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THE EFFECT OF TEMPERATURE ON THE POTENTIAL DIFFERENCE AND INPUT RESISTANCE OF RAT SEMINIFEROUS TUBULES

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SUMMARY

1. The p.d. of rat seminiferous tubules was $5{\cdot}86 \pm 0{\cdot}15$ mV, lumen negative, at 33° C and varied linearly with temperature between 24 and 37° C, exhibiting an apparent $Q_{10}^{25-35^\circ C}$ of 2·0 with a slope of 0·43 mV/° C. Exposing testes to a temperature of 37–43° C resulted in an initial hyperpolarization followed by depolarization of the tubules. These changes were more rapid in testes exposed to 41–43° C than in testes exposed to 37–39° C.

2. The R_{in} of seminiferous tubules was $198 \pm 7{\cdot}8$ kΩ at a testis temperature of 33° C. The R_{in} decreased when testes were maintained at 37 and 41° C, the rate of decrease being similar to the rate of depolarization.

3. Exposing testes to deep body temperature by unilateral surgical cryptorchidism caused a reduction of 30 and 64% in tubular p.d. and R_{in} respectively when measured 24 hr after surgery. Exposure to deep body temperature for up to 4 days did not cause any further change in either parameter.

4. There was no evidence that lanthanum penetrated through the Sertoli cell tight junctions after exposing testes to 41° C for up to 30 min.

5. The results indicate that the seminiferous tubule p.d. is maintained by a temperature-sensitive, cellular mechanism. Exposing testes to deep body temperature or above depolarized the tubules and increased their permeability.

INTRODUCTION

A potential difference of 5–7 mV (lumen negative) has been reported for the seminiferous tubules of the rat (Tuck, Setchell, Waites & Young, 1970; Levine & Marsh, 1971). Analysis of fluid withdrawn from the lumen of the tubules indicated that the germinal epithelium was able to elaborate a fluid which differed significantly from blood plasma; it contained relatively high concentrations of potassium and some amino acids,

and low concentrations of sodium and proteins. A number of compounds, including albumen and steroids, possess differential abilities to be transferred from the circulation to the rete testis fluid (RTF) of rams (Setchell, Voglmayr & Waites, 1969) and rats (Waites, Jones, Main & Cooper, 1973); there is thus good physiological evidence for the blood–testis barrier in the rat (see Setchell & Waites, 1975). The morphological nature of this barrier has been investigated using lanthanum as an electron opaque tracer (Dym & Fawcett, 1970). The lanthanum freely entered the basal parts of the germinal epithelium, but was unable to penetrate into the tubule lumen due to specialized tight junctions between adjacent Sertoli cells which form a columnar epithelium. On the basis of this it was suggested that the germinal epithelium could be divided into two compartments: a 'basal compartment' containing the spermatogonia and the Sertoli cells, and an 'adluminal compartment' around and between the Sertoli cells and developing spermatocytes (Dym & Fawcett, 1970).

The deleterious effect of local heating on the testis of scrotal mammals is well established. The first signs of histological damage to the germinal epithelium of a number of species after local heat treatment occur in the pachytene primary spermatocytes (Waites & Ortavant, 1968; Mazzari, du Mesnil due Buisson & Ortavant, 1968; Chowdhury & Steinberger, 1964, 1970; VanDemark & Free, 1970; Samisoni, 1972). Histological damage to the pachytene primary spermatocytes occurred within one hour of immersing rat testis in water at 42° C for 30 min (Blackshaw, Hamilton & Massey, 1973); changes in the distribution of enzymes were observed which appeared to be correlated to this damage, possibly due to the increased fragility of testicular lysosomes after heating (Blackshaw & Hamilton, 1970). It was suggested therefore that the cytoplasmic membranes of the late meiotic prophase of pachytene spermatocytes are damaged by heat so that intracellular ionic imbalance occurs causing mitochondrial damage and activation of lysosomes leading to autolysis of the cell (Blackshaw *et al.* 1973).

The effect of local heat on the blood–testis barrier has not received so much attention. Main & Waites (1973) have reported that heating rat testes to 41° C for up to 90 min produces subtle changes in the blood–testis barrier. Although the sodium, potassium and total protein contents of the RTF were unaffected, the entry of [^{86}Rb] rubidium was almost doubled, and significant reductions were observed in the volume of RTF from heated testes compared with unheated control testes.

In view of the complexity of the blood-testis barrier and the metabolic energy which must be expended for its maintenance, it seems likely that the developing spermatocytes require a specific extracellular medium for their proper development. Although the origin of the transepithelial

potential of the seminiferous tubules has not been established, it is possible that, as in other epithelia (Keynes, 1973; Schultz, 1973*a*), the potential is generated by some metabolically driven rheogenic transport mechanism. This study was undertaken therefore to examine the effects of local heating on the potential and conductance of the germinal epithelium in order to examine the possibility of a heat induced lesion of the blood-testis barrier.

METHODS

Animals. Rats of a Sprague–Dawley strain (ASH/CSE/1, Charles River Ltd, Margate, Kent) weighing 250–400 g were anaesthetized with ethyl carbamate (225 mg/kg, I.P.). The animals were placed in a supine position on a heated rat table and the right testis exposed through a scrotal incision. The testis was delivered into a perspex bath surrounded by a water jacket and the bath filled with 0·9% NaCl. The tunica albuginea was dissected to expose the seminiferous tubules. The temperature of the testis was monitored by a fine thermocouple connected to an electric thermometer (Ellab, Copenhagen) and controlled to within $\pm 0{\cdot}5°$ C by circulating water through the water jacket. The rectal temperature of the rat was monitored by a second thermocouple and maintained at $37 \pm 0{\cdot}5$ °C.

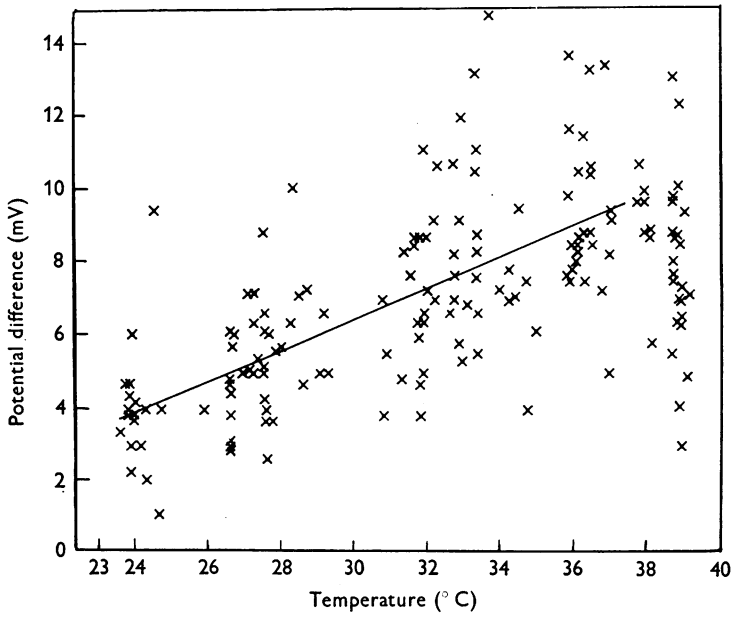
A group of eight rats was anaesthetized with Fluothane (I.C.I. Ltd) and made unilaterally cryptorchid. The left testis was returned to the body cavity by means of a mid line, abdominal incision and secured to the dorsolateral abdominal wall with a single ligature of fine surgical silk; the right testis remained in the scrotum as an experimental control. The effect of cryptorchidism was examined 1, 2, 3 and 4 days after surgery.

Measurement of potentials and resistances. The trans-tubular potential difference (p.d.) was measured with glass micro-electrodes manufactured from pyrex tubing (Joblings H 15/10 glass) on a horizontal micro-electrode puller (C. F. Palmer Ltd, Bucks). The micro-electrodes were filled with 3 M-KCl by the method of Tasaki, Polley & Orrego (1954) and selected for tip potentials of < 1 mV and impedances of 2–5 MΩ. Micro-electrodes were inserted into seminiferous tubules under visual control using a binocular microscope (Olympus) and a micro-manipulator (T.V.C. 500, Research Instruments Ltd). Penetration was made at an acute angle to the tubules and along their longitudinal axes so that the tip of the electrode could be advanced at least 1 mm beyond the site of penetration. The p.d. between the micro-electrode tip and an Ag/AgCl indifferent electrode in the bath was measured with a high input impedance negative capacitance amplifier and an oscilloscope (Tetronix 502 A).

The input resistance (R_{in}) of the seminiferous tubules was measured by injecting a constant current, square-wave pulse through a single micro-electrode and analysing the recorded transient (Schanne & de Ceretti, 1971). The negative capacitance amplifier was adjusted for minimum deformation of the square wave in the 0·9% NaCl bathing solution prior to each measurement of tubule R_{in} and checked after each impalement to ensure that the micro-electrode characteristics had not altered. All oscilloscope traces were photographed (Nihon Kohden PC-2A camera) for analysis, and R_{in} computed from the deformation of the square wave as described by Schanne & de Ceretti (1971).

Only tubules at the surface of the testis were used so that their temperature could be predicted from the bath temperature and penetration was under visual control. The exposed portions of the tubules were only penetrated once to avoid any deleterious effects due to insertion of the micro-electrode.

Histology. In three rats the right testis was immersed at 41 ± 0·5° C while the left testis was simultaneously maintained at 33 ± 0·5° C. The right testis was heated to 41° C for 10, 20 and 30 min in rats 1, 2 and 3; and the left testes were held at 33° C for 35, 45 and 50 min. Testes were fixed by perfusion of glutaraldehyde and lanthanum hydroxide into the spermatic artery for examination of the blood-testis barrier (Dym & Fawcett, 1970). Thin sections were post fixed in osmium tetroxide and examined on an electron microscope (Hitachi, 7S).



Text-fig. 1. The transepithelial p.d. of seminiferous tubules from the right testis of three rats plotted against testis temperature. The curve was fitted by regression analysis of the data obtained between 24 and 37° C.

RESULTS

The effect of temperature on the p.d. of the seminiferous tubules. The p.d. observed in 331 tubules from sixteen rats was 5·86 ± 0·15 mV (± S.E. of mean), lumen negative, at a testis temperature of 33 ± 0·5° C. The range of potentials varied from 2·2 to 11·2 mV.

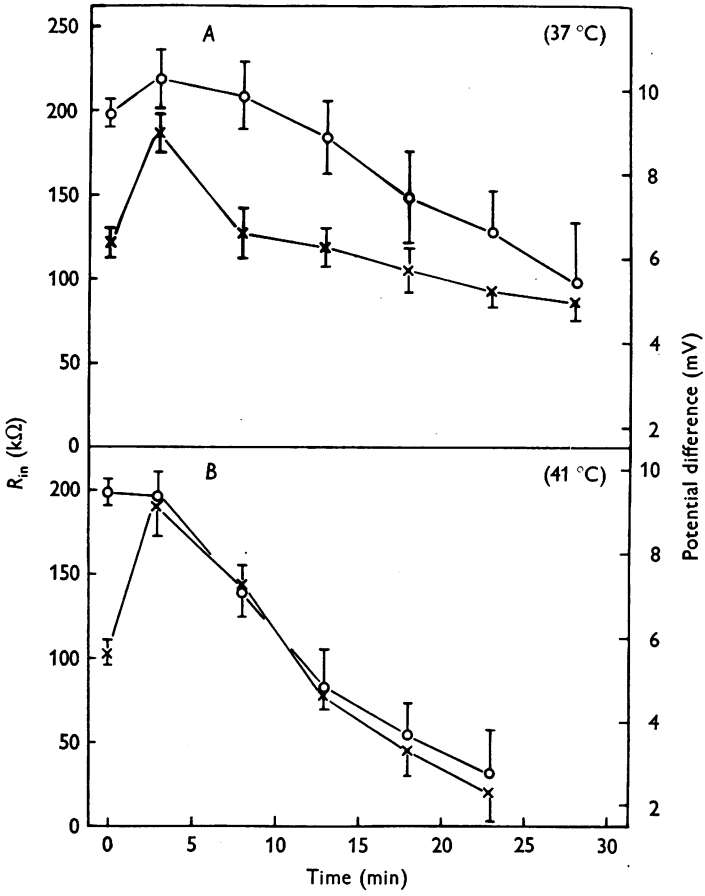
The temperature of one testis was raised from 24 to 39° C at approximately 0·5° C/min in three rats and the p.d. measured at recorded temperatures. The mean p.d. rose from 3·8 mV at 24° C to 9·3 mV at 37° C (Text-fig. 1); the mean p.d. at 39° C was 7·7 mV. Regression analysis of the data obtained between 24 and 37° C gave a linear plot ($P < 0·01$) with a slope of 0·43 mV/° C; and an apparent $Q_{10}^{25-35° C}$ of 2·0.

In a more detailed study the testes of anaesthetized rats were exposed

TABLE 1. The effect of heating the testis of the rat on the p.d. of the seminiferous tubules. All potentials are lumen negative ± S.E. of mean (n)

Controls		Experimental				
Exposure time (min)	32·5° C (mV)	Exposure time (min)	37° C (mV)	39° C (mV)	41° C (mV)	43° C (mV)
		0	6·38 ± 0·34 (41)	5·86 ± 0·52 (40)	5·64 ± 0·30 (40)	6·06 ± 0·27 (56)
0–10	5·74 ± 0·36 (32)					
		0–5	8·93 ± 0·45 (14)	8·15 ± 0·53 (21)	9·26 ± 0·79 (8)	8·91 ± 0·53 (30)
		6–10	6·59 ± 0·60 (27)	6·57 ± 0·51 (26)	7·24 ± 0·51 (28)	6·37 ± 0·39 (43)
30–40	5·57 ± 0·32 (34)					
		11–15	6·26 ± 0·45 (21)	6·02 ± 0·58 (24)	4·70 ± 0·42 (22)	4·87 ± 0·54 (46)
		16–20	5·72 ± 0·54 (19)	4·47 ± 0·38 (23)	3·31 ± 0·63 (20)	3·51 ± 0·37 (36)
60–70	5·75 ± 0·36 (37)					
		21–25	5·29 ± 0·43 (24)	3·08 ± 0·36 (8)	2·30 ± 0·69 (5)	2·98 ± 0·82 (5)
		26–30	5·00 ± 0·41 (24)			
95–105	5·90 ± 0·42 (37)					
		31–35	5·06 ± 0·89 (10)			

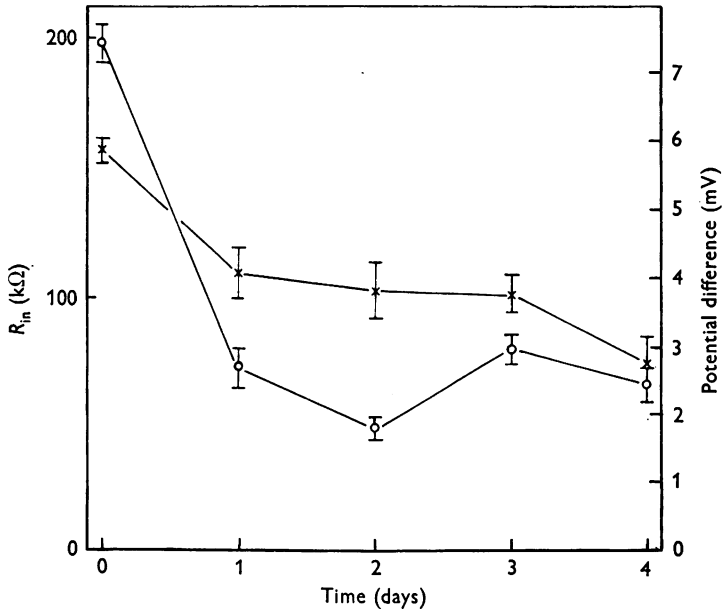
to a constant temperature between 33 and 43° C and the p.d. of the tubules measured against exposure time (Table 1). There was no change in the p.d. of tubules exposed to 33° C for up to 105 min. Exposing testes of 37, 39, 41 or 43° C gave a similar pattern of response in all experiments. The initial phase was a hyperpolarization of the tubules of



Text-fig. 2. The effect of exposing rat testes to (*A*) 37° C, (*B*) 41° C on the p.d. (×) and R_{in} (○) of the seminiferous tubules. Each point represents the mean potential over a 5 min period obtained from seven experiments. Vertical bars represent ± S.E. of mean.

about 3 mV which was maximal in the first 5 min, returning to the control values after 10–15 min. Thereafter the tubules became depolarized, the rate of depolarization being more rapid at 41 and 43° C than at 39° C, and slowest at 37° C.

The effect of temperature on the R_{in} of the seminiferous tubules. The mean R_{in} observed in 103 tubules from fourteen rats was $198{\cdot}0 \pm 7{\cdot}8$ kΩ at a testis temperature of $33 \pm 0{\cdot}5°$ C, the germinal epithelium is therefore an effective barrier to the movement of small ions. The R_{in} of the tubules changed in a similar fashion to the p.d. when their temperature was raised to 37 or 41° C (Text-fig. 2). There was a small initial increase in the mean R_{in} at both temperatures but it was not significantly different from the control values. The R_{in} then fell at a rate similar to that of the depolarization of the tubules at 37 and 41° C. Thus the mean R_{in} was only 28 % of the mean control value after 16–20 min at 41° C but still 68 % of the mean control value after 16–20 min exposure to 37° C.



Text-fig. 3. The effect of unilateral cryptorchidism on the p.d. (×) and R_{in} (○) of the seminiferous tubules. Each point represents the mean of fifty-three to sixty-four measurements on two rats. Vertical bars represent ± S.E. of mean.

The effect of cryptorchidism on the p.d. and R_{in} of the seminiferous tubules. The p.d. and R_{in} of the control testes in the scrotum of eight unilaterally cryptorchid rats did not differ significantly from the control values given above. The p.d. of the cryptorchid testes after 24 hr at body temperature was 4·1 mV, a significant reduction ($P < 0{\cdot}02$) of 30 % relative to the p.d. of tubules in the control testes of the same rats (Text-fig. 3). Measurements made 2, 3 and 4 days after returning testes

to the abdomen indicated that the p.d. of the tubules did not decrease significantly beyond this value, although a constant but slight trend to depolarization was evident. The R_{in} was reduced from 198 to 71·3 kΩ, i.e. to 36 % of its control value by 24 hr at body temperature ($P < 0{\cdot}001$). Exposure to body temperature for 2, 3 or 4 days had no further significant effect on the R_{in}.

The effect of temperature on the blood–testis barrier. Thin sections of each testis were examined in the electron microscope. The appearance of sections from the control testes was as described by Dym & Fawcett (1970), with lanthanum largely excluded by tight junctions between myoid cells. Lanthanum occasionally penetrated the myoid layer to the interspaces of the basal regions of the Sertoli cells but was always excluded from the lumen by tight junctions between adjacent Sertoli cells. In heated testes the incidence of lanthanum penetration of the myoid layer was much greater, but the ability of the Sertoli cell tight junctions to exclude lanthanum from the lumen was not impaired (see Pl. 1). In no section was lanthanum observed to penetrate the adluminal compartment.

DISCUSSION

The mean p.d. of 5·86 ± 0·15 mV (lumen negative) measured in these experiments was similar to the values reported before for the seminiferous tubules of the rat (7·4 ± 0·5 mV, Tuck *et al.* 1970; 4·8 ± 0·1 mV, Levine & Marsh, 1971). The p.d. varied in a predictable manner with temperature, increasing by 0·43 mV/° C between 25 and 35° C. This is much greater than predicted by the Nernst equation and suggests a cellular origin for the transepithelial p.d. This is of particular interest in view of the sensitivity of testicular function to elevated temperature. Exposing testes to temperatures of 37–43 °C caused an initial hyperpolarization followed by a depolarization of the seminiferous tubules, these changes occurred more rapidly at the higher temperatures (Table 1). The initial hyperpolarization of the tubules is of short duration and probably represents thermal stimulation of the metabolism of the tubules, since it is too large to be accounted for by thermal effects on diffusion or equilibrium potentials. The depolarization observed during the later phase of the experiments could be explained in several ways: a partial break-down of the tight junctions between adjacent Sertoli cells, an increase in the passive permeability, inactivation of an active rheogenic pump mechanism, or a combination of these.

The R_{in} of seminiferous tubules was 198·0 ± 7·8 kΩ at a testis temperature of 33° C. Tubule R_{in} fell on exposing the testes to 37–41° C (Text-fig. 2) indicating an increase in the permeability of the germinal epithelium

to small ions. This would allow ions to move down their electrochemical gradients, so depolarizing the tubules, it is therefore not surprising that the R_{In} and p.d. fell at very similar rates on exposing the testes to local heat.

The seminiferous tubules are known to secrete a fluid into the lumen (Tuck *et al.* 1970; Setchell & Waites, 1971; Cheung, Hwang & Wong, 1976). The primary secretion of tubular fluid contains high potassium and bicarbonate and low sodium and chloride concentrations relative to the plasma (Tuck *et al.* 1970). These ions must traverse the germinal epithelium in one of two ways, either a transcellular movement through the basal and adluminal cell membranes of the Sertoli cells, or via an extracellular ('shunt') pathway (Schultz, 1973*b*). The observed increase in conductance on exposing the seminiferous tubules to heat could be caused by increased 'shunt' conductance and/or an increase in the membrane conductance of the Sertoli cells. The failure of lanthanum to penetrate the shunt pathway between adjacent Sertoli cells, even after extreme heat treatment (41° C for 30 min) indicates the tenacity of these tight junctions. Whilst this cannot be taken to mean that there is no increase in the conductance of the shunt pathway, it seems probable that the large increase in epithelial conductance observed must involve an increase in the conductivity of the Sertoli cell membranes. This could occur exclusively in the basal or adluminal membrane, or in both.

The volume of fluid secreted by the testis is known to be reduced during and following local heating or cryptorchidism (Setchell, 1970; Main & Waites, 1973). Secretion is abolished in *in vitro* preparations of rat seminiferous tubules by temperatures below 25° C (Cheung *et al.* 1976). These results suggest that exposing seminiferous tubules to conditions which reduce fluid secretion also depolarize the tubules. It is therefore plausible to argue that the origin of the transepithelial p.d. is a rheogenic ion pumping mechanism, concerned with the movement of ions and water into the tubules. The inactivation of such a mechanism by exposing testes to temperatures of 37–43° C could increase the ionic permeability of the germinal epithelium, leading to depolarization of the tubules and a reduction or cessation of tubular fluid secretion. This would cause a deleterious change in the environment of the developing spermatocytes.

It is possible that the primary heat lesion is hypoxia. In the ram, testicular blood flow is not altered by temperatures of 40–41° C for periods sufficient to damage spermatogenesis, but oxygen uptake by the testis and epididymis is increased by about 70% (Waites & Setchell, 1964; Linzell & Setchell, 1969). In the rat testicular blood flow was reasonably constant at temperatures between 28 and 40° C (Waites,

Setchell & Fowlar, 1968; Waites, Setchell & Quinlan, 1973) while oxygen uptake doubled in a similar fashion to ram at testis temperatures of 41° C (Main, 1975). It is therefore possible that hypoxia in the germinal epithelium is a primary lesion of heat inactivation of the testes, and that the depolarization and associated secretory changes of heated seminiferous tubules result from metabolic dysfunction due to hypoxia.

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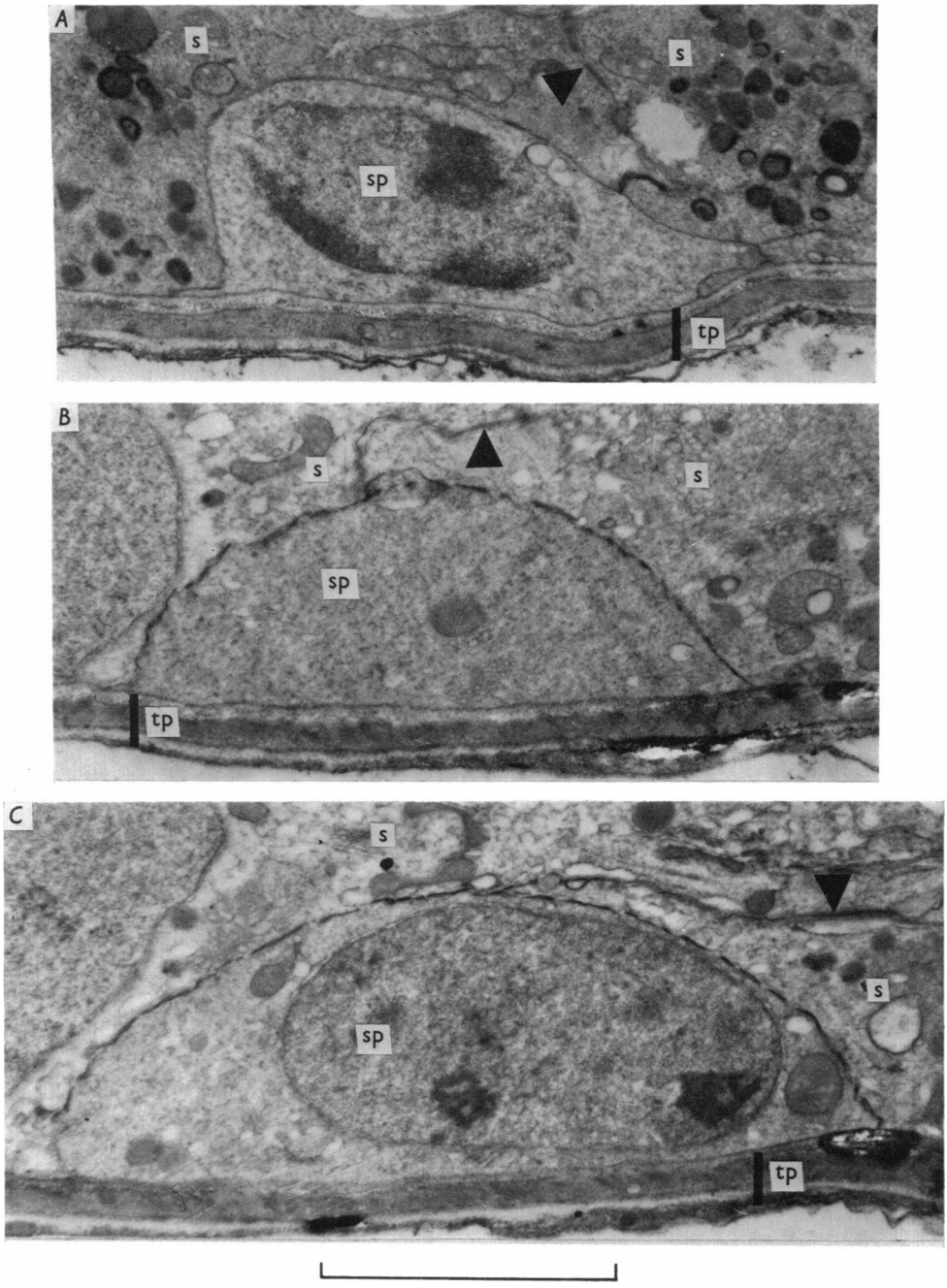

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EXPLANATION OF PLATE

Electron micrographs of seminiferous tubules from lanthanum perfused rat testes showing spermatagonia (sp) and Sertoli cells (s) resting on the tunica propria (tp). The intercellular spaces around the spermatagonia of unheated testes were frequently free of lanthanum (*A*) but in heated testes (*B* and *C*, 41° C) normally contained the electron-dense marker (*B* and *C*). Lanthanum was never observed to penetrate the tight junctions (arrows) between adjacent Sertoli cells in heated or control testes. Calibration bar 5 μm.