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PERFUSION–SECRETION RELATIONSHIPS IN THE ISOLATED ELASMOBRANCH RECTAL GLAND

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SUMMARY

Perfusion and sodium secretion parameters were measured in the isolated rectal gland of *Scyliorhinus canicula* L. perfused at *in vivo* pressures, and the effect of stimulation of secretory activity by cyclic AMP and theophylline on these parameters was determined. Stimulation resulted in large increases in secretion flow rate, percentage extraction of sodium from the perfusing fluid, and arteriovenous sodium concentration difference, but did not affect perfusion flow rate or the sodium concentration of the secreted fluid. Reduction of perfusion flow rate to values below 65% of the control level, achieved by reducing perfusion pressure, produced a marked decline in sodium secretion – a process accompanied by increases in the percentage extraction of sodium and arteriovenous concentration difference of sodium, but again without any change in the sodium concentration of the secreted fluid. The *in vivo* consequences of these findings are discussed with reference to related findings for the avian nasal salt gland.

The normal rate of secretion, its sodium concentration, and the nature of the dependence of secretion rate on perfusion flow below certain levels, were essentially unaffected by a reduction in the availability of oxygen to the gland by approximately 80%. It is concluded that the observed relationship between perfusion flow and sodium secretion rate in the stimulated gland is not related to oxygen availability, and hence that the primary underlying function of the synchronized secretion-related vasodilation seen in the gland is not to increase the supply of oxygen to the stimulated secretory tissue. We discuss possible reasons why this erroneous conclusion has been reached by other workers.

INTRODUCTION

An intimate relationship between secretion rate and blood flow is a widely reported feature of a variety of exocrine glands; a marked vasodilation, synchronous with a stimulation of secretion rate, has been described in several such tissues. Included among these are certain extrarenal salt-secreting glands of non-mammalian vertebrates, such as the avian nasal gland and the elasmobranch rectal gland. In the avian gland, several studies have determined the magnitude and control of these haemodynamic changes (Fänge, Schmidt-Nielsen & Robinson, 1958; Fänge, Krog & Reite, 1963; Hanwell, Linzell & Peaker, 1971) and also the detailed quantitative

Key words: elasmobranchs, rectal gland, haemodynamics, salt secretion, blood flow.

relationships between secretion rate and the rate of blood flow to the gland (Kaul, Gerstberger, Meyer & Simon, 1983). Despite the original suggestion by Burger (1962) that vasomotor changes may be a significant factor controlling secretion rate in the elasmobranch rectal gland, relatively few investigations have been made of the relationships between these two parameters in this animal group. It has been shown that increases in blood flow to the gland are indeed likely to be a feature of the overall stimulation of secretion *in vivo*. A mechanism that would explain such a secretion-related vasodilation, together with the integrated control of the relevant parameters, has been described (Shuttleworth, 1983*a*,*b*). In addition, some indications of increases in blood flow to the gland following stimulation by a variety of means have been described by Solomon *et al.* (1984, 1985; but see Discussion). What is not clear, however, is the exact nature of the relationship between blood flow and secretion rate, and to what extent the latter is dependent on the former.

MATERIALS AND METHODS

Specimens of the dogfish *Scyliorhinus canicula* weighing 0·6–1·1 kg were obtained from the Laboratory of the Marine Biological Association, Plymouth and were maintained at a temperature of 11 °C in large fibreglass tanks supplied with sea water. The technique used for the preparation and perfusion of the rectal gland was essentially the same as that described earlier (Shuttleworth, 1983*a*). Briefly, the gland was perfused *in situ via* the modified posterior mesenteric artery that supplies the gland. Perfusion was at constant pressure, achieved by a pump/overflow system supplied from a reservoir of dogfish Ringer solution (Shuttleworth, 1983*a*). In most experiments, this solution was equilibrated with 0·3 % carbon dioxide in oxygen, but for some experiments (see Results – Oxygen availability) this was changed to equilibration with air. Efferent perfusion flow – equivalent to secretory parenchyma perfusion flow (Kent & Olson, 1982) – was obtained by cannulation of the rectal gland vein. Efferent venous pressure was maintained at zero with respect to the gland. Perfusion flow rate (efferent) was determined in these experiments by means of an infrared drop sensor connected to a microprocessor-controlled digital flow meter (University of Exeter Microprocessor Unit).

In addition, in the experiments reported here, secretory fluid was collected by cannulation of the secretory duct. In *Scyliorhinus*, this duct is extremely short and opens into a small pocket-like structure on the mucosal face of the posterior intestine. Timed samples of secreted fluid were collected and weighed to determine flow rate, assuming a fluid specific gravity of 1. Sodium concentrations in the secreted fluid were obtained by flame photometric analysis (Corning 400) and sodium secretion rates determined by multiplying the sodium concentration by the secretion flow rate. All experiments were carried out in a constant temperature room at 11 °C.

The extraction of sodium from the perfusing saline during passage through the gland (%X) was calculated as a percentage of the sodium being delivered to the gland, as follows:

$$\%X = [R/C(P+S)] \times 10^5, \quad (1)$$

where R is the secretion rate in μmol min^{-1}, C is the concentration of sodium in the perfusing saline in mmol l^{-1}, and P is the efferent perfusion flow rate and S the secretion flow rate, both in μl min^{-1}. Similarly, the sodium concentration in the efferent perfusing saline (C′) was calculated as follows:

$$C' = [C(P+S) - 1000R]/P, \quad (2)$$

and the arteriovenous concentration difference was therefore equal to C minus C′.

RESULTS

Glands were initially perfused at a pressure of 17 mmHg, which is within the physiological range for dorsal aortic blood pressure in this species (Butler & Taylor, 1975). The measured and calculated parameters of secretion at this pressure are given in Table 1, together with the effect on these parameters of stimulating secretory activity in the gland by the addition of dibutyryl cyclic AMP (0·05 mmol l^{-1}) and the phosphodiesterase inhibitor theophylline (0·25 mmol l^{-1}) to the perfusing saline. Even in the unstimulated condition, perfusion of the gland at the flow rate indicated results in the production of a small volume of secreted fluid, which has a sodium concentration equivalent to 1·87 times that of the perfusing saline. Stimulation of secretory activity by the addition of cyclic AMP and theophylline to the Ringer solution produces a greater than 20-fold increase in the flow rate of this secreted fluid with no significant change in its sodium concentration. In the isolated gland perfused without catecholamines, as in this case, no change in efferent perfusion flow occurs at the same time as the stimulation of secretion (Table 1) and the enhanced secretion rate results in a marked increase in the percentage extraction of sodium by the gland.

Changes in perfusion flow

The effect of changes in perfusion flow on secretory parameters of the isolated gland were studied by changing perfusion pressure. Previous studies on the perfused

Table 1. *The effect of stimulation with cyclic AMP and theophylline and the effect of a reduction in the availability of oxygen in the perfusate on normal perfusion and sodium secretion parameters in isolated rectal glands*

	Unstimulated	Stimulated	
	Oxygen*	Oxygen*	Air†
Perfusion flow (μl g^{-1} min^{-1})	7093 ± 607	7437 ± 312	7864 ± 417
Secretion flow (μl g^{-1} min^{-1})	3·6 ± 1·9	78·9 ± 2·6	80·0 ± 1·0
Secretion concentration (mmol l^{-1})	516·8 ± 14·2	504·0 ± 6·4	515·0 ± 4·8
Secretion rate (μmol g^{-1} min^{-1})	1·8 ± 0·9	39·7 ± 1·4	41·2 ± 0·8
% extraction	0·09 ± 0·04	1·95 ± 0·13	1·89 ± 0·10
A/V difference (mmol l^{-1})	0·11 ± 0·05	2·45 ± 0·17	2·45 ± 0·15

Values represent means ± S.E. $N = 5$ (unstimulated and stimulated/air-equilibrated) and 9 (stimulated/oxygen-equilibrated).

* Glands perfused with saline equilibrated with 99·7% oxygen in carbon dioxide.

† Glands perfused with air-equilibrated saline.

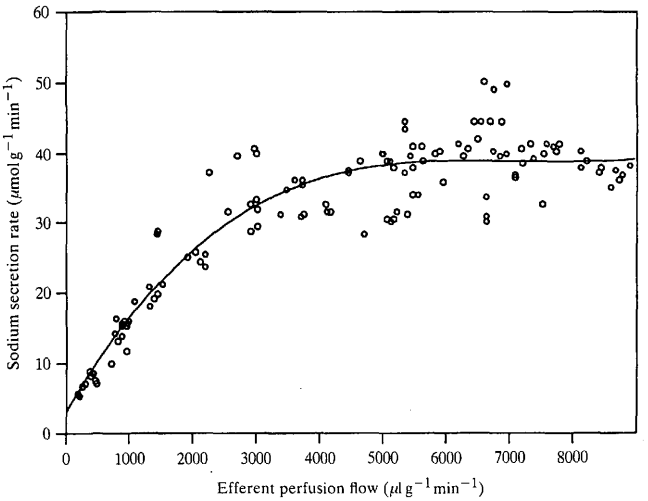

Fig. 1. Sodium secretion rates at different perfusion flows in the isolated rectal gland stimulated with cyclic AMP and theophylline. Data points represent pooled values obtained from 15 separate fish. The curve shown was obtained by computer using a polynomial regression analysis.

gland of *Squalus* (Shuttleworth & Thompson, 1983) have shown that perfusion flow is the critical haemodynamic factor determining secretion rate rather than perfusion pressure, within the physiological range, or vascular resistance. In the isolated gland of *Scyliorhinus*, it has been found that there is an essentially linear increase in flow with increasing pressure between approximately 8 mmHg and 35 mmHg (unpublished data). In Fig. 1, the effect of changing perfusion flow on sodium secretion rate in glands stimulated with cyclic AMP and theophylline is depicted. The data show that sodium secretion rate is progressively reduced at perfusion flows below 5000 $\mu l\, g^{-1}\, min^{-1}$. Despite the low secretion rates often being recorded (secretion flows as low as 1 $\mu l\, min^{-1}$) and the fact that the data points for Fig. 1 are derived from several different animals, there is a remarkable consistency in the response of secretion rate to reductions in perfusion flow. The data presented in Fig. 2 show that the sodium concentration of the secreted fluid remains essentially constant over the whole range of perfusion flows, and hence secretion rates, studied. This measured concentration is very similar to that seen in the very small volumes of secreted fluid produced by the unstimulated gland (Table 1). It would appear that the large changes in sodium secretion rate, both following stimulation by cyclic AMP and theophylline (see above) or by changes in perfusion flow in stimulated glands, are not accompanied by changes in the concentration of the secreted fluid but simply represent changes in the volume of fluid produced. For each of the data points shown in Fig. 1, the percentage extraction (equivalent to the percentage of the sodium

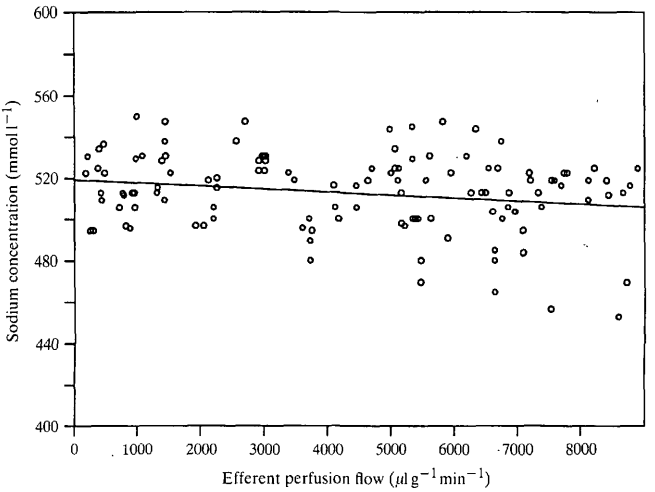

Fig. 2. Sodium concentration of secreted fluid produced at different perfusion flows in the isolated rectal gland stimulated with cyclic AMP and theophylline. Individual data points obtained from a total of 15 fish. The line was obtained by computer using linear regression analysis and has a slope not significantly different from zero.

arriving at the gland in the perfusing fluid that is actually secreted) was calculated from equation 1 and plotted against the rate of efferent perfusion flow (Fig. 3) or the simultaneous sodium secretion rate (Fig. 4). As perfusion flow is reduced, the extraction of sodium increases from a value equivalent to approximately 2% of the sodium arriving at the gland, up to more than 10%. Thus at the lowest rates of sodium secretion, the percentage extraction of sodium from the perfusing fluid is actually at its highest. This increase in the percentage extraction of sodium as the rate of sodium secretion decreases is, of course, paralleled by an increase in the calculated arteriovenous difference in sodium concentration, which rises from the normal level in stimulated glands of 2·43 mmol l^{-1} to a value in excess of 14 mmol l^{-1} at the lowest rates of sodium secretion (data not shown).

Oxygen availability

The supply of oxygen to the perfused gland was reduced by changing from a perfusing saline equilibrated with 99·7% oxygen to one equilibrated with air (see Materials and Methods). In the gland stimulated to secrete using cyclic AMP and theophylline and perfused at 17 mmHg, this reduction in oxygen availability produced no significant change in any of the secretory parameters determined (Table 1). Fig. 5 shows the effect of reducing perfusion flow, achieved by reducing the afferent perfusion pressure, on sodium secretion rate in stimulated glands perfused with air-equilibrated saline. Reduction in perfusion flow below 4000 $\mu l\, g^{-1}\, min^{-1}$ produces a

progressive decline in secretion rate. The response seen, however, is essentially similar both qualitatively and quantitatively to that observed in stimulated glands perfused with saline equilibrated with 99·7% oxygen (see Fig. 1), with the possible exception that at the lower perfusion flows, sodium secretion rate with the air-equilibrated saline appears to be approximately 20% lower than with the oxygen-equilibrated saline at the same perfusion flow. As with glands perfused with the oxygen-equilibrated saline, this reduction of sodium secretion rate at low perfusion flows in air-equilibrated perfusions is accompanied by increases in the percentage extraction of sodium by the gland (increasing to 6·8%) and parallel increases in the arteriovenous concentration difference for sodium (increasing to 8·7 mmol l^{-1}). Both of these changes are some 40% lower than the corresponding values for glands perfused with oxygen-equilibrated saline (see above). As before, no significant change in the concentration of the secreted fluid was noted at any of the perfusion flows or secretion rates measured in these air-equilibrated perfusions.

DISCUSSION

This paper describes the first detailed measurement of secretion rate in the perfused rectal gland of an elasmobranch species other than *Squalus*. The sodium secretion rate measured for the gland from *Scyliorhinus* following stimulation with cyclic AMP and theophylline (39·7 ± 1·4 μmol g^{-1} min^{-1}; Table 1) is similar to the value of 24·6 ± 0·9 μmol g^{-1} min^{-1} for *Squalus* (Shuttleworth & Thompson, 1983).

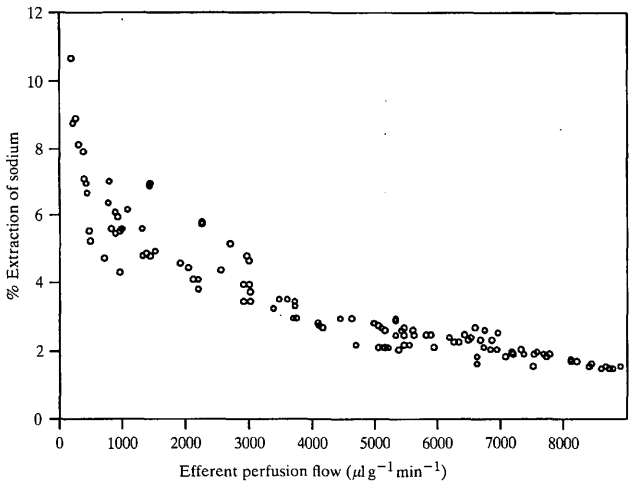

Fig. 3. Sodium extraction rate (expressed as percentage of total sodium being delivered to the gland) at different perfusion flows in the isolated rectal gland stimulated with cyclic AMP and theophylline. Individual values from 15 fish calculated using equation 1.

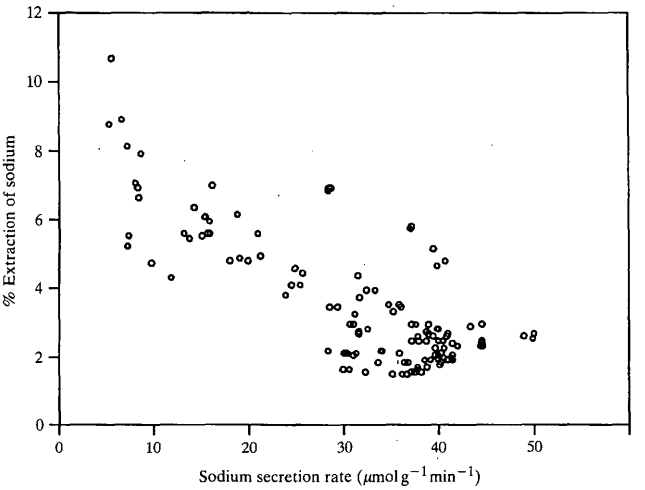

Fig. 4. Relationship between sodium extraction rate and its secretion rate in the isolated, stimulated rectal gland. Other information as in Fig. 3.

Even at the very low secretion rates seen in unstimulated glands, the values obtained per weight of gland are also similar in the two species ($1{\cdot}8 \pm 0{\cdot}9\ \mu mol\ g^{-1}\ min^{-1}$ in *Scyliorhinus*, $1{\cdot}3 \pm 0{\cdot}5\ \mu mol\ g^{-1}\ min^{-1}$ in *Squalus*). It should be noted that this similarity in secretion rate exists despite very large differences in both relative and absolute size between the glands of the two species. A typical specimen of *Scyliorhinus* weighing 800 g has a gland weighing approximately 0·07 g, compared to a gland weight of 2·5 g in a typical specimen of *Squalus* weighing 6000 g. The sodium concentration of the secreted fluid is also similar in the two species ($504{\cdot}0\ mmol\ l^{-1}$ in *Scyliorhinus*, $529{\cdot}6\ mmol\ l^{-1}$ in *Squalus*). This concentration remains remarkably constant whether the gland has been stimulated or not (see Table 1), over a wide range of perfusion flows (see Fig. 2), and whether it is being perfused with oxygen-equilibrated saline or air-equilibrated saline (see Table 1). Thus, under a wide range of different conditions of stimulation, perfusion rate or oxygen supply, resulting in individual secretion rates varying from $0{\cdot}22\ \mu mol\ g^{-1}\ min^{-1}$ to $50{\cdot}0\ \mu mol\ g^{-1}\ min^{-1}$, the sodium concentration of the secreted fluid remains remarkably consistent at a value of $509{\cdot}7 \pm 1{\cdot}42$ (mean ± S.E. of 177 separate readings from 30 different glands). This supports our previous findings (Shuttleworth & Thompson, 1983) and those of several other workers (Burger & Hess, 1960; Burger, 1962; Siegel, Schon & Hayslett, 1976; Stoff *et al.* 1977) on *Squalus*. For example, in *Squalus* it was found that only under conditions of perfusion at pressures above the physiological range (>20 mmHg) did the concentration of the secreted fluid fall significantly (Shuttleworth & Thompson, 1983). Under such conditions, the reduced concentration was

associated with an increase in the volume of secreted fluid and probably indicates a deterioration in the integrity of the secretory epithelium.

In any event, it is clear from this study and the several other investigations both on the isolated perfused gland, and the gland *in vivo*, referred to above, that changes in ion secretion rate are achieved entirely by changes in the volume of secreted fluid and not in its concentration. This is in contrast to the situation in birds where positive correlations have been reported between secretion flow rate and the concentrations of sodium and chloride (Hughes, 1970; Hanwell, Linzell & Peaker, 1971), although Hanwell *et al.* (1971) report a negative correlation between secretion flow rate and ionic concentration when studied in *individual* birds. Hence the exact nature of the contribution of changes in the concentration of the secretion to the overall secretion rate in birds is far from apparent, although that such a contribution can occur is clear. As in other exocrine glands, these changes in concentration of the secreted fluid at different secretion flow rates have led some authors to propose that the formation of the final secretion in the avian gland involves a subsequent modification of an initially secreted fluid – the so-called post-tubular modification hypothesis (Marshall, Hyatt, Phillips & Condron, 1985). The evidence suggests that such a secondary modification of the secreted fluid does not occur in the elasmobranch gland.

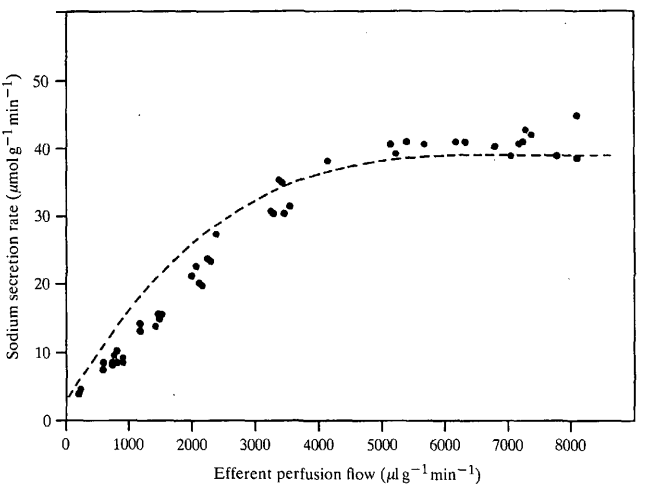

Fig. 5. Sodium secretion rates at different perfusion flows in the stimulated rectal gland perfused with air-equilibrated saline (see text for details). Individual data points from 10 separate fish. The broken curve is the corresponding relationship found for glands perfused with saline equilibrated with 99·7% oxygen + 0·3% carbon dioxide and is the same as that shown in Fig. 1.

In this study, we have found that the rate of sodium secretion in the isolated perfused gland is reduced at perfusion flows below 60% of the flow in the normal perfused gland (i.e. glands perfused at 17 mmHg). This supports our previous findings in *Squalus* (Shuttleworth & Thompson, 1983), where a qualitatively similar relationship between perfusion flow and sodium secretion was found and where evidence was presented to show that the critical haemodynamic parameter determining secretion rate was perfusion flow itself, as opposed to perfusion pressure or the vascular conductance of the tissue. In the study presented here, it has also been shown that this decline in sodium secretion rate at low perfusion flows is associated with a progressive increase in the percentage extraction of sodium and hence in the induced arteriovenous concentration difference for this ion. These relationships between secretion rate, perfusion flow and percentage extraction for the rectal gland appear, superficially, to be very different from those reported by Kaul *et al.* (1983) for the avian salt gland. These authors demonstrated a direct linear relationship between secretion rate and blood flow in the avian gland resulting in an essentially constant percentage extraction over the working range (compare our Fig. 4 with their fig. 7). However, this apparent conflict is resolved when the precise nature of the experiments in the two cases is compared. In the study by Kaul *et al.* (1983), the gland was stimulated to varying degrees, by infusing hypertonic NaCl into the bird at different rates, and then blood flow and secretion rate were determined. In most of the experiments in the study reported here, the gland was stimulated to essentially a constant (i.e. maximal) rate and perfusion flow was reduced to test its effect on secretion rate. Under these conditions, we have found that the percentage extraction increased. In other words, in our experiments increases in secretion rate induced by changing perfusion flow at a constant degree of stimulation are associated with decreases in the percentage extraction. However, when perfusion flow was held constant and the unstimulated gland was stimulated by the addition of cyclic AMP and theophylline (i.e. the degree of stimulation was increased), the observed increase in secretion rate was associated with an increase in percentage extraction (see Table 1). As stated above (see Introduction), the stimulation of rectal gland secretory activity *in vivo* is believed to be associated with a synchronous increase in blood flow. According to the above argument, the simultaneous stimulation of secretory activity (increasing extraction rate at constant blood flow) and increased blood flow (decreased extraction rate at constant secretion rate) would lead to an enhanced secretion with little or no change in the percentage extraction, which would exactly correspond with the findings of Kaul *et al.* (1983) on the avian gland *in vivo*.

That such a vasodilation must occur synchronously with the stimulation of secretion has been suggested before (Shuttleworth, 1983*a*) and, in a series of studies, Solomon *et al.* (1984, 1985) have attempted to demonstrate its existence *in vivo*. Unfortunately, scrutiny of their data suggests some problems regarding the condition of the gland in their experiments. For example, these authors report (Solomon *et al.* 1985) that following as much as a 63% increase in plasma volume (the parameter they consider to be the principal factor determining secretory activity) chloride secretion rate increased to only 422 μmol g^{-1} h^{-1} or 7 μmol g^{-1}

min^{-1}. This is considerably lower than the 20–78 $\mu mol\,g^{-1}\,min^{-1}$ recorded by other workers following plasma volume expansion in *Squalus in vivo* (Erlij, Rubio & Silva, 1980; Swenson & Maren, 1984). Furthermore, after volume expansion, the rate of blood flow to the gland reported by Solomon *et al.* (1985) was only 36 $ml\,g^{-1}\,h^{-1}$ or 600 $\mu l\,g^{-1}\,min^{-1}$ which, from the data presented here, and on *Squalus* by Shuttleworth & Thompson (1983), would suggest a gland in a highly vasoconstricted state. Of even greater concern is the fact that calculations from the data presented (see table 1 in Solomon *et al.* 1985) indicate that, in their experiments, chloride concentrations in the secreted fluid ranged from only 303–393 $mmol\,l^{-1}$. Thus, in the control fish, secretion flow is reported as 0·27 $ml\,g^{-1}\,h^{-1}$ and chloride secretion rate as 82 $\mu mol\,g^{-1}\,h^{-1}$, indicating a secretion concentration of only 303·7 $mmol\,l^{-1}$. Similarly, after volume loading, the reported secretion flow increases to 1·11 ml $g^{-1}\,h^{-1}$ and the chloride secretion rate to 422 $\mu mol\,g^{-1}\,h^{-1}$ but this still only indicates a secretion concentration of 380·2 $mmol\,l^{-1}$. Such abnormally low concentrations, which were in some instances only 11% higher than the corresponding plasma concentration, indicate a failure of active secretion (Siegel *et al.* 1975) and a breakdown of epithelial integrity. It would appear that their preparation, involving perfusion of an explanted gland by a 'donor' fish, possesses some major problems probably resulting from a vast excess of circulating catecholamines. It has been shown that the rectal gland is very sensitive to vasoconstriction by catecholamines (Shuttleworth, 1983*a*; Shuttleworth & Thompson, 1981, 1983). Perfusion flow through the gland can be reduced to as little as 5–10% of control values by the addition of noradrenaline at concentrations of 5×10^{-7}–10^{-6} $mol\,l^{-1}$ and, at such low perfusion rates, secretion is markedly inhibited.

These same authors (Solomon *et al.* 1984) have claimed that the observed, or predicted, increases in blood flow following stimulation of the gland are necessary to provide sufficient oxygen to support the increased rate of ion secretion. That the vasomotor changes do not directly arise as a result of increases in metabolic activity of the gland but result from an independently regulated response, has been demonstrated by us (Shuttleworth, 1983*a*,*b*) and subsequently supported by Solomon *et al.* (1984). Nevertheless, on the basis of measurements of partial pressure of oxygen in the blood leaving the gland in their 'semi-pithed' fish preparation, Solomon *et al.* (1984) have claimed that the gland extracts almost all of the oxygen supplied in the blood under both unstimulated and stimulated conditions. However, as discussed above, the gland in their preparation is apparently in a highly vasoconstricted or, at least, poorly perfused state and, as such, it is perhaps not surprising that much of the oxygen in the very low blood flow observed is extracted by the gland, but this is unlikely to be representative of the true situation *in vivo*. In contrast, the data presented in this paper do not support the contention that the increased blood flow is necessary to supply sufficient oxygen for the functioning of the gland. Despite the high partial pressure of oxygen in the oxygen-equilibrated saline used in our studies, it can be calculated that the actual oxygen content of this saline is somewhat lower than that typical of arterial blood in elasmobranchs. However, no effect of reducing perfusion rate was seen until the perfusion flow had been reduced to approximately

60% of its normal level. Of even greater significance is the finding that an 80% reduction of the oxygen supply to the gland, by perfusion with air-equilibrated saline instead of oxygen-equilibrated saline, had no significant effect on sodium secretion rate or on the relationship between perfusion flow and secretion rate. Clearly then, the reduction in sodium secretion rate at low perfusion flows does not result from any limitation in the supply of oxygen to the gland. Indeed, it could be claimed that the demonstration by Solomon *et al.* (1984) that the gland is, at least, *capable* of reducing the partial pressure of oxygen in the perfusing blood to values as low as 10 Torr makes it highly unlikely that the demonstrated dependence of secretion rate on blood flow is related, either directly or indirectly, to oxygen supply. It is concluded that limitations in the supply of oxygen are not the cause of the profound effect of low perfusion flows on secretion rate. The true nature of this phenomenon is currently under investigation in the authors' laboratory.

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