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A BLOOD–TESTIS BARRIER RESTRICTING PASSAGE FROM BLOOD INTO RETE TESTIS FLUID BUT NOT INTO LYMPH

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SUMMARY

1. A permeability barrier in or around the seminiferous tubules of rams has been demonstrated by studying the rate of passage of a variety of substances from blood plasma into fluid collected from the rete testis and into testicular lymph.

2. All substances studied passed readily into testicular lymph.

3. Tritiated water, urea, ethanol and bicarbonate in rete testis fluid equilibrated with blood plasma within 3 hr; Na^+, K^+, Rb^+, Cl^-, I^-, CNS^-, creatinine and galactose entered slowly and *p*-aminohippurate (PAH), glutamate, iodinated albumin, inulin and [^{51}Cr]EDTA did not appear in rete testis fluid at all.

4. Rubidium was excluded relative to iodoantipyrine from the testes of control and hypophysectomized rats and from rat testes heated to 37, 40, 43 and 45° C; no such exclusion was seen in testes of rats which had been given cadmium chloride 5 months earlier so as to destroy the seminiferous tubules.

5. It is suggested that this permeability barrier will regulate the access to the seminiferous epithelium of some constituents of blood plasma, isolate the germinal cells immunologically and help to maintain the concentration differences between rete testis fluid and lymph or blood plasma.

INTRODUCTION

During early studies which led to the concept of the blood–brain barrier, it was noticed that some of the dyes injected did not pass readily into the seminiferous tubules (Ribbert, 1904; Bouffard, 1906; Goldmann, 1909;

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Pari, 1910). This was later confirmed with other dyes (de Bruyn, Robertson & Farr, 1950; Goldacre & Sylven, 1959, 1962), and subsequently it was found that a number of other compounds are also excluded from the seminiferous tubules (Ro & Busch, 1965; Hammarström, 1966; Kormano, 1967*a*, *b*, 1968). However, this impermeability is not found in the testes of new-born rats but develops at the age of about 15 days (Kormano, 1967*b*).

When testicular blood flow was measured by the indicator fractionation technique using [^{86}Rb]rubidium chloride and [^{131}I]iodoantipyrine, it was found that rubidium gave much lower results than iodoantipyrine, whereas, in most other organs except brain, the two indicators yielded similar values. It was therefore concluded that there were areas in the testis, as in the brain, into which rubidium penetrated only slowly (Waites & Setchell, 1966).

In a further investigation of this subject, the present paper reports the results of some experiments on rats with altered testicular function and on conscious rams; in the rams, we measured the rate of penetration of a variety of substances from blood plasma into testicular lymph and into fluid collected from the rete testis. An examination of the differing rates at which these substances pass into the seminiferous tubules should throw light on the nature of the permeability barrier in the testis. A preliminary report has been presented to the Physiological Society (Setchell, 1967).

METHODS

Materials

Animals. Twenty-one Merino rams aged 2–4 yr and weighing 52–64 kg were used. They were kept in a room at 21° C with 12 hr of light per day and fed 1 kg of a mixture of oat grain and chaffed lucerne hay daily.

The rats were from the same colony as those used by Waites & Setchell (1966).

Surgical preparation of rams. Catheters were placed into some or all of the following: (1) the rete restis via the efferent ducts (Voglmayr, Scott, Setchell & Waites, 1967); (2) a testicular lymphatic duct in the spermatic cord (Lindner, 1963, 1966; Cowie, Lascelles & Wallace, 1964); (3) the testicular artery; (4) an internal spermatic vein; (5) a femoral artery; (6) the posterior vena cava via a recurrent tarsal vein (see Setchell & Hinks, 1967). Because of the tendency of testicular lymph to clot, a finer PVC tube (0·2 mm inside diameter (i.d.), 0·5 mm outside diameter (o.d.) Dural Plastics, (Dural, N.S.W.) was passed to within a few mm of the tip of the lymphatic catheter (PVC 0·87 mm i.d., 1·27 mm o.d.) and heparin solution (10,000 units/ml.) was pumped at a rate of 0·5 ml./hr through the finer tube.

Experimental procedure

Ram experiments. All experiments were done at least 24 hr after surgery was completed. The basic procedure was similar to that used by Davson (1955) for studying the rate of passage of substances into cerebrospinal fluid (c.s.f.) and aqueous humour. The plasma concentration of the substance being studied was raised and then held approximately constant by a suitable combination of priming injection and continuous infusion into the catheter in the posterior vena cava. It was possible to study a number of substances in one

experiment (provided that no interference occurred during analysis), but each substance was studied only once in each animal. While the plasma concentration was constant, rete testis fluid and testicular lymph were collected usually for six half-hourly periods and blood samples were collected from the femoral artery or jugular vein usually after ½, 1½ and 2½ hr. In some experiments with [^{42}K] potassium chloride and [^{86}Rb] rubidium chloride, infusions were given into the testicular artery at the rate of 0·15ml./min with no priming dose. In these experiments, blood samples were collected from the ipsilateral internal spermatic vein.

All injections and infusions were made in pyrogen-free physiological saline (Baxter-D. H. A., Rosebury, N. S. W.).

Rat experiments. Rats aged 24 weeks were treated as follows: (1) four were hypophysectomized by the parapharyngeal approach; (2) seven were given one subcutaneous injection of cadmium chloride (3 μ-moles/100 g body wt.); (3) the scrotum of four to six rats anaesthetized with pentobarbitone sodium were immersed for 15 min in water at each of the following temperatures: 33, 37, 40, 43 and 45° C. Controls were included for each group. Relative blood flow, as defined by Waites & Setchell (1966), was measured, using both [^{131}I]iodoantipyrine and [^{86}Rb]rubidium chloride, 4–5 months after hypophysectomy or the injection of cadmium or at the end of the period of heating.

Analytical methods. Radioactivity was measured by liquid scintillation for β-emitters and a sodium iodide well counter for γ-emitters (Packard, La Grange, Illinois). The following chemical methods were used: (1) ethanol, Williams, Linn & Zak (1958); (2) bicarbonate, Hinks, Mills & Setchell (1966), and the Auto Analyser (Technicon, Chauncey, N.Y.) method involving decoloration of buffered phenolphthalein; (3) thiocyanate, Bowler (1944); (4) creatinine, Hare (1950); (5) galactose, the difference between total reducing sugars with *o*-toluidine (Hyvärinen & Nikkilä, 1962) and glucose by glucose oxidase (Huggett & Nixon, 1957); (6) inulin, Roe, Epstein & Goldstein (1949); (7) *p*-aminohippurate, Bratton & Marshall, (1939); (8) sodium and potassium, flame photometer attachment of the Auto Analyser; (9) chloride, a colorimetric method with the Auto Analyser using mercuric thiocyanate, ferric nitrate and mercuric nitrate; (10) phosphate, Fiske & Subbarow (1925).

RESULTS

Ram experiments

The substances studied can be classified into three distinct groups according to the rate at which they passed into rete testis fluid; all substances passed readily into testicular lymph.

Group 1. *Substances passing readily into both rete testis fluid and testicular lymph.* Tritiated water passed rapidly from the blood plasma into both rete testis fluid and testicular lymph so that rete testis fluid (5 expts.) and testicular lymph (1 expt.) collected during the second half-hour of the experiment contained the same concentration of tritiated water (TOH) as did the blood plasma (Fig. 1). Ethanol, bicarbonate and urea (1 expt. on each) passed into rete testis fluid less rapidly than tritiated water but equilibrium with blood plasma was reached after 2 hr of infusion (Fig. 1, Table 1).

Group 2. *Substances passing readily into testicular lymph but slowly into rete testis fluid.* With substances in this group, differences in concentration remained, even after 3 hr, between rete testis fluid and either testicular

lymph or blood plasma (Fig. 1, Table 1). Substances in this group included potassium (2 expts., rete testis fluid (RTF) and testicular lymph (TL)), rubidium (1 expt. RTF and TL, 1 expt. RTF), sodium (1 expt. RTF), chloride (1 expt. RTF), iodide (2 expts. RTF and TL), thiocyanate (1 expt. RTF and TL, 2 expts. RTF), creatinine (2 expts. RTF and TL, 3 expts. RTF), galactose (2 expts. RTF) (Fig. 1, Table 1). The transfer constant (K_{out}) as

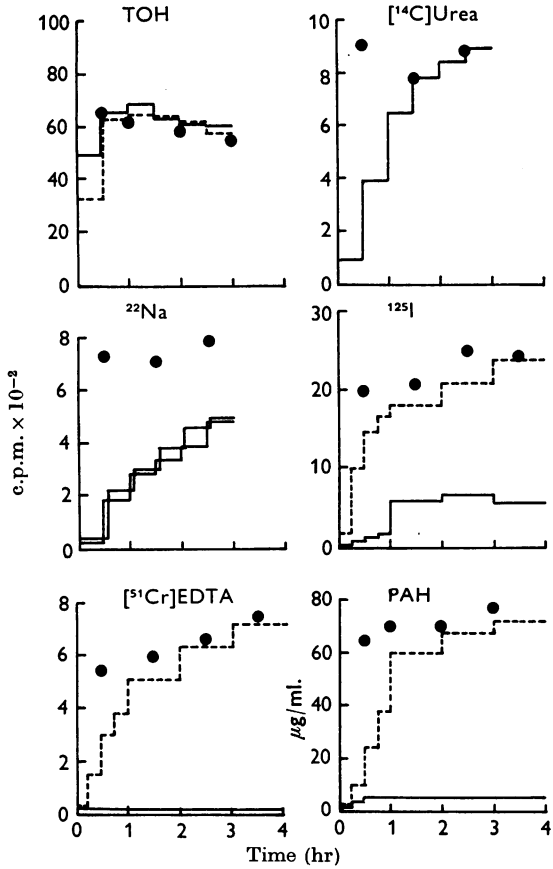

Fig. 1. The concentration of various substances in blood plasma (●), testicular lymph (- - -) and rete restis fluid (——) during intravenous infusions, in most instances after a priming dose. The graph for ^{22}Na shows the results for both testes of one ram. Ordinates: counts per minute (c.p.m.) × 10^{-2}, except for PAH which is μg/ml. Abscissae: time in hours.

defined by Davson (1956) for ^{22}Na was 0·0072 min^{-1}; the equivalent figures for c.s.f. and aqueous humour given by Davson (1956) for a variety of species ranged from 0·0041 to 0·023 min^{-1} for c.s.f. and from 0·009 to 0·025 min^{-1} for aqueous humour. The transfer constant for ^{36}Cl from rete testis fluid was 0·0060 min^{-1}.

It was peculiar to both rubidium and potassium that the radioactivity in the rete testis fluid continued to rise after the infusion had been stopped and the radioactivity in the plasma had begun to fall. This trend continued

TABLE 1. The ratio of concentrations in rete testis fluid or testicular lymph to that in blood plasma when plasma concentration had been raised and held approximately constant for 3 hr. Equivalent figures for aqueous humour and c.s.f. of the rabbit or dog (D) are given where available. Individual values or means ± S.E. of mean with number of observations in parentheses

Substance	Testicular lymph	Rete testis fluid	Aqueous† humour	Cerebrospinal† fluid
Tritiated water	1·05	1·07 ± 0·025 (6)	1	1
Ethanol	—	0·98, 1·06	1	1
Urea	—	1·01	0·26	—
Bicarbonate	—	0·97	—	—
Thiocyanate	0·80	0·77, 0·81, 0·93	0·78	0·095 (120 min)
^{42}Potassium	—	0·59, 0·81	0·55 (120 min)	0·16 (120 min)
^{22}Sodium	—	0·63, 0·65	{0·74 {0·90 D‡	{0·50 {0·60 D‡
^{36}Chloride	—	0·61	0·90 D‡	0·80 D‡
^{86}Rubidium	1·13	0·41 ± 0·11 (4)	—	—
^{125} Iodide	0·95, 0·97	0·28	0·54	0·018 (120 min)
Creatinine	0·96, 1·04	0·16, 0·20, 0·39	0·17	0·08
Galactose	—	0·06, 0·18	—	—
p-aminohippurate	0·80, 1·02	< 0·05 (6)	0·07 (120 min)	0·004 (120 min)
Inulin	0·96	< 0·05 (2)	—	—
[^{51}Cr]EDTA	0·76, 0·94	< 0·05 (2)	—	—
[^{14}C]glutamic acid	—	< 0·05 (1)	—	—
[^{131}I]albumin	1·0*	< 0·05 (1)	—	—

* Cowie *et al.* 1964; † Davson, 1956; ‡ Wang, 1948.

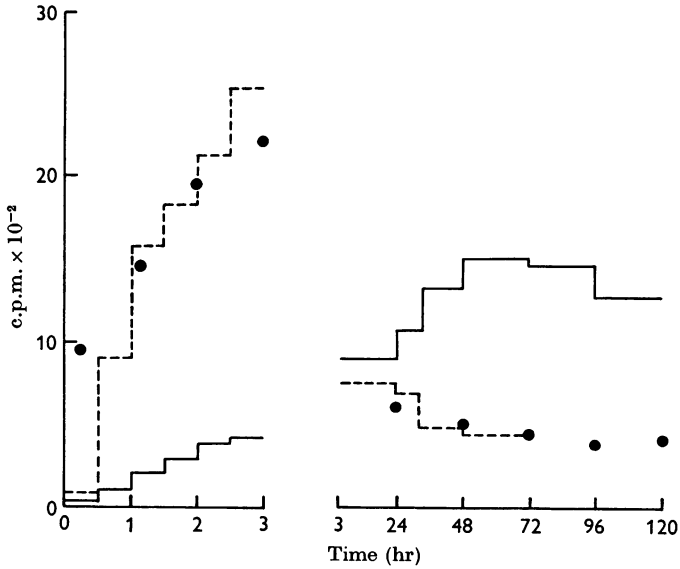

Fig. 2. The radioactivity in blood plasma (●), testicular lymph (- - -) and rete testis fluid (——) during and after an intravenous infusion of [^{86}Rb]rubidium chloride between 0 and 3 hr.

until rete testis fluid contained three times as much radioactivity as blood plasma (Fig. 2); i.e. comparable to the ratio of the concentration of non-radioactive potassium present in these fluids.

When rubidium or potassium was infused into the testicular artery, it was not possible to express the results in the same way. Instead, the ratio of the concentration of radioactive rubidium to tritiated water in the rete testis fluid, testicular lymph or blood plasma from the internal spermatic vein was compared with an equivalent ratio for the infusate. The ratio of rubidium to tritiated water in the rete testis fluid rose slowly during the 5 hr infusions (2 expts.) but always remained below the ratio in the infusate or blood plasma from the internal spermatic vein (Fig. 3).

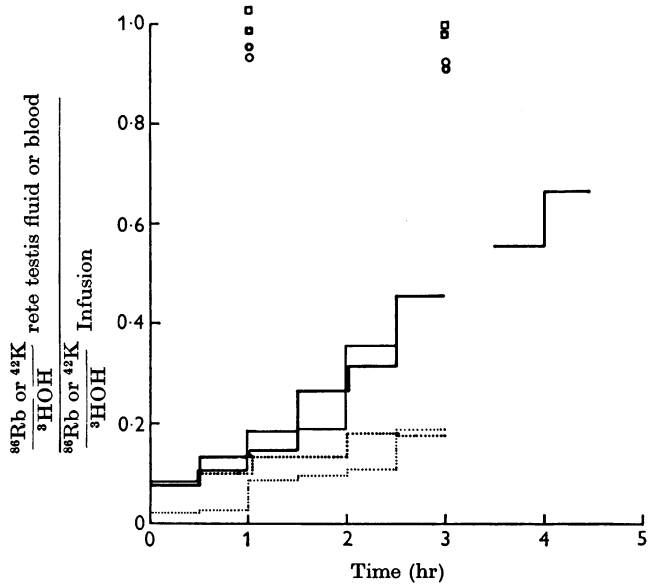

Fig. 3. The ratio of ^{86}Rb or ^{42}K to ^{3}HOH in rete testis fluid (^{86}Rb: ——; ^{42}K: . . . .) or blood from the internal spermatic vein (^{86}Rb: □; ^{42}K: ○) compared with the ratio of the isotopes in the infusion given into the testicular artery between 0 and 5 hr.

The ratio of potassium to tritium in the rete testis fluid remained below that of the infusate (2 expts.), whereas the ratio in testicular lymph rapidly became similar to the ratio in the infusate.

Group 3. *Substances passing readily into testicular lymph but not at all into rete testis fluid.* Even after infusions longer than 3 hr, some substances were present in rete testis fluid in only insignificant concentrations. In this category were *p*-aminohippurate (2 expts. RTF and TL, 4 expts. RTF), [^{14}C]glutamic acid (1 expt. RTF), [^{131}I]iodinated albumin (1 expt. RTF), inulin (1 expt. RTF and TL, 1 expt. RTF), and [^{51}Cr]chromium EDTA

(2 expts. RTF and TL) (Fig. 1, Table 1). [^{51}Cr]EDTA was used because it has been shown that this substance is filtered by the kidney glomeruli but neither resorbed nor secreted in the tubules (Stacy & Thorburn, 1966) and is not absorbed from the gastrointestinal tract (Downes & McDonald, 1964; Warner & Stacy, 1968).

Composition of rete testis fluid. Rete testis fluid is quite different in composition from testicular lymph or blood plasma. The much higher concentrations of potassium, inositol and glutamic and aspartic acids in rete testis fluid have already been reported (Voglmayr, Waites & Setchell, 1966; Voglmayr *et al.* 1967; Setchell, Hinks, Voglmayr & Scott, 1967; Setchell, Dawson & White, 1968), and there are also important differences in the concentrations of chloride, bicarbonate and phosphate (Table 2).

Rat experiments

Relative blood flow as defined by Waites & Setchell (1966) measured with rubidium was only about one quarter of the value found with iodoantipyrine in the same control rats, confirming previous observations (Waites & Setchell, 1966). This difference had disappeared in rats given cadmium 5 months earlier; the estimates using rubidium were correspondingly higher and similar to those with iodoantipyrine. The histological appearance of the testes of the control rats was normal but those of the cadmium-treated rats consisted only of regrown interstitial cells and were without organized seminiferous tubules. The difference between the uptake of rubidium relative to iodoantipyrine was greater in testes of rats locally heated to 43° C and this discrepancy became even greater at 45° C. In the hypophysectomized rats, there was still a discrepancy between the two measurements, but less than in the control animals, and the over-all figures were lower (Table 3).

DISCUSSION

Our experiments show that, of the substances that pass readily into testicular lymph, only a few pass equally freely into the seminiferous tubules. These tubules are normally filled with fluid which has been assumed to be that fluid which is collected from the rete testis, since no blood or lymph vessels enter the tubules. Testicular lymph is believed to be derived from the extracellular fluid of the interstitial tissue. It would therefore appear that there is a permeability barrier in or around the seminiferous tubules. This finding is supported by microscopic studies with acriflavine (Kormano, 1967*b*), DOPA (Kormano, 1967*a*) and [^{131}I]-albumin (Mancini, Vilar, Alvarez & Seiguer, 1965); all these materials pass readily into the interstitial tissue but not into the tubules.

A number of such permeability barriers have been described elsewhere in the body: between the blood plasma and the c.s.f., aqueous humour (see

TABLE 2. The concentration of various substances in rete testis fluid, and blood plasma from the internal spermatic vein of rams, the ratio R_{RTF} between paired samples of rete testis fluid and blood plasma from the same animals and the equivalent figures (for testicular lymph R_{TL}), dialysate of plasma (R_{dial}), cerebrospinal fluid ($R_{c.s.f.}$) and aqueous humour (R_{aq}) where known for the sheep, goat (G) or ox (Ox), otherwise rabbit (R). Except where stated otherwise, concentrations are given in m-equiv/l. and as mean ± S.E. of mean

Substance	Rete testis fluid	Blood plasma from internal spermatic vein	R_{RTF}	R_{TL}	R_{dia}	$R_{c.s.f.}$	R_{aq}
Ions							
Sodium	118 ± 3·5 (30)	145 ± 0·98 (14)	0·91	1·00*	0·945 (R)‡	0·94 (G)†	0·93‡
Potassium	12·50 ± 0·51 (30)	4·26 ± 0·11 (14)	3·16	1·00*	0·96 (R)‡	0·69 (G)†	0·96 (Ox)‡
Calcium	2·09 ± 0·31 (4)	5·50 ± 0·12 (4)	0·38	0·91*	0·65 (R)‡	0·48 (G)†	0·55 (Ox)‡
Magnesium	0·67 ± 0·05 (4)	1·87 ± 0·16 (4)	0·36	0·89*	0·80 (R)‡	0·86 (G)†	0·66 (Ox)‡
Chloride	128 ± 2·4 (22)	112 ± 1·5 (6)	1·20	1·03*	1·04 (R)‡	1·15 (G)† 1·09 (G)‡	1·16‡
Bicarbonate	8·1 ± 0·38 (40)	26·3 ± 0·40 (25)	0·33	1·09*	1·04 (R)‡	0·81 (G)‡	0·67 (G)‡
Phosphate	0·075 ± 0·001 (6)	1·54 ± 0·22 (6)	0·049	1·06*	—	0·35 (R)‡	0·60 (Ox)‡
Total inorganic salts (mg/ml.)	8·79	—	—	—	—	—	—
Ash (mg/ml.)	8·19 ± 0·68 (3)	—	—	—	—	—	—
Organic material							
Protein g/100 ml.	0·14 ± 0·017 (22)	8·8	0·018	0·66*	—	0·0038 (R)‡	0·0048 (R)‡
Urea	4·8 ± 0·053 (5)	4·9 ± 0·49 (5)	0·98	—	1·00 (R)‡	0·81 (R)‡	0·85 (Ox)‡
Osmolality (m-osmole/kg)	291 ± 3·1 (14)	297 ± 1·8 (14)	0·98	—	0·995 (R)‡	—	—

* Wallace & Lascelles, 1964; † Pappenheimer, Heisey, Jordan & Donner, 1962; ‡ Davson, 1956, 1962, 1967.

Davson, 1956, 1962, 1963), saliva (Burgen & Emmelin, 1961), or follicular fluid (Zachariae, 1958). The rate at which substances enter rete testis fluid is intermediate between the rates of entry into c.s.f. and aqueous humour; no quantitative studies have been made on saliva or follicular fluid. Rete testis fluid also has many similarities in composition to c.s.f. and aqueous humour. The finding that substances like inulin do not readily enter any of these fluids is strong evidence that they are actively secreted, not filtered, and this is supported by certain characteristics of their composition, particularly the high chloride concentration.

TABLE 3. Effect of local heating, hypophysectomy or previous treatment with cadmium chloride on relative blood flow as defined by Waites & Setchell (1966) (mean ± S.E. of mean) determined with [^{131}I]iodoantipyrine and [^{86}Rb]rubidium chloride, in the testes of rats

Testis immersed in water at (°C)	No. of observations	Relative blood flow: [^{131}I]iodoantipyrine	Relative blood flow: [^{86}Rb]rubidium chloride	P of difference between I and Rb by t test on paired values
33	12	$0{\cdot}70 \pm 0{\cdot}030$ N.S.	$0{\cdot}32 \pm 0{\cdot}002$ N.S.	***
37	10	$0{\cdot}69 \pm 0{\cdot}034$**	$0{\cdot}32 \pm 0{\cdot}014$**	***
40	8	$0{\cdot}81 \pm 0{\cdot}013$***	$0{\cdot}38 \pm 0{\cdot}019$***	***
43	8	$1{\cdot}30 \pm 0{\cdot}014$***	$0{\cdot}40 \pm 0{\cdot}009$*	***
45	8	$2{\cdot}20 \pm 0{\cdot}10$	$0{\cdot}68 \pm 0{\cdot}093$	***
After hypophysectomy	8	$0{\cdot}39 \pm 0{\cdot}049$*	$0{\cdot}25 \pm 0{\cdot}017$ N.S.	*
Control	6	$0{\cdot}52 \pm 0{\cdot}036$	$0{\cdot}28 \pm 0{\cdot}013$	***
After cadmium chloride injection	14	$0{\cdot}69 \pm 0{\cdot}062$*	$0{\cdot}73 \pm 0{\cdot}058$ **	N.S.
Control	10	$0{\cdot}88 \pm 0{\cdot}019$	$0{\cdot}48 \pm 0{\cdot}012$	***

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$; N.S. = not significant. Probabilities in body of table are by t test on two adjacent means.

Analysis of variance: for temperatures, between indicators***,
between temperatures***,
temperature × indicator interaction***.
for hypophysectomy, between indicators***,
between hypophysectomy and control*,
hypophysectomy × indicator interaction N.S.
for cadmium, between indicators*,
between cadmium and control N.S.,
cadmium × indicator interaction***.

On morphological grounds, two possible sites for the blood–testis barrier can be suggested. There is a well-defined boundary or limiting tissue around the tubules (Clermont, 1958; Lacy & Rotblat, 1959; Leeson & Leeson, 1963; Gardner & Holyoke, 1964; Schmidt, 1964; Kagayama, Irisawa, Shirai & Matsushita, 1965; Ross & Long, 1966; Ross, 1967) composed of four concentric layers, two cellular and two non-cellular, which reaches full development in the rat only after birth (Leeson & Leeson, 1963) at about the age at which the impermeability to dyes develops (Kormano, 1967*b*). This structure is particularly well-developed in the ram, especially the inner non-cellular layer, which consists of many

electron-dense lamellae (Lacy, 1967). There are also narrow junctions between adjacent Sertoli cells (Nicander, 1963, 1967; Bawa, 1963; Brökelmann, 1963; Gardner & Holyoke, 1964; Flickinger & Fawcett, 1967). The increased permeability in heated testes may be due to the failure of the secretory processes at the higher temperatures and it is interesting that Pari (1910) noticed that the reverse was also true, namely, that cells within the tubules stained more readily when the testes were cooled.

The suggestion, from the rapid passage of indicators into testicular lymph, that there is little restriction to passage at the testicular capillary wall, is supported by the findings in the cadmium-treated rats. Their testes can be regarded as consisting entirely of interstitial tissue with capillaries but no tubules or basement membranes. It has been shown that [^{131}I]albumin equilibrates rapidly between blood plasma and testicular lymph (Cowie *et al.* 1964) and, by autoradiographic techniques, that [^{131}I]albumin passes readily into the interstitial tissue (Mancini *et al.* 1965). However, the capillaries of the testis have an endothelium of the A-1-α type (Crabo, 1963; Ross, 1963; Murakami, 1966) and, unlike those of all other endocrine tissues, they are unfenestrated (Wolff & Merker, 1966). Furthermore, particles of saccharated iron oxide penetrated the testicular capillaries only when the animal had been given cadmium (Clegg & Carr, 1967) and the rapid passage of substances into the lymph must be due to rapid turnover of the relatively small volume of extracellular fluid in the interstitial tissue.

The existence of the blood–testis barrier has many important consequences for the function of the seminiferous tubules. The composition of the fluid around the cells differs from, and is presumably more stable than, that elsewhere in the body. The low protein content of the fluid would suggest that protein hormones, such as those from the pituitary, may have limited access. The access of drugs would also probably be affected and the dependence of the testis on blood glucose for carbon dioxide production (Setchell & Hinks, 1967) suggests that other possible metabolic fuels may be excluded. The sequestration of germinal cells behind the barrier might also partly explain why they are not recognized as 'self' by the body's immunological system when they are injected but, nevertheless, do not provoke an autoimmune response under normal conditions. Likewise, the germinal cells are probably not exposed in the usual way to any circulating induced or naturally occurring antibodies (Johnson & Setchell,1968). The barrier would also facilitate the maintenance of a high concentration of glutamic acid and other amino acids with important consequences in nucleic acid metabolism (Setchell *et al.* 1967).

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