The blood–testis barrier and temperature damage to the testis of the rat

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Summary. The integrity of the blood–testis barrier was investigated during and after local heating of rat testes sufficient to produce a temporary cessation of spermatogenesis. The flow, ionic composition and protein content of rete testis fluid (RTF) collected from testes maintained at 33 or 41°C were unaffected either at the time of treatment or up to 2 days later when the major cytological consequences of heating occurred. The normally low rate of transfer of albumin from blood to RTF was unaffected during and after heating. Transfer constants for radioactive K, Rb, Na and lysine consistently increased during heating although there were time-dependent differences between the patterns of response for each molecule. The normally rapid transfer of testosterone was unaffected by heating, but the entry rates of radioactivity into RTF after the infusion of more slowly diffusing steroids were enhanced at 41°C. The clearest effects of heating were an approximate doubling in the uptake of oxygen and decrease in the net synthesis of protein by the testis. It is concluded that heating sufficient to damage spermatogenesis was not associated with dramatic alterations in the integrity of the blood–testis barrier but more with changes in testicular metabolism.

Introduction

In the male mammal, meiosis and spermatid differentiation occur inside a continuous barrier formed by tight junctions between the Sertoli cells of the seminiferous tubules (see Fawcett, 1975; Setchell & Waites, 1975, for references). Small molecules, such as ethanol and urea, are able to cross freely into this adluminal compartment of the germinal epithelium (Setchell, Voglmayr & Waites, 1969) and a specific carrier mechanism exists for the uptake of glucose (Middleton, 1974). However, most serum proteins, and smaller molecules of the type normally excluded from intracellular spaces (including inulin, sucrose and ⁵¹Cr-labelled EDTA), are almost entirely prevented from entering the fluid inside the seminiferous tubules and rete testis. This differential permeability is termed the ‘blood–testis barrier’ and is formed before the first meiotic divisions occur (Setchell, 1970a; Vitale, Dym & Fawcett, 1973). If the barrier is disrupted, as for example after iso-immunization with testis homogenates (Johnson, 1970, 1972) or by administration of small doses of cadmium salts (Koskimies, 1973a,b), then so also is spermatogenesis.

The testis is damaged by small rises from its normal temperature (see VanDemark & Free, 1970, for references). Since the nature of thermal damage is poorly understood it is of interest to establish whether the permeability of the ‘blood–testis barrier’ is affected by local heating in such a way as to contribute to the consequent damage to spermatogenesis. Preliminary results have been reported previously (Main & Waites, 1973).

Materials and Methods

Adult rats of the Sprague–Dawley strain (240–400 g; Charles River (U.K.) Ltd) were used. They had free access to food and water and were kept at a temperature of 23°C in 12 h light/12 h darkness.

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Preliminary experiments

Groups of 4–6 rats were anaesthetized with sodium pentobarbitone (50 mg/kg body weight, i.p.). The scrota were immersed in a temperature-controlled water bath. In this way the testes were exposed for 1 h to temperatures from 38 ± 0.1 to $41 \pm 0.1^\circ\text{C}$. Control animals were exposed to 33°C . Subcutaneous scrotal and rectal temperatures were monitored during heating by means of thermistor probes (Grant Instruments Ltd, Toft, Cambridge, U.K.). Testicular wet weight and histological appearance were observed at autopsy, 1, 2, 3, 4, 10 and 21 days after heating.

The proportion of the cardiac output received by the testis was obtained by an indicator fractionation technique (Sapirstein, 1958) using ^{125}I -iodoantipyrine. Simultaneously an index of vascular permeability was obtained by measuring the uptake by the testis of ^{131}I -labelled human serum albumin from the blood (Waites & Setchell, 1966). Measurements were made either at the end of the period of heating or up to 4 days later. In some animals samples of blood and rete testis fluid (RTF, see below) were collected 2 days after heating.

The concentrations of sodium and potassium in plasma and RTF were measured by flame photometry and that of total protein by the method of Lowry, Rosebrough, Farr & Randall (1951).

Experiments on the 'blood-testis barrier'

At a preliminary operation rats were anaesthetized by inhalation of a halothane: oxygen mixture and the testes were exposed through a midline abdominal incision. The efferent ducts were ligated with fine braided silk sutures at their point of origin from the tunica albuginea. The testes were then returned to the scrotum, the incision closed and the animals allowed to recover. Between 18 and 24 h later, the rats were anaesthetized with sodium pentobarbitone and polyvinylchloride cannulae were placed in one jugular vein and the opposite common carotid artery. Each testis was brought out through a scrotal incision to lie immersed in a bath of liquid paraffin. The baths were constructed with water-heated walls so that the temperature of each testis could be controlled independently.

Cannulae were then inserted into each rete testis (Tuck, Setchell, Waites & Young, 1970) and RTF collected into glass capillary tubes. With one testis maintained at 33°C (control) and the other at 37 or 41°C , a number of radioactively labelled molecules (see Table 1) were introduced into the blood stream through the jugular venous cannula. Levels of radioactive tracer were assayed, by appropriate gamma or liquid scintillation counting methods, in samples of whole arterial blood or

Table 1. Radioactively labelled molecules obtained from the Radiochemical Centre, Amersham, and used in this study

Molecule	Specific activity as supplied (mCi/mmol unless otherwise stated)
^{125}I -labelled human serum albumin	2.5 $\mu\text{Ci}/\text{mg}$ albumin
^{131}I -labelled human serum albumin	20–80 $\mu\text{Ci}/\text{mg}$ protein
$^{86}\text{RbCl}$	1–12 mCi/mg Rb
^{42}KCl	16 $\mu\text{Ci}/\text{mg}$ K
$^{24}\text{NaCl}$	340 $\mu\text{Ci}/\text{mg}$ Na
L-[5- ^3H]Arginine hydrochloride	5000
L-[4,5- ^3H]Leucine	30,000
L-[U- ^{14}C]Lysine hydrochloride	312
L-[4,5(n)- ^3H]Lysine hydrochloride	5000
L-[2,3- ^3H (n)]Valine	10,000
[7(n)- ^3H]Androst-4-ene-3,17-dione	3000
[1,2- ^3H]Cortisol	40–60
[7- ^3H]Dihydrotestosterone	—*
[2,4,6,7(n)- ^3H]Oestradiol-17 β	80,000–110,000
[7(n)- ^3H]Pregnenolone	10,000–25,000
[7(n)- ^3H]Progesterone	5000
[1,2,6,7(n)- ^3H]Testosterone	80,000–110,000

* Sp. act. unknown; gift from Dr B. J. A. Furr.

plasma and whole or cell-free RTF collected at 5–15 min intervals over 1–1.5 h (and occasionally up to 5 h) after cannulation of the rete. Plasma and RTF were immediately separated from cells by centrifugation. When radiolabelled amino acids were infused the samples were deproteinized by precipitation with 6% perchloric acid before radioactivity estimation.

The entry of radioactive molecules into RTF was assessed by plotting the ratio of radioactivity concentration ($(\text{d/min})/\mu\text{l}$) in RTF to that in plasma collected simultaneously. When the equilibrium concentrations in RTF and plasma were known a quantitative index of exchange rate was obtained by calculating the transfer constant (k_{out}) from the relationship: $\log_e (1 - C_{\text{RTF}}/r_{\text{eq}} C_p) = -k_{\text{out}} t$ (Davson, 1967), in which C_{RTF} = concentration of marker molecule in RTF, C_p = concentration in plasma, r_{eq} = equilibrium ratio of concentration of marker in RTF to that in plasma, k_{out} = transfer constant (min^{-1}), and t = time (min) after raising the circulating level of the marker under study. Following the sudden elevation of the level of a marker molecule in the blood stream, the function $\log_e (1 - C_{\text{RTF}}/r_{\text{eq}} C_p)$ plotted against time gives a straight line of slope equal to $-k_{\text{out}}$. As equilibrium is approached this plot deviates from linearity and therefore only the initial slope after raising the plasma concentration of the marker molecule must be considered. When C_{RTF}/C_p reaches half the value of r_{eq} , then $1 - C_{\text{RTF}}/r_{\text{eq}} C_p = 0.5$ and the half-time for turnover may be calculated.

The transfer constant k_{out} is related to the kinetic rate constant for exchange between plasma and RTF, and is affected by concentration if any saturable carrier transport mechanism is involved. The values obtained remain independent of concentration only for passively transferred molecules whose diffusion is not limited by blood flow. However, in our experiments, by using radioactively labelled molecules of high specific activity, the normal concentrations of the substances under study were not appreciably altered. The simple transfer constant was therefore used as an empirical measure of the effects of temperature on transfer rates between plasma and RTF.

The effect of temperature on testis metabolism in anaesthetized rats

Oxygen consumption. Polyvinylchloride cannulae were placed in a carotid artery and a major subcapsular vein draining each testis. One testis was kept at 33°C and the other at 41°C for up to 1.5 h as described above. Samples of arterial and testicular venous blood (10 μl) were collected at approximately 15 min intervals and the oxygen contents determined (Tazawa, 1970).

Net protein synthesis. Net protein synthesis in the testis was estimated by measuring the incorporation of radioactivity into ethanol-precipitable material 1–1.5 h after injection or infusion of ^3H - and ^{14}C -labelled amino acids (see Table 1). One testis of each anaesthetized rat was maintained at 33°C serving as a control for the contralateral organ kept at 41°C. At the end of each period of treatment an arterial blood sample was taken, both testes were removed, weighed and quickly frozen to -20°C . Blood plasma was separated at once and also stored at -20°C . Upon thawing, plasma and decapsulated testes were homogenized in four volumes of absolute ethanol. The homogenates were centrifuged and the supernatant and precipitate were extracted with chloroform:methanol (2:1 v/v) to remove lipid-soluble material. The radioactivity remaining in the precipitates was designated 'protein-bound' while that remaining in the corresponding supernatant was termed the 'free amino acid' fraction. At least 70% of the total supernatant radioactivity ran with authentic infused amino acid when chromatographed on cellulose thin layers in *n*-butanol:acetic acid:water (80:20:20 by vol.) and 96% ethanol:34% ammonia (70:30 v/v).

Results

Temperature and testicular weight

Heating the testis of a rat to 41°C for 1 h damaged late primary spermatocytes and young spermatids (Setchell & Waites, 1972) and caused a marked fall in testicular weight within 2 to 4 days (Table 2). There was no significant effect on either the weight or histological appearance of the testis when lower temperatures (38–40°C) were applied for 1 h.

Table 2. Testis weights of rats (mean $\pm$ S.E.M.; 4–8 animals per group) up to 21 days after maintaining the scrotum at 33°C (control) or 41°C (heated) for 60 min

Days after treatment	Testis wt (g)	
	Control	Heated
0	1.59 $\pm$ 0.03	1.60 $\pm$ 0.06
1	1.69 $\pm$ 0.04	1.42 $\pm$ 0.06
2	1.64 $\pm$ 0.04	1.36 $\pm$ 0.02
3	1.62 $\pm$ 0.06	1.22 $\pm$ 0.04
4	1.49 $\pm$ 0.05	1.07 $\pm$ 0.02
10	1.71 $\pm$ 0.02	0.96 $\pm$ 0.05
21	1.73 $\pm$ 0.04	0.89 $\pm$ 0.05

Vascular response of the testis to heating

The fraction of the cardiac output perfusing the testes of rats was significantly greater when the scrotum was immersed for 1 h at 41°C than at 33°C (Table 3). The increase observed was small (36%) and confirmed previous observations that the testicular vascular bed is relatively unresponsive to increased temperature (Waites & Setchell, 1964; Glover, 1966; Waites, Setchell & Quinlan, 1973). Vascular permeability in the testis, as indicated by uptake of ^{131}I -labelled albumin, was unaffected by the local heating applied. Blood flow and vascular permeability were at control levels within 6 h of heating and remained so for 2 days. By 4 days after heating, when testicular weight had fallen to about half the initial and the control values, blood flow per unit weight was still in the normal range (Table 3). The statistically significant ($0.02 < P < 0.05$; Student's *t* test) difference between heated and control groups for the vascular permeability index at 4 days after treatment (Table 3) may be artefactual, because the index is obtained by measuring whole tissue uptake of ^{131}I -labelled albumin, which is excluded both from rete testis fluid (see below) and the seminiferous tubules (Setchell & Wallace, 1972; Setchell, Hinton, Jacks & Davies, 1976). The loss of seminiferous tubular

Table 3. Relative blood flow and an index of vascular permeability in the testes of rats after exposure for 1 h to 41°C (heated) or 33°C (control)

Time after heat exposure	Testis	Relative blood flow†	Vascular permeability index‡
0 h	Heated	0.98 $\pm$ 0.05**	1.5 $\pm$ 0.2
	Control	0.72 $\pm$ 0.06	1.8 $\pm$ 0.3
6 h	Heated	0.82 $\pm$ 0.07	2.0 $\pm$ 0.4
	Control	0.86 $\pm$ 0.03	1.8 $\pm$ 0.2
1–2 days	Heated	0.87 $\pm$ 0.05	1.8 $\pm$ 0.2
	Control	0.90 $\pm$ 0.08	1.6 $\pm$ 0.2
4 days	Heated	0.99 $\pm$ 0.14	2.0 $\pm$ 0.2*
	Control	1.10 $\pm$ 0.09	1.4 $\pm$ 0.2

Values are mean $\pm$ S.E.M. of 4–5 observations/group. Significantly different from control value, * $P < 0.05$; ** $P < 0.01$ (Student's *t* test).

† Relative blood flow (RBF: Waites & Setchell, 1966), using ^{125}I -iodoantipyrine:

$$\text{RBF} = \frac{\text{tissue } ^{125}\text{I ((ct/min)/mg)}}{\text{whole body (injected dose) } ^{125}\text{I ((ct/min)/mg)}}$$

‡ Vascular permeability index (VP), using ^{131}I -labelled human serum albumin:

$$\text{VP} = \left(\frac{\text{tissue } ^{131}\text{I ((ct/min)/mg) / blood } ^{131}\text{I ((ct/min)/mg)}}{\text{RBF}} \right) \times 10^2.$$

components of the testis after heating would have reduced the proportion of the testis unavailable to albumin and account for the observed increase in uptake of radioactive albumin.

RTF composition and flow rate

RTF flow from heated (41°C) and control (33°C) testes declined approximately exponentially after cannulation and approached the secretion rate of 0.33 $\mu\text{l}/\text{testis min}^{-1}$ (Setchell & Waites, 1971; Main, 1976) after 1.5–2.5 h (Text-fig. 1). Although RTF tended to flow more slowly from heated

Table 4. The concentrations of sodium, potassium and protein in rete testis fluid (RTF) collected from anaesthetized rats (a) during maintenance of the testes at 33°C (control) or 41°C (heated), or (b) during maintenance of testes at 33°C 2 days after exposure to 33 or 41°C

	Time (min) after rete testis cannulation	Treatment	Sodium (mmol/l)	Potassium (mmol/l)	Protein (mg/ml)
(a) Heat exposure during RTF collection	0–30	Control	139 $\pm$ 4	11.9 $\pm$ 0.6	1.49 $\pm$ 0.31
		Heated	142 $\pm$ 3	12.9 $\pm$ 0.7	1.85 $\pm$ 0.42
	31–60	Control	138 $\pm$ 6	11.9 $\pm$ 0.5	1.71 $\pm$ 0.41
		Heated	146 $\pm$ 4	13.7 $\pm$ 0.7	1.77 $\pm$ 0.69
	61–90	Control	149, 142	12.2, 12.8	1.28
		Heated	152	13.8	1.35
(b) Heat exposure 2 days before RTF collection	0–90	Control	156 $\pm$ 3	17.0 $\pm$ 1.3	1.61 $\pm$ 0.20
		Heated	153 $\pm$ 4	15.9 $\pm$ 0.7	2.26 $\pm$ 0.22

Values are single samples or the mean $\pm$ S.E.M. of 4–5 samples from 5 rats. No significant differences ($P > 0.05$) between heated and control groups by Student's *t* test.

Text-fig. 1. The flow rate of RTF from cannulae placed in rat testes maintained at 33°C (○) or 41°C (●). Arrow indicates the time of cannulation and the start of heating. The horizontal broken line represents the secretion rate of testicular fluid estimated by weight increase after efferent duct ligation (Main, 1976). Vertical bars indicate the S.E.M. for pooled data from 20 rats.

Text-fig. 2. The entry of radioiodinated human serum albumin into rete testis fluid (RTF) of rats. (a) Levels of radioactivity in whole arterial blood (●) and RTF (○) up to 5 h after cannulating the rete testis of gonads maintained at 33°C and injecting 125 I-labelled albumin intravenously. The sample of RTF labelled with an asterisk was slightly contaminated with red blood cells: all other samples were free of blood contamination. (b) A similar experiment with 131 I-labelled albumin and RTF collection from testes maintained at 33°C (○) or 41°C (▲).

testes, this difference was not statistically significant overall. The concentrations of sodium, potassium and total protein in RTF remained constant over the collection period and no significant differences were observed between heated and control testes (Table 4a). The amount of fluid collected from the testes over the 2.5 h period after cannulation was approximately 90% of the estimated volume of fluid accumulating after ligation of the efferent ducts 18–24 h previously. There was a slight rise in total protein concentration after 2 days but this was not statistically significant (Table 4b).

Temperature and exchanges between blood and RTF

Albumin. When radioiodinated human serum albumin was injected i.v. radioactivity in RTF was barely detectable up to 5 h after rete testis cannulation in testes maintained at 33°C. There was no increase in the amount of radioactivity when RTF was collected from testes maintained at 41°C for up to 2 h (Text-fig. 2).

Text-fig. 3. Effect of local heating to 41°C on the entry of (a) ^{24}Na , (b) ^{86}Rb , (c) L-[^3H]lysine and (d) ^{42}K into rete testis fluid (RTF) of rats. t = radioactivity ((d/min)/ μl) in RTF from heated testes divided by mean radioactivity in plasma ((d/min)/ μl) during the sampling period and c is the same ratio for RTF collected simultaneously from contralateral control testes maintained at 33°C . A value of t/c (ordinate) exceeding 1.0 indicates an increased rate of entry due to heating. Points represent means $\pm$ S.E.M. from 4–5 animals for each molecule. Time 0 = start of heating and infusion.

Potassium, rubidium, sodium and lysine. These molecules entered RTF from the blood at moderate rates in control testes maintained at 33°C and more rapidly at higher temperatures; transfer constants (Davson, 1967) consistently increased between 33°C and 41°C (Table 5). However, a complex picture emerged when the apparent heat-induced stimulation of transport was plotted against time (Text-fig. 3). A maximum effect occurred at 10–15 min after starting heating and the infusion of

Table 5. Effect of temperature on the transfer constant (k_{out}) for ^{24}Na , ^{42}K , ^{86}Rb and [^3H]lysine between rete testis fluid and plasma of rats

Molecule	Transfer constant ($k_{\text{out}} \times 10^2$)		
	33°C	37°C	41°C
Sodium (^{24}Na)	0.33 ± 0.02 (4)	$0.45 \pm 0.05^*$ (3)	$0.53 \pm 0.05^{**}$ (4)
Potassium (^{42}K)	0.33 ± 0.03 (4)	—	0.44 ± 0.09 (4)
Rubidium (^{86}Rb)	0.31 ± 0.03 (8)	0.47 ± 0.11 (3)	$0.54 \pm 0.11^*$ (5)
L-[4,5- ^3H]Lysine	0.71 ± 0.08 (4)	—	$1.26 \pm 0.25^*$ (4)

Values are mean $\pm$ S.E.M., no. of rats in parentheses. The equilibrium concentrations for sodium and potassium were taken as the control values from Table 4; rubidium was assumed to behave like potassium. Concentrations of lysine in plasma and RTF of rats were determined by ion-exchange chromatography (Main, 1976). Student's t test of significance of difference from control value at 33°C : * $P < 0.05$; ** $P < 0.01$.

Table 6. Effect of temperature on the ratio (single determinations or mean $\pm$ S.E.M. of 4 determinations) of radioactivity in RTF (C_t) to that in plasma (C_p) at 30 min after exposure of the testes to 33°C (c) or 41°C (t) and the start of intravenous infusions of ^3H -labelled steroids

Steroid infused	C_t/C_p		Temperature coefficient†
	33°C (c)	41°C (t)	
Androstenedione	0.71 $\pm$ 0.07	0.49 $\pm$ 0.07*	-1.38
Testosterone	0.64, 0.65	0.60, 0.69	-1.08, +1.08
Pregnenolone	0.47	0.41	-1.16
Progesterone	0.32, 0.42	0.27, 0.39	-1.20, -1.09
Dihydrotestosterone	0.13, 0.16	0.18, 0.22	+1.48, +1.47
Oestradiol-17 β	0.14	0.17	+1.27
Cortisol	0.032, 0.036	0.053, 0.053	+1.82, +1.59

* Significantly less ($P < 0.05$) than value at 33°C; Student's paired t test.

† Calculated as $(t - c)/c$.

Table 7. The effect of temperature on the uptake of oxygen (ml/ml at s.t.p.) by the testes, maintained at 41°C (heated) or 33°C (control) for up to 1.5 h, of anaesthetized rats

Time (min)	O_2 content of blood from:		
	Carotid artery	Testicular vein	
		Control	Heated
0-30	0.130 $\pm$ 0.005	0.085 $\pm$ 0.009*	0.055 $\pm$ 0.008
31-60	0.143 $\pm$ 0.006	0.070 $\pm$ 0.009**	0.027 $\pm$ 0.010
61-90	0.138 $\pm$ 0.005	0.087 $\pm$ 0.008**	0.051 $\pm$ 0.009

Values are mean $\pm$ S.E.M. for 4-6 samples from 3 rats.

Student's t test for significance of difference between testicular venous O_2 contents of heated and control testes; * $P < 0.05$; ** $P < 0.02$.

Table 8. Incorporation ((d/min)/g testis/ μCi injected) of ^3H into ethanol-precipitated and supernatant fractions of homogenates of rat testes, one maintained at 33°C and the other at 41°C, 18-24 h after ligation of the efferent ducts and 31-34 min after a 60-min intravenous infusion of ^3H -labelled amino acid

Amino acid infused	Precipitate (P)		Supernatant (S)		P/(P + S)*		t/c
	33°C	41°C	33°C	41°C	33°C (c)	41°C (t)	
L-Lysine	550	740	560	870	0.50	0.46	0.92
L-Lysine	800	1000	690	870	0.54	0.53	0.98
L-Arginine	890	960	730	1040	0.55	0.48	0.87
L-Arginine	729	880	500	880	0.59	0.50	0.85
L-Leucine	1120	730	380	730	0.75	0.50	0.67
L-Leucine	870	600	310	550	0.74	0.52	0.70
L-Valine	320	330	120	330	0.73	0.50	0.69
L-Valine	600	400	230	500	0.72	0.44	0.61

* Student's paired t test of difference between the values of $(P/(P + S))$ at 33°C compared with 41°C: combined lysine and arginine experiments: $P < 0.02$; combined leucine and valine experiments: $P < 0.001$.

potassium (^{42}K) or rubidium (^{86}Rb), and was followed by a progressive decline towards control levels. This pattern was far less marked for [^3H]lysine and was not seen for sodium (^{24}Na).

Steroids. During i.v. infusion of labelled steroids, the normally rapid appearance of [^3H]testosterone in RTF (Cooper & Waites, 1972, 1975) was not affected by heating the testes to 41°C , but for the more slowly penetrating steroids, cortisol, oestradiol and dihydrotestosterone, a positive effect of elevated temperatures was seen (Table 6). When [^3H]androstenedione, [^3H]progesterone and [^3H]pregnenolone were infused, radioactivity tended to appear more slowly in RTF from heated than from control testes (see Discussion).

Metabolism

The arteriovenous difference for oxygen across the heated testes of anaesthetized rats was increased by 66% over that for control testes (Table 7). Therefore, testicular oxygen consumption was approximately doubled at 41°C compared with the control temperature if the slight increase in testicular blood flow is taken into account. The increased arteriovenous difference for oxygen was sustained for at least 60 min. The fraction of the total uptake of circulating radioactive amino acid which was converted into ethanol-precipitable material in the testis was consistently less in the heated than in the control testis from the same rat (Table 8), indicating a reduced net synthesis of protein at the higher temperature.

Discussion

Heating the testes of rats to 41°C for 1 h is sufficient to cause a temporary but severe reduction of sperm output (Setchell & Waites, 1972) and a reduction in testis weight. However, judging from the failure of circulating ^{125}I - and ^{131}I -labelled albumin to enter RTF, it appears that the blood-testis barrier to proteins does not break down at this temperature. The normally low concentration of protein in RTF was maintained both during the period of heating and 2 days later (Table 4), by which time clear signs of cytological damage have been observed after similar acute heat exposures (Chowdhury & Steinberger, 1964, 1970; Blackshaw & Hamilton, 1970, 1971) and in cryptorchidism (Davis & Firlit, 1966). Since in addition the ionic composition of RTF remains in the normal range throughout the onset of testicular damage caused by local heating in rams (Setchell, Voglmayr & Hinks, 1971) and rats (Setchell & Waites, 1972), a gross breakdown of the 'blood-testis barrier' cannot be the cause of heat-induced aspermatogenesis.

Nevertheless, there appear to be subtle changes in the rates of exchange of a number of molecules between blood plasma and RTF when testis temperature is raised to 41°C . The increase in rates of exchange of ^{86}Rb , ^{42}K , ^{24}Na and [^{14}C]- and [^3H]lysine and some ^3H -labelled steroids with temperature is not unexpected. However, the early maximal stimulation of ^{86}Rb and ^{42}K movements followed by a decline in apparent exchange rate is less readily explicable and merits further investigation, especially as a pattern of initial hyperpolarization followed by depolarization of the seminiferous tubules has been observed at the same temperature (Gladwell, 1977).

Calculations (Setchell, 1970b) and measurements with an oxygen microelectrode (Free, Schluntze & Jaffe, 1976) have indicated that the oxygen tension within the seminiferous tubules is low (around 12 mmHg). Given the marked fall in testicular venous oxygen content, therefore, oxygen supply to the germinal epithelium of the rat may become limiting at high temperatures, as also suggested for the ram (Waites & Setchell, 1964). Thus the heat-induced stimulation followed by a decline in potassium exchange and the same pattern of change in transtubular potential (Gladwell, 1977) could both be due to a progressive inhibition of the active transport of potassium because of oxygen deficiency. This would explain reports that the uptake of ^{86}Rb by the whole testis (Setchell *et al.*, 1969) and also by isolated seminiferous tubules *in vitro* (Setchell & Singleton, 1971) was reduced at temperatures higher than 33°C . However, the effects of directly reducing the supply of oxygen to the testis at normal temperatures still need investigation.

Normally, testosterone gains rapid access to RTF (Cooper & Waites, 1972, 1975) and to seminiferous tubular fluid (Setchell & Main, 1975). This rapid access is unimpaired during heating (see

Table 6), an important point since testosterone is believed to play a major role in the maintenance of spermatogenesis in the rat (Steinberger, 1971). When [^3H]androstenedione was infused intravenously, the appearance of radioactivity in RTF was inhibited by heating. However, during such an infusion testosterone is the principal radiometabolite in testicular venous plasma (Setchell & Main, 1975) and RTF (Cooper & Waites, 1975). Because the synthesis of testosterone from its precursors in testis slices is inhibited by raising the incubation temperature (LeVier & Spaziani, 1968a), it is possible that the reduced penetration of radioactivity from blood plasma to RTF at 41°C during infusions of [^3H]androstenedione is due to reduced conversion to testosterone, if it is assumed that the permeability of the blood–testis barrier to androstenedione is much less than to testosterone. Minor differences in the structure of steroid molecules can result in large differences in their ability to enter RTF from the bloodstream (Cooper & Waites, 1975), and a similar argument might therefore be applied to explain the negative temperature effect when [^3H]progesterone and [^3H]pregnenolone were infused.

Another consequence of high temperature appears to be a marked impairment of net testicular protein synthesis (Table 8), as observed in testis slices *in vitro* (Davis, Firlit & Hollinger, 1963). This could be due to an intrinsically thermolabile enzyme system in the testis or be a consequence of the effects of hypoxia on energy substrate levels. The same explanation might apply to the inhibition of steroid conversions in the testis at high temperatures, although non-steroidal lipid synthesis in the testis does not appear to be heat-sensitive (LeVier & Spaziani, 1968b).

In conclusion it appears that the blood–testis barrier is resistant to heat, even though high temperature alters testicular metabolism and ion movements. Hypoxia or substrate deficiency has been invoked as the possible mechanism of heat-induced damage to mammalian testes by many authors (see Moore, 1926; Harrison, 1956; Ewing & VanDemark 1963; Waites & Setchell, 1964). More recently, it has been suggested that changes in membrane permeability at the cellular or subcellular level may be the primary lesion in heat-induced aspermatogenesis (Blackshaw & Hamilton, 1970, 1971; Lee & Fritz, 1972; Blackshaw, 1974; Lee, 1974), but such permeability changes could result secondarily from local hypoxia or energy substrate deficiency. Neither explanation is satisfactory and a wider basis must be sought to explain the heat sensitivity of the scrotal testis.

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