the initial segment with some further acidification occurring in the intermediate zone. The difference in pH between the beginning and end of the initial segment was highly significant ($P < 0.0005$), but there was no significant difference between the pH of the beginning of the initial segment and that of the seminiferous tubules ($P < 0.15$). The additional acidification that occurred between the end of the initial segment and the intermediate zone was also significant ($P < 0.0025$).

Table 1. Luminal pH (mean $\pm$ s.e.m.) in different regions of the rat epididymis and seminiferous tubules measured *in vivo* with antimony microelectrodes

Region	No. of animals	pH
Seminiferous tubules	8	7.30 ± 0.11
Beginning of initial segment	14	7.20 ± 0.06
End of initial segment	20	6.79 ± 0.03
Intermediate zone	24	6.57 ± 0.08
Caput epididymidis	14	6.64 ± 0.03

Discussion

The results presented in this paper describe the first measurement of pH made *in vivo* in the male reproductive tract and the values recorded in seminiferous tubules and in the caput epididymidis are similar to those previously measured *in vitro* (Levine & Marsh, 1971). However, the technique described here enables measurements to be made in inaccessible but functionally highly active regions of the epididymis and was able to localize precisely the primary site of acidification in the initial segment.

Cohen *et al.* (1976) reported substantial carbonic anhydrase activity in the efferent ductules. The present results show that no appreciable acidification occurs in this region. In most tissues carbonic anhydrase is required for optimal H^+ or HCO_3^- transport, although some transport does continue

when the enzyme is inhibited, especially in the kidney (Giebisch *et al.*, 1977; Maren, 1977). The present technique of measurement of pH *in vivo* allows perfusion studies to be made of rates of acidification in different regions of the epididymis before and after inhibiting carbonic anhydrase activity. These experiments are currently being performed.

Since spermatozoa mature and are stored in the epididymis it is quite possible that the relative acidity of the epididymis is involved in these processes. In addition, epididymal fluid contains primarily organic molecules which may be transported across the epithelium (Levine & Marsh, 1971). Jackson, Schiau, Bane & Fox (1974) demonstrated that for transport of weak electrolytes a transmural pH gradient of 0.5 could substantially affect fluxes across intestinal epithelium. The epididymis maintains pH gradients greater than 0.5 and it is quite possible that alterations in luminal pH could alter the composition of epididymal fluid with profound effects on sperm viability.

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