J. Physiol. (1969), **201**, 129–143
With 8 text-figures
Printed in Great Britain

METABOLISM, SPERM AND FLUID PRODUCTION OF THE ISOLATED PERFUSED TESTIS OF THE SHEEP AND GOAT

BY J. L. LINZELL AND B. P. SETCHELL*

From the Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge, England

(*Received* 31 *July* 1968)

SUMMARY

1. A total of nineteen ram and three goat testes have been perfused in isolation at 34–35° C for 3½–9 hr with heparinized blood and an added 5-HT antagonist (bromolysergic acid diethylamide) and their function compared with that of the normal ram testes *in vivo*.

2. The metabolism of the perfused testes and the testes *in vivo* was similar but blood flow through the perfused testes was two to three times normal.

3. Vasoconstriction was produced by adrenaline and noradrenaline (10–20 μg I.A.) and by electrical stimulation of nerves in the spermatic cord or of the lumbar sympathetic chain; these responses were abolished or reduced by phenoxybenzamine.

4. Flow of fluid from the rete testis continued only if ischaemia was reduced to a minimum and glucose concentration in the blood perfusing the testis was kept above about 25 mg/100 ml.; the fluid secreted during perfusion was of normal composition.

5. The perfused testis showed no evidence of autoregulation and the flow of fluid was not affected by changes in perfusion pressure.

6. When the temperature of three testes was raised to 40° C for 2 hr, metabolism increased but blood flow was unaltered; the flow of fluid and the concentration of spermatozoa decreased during heating.

7. The testes perfused at normal scrotal temperatures (34–35° C) were histologically normal but some abnormalities were observed in the heated testes.

INTRODUCTION

A number of studies have been made on the production of steroids by the isolated perfused testis (Ssentjurin, 1926; Sakamoto, 1936; Danby 1940; Nyman, Geiger & Goldzieher, 1959; Ewing & Eik-Nes, 1966), and

* Present address: C.S.I.R.O., Division of Animal Physiology, The Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia.

some aspects of the metabolism of this preparation have also been investigated (Vandemark & Ewing, 1963). However, in these studies the function of the perfused testis was not compared with that in the intact conscious animal as has been done with the mammary gland (Hardwick & Linzell, 1960). Indeed, the functions of the testis have been measured only recently in conscious animals, in terms of blood flow and metabolism (Setchell & Waites, 1964), steroid production (Setchell, Waites & Lindner, 1965) or sperm production and fluid secretion from the rete testis (Voglmayr, Waites & Setchell, 1966; Voglmayr, Scott, Setchell & Waites, 1967; Setchell, 1967*a*). Since all these measurements have been made in rams, it was decided to attempt to perfuse the isolated testis of this species and to compare its function with that in the conscious animal. It was then hoped to use the perfused preparation for other studies not possible in the conscious ram and the results of these investigations are described. A preliminary communication has been presented to the Physiological Society (Linzell & Setchell, 1968).

METHODS

Animals. Twelve adult Clun Forest rams weighing between 34 and 102 kg and two 1 yr old Saanen billygoats each weighing 45 kg were used. They were brought in from pasture a few days before the perfusion and fed pasture hay and a standard cereal mixture. Food was withheld for 24 hr and water for 12 hr before surgery. Their testes weighed between 100 and 325 g and the epididymides between 22 and 60 g.

Surgical procedure. The procedure varied as the period of ischaemia during setting up of the perfusion was reduced: the final routine adopted was as follows.

The ram was anaesthetized with halothane (Fluothane, I.C.I.) and oxygen, laid on his back and the scrotum divided along the median raphe. If possible, skin was resutured around each testis or exposed areas were covered with polyethylene sheet. The skin incisions were then continued around the neck of the scrotum and the penis divided so that the testes remained attached only by the spermatic cords. Cannulae (P.V.C. 0·76 mm internal diameter, 1·20 mm outside diameter, Dural Plastics, Dural, N.S.W., Australia) were inserted into the rete testis through the efferent ducts (Voglmayr *et al.* 1967) and collection of the testicular secretion begun.

The abdominal wall was opened with a transverse incision immediately anterior to the pubic symphysis to expose the internal spermatic artery and vein. The cremaster muscle, the vas deferens and the deferential vessels were cut and the internal spermatic cord freed from the abdominal wall, with the minimum of handling to avoid arterial spasm. For fear of damaging the vascular cone and the testis, the epididymis was not removed; the injection of Indian ink at the end of a perfusion confirmed that the testis and the head and body of the epididymis but not the tail were perfused. However, most of the venous effluent came from the testis, since clamping the head of the epididymis, the region of highest blood flow (Setchell, Waites & Till, 1964), during two perfusions lowered the venous outflow by less than 5%.

The spermatic vessels were clamped with a pair of small haemostats where the multiple veins of the spermatic cord had united to form either a single or a double vessel, and the artery quickly cannulated with a needle cannula (2 mm o.d.). As soon as possible, perfusion was begun from a syringe containing 20 ml. blood plus the 5-HT antagonist bromolysergic acid diethylamide (B.O.L. 148, Sandoz, Basel, 0·5 mg) at about 20 ml./min and continued at this rate while the vein was cannulated with a glass cannula. Ischaemia lasted for 30–90 sec, with a further 30–45 sec while the organ was transferred to the apparatus.

Perfusion. The apparatus used was basically for a constant-pressure perfusion (110 mm Hg, mean) as described by Hardwick & Linzell (1960) but with no dialyser (see Fig. 1). Blood temperature was initially 32–35° C and was raised to 37–39° C. Air temperature was controlled so that testis temperature remained within the normal range of 33–34° C unless otherwise stated. The testis was perfused with 500 ml. of the animal's own blood drawn from its

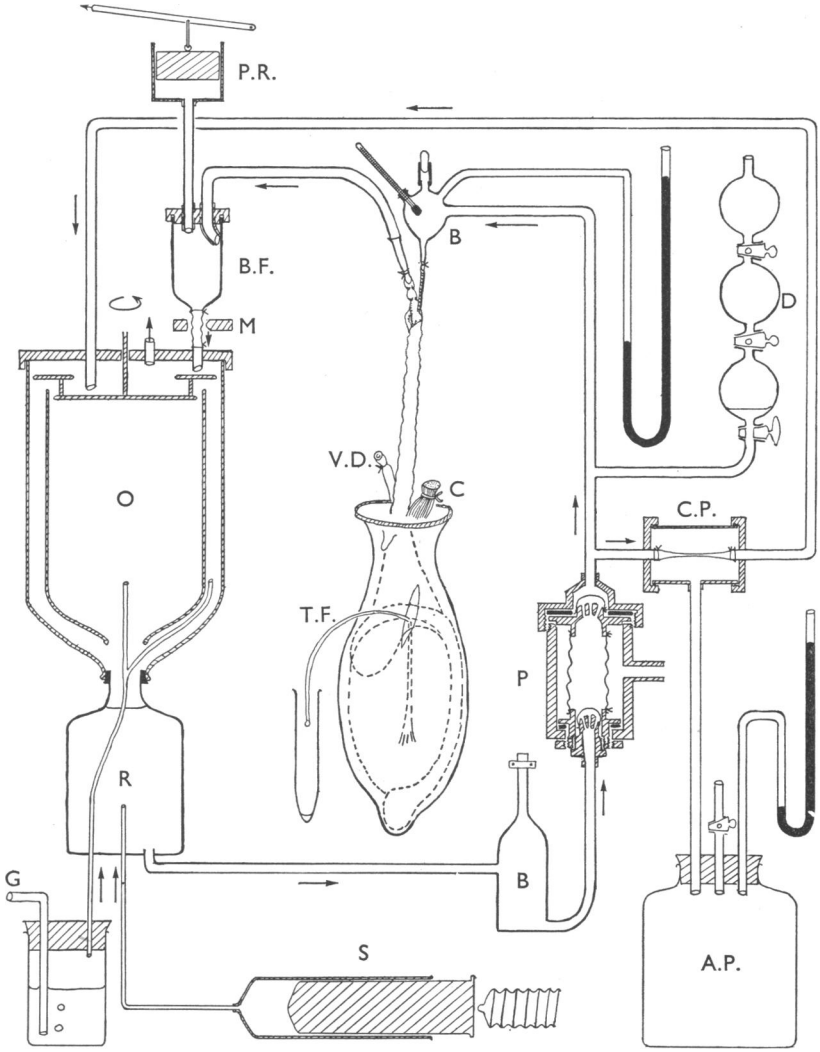

Fig. 1. Apparatus used for perfusion of the ram's testis at constant pressure. P, valve chamber of Dale–Schuster pump; C.P., Starling by-pass, with air pressure at 120 mm Hg (A.P.); D, air damper to control pulse pressure; B, bubble traps; B.F., modified Gaddum flow recorder with piston recorder (P.R.), recording, by air transmission, the rise of blood in the measuring cylinder, when the magnetic clamp (M) is closed; O, double Hooker oxygenator; R, blood reservoir; G, inflow of 7% CO_2 in air; S, motor-driven syringe or peristaltic pump supplying glucose; T.F., catheter in rete testis; C, cremaster muscle; V.D., vas deferens.

carotid into heparin (10 u./ml.) and B.O.L. 148 (1·5–2 mg.) while Krebs-Henseleit (1932) solution was infused intravenously. Glucose was infused into the reservoir, usually at a rate of 1 or 2 mg/min as a 0·1–0·2% solution in saline or Krebs solution, and adenosine at a rate of 1 mg/min for 1 hr. Blood samples were taken from the arterial and venous cannulae at half-hourly intervals and the testicular secretion collected. To study vasomotor responses, the perfusion was changed to constant volume flow at about 100 mm Hg and then perfusion pressure recorded during nerve stimulation and the addition of drugs. In two experiments one testis was perfused *in situ* at constant volume inflow. The animal was heparinized (1000 u./kg) and blood was pumped into the testicular artery near the aorta without interfering with the veins and nerves. The pump was supplied by a reservoir kept filled from a carotid artery using a magnetic clamp controlled by the blood level.

Radioactive isotopes. In two experiments [U-^{14}C]glucose was infused at 0·1 μc/min following a priming dose of 15 μc. In two others [1-^{14}C]acetate (30 and 48 μc sodium acetate, 1 μc/mg acetate) (Radiochemical Centre, Amersham, Bucks) was added as a single injection. The specific activity of glucose was determined by recrystallizing glucose pentaacetate as described by Jones (1965), that of acetate after steam distillation as described by Annison & White (1962) and that of carbon dioxide by the method of Hinks, Mills & Setchell (1966) or Annison & Lindsay (1961).

Analytical methods. Oxygen and carbon dioxide content were determined as described by Peters & Van Slyke (1932), oxygen saturation by a reflectance oximeter (American Optical Company), glucose by the toluidine method (Hyvarinen & Nikkila, 1962), glutamate by the enzymatic method of Sowerby & Ottaway (1966), with the modification that the final colour was extracted with 5 ml. ethyl acetate and dried with anhydrous sodium sulphate before reading instead of stabilizing the colour with acid acetone. Spermatozoa were counted with a haemocytometer and electrolytes in the rete testis fluid determined with an autoanalyser (Technicon, Chauncey, N.Y.) using flame photometry for Na and K, decoloration of buffered phenolphthalein for bicarbonate and a colorimetric method using mercuric thiocyanate, ferric nitrate and mercuric nitrate for chloride.

RESULTS

Blood flow. With constant pressure perfusion, blood flow through the testis was remarkably uniform but at about three times the rate through the testis of the conscious ram (Table 1). In one experiment the 5-HT antagonist was omitted and the initial flow was very low (about 2 ml./100 g/min). Within a few minutes of adding B.O.L. 148, blood flow increased and within 30 min reached 30 ml./100 g/min, a value similar to those found if B.O.L. was present from the start. Blood flow and perfusion pressure were linearly related over a wide range (Fig. 2), even during stimulation of the spermatic nerves at 10 impulses/sec.

Vasomotor responses. When the testis was perfused at constant volume inflow, stimulation of the nerves in the spermatic cord was followed by a sharp increase in perfusion pressure. Responses of similar magnitude were produced by single intra-arterial injections of adrenaline or noradrenaline (100 μg) (Fig. 3) with smaller responses to smaller doses. Injections of isopropyl noradrenaline (10–100 μg), acetylcholine (10–100 μg), eserine (2·5 mg), atropine (1–10 mg), oxytocin (1 u.) or phenoxybenzamine (6 mg)

had no effect but after phenoxybenzamine the response to the catecholamines was abolished and that to nerve stimulation greatly reduced.

Two testes, perfused *in situ*, showed similar responses but were more sensitive to catecholamines (Fig. 4). Vasoconstriction was elicited by electrical stimulation of the sympathetic chain, maximal responses being obtained from between fourth and fifth lumbar ganglia. Prostaglandin F-1-α (1–5 μg) had negligible effects.

TABLE 1. A comparison of the functions of isolated perfused testes of rams with those of the testes of rams *in vivo*

	In vivo	Perfused
Blood flow (ml./100 g/min)	9·6*	22·4 ± 2·0 (22)
Metabolism		
Oxygen uptake (ml./100 g/min)	0·59*	0·50 ± 0·049 (20)
CO_2 production (ml./100 g/min)	0·53*	0·54 ± 0·059 (20)
R.Q.	0·90*	1·08
Glucose uptake (mg/100 g/min)	0·74*	0·75 ± 0·073 (18)
Percentage of oxygen uptake which could be accounted for by oxidation of all glucose taken up	95 %*	112 %
$^{14}CO_2$ derived from glucose	71 %†	42 % ± 4 (2)
Rete testis fluid secretion (ml./100 g/hr)	1·0‡	0 to 1·1
Composition of rete testis fluid		
Spermatozoa/ml.	1×10^8‡	$0{\cdot}1–0{\cdot}96 \times 10^8$
Glutamic acid (μ-mole/ml.)	1·94 ± 0·18 (22)	0·1–4
Sodium (m-equiv/l.)	118§	137 ± 2·1
Potassium (m-equiv/l.)	12·5§	12·7 ± 0·64
Chloride (m-equiv/l.)	128§	139 ± 2·8
Bicarbonate (m-equiv/l.)	8·1§	10·5 ± 0·67

* Setchell & Waites, 1964. † Setchell & Hinks, 1967. ‡ Voglmayr *et al.* 1966.
§ Setchell, Voglmayr & Waites, 1969.

Oxygen and glucose uptake and carbon dioxide production. Despite the much higher blood flow the metabolism of the isolated perfused testis plus epididymis, in terms of oxygen and glucose uptake and carbon dioxide production, was very similar to metabolism of the testis *in vivo* (Table 1). Glucose was removed from the perfusate continuously as revealed by consistent arteriovenous differences and, if the rate of input was too low, blood glucose concentration was reduced to zero. There was also a small uptake of acetic acid, quantitatively much less important than that of glucose. The uptake of glucose was unaffected by high concentrations of acetate in the blood (up to 6·0 m-equiv/l.).

Radioactive glucose was infused in two experiments. The specific radioactivity of the carbon dioxide produced by the testis was 38 and 46 % of that of the glucose taken up, indicating that about 40 % of the carbon dioxide produced by the testis was derived from blood glucose. Radioactive acetate was formed by the testis from radioactive glucose, and the specific radioactivity of the acetate after 7 hr, including 2 hr heating, was about 50 % of that of the glucose.

The fraction of CO_2 derived by the testis from acetate could not be calculated in the experiments in which radioactive acetate was infused because the specific radioactivity of the acetate fell steadily during the experiment from about 2000 μc/g of carbon to about 50 μc/g of carbon, presumably due to non-radioactive acetate being made by the testis from glucose.

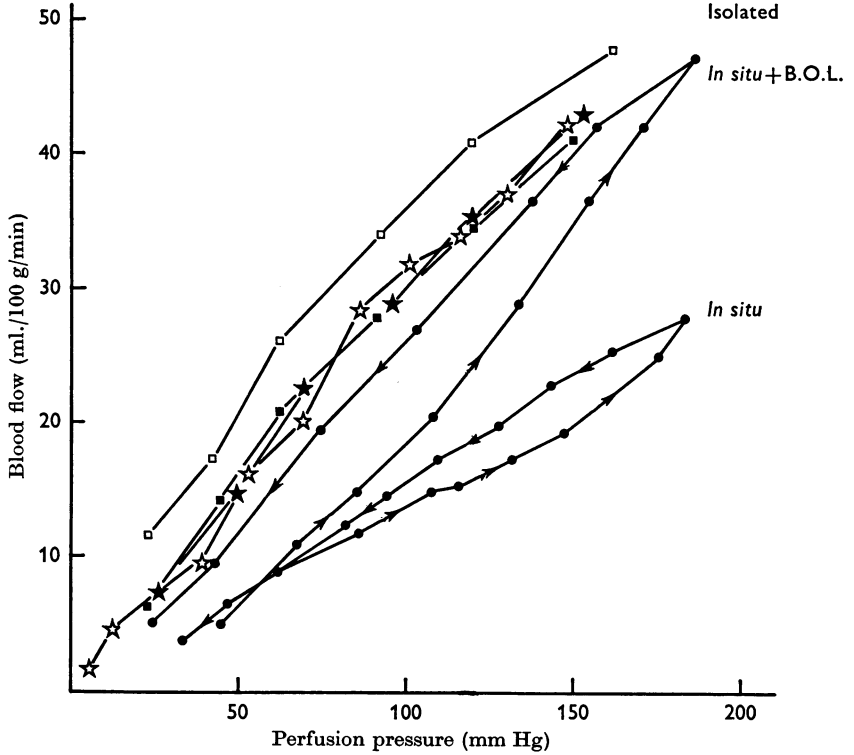

Fig. 2. Pressure–flow diagrams of ram testes perfused at constant volume inflow. Blood flow was measured from a cannula in the vein, at a constant pressure of 5 cm of blood. The two bottom curves are from the same testis perfused *in situ* with the nerves and lymphatics intact, before and after bromolysergic acid diethylamide (B.O.L.), 1·4 mg I.A. The other curves are from four testes perfused in isolation with 500 ml. autologous blood and 1·5–2·0 mg B.O.L. Different symbols refer to different rams.

Fluid secretion. Until the ischaemic period during the setting up of the perfusion had been reduced to less than 2 min, no fluid was secreted by the testes through the rete testis. However, in the later perfusions when the testis was subjected to minimal ischaemia, rete testis fluid was secreted at rates up to those seen in conscious animals, although there was always a lag of about an hour after the beginning of perfusion before fluid flow

began (Fig. 5); this lag was longer than the time (20–30 min) taken for testis temperature to rise to normal values.

In one experiment, due to an arterial air embolus, the testis was subjected to a second period of anoxia when fluid flow had reached near-normal rates. Again fluid secretion fell and only slowly returned to pre-ischaemia values although blood flow was restored within 10 min of the embolus occurring (Fig. 6) and temperature was unaffected.

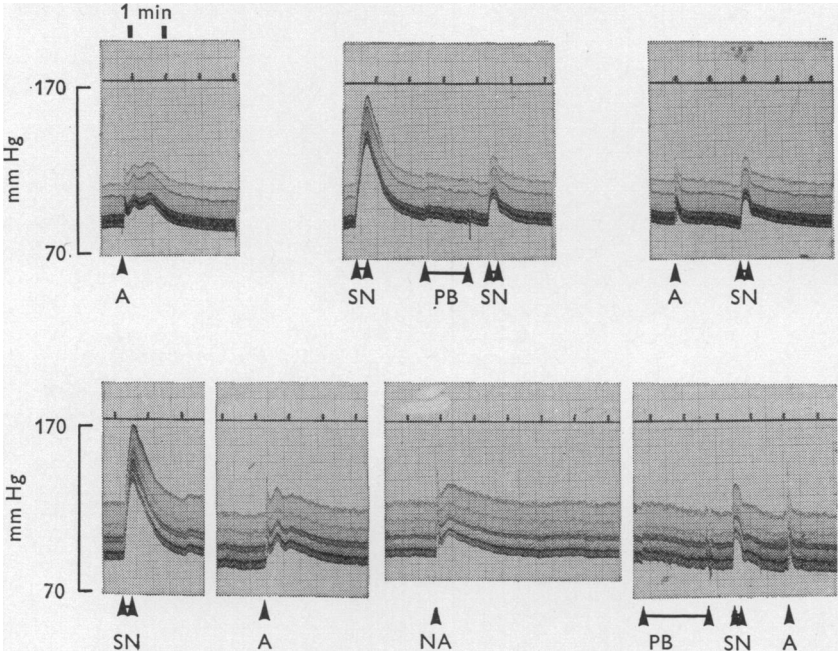

Fig. 3. Vasomotor responses of the isolated perfused ram's testis. The two testes had been perfused at constant pressure 6·5 and 9 hr. When perfused at constant volume inflow both showed similar responses to stimulation of the spermatic nerve (SN) (5 V, 10 msec, 50/sec) adrenaline (A) and noradrenaline (NA) 100 μg I.A. Phenoxybenzamine (PB), 6 mg, abolished the responses to the catecholamines and reduced that to nerve stimulation.

Fluid secretion was unaffected by altering the perfusion pressure between 100 and 50 mm Hg although a temporary rise in flow rate did occur when the pressure was increased from 50 to 100 mm Hg (Fig. 7).

The secretion of rete testis fluid appeared to be related to the blood glucose concentration. Glucose was infused continuously in all experiments at the mean rate at which it is removed *in vivo* (Setchell & Hinks, 1967). However, due to variations in initial concentration and testis size, the actual concentration of glucose varied, and occasionally fell to very low levels. A graph of glucose concentration and fluid flow showed that

rete testis fluid was only produced at appreciable rates when blood glucose concentration was above 25 mg/100 ml. although normal arteriovenous differences were maintained down to 7 mg/100 ml. (Fig. 8).

Sperm concentration. The concentration of spermatozoa in the testicular

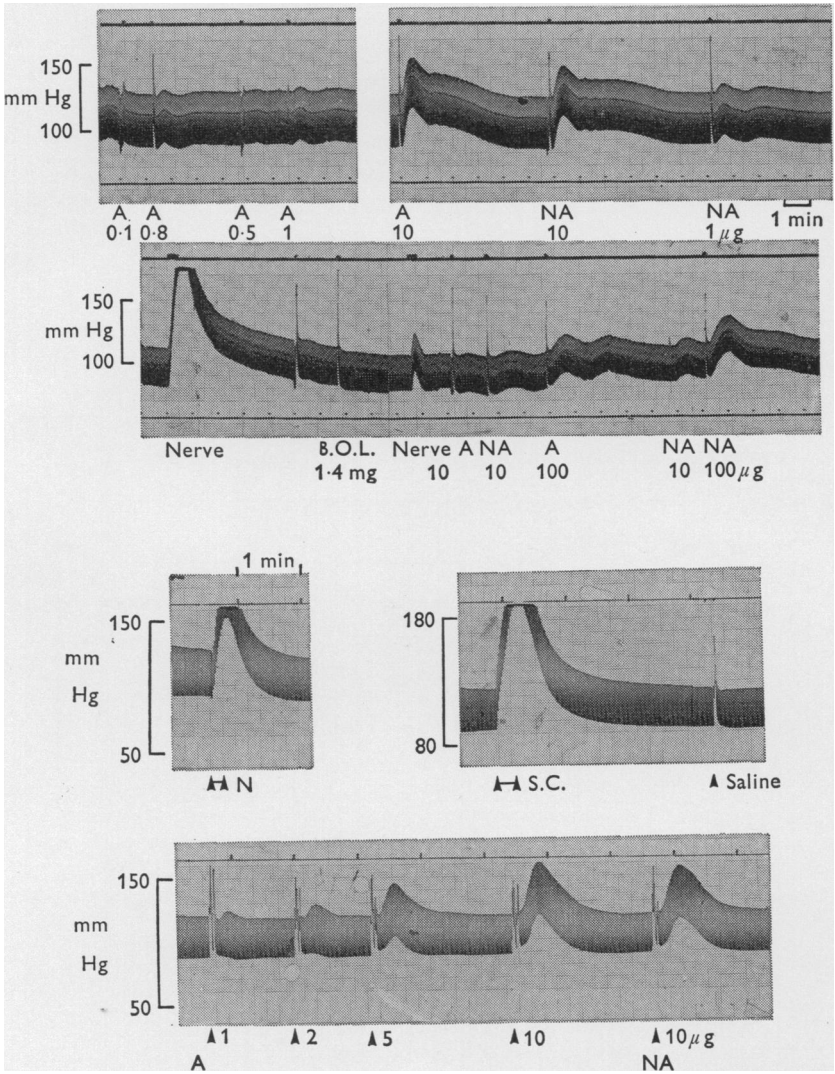

Fig. 4. Vasomotor responses of the ram's testis perfused *in situ*, with nerves intact. *Above*, comparison of responses to adrenaline (A), and noradrenaline (NA) and spermatic nerve stimulation (N) (5 V, 10 msec, 50/sec) and the partial adrenolytic effect of bromolysergic acid diethylamide (B.O.L.) 1·4 mg I.A. *Below*, comparison of vasoconstrictor responses to stimulation of spermatic nerve (N) and lumbar sympathetic chain (S.C.) between ganglia 4 and 5 (10 V, 10 msec, 50/sec) and to adrenaline (A) and noradrenaline (NA).

secretion was even more sensitive to ischaemia than the flow of fluid, in that in some experiments fluid was secreted with a low and falling concentration of spermatozoa, but again, by reducing the period of ischaemia to less than 2 min, concentrations of spermatozoa were obtained within the range found in conscious rams.

The production of spermatozoa continued for a number of hours at a fairly constant rate in four experiments but fell off towards the end of perfusions (6–8 hr).

Composition of rete testis fluid. The composition of the rete testis fluid secreted by the isolated perfused testis resembled very closely that

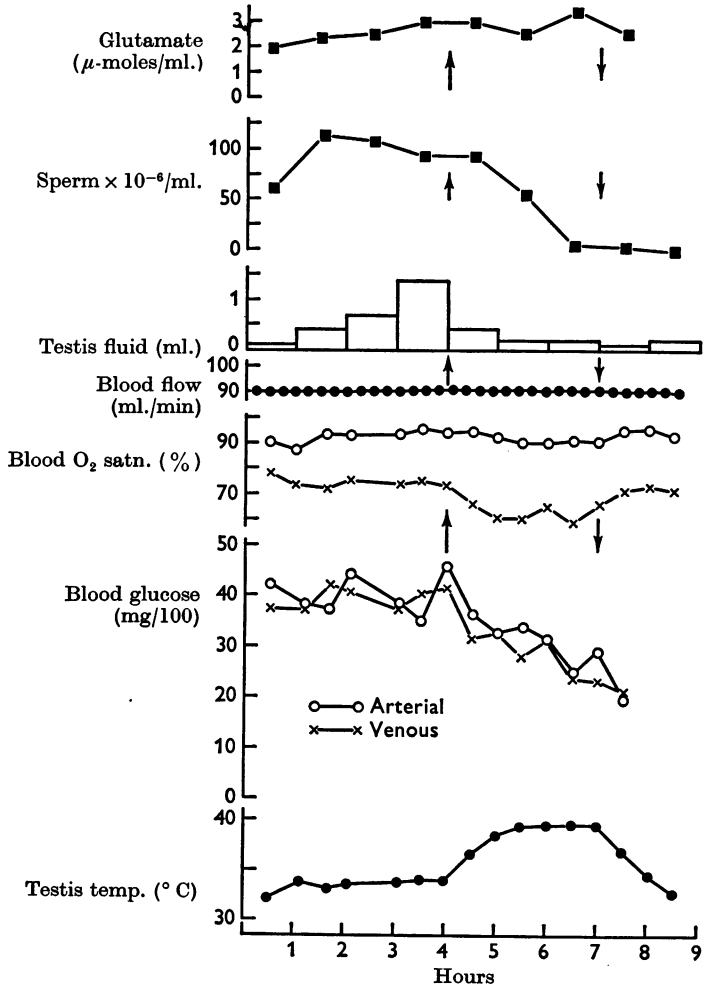

Fig. 5. Effect of heat on the metabolism and rete testis fluid production of the isolated perfused ram's testis.

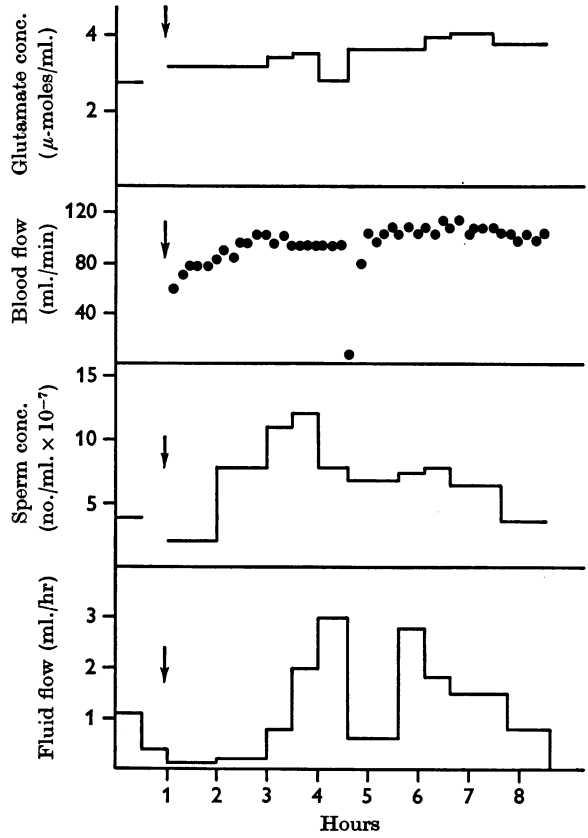

Fig. 6. The flow and composition of rete testis fluid during the perfusion in which a fall in blood flow due to air embolism occurred. Perfusion started at one hour.

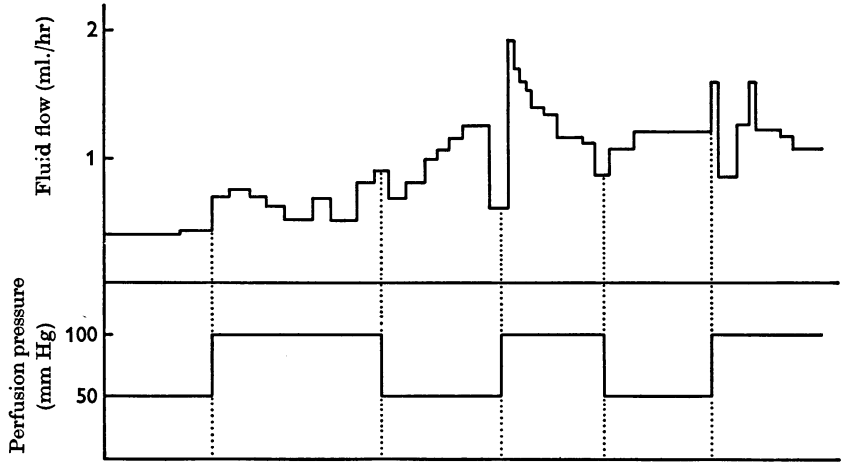

Fig. 7. The effect of perfusion pressure on rete testis fluid flow in an isolated perfused ram's testis. The flow was calculated from the interval between drops, each 0·018 ml.

collected from the rete testis of the conscious ram (Table 1) in its high concentration of glutamic acid, potassium and chloride and the low concentration of sodium, bicarbonate and glucose, compared with blood plasma.

Effect of heat. When the temperature of the perfusion chamber was increased in three experiments so that testis temperature rose from 33–34° C to about 39° C (the normal deep body temperature for rams) for 2 hr, there was a rapid decrease in the rate of flow of rete testis fluid and a slower fall in the concentration of spermatozoa. By the end of the 2 hr heating the

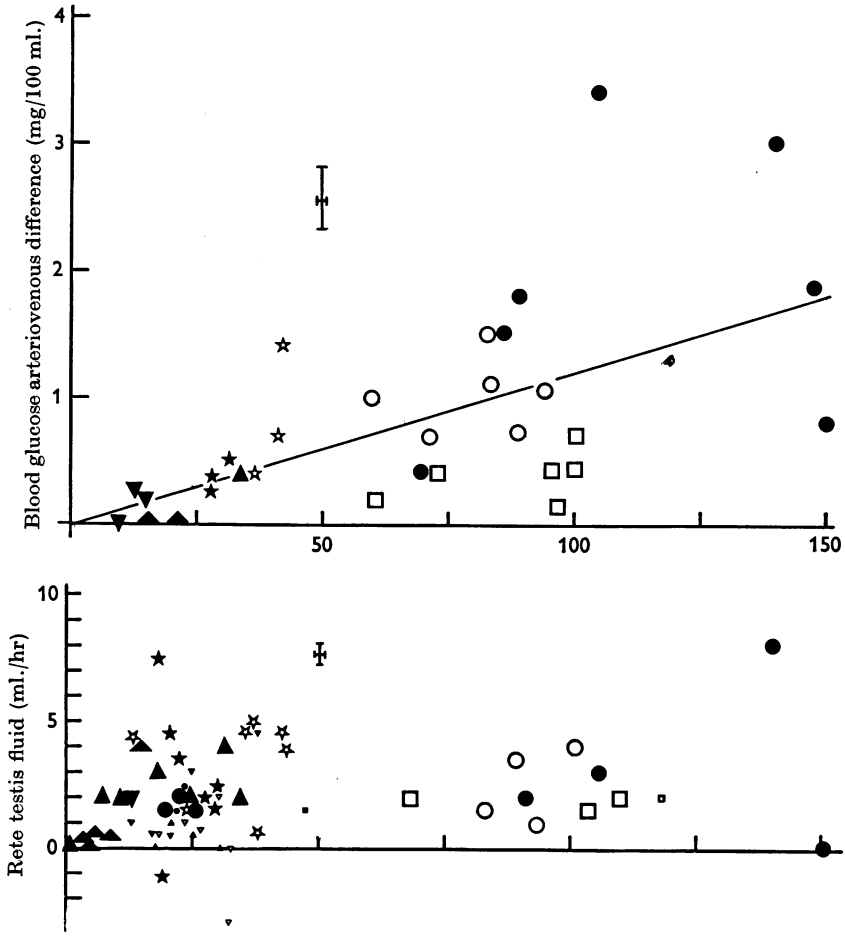

Fig. 8. The relation between blood glucose concentration and glucose arteriovenous difference and the flow of rete testis fluid in isolated perfused ram's testes. The crosses show mean ± S.E. values found in conscious animals. The line was calculated by the method of least squares (r = 0·6). Each experiment is represented by a different symbol.

fluid secreted was practically azoospermic. There was no change in blood flow but an increase in oxygen and glucose uptake (Fig. 5), and at the end of the 2 hr the testis was deriving 58 % of its carbon dioxide from blood glucose, compared with 46 % before heating.

Histological appearance of testis after perfusion. In the testes perfused at 33–34° C, the normal scrotal temperature, for 3½–9 hr, the seminiferous tubules presented normal spermatogenesis with no loss of cells of any particular type. In the three perfusions in which the temperature of the testis was raised to 39° C for 2 hr and then lowered to 33–34° C for a further hr, some regions of the organ contained tubules surrounded by extravasated blood. The germinal epithelium of these tubules was detached from the boundary tissue whereas the germinal epithelium was normal in appearance in other areas.

Goat testes. Three testes of two 1 year old goats were perfused for 4–5·5 hr and the results were similar to those in rams. Two testes perfused with homologous blood had blood flows of 9 and 38 ml./100 g/min and one with autologous blood, 30 ml/100 g/min. Rete testis fluid flowed from two testes of one animal. During the operation at first the flow rates were 2·4 and 2·6 ml/hr, the concentration of sperm $4{\cdot}07 \times 10^7$ and $1{\cdot}86 \times 10^7$/ml. and of glutamate 1·6 and 1·3 μ-mole/ml., but after 6 hr of anaesthesia these values had all declined markedly; during perfusion after ischaemic intervals of 10 and 6 min the fall in sperm and glutamate concentration continued to negligible levels, but the flow of fluid was maintained at 0·4–0·6 ml./hr. In these two perfusions four measurements at hourly intervals gave the following mean data: O_2 uptakes 0·5 and 0·33 ml./100 g/min, R.Q. 1·14 and 1·11 and the glucose uptakes 0·21 and 0·13 mg/min (arterial blood concentration 17–37 mg/100 ml.).

DISCUSSION

It is clear from these results that the functions of the perfused testis can approach those of the testis *in vivo* provided the period of ischaemia to which the perfused testis is subjected is kept to a minimum and the testis is supplied with adequate amounts of glucose. The secretion of rete testis fluid and the production of spermatozoa seem to be especially sensitive to even the short periods of ischaemia usual in setting up a perfusion. This and the fact that the secretion of fluid seems to be independent of perfusion pressure but dependent on glucose concentration is further evidence that the secretion of fluid by the seminiferous tubules is an active process, not passive filtration (Setchell, 1967*a*; Waites & Setchell, 1968).

The spermatogenic function of the testis certainly seems to be more critical of conditions of perfusion than the production of steroids and this

may be related to the fact that the interstitial cells are close to blood capillaries and bathed by an extracellular fluid whose composition, as judged by the analysis of testicular lymph, is very similar to blood plasma (Lindner, 1963, 1966; Cowie, Lascelles & Wallace, 1964; Wallace & Lascelles, 1964). The seminiferous epithelium on the other hand lies behind a permeability barrier (Kormano, 1967; Setchell, 1967*b*; Setchell *et al.* 1969) and is bathed in a fluid of quite different composition (Waites & Setchell, 1968).

Whatever the explanation of the greater sensitivity of the germinal epithelium to ischaemia, the latter does not seem to affect the metabolism of the testis and epididymis as a whole to any great extent, unless it is to reduce slightly the fraction of the carbon dioxide produced by the testis which comes from blood glucose. Normal metabolism in the perfused testis is maintained despite the higher than normal blood flow. This high blood flow is interesting because blood flow through the testis in the conscious ram cannot be increased by a number of means which increase blood flow through other organs, for example, acetylcholine phenoxybenzamine or heat (Waites & Setchell, 1964; Setchell, Waites & Thorburn, 1966). The use of the 5-HT antagonist bromolysergic acid diethylamide (B.O.L. 148), which, at the concentration used, has some antagonism to noradrenaline and sympathetic nerve stimulation (Fig. 4), may be responsible, at least in part, for the supranormal blood flow, for the lack of autoregulation in the isolated perfused testis, and for its insensitivity to catecholamines. The testes perfused *in situ*, where the concentration of B.O.L. 148 was lower or nil, were much more sensitive to catecholamines but still showed no evidence of autoregulation, but blood flow was still higher than normal, suggesting that there is normally some vascular tone in the testis in conscious animals.

It has been suggested that at least part of the testicular damage caused by heat *in vivo* may be due to hypoxia induced by an increase in oxygen consumption without a corresponding increase in testicular blood flow (Waites & Setchell, 1964; Setchell *et al.* 1966). The degree and duration of scrotal heating used in these earlier studies was sufficient to cause moderate to profound seminal degeneration and the same heating was used in the present studies with the isolated perfused testis. Sperm production *in vivo* was quickly reduced during the heating but, as *in vivo*, oxygen and glucose uptake increased without a corresponding increase in blood flow. These results are in contrast with those of Ewing & Vandemark (1963) in rabbits, who observed a decrease in perfusate flow in the third hour of perfusion at 39·5° C, with a concomitant decrease in glucose uptake; they did not measure oxygen consumption. From their evidence they concluded that the effect of heat was to decrease glucose available to the germinal

epithelium but this explanation is not supported by our results in the ram.

The histological findings after heating the perfused ram testis suggest that non-specific damage was observed rather than the specific lesions in spermatogonia and spermatocytes described by Waites & Ortavant (1968) after the application of heat to the testes of conscious rams. However, the specific lesions were not observed until 12 hr after heating, and none of the perfusions was continued for as long as that after heating the testis.

The failure of fluid secretion and sperm production after a period of ischaemia usual in the setting up of perfusions means that earlier results with the perfused testis must be treated with some caution, particularly if the results are taken to apply to the testis as a whole and not just the interstitial tissue. Nevertheless, the fact that an isolated perfused testis under the right conditions can perform, at least temporarily, the normal functions of steroidogenesis, spermatogenesis and fluid secretion does mean that the isolated perfused testis will be a useful tool for studying these processes.

We wish to thank Dr G. M. H. Waites for his help in the assessment of the histological material, and Messrs I. R. Fleet and R. D. Burton for their enthusiastic and skilled assistance. One of us (B. P. Setchell) was visiting England as a Royal Society and Nuffield Foundation Commonwealth Bursar.

REFERENCES

ANNISON, E. F. & LINDSAY, D. B. (1961). Acetate utilisation in sheep. *Biochem. J.* **78**, 777–785.

ANNISON, E. F. & WHITE, R. R. (1962). Further studies on the entry rates of acetate and glucose in sheep, with special reference to endogenous production of acetate. *Biochem. J.* **84**, 546–552.

COWIE, A. T., LASCELLES, A. K. & WALLACE, J. C. (1964). Flow and protein content of testicular lymph in conscious ram. *J. Physiol.* **171**, 176–187.

DANBY, M. (1940). Formation and destruction of male hormone by surviving organs. *Endocrinology* **27**, 236–241.

EWING, L. L. & EIK-NES, K. B. (1966). On the formation of testosterone by the perfused rabbit testis. *Can. J. Biochem. Physiol.* **44**, 1327–1344.

EWING, L. L. & VANDEMARK, N. L. (1963). Factors affecting testicular metabolism and function. III. Effect of *in vitro* temperature elevation on tissue slices and perfused testes in the rabbit. *J. Reprod. Fert.* **6**, 17–24.

HARDWICK, D. C. & LINZELL, J. L. (1960). Some factors affecting milk secretion by the isolated perfused mammary gland. *J. Physiol.* **154**, 547–571.

HINKS, N. T., MILLS, S. C. & SETCHELL, B. P. (1966). A simple method for the determination of the specific activity of carbon dioxide in blood. *Analyt. Biochem.* **17**, 551–553.

HYVARINEN, A. & NIKKILA, E. O. (1962). Specific determination of blood glucose with *o*-toluidine. *Clinica chim. Acta* **7**, 140–143.

JONES, G. B. (1965). Determination of the specific activity of labelled blood glucose by liquid scintillation using glucose pentaacetate. *Analyt. Biochem.* **12**, 249–258.

KORMANO, M. (1967). Dye permeability and alkaline phosphatase activity of testicular capillaries in the post natal rat. *Histochemie* **9**, 327–338.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z. physiol. Chem.* **210**, 33–66.

LINDNER, H. R. (1963). Partition of androgen between the lymph and venous blood of the testis in the ram. *J. Endocr.* **25**, 483–494.

LINDNER, H. R. (1966). Participation of lymph in the transport of gonadal hormones. *Proc. 2nd Int. Congr. horm. Steroids*, 1966. Exc. Med. Int. Conf. Ser. No. 132, 821–827.

LINZELL, J. L. & SETCHELL, B. P. (1968). The output of spermatozoa and fluid by, and the metabolism of, the isolated perfused testis of the ram. *J. Physiol.* **195**, 25–26*P*.

NYMAN, M. A., GEIGER, J. & GOLDZIEHER, J. W. (1959). Biosynthesis of estrogen by the perfused stallion testis. *J. biol. Chem.* **234**, 16–18.

PETERS, J. P. & VAN SLYKE, D. D. (1932). *Quantitative Clinical Chemistry*, vol. 2. Baltimore: Williams and Wilkins.

SAKAMOTO, M. (1936). Ein Experiment über den Einfluss der Testicularflüssigkeit auf die Hoden und die Samenblase der infantilen männlichen Mäuse. *Folia endocrin. jap.* **12**, 59–60.

SETCHELL, B. P. (1967*a*). Fluid secretion by the testis. *J. Reprod. Fert.* **14**, 347–348.

SETCHELL, B. P. (1967*b*). The blood-testicular fluid barrier in sheep. *J. Physiol.* **189**, 63–65*P*.

SETCHELL, B. P. & HINKS, N. T. (1967). The importance of glucose in the oxidative metabolism of the testis of the conscious ram and the role of the pentose cycle. *Biochem. J.* **102**, 623–630.

SETCHELL, B. P., VOGLMAYR, J. K. & WAITES, G. M. H. (1969). A blood-testis barrier restricting passage from blood into rete testis fluid but not into lymph. *J. Physiol.* **200**, 73–85.

SETCHELL, B. P. & WAITES, G. M. H. (1964). Blood flow and the uptake of glucose and oxygen by the testis and epididymis of the ram. *J. Physiol.* **171**, 411–425.

SETCHELL, B. P., WAITES, G. M. H. & LINDNER, H. R. (1965). Effect of undernutrition on testicular blood flow and metabolism and the output of testosterone in the ram. *J. Reprod. Fert.* **9**, 149–162.

SETCHELL, B. P., WAITES, G. M. H. & THORBURN, G. D. (1966). Blood flow in the testis of the conscious ram measured with krypton^{85}-effects of heat, catecholamines and acetylcholine. *Circulation Res.* **18**, 755–765.

SETCHELL, B. P., WAITES, G. M. H. & TILL, A. R. (1964). Variations in blood flow in the testis and epididymis of the sheep and rat. *Nature, Lond.* **203**, 317–318.

SOWERBY, J. M. & OTTAWAY, J. H. (1966). The enzymic estimation of glutamate and glutamine. *Biochem. J.* **99**, 246–252.

SSENTJURIN, B. S. (1926). Über die nach der Methode von N. P. Kravkov gewonnene Testicularflüssigkeit. *Z. ges. exp. Med.* **48**, 712–723.

VANDEMARK, N. L. & EWING, L. L. (1963). Factors affecting testicular metabolism and function. I. A simplified perfusion technique for short-term maintenance of rabbit testis. *J. Reprod. Fert.* **6**, 1–8.

VOGLMAYR, J. K., SCOTT, T. W., SETCHELL, B. P. & WAITES, G. M. H. (1967). Characteristics of testicular spermatozoa and fluid collected from conscious rams; glucose dissimilation, resistance to cold and factors affecting motility. *J. Reprod. Fert.* **14**, 87–99.

VOGLMAYR, J. K., WAITES, G. M. H. & SETCHELL, B. P. (1966). Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. *Nature, Lond.* **210**, 861–863.

WAITES, G. M. H. & ORTAVANT, R. (1968). Effets précoces observés sur les testicules soumis à une action brève de température élevée. *Annls Biol. anim. Biochim. Biophys.* (In the Press.)

WAITES, G. M. H. & SETCHELL, B. P. (1964). Effect of local heating on blood flow and metabolism in the testis of the conscious ram. *J. Reprod. Fert.* **9**, 339–349.

WAITES, G. M. H. & SETCHELL, B. P. (1968). Some physiological aspects of the function of the testis. In *The Gonads*, ed. MCKERNS, K. W. New York: Appleton Century Crofts.

WALLACE, J. C. & LASCELLES, A. K. (1964). Composition of testicular and epididymal lymph in the ram. *J. Reprod. Fert.* **8**, 235–242.