EFFECT OF LOCAL HEATING OF THE SCROTUM, TESTES AND EPIDIDYMIDES OF RATS ON CARDIAC OUTPUT AND REGIONAL BLOOD FLOW

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(Received 2nd June 1972)

Summary. The effects of immersing the scrotum, tail, testes and epididymides of rats in water at temperatures in the range 28 to 45°C on the cardiac output and blood flow through various tissues have been examined to assess the possible rôle of the cardiovascular system in the cytological responses of the testis to heat. The cardiac output recorded before immersion was 33·9 ± 0·8 (mean ± S.E.M.; 110 determinations) ml/100 g/min. Apart from the blood flow in the scrotal skin, the cardiovascular parameters were not consistently affected by temperatures of 28, 33, 37 and 40°C. The cardiac output was slightly elevated when the scrotum was immersed in water at 43 and 45°C, but blood flow through the brain, testes and all regions of the epididymides was markedly increased. Blood flow through the epididymal fat pad and mid-side abdominal skin was virtually unchanged at all temperatures. In contrast to all other tissues, the blood flow in the scrotal skin increased with temperature, being almost twice as great at 37°C as at 33°C, and with further increases at higher temperatures. There were no significant differences in cardiovascular parameters on immersing the tail alone in water at either 33 or 43°C.

The results draw attention to the cardiovascular repercussions of immersing the scrotum of anaesthetized rats in water at high temperatures, and to the need for a re-evaluation of the cytological responses using more physiological temperatures.

INTRODUCTION

In previous experiments to determine the action of local heat on spermatogenesis in rats, it has been usual to immerse the scrotum in water, inevitably heating the tail and part or all of the hind limbs as well. The time of immersion and temperature of the water has varied from very hot temperatures (43 to 46°C) for short times (10 to 20 min) to more moderate temperatures for longer times (for review, see VanDemark & Free, 1970). In such experiments on anaesthetized rats, general body temperature is hard to control, and it seems...
possible that hyperthermia with consequent cardiovascular repercussions may have contributed to the results obtained by others with the high temperatures used. It is important to know about such repercussions, as the changes in testicular blood flow during heating are of significance for metabolic changes which may well affect the cellular response.

For example, after locally heating the scrotum of conscious rams to 40°C for 2½ hr, certain pachytene spermatocytes were destroyed and type-B spermatogonia began an abortive division in which many were arrested between prophase and metaphase (Waites & Ortant, 1968). By contrast, spermatogonia of rats were apparently unaffected by local heating to 43°C for 15 or 20 min, although, again, certain of the pachytene spermatocytes were destroyed (Chowdhury & Steinberger, 1964, 1970; Collins & Lacy, 1969).

Whereas testicular blood flow in conscious sheep did not increase when the scrotum was heated to 40°C (Waites & Setchell, 1964; Setchell, Waites & Thorburn, 1966), except in a strain of Merino rams which are relatively resistant to the effects of heat on fertility (Fowler & Setchell, 1971), in anaesthetized rats, testicular blood flow, expressed as a fraction of the assumed cardiac output, increased at temperatures above 41°C (Glover, 1966; Waites, Setchell & Fowler, 1968). The spermatogonia of sheep may therefore be damaged by hypoxia whereas, in rats, the local hyperaemia may bring sufficient oxygen to protect the spermatogonia. However, the measurements of testicular blood flow in rats reported so far have depended on the assumption that the cardiac output remained unchanged whereas the values from sheep are absolute.

In the present experiment on rats, by measuring the cardiac output and ‘relative’ blood flow simultaneously, the absolute blood flow through the testes, epididymides and scrotum was determined after heating the scrotal region and the tail to levels which included the higher temperatures used by other workers. Testicular vascular control was maintained at temperatures below 40°C, but the character of cardiovascular responses throughout the body altered when temperatures of 43 and 45°C were applied. The rôle of the tail in such responses has been examined.

**MATERIALS AND METHODS**

**Animals**

The rats (Wistar strain) were 60 to 90 days old and weighed 250 to 440 g. They were anaesthetized with pentobarbitone sodium (50 mg/kg, intraperitoneally), and a polyvinylchloride cannula (0.5 mm i.d., 0.8 mm o.d., Dural Plastics, Dural, N.S.W., Australia) was inserted into one jugular vein so that the tip lay in the superior vena cava near to the right atrium. A copper–constantan thermocouple or a thermistor mounted in a polythene or metal tube (0.5 mm or 0.75 mm o.d.) was inserted into the opposite carotid artery and advanced to lie in the arch of the aorta.

**Cardiac output**

This was measured by thermodilution (Fegler, 1954, 1957) with the rats lying on their backs on a wooden board (see Text-fig. 1, Setchell & Waites, 1972).
Experiment 1
Arterial blood temperature was recorded by thermocouple and 0·5 ml 0·9% NaCl at room temperature was rapidly injected through the venous cannula, the resultant thermal dilution curves being recorded on a fast recorder (Kipp & Zonen, 1 mV range). Usually, three control curves, 2 min apart, were obtained before the board was tilted just sufficiently to immerse the tail and scrotum in a water bath, the temperature of which was controlled to within 0·1° C (Thermomix pump: Braun, Melsungen, Germany). Preliminary experiments revealed that the testicular temperature rose to within 0·5° C of that of the water bath within 5 min of immersion. Four rats were examined at each of the following temperatures: 33, 37, 40, 43 and 45° C.

Experiment 2
Thermistors (Stantel U23 UD: Standard Telephone and Cables Pty Ltd) were used to record blood temperature and 0·1 ml 0·9% NaCl at room temperature was injected to obtain thermal dilution curves (Rikadenki Koygo Co., Ltd, 1 mV full scale deflection). Three rats were examined with their tails and scrota immersed at each of the following temperatures: 28, 33, 37, 40, 43 and 45° C.

Experiment 3
Cardiac output was measured as in Exp. 2 except that 0·4 ml 0·9% NaCl at room temperature was injected. Three control thermal dilution curves were obtained before the rats were lowered so that their tails alone were immersed in water at 33° C (four rats) or at 43° C (four rats).

Blood flow
Fifteen minutes after the scrotum had been immersed in the water bath, three thermodilution curves were obtained at approximately 1-min intervals and then 10 μCi [131I]iodoantipyrine was rapidly injected through the venous cannula. Twenty-five seconds after the injection, the rat was removed from the bath, still attached to the board and at 30 sec, it was killed either with a double-bladed guillotine which isolated the heart from the rest of the body or with an overdose of pentobarbitone sodium. The testes, epididymides, portions of skin from the scrotum and mid-side abdomen and half the brain were removed. The epididymides were cut into four portions as described in Waites & Setchell (1966). In Exp. 3, instead of mid-side abdominal skin, 2-cm long segments of the tip and middle portion of the tail were taken. All tissues were weighed and their contents of isotope determined by counting in an automatic-spectrometer (Packard, La Grange, Illinois). 'Relative blood flow' was calculated (Waites & Setchell, 1966) and then multiplied by cardiac output expressed per unit body weight to obtain absolute values for blood flow.

RESULTS
Cardiac output was measured using injection volumes varying from 0·5 ml and 0·4 ml (Exps 1 and 3, respectively) to 0·1 ml (Exp. 2). As Hanwell & Linzell
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(1972) reported that injection volumes below 0.2 ml may underestimate the cardiac output in rats, the results from the three experiments are given separately.

The mean cardiac outputs ± S.E.M., recorded before immersing the scrotum in Exps 1 to 3, were 34.8±2.8, 33.7±0.74 and 32.6±4.3 ml/100 g/min, respectively (twenty determinations on twenty rats, ninety determinations on eighteen rats and twenty-three determinations on eight rats).

Experiments 1 and 2
Mean aortic temperatures were 36.5±0.22 and 36.6±0.18°C respectively. The cardiac output was unchanged until the temperature of the scrotum was raised to 43 or 45°C, when it tended to increase in parallel with the aortic blood temperature which rose significantly (Text-fig. 1).
Scrotal heating, blood flow and cardiac output

The mean blood flow in the testes when the scrotum was immersed in water at 33°C (the normal scrotal temperature) was 24.4±1.2 (six rats) and 21.8±0.8 (three rats) ml/100 g/min in Exps 1 and 2, respectively. The testicular blood flow, expressed as either relative or absolute values, was not consistently affected when the testes were kept at 37 or 40°C for 20 min although, in Exp. 2, the testicular blood flow was lower than the other values when the testes were at 28°C. At 43°C, there was a sharp increase in the testicular blood flow, which rose even further at 45°C, due to changes in both the cardiac output and the relative blood flow (Text-fig. 1).

![Text-Fig. 2. Blood flow in four regions of the epididymis of anaesthetized rats with the scrotum immersed at temperatures in the range 28 to 45°C for 20 min. Values expressed, and symbols used, as in Text-fig. 1.](image)

The blood flow in all regions of the epididymides did not change consistently until the temperature applied rose to 43 and 45°C when large increases were recorded (Text-fig. 2).

In contrast to these results, the mean blood flow through the scrotal skin increased from 16.3±1.3 (Exp. 1) and 13.9±0.77 (Exp. 2) ml/100 g/min at 33°C to 30.8±5.2 and 29.9±3.7 when the temperature rose to 37°C, but the blood flow through the mid-side skin did not change significantly with tempera-
ture (Text-fig. 3). The blood flow through epididymal fat, the only other tissue heated directly, did not increase with temperature.

The brain blood flow in Exp. 2 increased from control values of 50.5 ± 3.9 and 51.8 ± 2.7 ml/100 g/min when the applied temperatures were 33 and 37°C, respectively, to 86.1 ± 19.5 ml/100 g/min when the testes were heated to 40°C, although this increase was due largely to the results from one rat. There were no increases in brain blood flow when the testes were heated to 40°C in Exp. 1, but in both experiments, brain blood flow was increased when the testes were heated to 43 or 45°C (Text-fig. 1).

![Text-fig. 3. Blood flow in the skin of the scrotum and mid-side, and in the epididymal fat of anaesthetized rats with the scrotum immersed at temperatures in the range 28 to 45°C for 20 min. Values expressed, and symbols used, as in Text-fig. 1.](image)

Experiment 3

Immersing the tail at 43°C did not significantly alter the cardiac output, or the blood flow in the testes, epididymides or scrotal skin when compared with immersing the tail at 33°C. The blood flow in the tail itself rose from 6.1 (range 2.1 to 9.5) and 6.7 (range 5.8 to 8.1) ml/100 g/min in the tip and mid-portions, respectively, at 33°C, to 74.0 and 19.5 ml/100 g/min at 43°C. The mean aortic temperatures, however, did not increase and indeed fell slightly from a mean of 36.5°C in all rats. The brain blood flow was variable and significantly elevated in three of four rats in both groups: means 101 and 93 ml/100 g/min after tail immersion at 33 and 43°C, respectively.
DISCUSSION

Our values for absolute blood flow in the testes of rats measured with the scrotum at 33°C compare favourably with values reported recently using 133Xenon-clearance (24.0±1.0 ml/100 g/min; Joffre & Joffre, 1971) and other estimates calculated from measured relative blood flow and assumed cardiac output values (for review, see Setchell, 1970). The testicular blood flow levels remained reasonably constant as the scrotal temperature was varied in the range 28 to 40°C. By contrast, the blood flow through the skin of the scrotum of rats, as in rams (Fowler & Setchell, 1971), increased progressively as the temperature increased above 33°C.

These results and those of Glover (1965, 1966) show that when testicular temperature is raised to body temperature, the blood vessels of the rat testis respond like those in the ram (Waite, Setchell, 1964; Setchell et al., 1966). Vasodilatation and elevated blood flow only occurred when the testicular temperature was well above the body temperature. In this respect, eutherian mammals appear to differ from marsupials in which the testicular blood flow increases when the testicular temperature is raised to the body temperature (Setchell & Thorburn, 1969). However, the seasonally breeding animals, dormouse, ferret and fox, all have augmented testicular blood flow during the period of spermatogenic activity (Joffre, 1971).

Greatly increased testicular blood flow was observed in the present experiments when the scrotal temperature was kept at 43 and 45°C for 20 min. The effect was presumably due to local loss of vascular tone, although the rats, even in this short time, became hyperthermic with aortic blood temperatures up to 4°C above normal. This general hyperthermia was associated with elevated cardiac output and increased blood flow in the brain and so some neurally mediated vasomotor responses may be involved in the testicular hyperaemia. Elevated brain blood flows were recorded in Exp. 3 without hyperthermia and this effect may be due to changes in gas tensions in blood due to anaesthetism and posture.

What is the relevance of these cardiovascular changes to the cytological effects of heat? Spermatogonia, unlike other cells in the germinial line, adjoin the basement membrane. Peroxidase and lanthanum salts can pass into some of the intercellular spaces between the spermatogonia and the Sertoli cells but not into the spaces between adjacent Sertoli cells or between the Sertoli cells and the other cells of the germinial epithelium (Fawcett, Leak & Heidger, 1970; Dym & Fawcett, 1970). Spermatogonia, therefore, probably depend on nutrients which diffuse through the boundary tissue, whereas the other germinial cells probably rely on what reaches them through the Sertoli cells. Two mechanisms may therefore be involved in producing testicular damage when the testes are heated to moderate temperatures for several hours. The abortive division which the spermatogonia undergo could be due to metabolically induced hypoxia, but the spermatocytes and spermatids could also be damaged by the failure of fluid secretion during heating observed by Setchell, Voglmayr & Hinks (1971). At higher temperatures maintained for shorter times, the increased blood flow may prevent the hypoxic effect on the spermatogonia but the persistent
effect on the spermatocytes and spermatids would suggest that fluid secretion is probably further reduced. However, a species difference may be involved as the different temperatures applied in the cytological studies quoted were in different species.

Furthermore, the general effects of the heating used differs for the two species. The results reported here show that a general hyperthermia is provoked by heating the scrotum and tail of anaesthetized rats to $43^\circ$C for 20 min which did not occur when the tail alone was heated. Aortic blood temperatures of $38-1^\circ$C (Exp. 1) and $39-0^\circ$C (Exp. 2) are high deep-body temperatures for rats and $43^\circ$C would be an abnormally high tissue temperature in any animal, whereas $40^\circ$C is within the possible range for deep-body temperature in sheep and heating the scrotum to this temperature in conscious rams caused a fall in body temperature by increasing respiration rate (Waites, 1962) but no change in cardiac output (Fowler & Setchell, 1971). In mammals, hypothalamic temperatures greater than $41^\circ$C provoke extreme thermoregulatory responses completely different in character from those produced at lower temperatures (Bligh, 1966). The cutaneous ‘warm’ receptors in the scrotum respond maximally around $40^\circ$C in both sheep (Waites, 1962) and rats (Iggo, 1969).

For these reasons and others expressed elsewhere (Setchell & Waites, 1972), we believe that, in experiments planned to examine the uniqueness of thermal effects on the testis and to make comparisons between species possible, the upper range of temperatures applied to the testes should not exceed 40 to $41^\circ$C, and deep-body temperature should be controlled within the normal range for the species. Tissue temperatures above this may well produce non-specific effects as well as undesirable repercussions elsewhere in the body.

ACKNOWLEDGMENTS

The work reported was supported by grants from The Population Council, New York, N.Y., Grant No. M70.0142C, and the Rural Credits Development Fund, Reserve Bank of Australia to G.M.H.W. We are grateful to Dr J. L. Linzell and to Mr S. J. Main, B.Sc., for help with Exp. 3.

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