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TRANSCAPILLARY EXCHANGE IN THE CAT SALIVARY GLAND DURING SECRETION, BRADYKININ INFUSION AND AFTER CHRONIC DUCT LIGATION

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SUMMARY

1. Capillary permeability-surface area products for ^{86}Rb, [^{51}Cr]EDTA (mol. wt. 357), [^{57}Co]cyanocobalamin (mol. wt. 1353) and [^{125}I]insulin (approximate mol. wt. 6000) have been measured using the single-circulation, multiple-tracer dilution technique in the *in situ* perfused submandibular salivary gland during parasympathetic nerve stimulation, close-arterial bradykinin infusion and following chronic duct ligation.

2. In glands with a natural blood supply, permeability-surface area for ^{86}Rb and [^{51}Cr]EDTA increased during parasympathetic stimulation, but this was shown to be related to the concomitant increase in blood flow rather than to a change in capillary permeability or in surface area.

3. In glands perfused at constant flow, parasympathetic stimulation led to a decrease in permeability-surface area for EDTA ($-19{\cdot}1 \pm 5{\cdot}2$ %, mean ± S.E., $n = 5$, $P < 0{\cdot}05$), cyanocobalamin ($-12{\cdot}3 \pm 6{\cdot}0$, $n = 12$, $P < 0{\cdot}05$), and insulin ($-15{\cdot}3 \pm 4{\cdot}8$, $n = 11$, $P < 0{\cdot}02$). It is suggested that this may be the result of a redistribution of flow from the acinar microcirculation to a less permeable ductal vasculature.

4. Bradykinin infusion had no significant effect on permeability-surface area for EDTA and cyanocobalamin in perfused glands.

5. In perfused glands, ligation of the submandibular duct for 3–12 days reduced permeability-surface area (ml. $\text{min}^{-1}.\text{g}^{-1}$) for [^{51}Cr]EDTA from $5{\cdot}26 \pm 0{\cdot}60$ (mean ± S.E., $n = 9$) to $4{\cdot}20 \pm 0{\cdot}12$ ($n = 4$, $P < 0{\cdot}30$), [^{57}Co]cyanocobalamin from $3{\cdot}22 \pm 0{\cdot}12$ ($n = 48$) to $2{\cdot}02 \pm 0{\cdot}08$ ($n = 15$, $P < 0{\cdot}001$) and [^{125}I]insulin from $1{\cdot}52 \pm 0{\cdot}07$ ($n = 39$) to $0{\cdot}72 \pm 0{\cdot}23$ ($n = 11$, $P < 0{\cdot}001$).

INTRODUCTION

During maximal parasympathetic nerve stimulation the cat submandibular salivary gland can secrete up to 1 ml./min of saliva which is accompanied by an increase in blood flow from about 0·5 to 5–10 ml./min. The regulation of such

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a rapid water and solute flux requires a readily controllable blood flow and an extremely permeable microvasculature.

The mechanisms controlling salivary blood flow have been vigorously debated. The vasodilator peptide bradykinin has been implicated by some (see Hilton, 1960) and there is no doubt that kinins can be released from their plasma substrate by salivary kallikrein into the gland's vasculature (Ferreira & Smaje, 1976). However, kallikrein is certainly not the only mediator of the vasodilatation (Schachter & Beilenson, 1967; Gautvik, 1970; Ferreira & Smaje, 1976) and it may play a role as yet undefined, in the secretory process (Smaje, 1974).

Whatever its role in the salivary gland, bradykinin clearly causes macromolecular leakage from venules in skeletal muscle (Majno, Gilmore & Leventhal, 1967), mesentery (Verrinder, Fraser & Smaje, 1974) and hamster cheek pouch (Svensjö, Persson & Rutili, 1977), and it is generally assumed that it can do so in the salivary gland microvasculature.

While it is now clear that the vasculature of the resting salivary gland is extremely permeable to small solutes (Mann, Smaje & Yudilevich, 1979) it is not known whether this changes during secretion since only preliminary findings have been reported in the literature. In the cat submandibular gland, Hilton (1960) suggested that parasympathetic stimulation could induce protein leakage, and subsequently Eliasson, Hilton & Folkow (1973) reported that bradykinin and parasympathomimetics increased capillary filtration capacity in the pig salivary gland. Lundvall & Holmberg (1978) confirmed the increase in filtration capacity on parasympathetic stimulation in the cat salivary gland but ascribed the increase to an increase in surface area, not permeability.

In view of the effects of bradykinin on microvascular permeability and the increase in filtration capacity during stimulation of the parasympathetic nerve to the cat submandibular gland, it seemed of interest to study the effects of parasympathetic stimulation and bradykinin infusion on capillary permeability-surface area products for a range of lipid-insoluble molecules. Parasympathetic stimulation was also studied in glands whose main excretory ducts had been ligated for 3–12 days, since ligation results in marked changes in salivary gland function and morphology. It is also known that following release of the ligature, stimulation still leads to secretion, albeit at a reduced rate (Darke & Smaje, 1973). This may reflect the reduction in acinar tissue accompanying ligation (Standish & Shafer, 1957) but there is also a striking fall in kallikrein concentration in the gland and saliva (see Darke & Smaje, 1973) and a reduction in ductal sodium reabsorption (Smaje, 1974; Fraser & Smaje, 1978).

In the present paper permeability-surface area products for ^{86}Rb, [^{51}Cr]EDTA, [^{57}Co]-cyanocobalamin and [^{125}I]insulin were measured using the single-passage, multiple-tracer indicator dilution technique (Crone, 1963; Martín de Julián & Yudilevich, 1964). Preliminary accounts of this work have been presented previously (Yudilevich & Smaje, 1976; Mann, Smaje & Yudilevich, 1976, 1977).

METHODS

Cats of either sex, weighing between 2·0 and 4·5 kg, were anaesthetized with Na pentobarbitone (Sagatal, May & Baker Ltd), 35 mg/kg I.P., supplemented as required, via a cannula in the femoral vein. Both the trachea and right femoral artery were cannulated, and rectal temperature was maintained between 37 and 38 °C using a heated dissection table. Blood pressure was monitored from a cannula in the femoral artery using a pressure transducer (Bell & Howell 4-326-L212) and recorded together with salivation and blood or perfusion flow on a Devices M19 pen recorder.

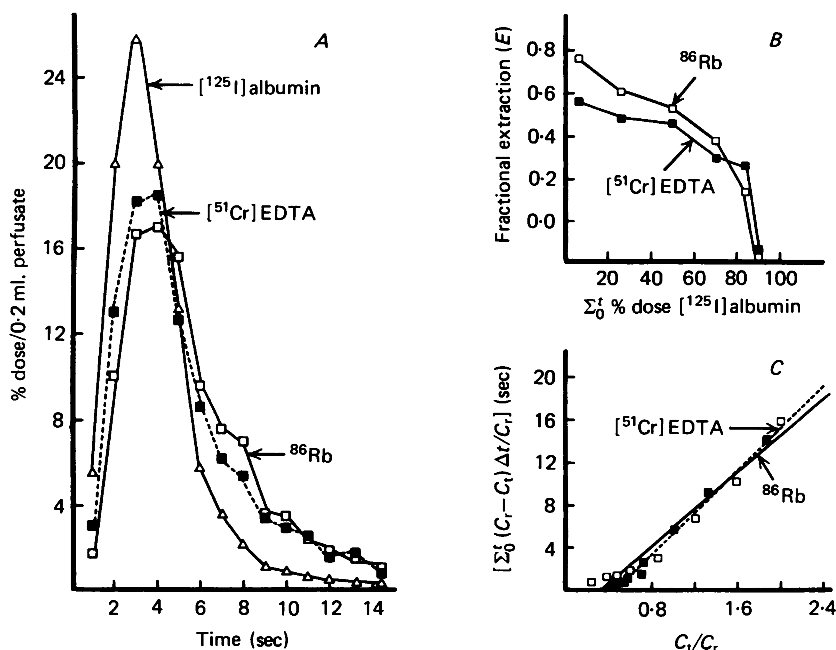

Fig. 1. Estimation of the initial capillary extraction. *A*, venous concentration-time curves for [^{125}I]serum albumin (intravascular reference tracer, C_r) and ^{86}Rb and [^{51}Cr]EDTA (test tracers, C_t) in a perfused gland. Concentrations have been normalized with respect to the injected tracer doses. *B*, fractional capillary extraction curves for ^{86}Rb and [^{51}Cr]EDTA. A ratio value (C_t/C_r) was calculated for each pair of data points in *A* and $E = 1 - C_t/C_r$. *E* is plotted as a function of the accumulated recovery of [^{125}I]albumin. *C*, graphical analysis (Martín de Julián & Yudilevich, 1964) used to estimate the initial capillary extraction (E_0) from 1 minus the *X*-intercept of the regression line.

Submandibular gland

The procedures for isolation and perfusion of the submandibular gland, stimulation of the parasympathetic nerve (chorda lingual) and collection of saliva were as described previously (Mann *et al.* 1979). Glands were studied either with an intact blood supply or perfused artificially at constant flow with a Krebs solution containing bovine serum albumin (60 g/l.).

Close-arterial bradykinin infusion

In the perfused glands, bradykinin (Sandoz Ltd) was infused into the perfusate via a three-way tap close to the gland using a Braun syringe pump. The dose of bradykinin given was sufficient to produce a 5–10 mmHg fall in the perfusion pressure at a perfusion rate of 1 ml./min.g.

Chronic duct ligation

Four cats were anaesthetized with sterile Na pentobarbitone (35 mg/kg I.P., 'Sagatal') and the neck was shaved. The right submandibular duct was ligated twice using full aseptic precautions (Darke & Smaje, 1973). The animals were kept for 3, 4, 7 or 12 days before capillary permeability-surface area measurements were made. Recovery was uneventful in all cases.

Measurement of capillary permeability-surface area product

Estimates of permeability-surface area (PS) were made using the following equation (Renkin, 1959; Crone, 1963):

$$PS = -F \ln (1-E_0),$$

where F is the plasma or perfusate flow in ml./min.g and E_0 is the initial fractional extraction of the labelled test solute. The single-passage, multiple-tracer dilution technique was used to estimate E_0. Following a close-arterial injection (50 μl. in 1 sec) of a Krebs albumin solution containing an intravascular reference tracer ([^{125}I] or [^{131}I]serum albumin) and one or more test tracers (^{86}Rb, [^{51}Cr]EDTA, [^{57}Co]cyanocobalamin or [^{125}I]insulin) the glandular venous effluent was sampled at 0·3–1·0 sec intervals for up to 30 sec. This method and the radioactive counting procedures are described in detail in the previous paper (Mann *et al.* 1979). A typical analysis of indicator dilution data is shown in Fig. 1. Since extraction for ^{86}Rb and [^{51}Cr]EDTA decreased with time due to tracer backflux from the interstitium into the circulation (Fig. 1*B*), the extrapolation procedure of Martín de Julián & Yudilevich (1964) was used to calculate E_0 (Fig. 1*C*). High correlation coefficients (0·95–0·99) were found for the linear analysis.

RESULTS

Effect of parasympathetic nerve stimulation on permeability-surface area products (PS)

In four glands with an intact blood supply, PS for ^{86}Rb and [^{51}Cr]EDTA (mol. wt. 357) were measured simultaneously in the absence and presence of maintained parasympathetic (chorda lingual) nerve stimulation. Fig. 2*A* illustrates two of these experiments and it can be seen that stimulation led to an increase in PS for both tracers and also a marked increase in plasma flow due to vasodilatation. For readily diffusing tracers, an increase in flow alone will increase PS (Alvarez & Yudilevich, 1969; Mann *et al.* 1979). Thus, it was not clear whether the increase in PS was due to a real change in PS, i.e. increased permeability or surface area, or to the increase in blood flow.

In order to study the effect of parasympathetic stimulation on PS independent of changes in flow, glands were perfused at constant flow with a Krebs solution containing 6% bovine serum albumin. Fig. 2*B* shows that, as expected, an increase in the perfusion flow led to an increase in PS but parasympathetic stimulation did not further increase PS relative to the non-stimulated control values. This implies that the increase in PS observed during stimulation in glands with a natural blood supply (Fig. 2*A*) was due to an increase in flow alone.

As the exchange of ^{86}Rb and [^{51}Cr]EDTA was flow-limited in non-stimulated perfused preparations (Mann *et al.* 1979), further experiments were performed using larger test solutes, [^{57}Co]cyanocobalamin (mol. wt. 1353) and [^{125}I]insulin (approximate mol. wt. 6000), at high flow rates where only diffusion would limit solute exchange. Table 1 summarizes these data and shows that parasympathetic stimulation had a variable effect in the ten animals but there was a significant reduction

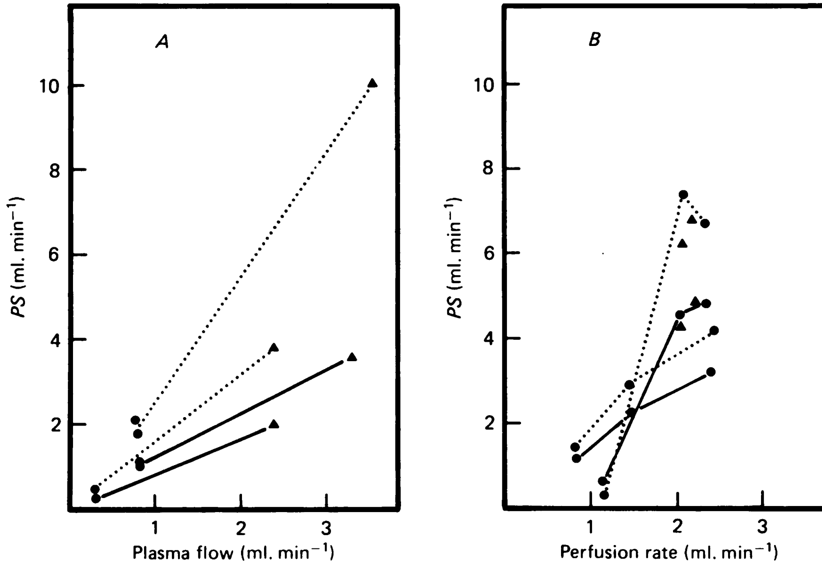

Fig. 2. Relationship of *PS* for ^{86}Rb (dotted lines) and [^{51}Cr]EDTA (continuous lines) against flow. *A*, in two cats *PS* estimates at the low flow (●) were obtained in the resting gland and *PS* at the higher flow (▲) was measured during maintained parasympathetic nerve stimulation. *B*, in two other cats *PS* measurements were made in resting glands perfused at constant low or high flow rates (●). The effect of stimulation (▲) was only studied at high perfusion flow rates. Further experiments on the effect of stimulation on *PS* at constant flow are summarized in Table 1.

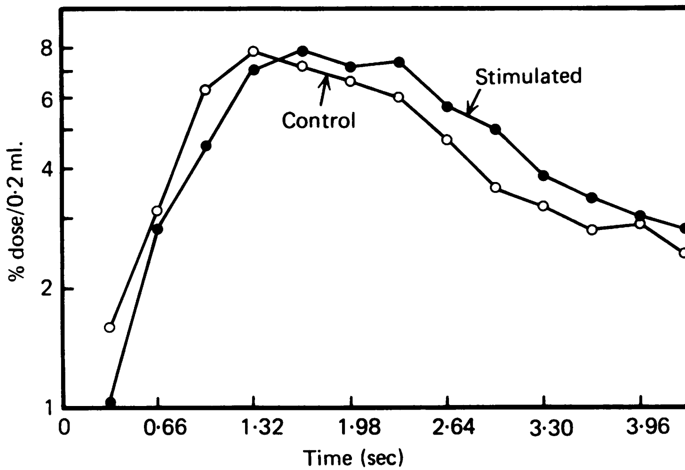

Fig. 3. Venous concentration-time curves for [^{131}I]albumin in paired non-stimulated (○) and parasympathetic nerve stimulated (●) experiments in one animal. The perfusion flow was identical in the two situations and the per cent dose has been plotted logarithmically to facilitate extrapolation of the downslope concentration to infinite time for the calculation of the vascular mean transit time, $\bar{t}$.

TABLE 1. Effect of parasympathetic nerve stimulation on vascular resistance and *PS* for [^{51}Cr]EDTA, [^{57}Co]cyanocobalamin and [^{125}I]-insulin. Unstimulated and stimulated experiments were performed at identical perfusion flow rates and a paired *t* test was used to assess the percent change in *PS* during stimulation

		Resistance (mmHg)/(ml. min^{-1}.g^{-1})		*PS* (ml. min^{-1}.g^{-1})					
				[^{51}Cr]EDTA		[^{57}Co]cyanocobalamin		[^{125}I]insulin	
Experiment	Perfusion flow (ml. min^{-1}.g^{-1})	Resting	% change	Resting	% change	Resting	% change	Resting	% change
6–6	2·12	20·8	−13·0	2·05	−28·8	—	—	—	—
7–6	3·95	16·7	0	2·82	−8·5	—	—	—	—
8–6	7·40	5·9	+2·3	7·36	−5·3	—	—	—	—
	4·82	7·0	+2·9	4·79	−21·9	—	—	—	—
23–6	3·40	25·8	−27·3	—	—	2·38	+16·8	1·01	−1·0
	4·89	20·4	−20·0	—	—	3·69	+22·2	1·68	−4·2
24–6	6·58	17·0	−10·7	—	—	3·50	−20·8	1·12	−8·0
14–7	8·27	6·3	+19·2	—	—	4·17	+1·9	1·60	−13·1
15–7	5·01	7·2	+44·4	—	—	4·12	−30·3	2·33	−27·9
	7·12	7·0	+12·0	—	—	3·87	−33·3	1·36	−39·7
16–7	5·21	7·7	+15·0	—	—	4·99	−39·5	2·52	−20·6
	6·63	8·4	−7·1	—	—	4·66	−18·0	2·38	−20·6
	5·21	9·6	−12·0	—	—	4·88	−28·0	2·78	−22·0
	6·39	9·4	−6·6	—	—	4·76	+4·6	2·40	+19·0
17–7	7·60	8·7	−6·0	—	—	3·11	+2·9	—	—
	9·05	7·7	+5·7	—	—	4·61	−26·0	2·40	−29·0
26–7	6·09	8·4	−20·0	4·94	−31·0	—	—	—	—
Mean % change			−3·48		−19·1		−12·3		−15·3
S.E. of mean			±4·13		±5·2		±6·0		±4·8
n			17		5		12		11
				$P < 0·05$		$P < 0·05$		$P < 0·02$	

in *PS* for all tracers studied. In these glands perfused at constant flow, the vascular resistance was 1/10 to 1/35th that of the normal gland (200 mmHg/[ml. $min^{-1}.g^{-1}$]) suggesting that there was little remaining vascular tone. There was no correlation between changes in *PS* and either initial vascular resistance or changes in vascular resistance.

Chorda stimulation also led to an increase in the mean transit time of the intravascular marker, [^{131}I] or [^{125}I]albumin, as calculated according to Meier & Zierler (1954). The mean transit time computation was referred to the appearance time of albumin in the venous effluent from the gland for the non-stimulated run. The same time delay after an isotope injection was used to assess the transit time for the stimulated runs. Non-stimulated experiments at a mean flow of 6·2 ml. $min^{-1}.g^{-1}$ gave a mean transit time ($\bar{t}$) of $1{\cdot}97 \pm 0{\cdot}13$ sec (mean ± S.E. of mean, $n = 12$) which increased to $2{\cdot}14 \pm 0{\cdot}15$ sec during maintained parasympathetic stimulation at the same flow. A paired *t* test on the twelve experiments in six cats revealed that the difference was significant ($P < 0{\cdot}01$), and it is apparent from Fig. 3 that the albumin curve in the stimulated gland lies below that in the non-stimulated gland on the upslope and is displaced in time after the peak of the control curve.

Effects of bradykinin on PS

For these experiments four glands were perfused at a constant flow between 5 and 8 ml. $min^{-1}.g^{-1}$. The reactivity of the vasculature to bradykinin was established in each animal by observing a fall in systemic arterial blood pressure on intravenous injection of the peptide. Control and bradykinin-containing perfusates were tested alternatively at identical flows and when bradykinin was infused, at least 2 min were allowed to establish steady-state conditions before *PS* for [^{57}Co]cyanocobalamin and [^{51}Cr]EDTA were measured.

TABLE 2. Effect of close-arterial infusion of bradykinin on *PS* for [^{51}Cr]EDTA and [^{57}Co]-cyanocobalamin. Control and bradykinin runs were conducted at identical flow rates (mean rate 6·4 ml. $min^{-1}.g^{-1}$). Values are mean ± S.E., *n* is the number of experimental runs and the number of animals follows in parentheses

	Perfusion pressure (mmHg)	*PS* (ml. min^{-1} g^{-1})	
		[^{51}Cr]EDTA	[^{57}Co]cyanocobalamin
Control	$52{\cdot}5 \pm 7{\cdot}4$	$5{\cdot}92 \pm 0{\cdot}64$	$4{\cdot}07 \pm 0{\cdot}70$
Bradykinin	$50{\cdot}9 \pm 6{\cdot}4$	$5{\cdot}95 \pm 0{\cdot}59$	$3{\cdot}65 \pm 0{\cdot}46$
n (animals)	7 (4)	7 (2)	6 (4)
P	$< 0{\cdot}40$	$< 0{\cdot}90$	$< 0{\cdot}30$

Bradykinin was infused close-arterially to produce an effective concentration of 0·2–2 μg/ml.g but even the highest doses had no consistent effect on perfusion pressure. As can be seen from Table 2, bradykinin had no significant effect on *PS* either. In individual experiments perfusion pressure either rose or fell and so did *PS*, but there was no significant correlation between the two variables. Furthermore, analysis of the [^{125}I]albumin reference dilution curves revealed no significant difference in the recovery of albumin in control and bradykinin-infused experiments nor was there a significant difference in the mean transit times in the two situations.

Effects of chronic duct ligation on PS

PS measurements for [^{51}Cr]EDTA, [^{57}Co]cyanocobalamin and [^{125}I]insulin were performed at flows from 4 to 7 ml. $min^{-1}.g^{-1}$ in four cats, 3–12 days after ligation of the main excretory duct of the gland. The vascular and secretory responses to parasympathetic nerve stimulation in glands with an intact blood supply following this procedure have been described by Darke & Smaje (1973). In the present experiments the normal secretion rate in perfused glands was 726 ± 149 μl./min (mean $\pm$ S.E., $n = 7$) and following duct ligation it fell to 371 ± 55 μl./min ($n = 5$) which is similar to the fall observed by Darke & Smaje (1973).

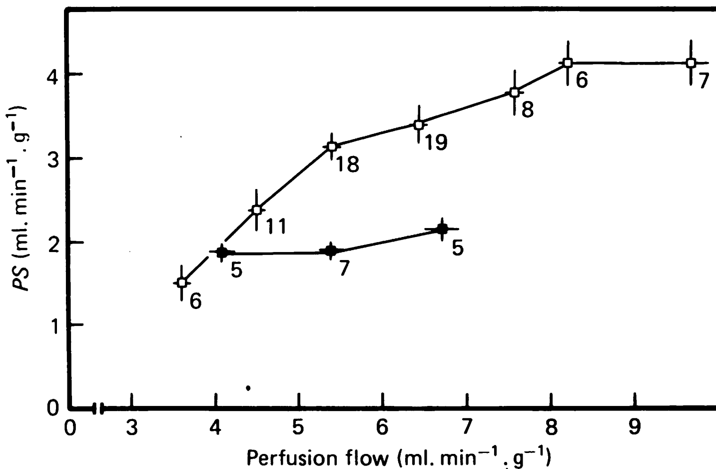

Fig. 4. The effect of flow on *PS* for [^{57}Co]cyanocobalamin in normal (□) and duct-ligated (■) glands. Individual data points reflect mean *PS* values grouped per flow range and the numbers beside each mean indicate the number of experimental observations. The horizontal and vertical standard error bars refer to the flow and *PS*, respectively.

The effect of perfusion flow on *PS* for [^{57}Co]cyanocobalamin was tested in duct-ligated glands and then compared with data obtained at a similar flow range in control glands (Fig. 4). It can be seen that in the controls, *PS* increased from a value of 1·5 towards a plateau value of about 4·1 ml. $min^{-1}.g^{-1}$ over a flow range from 3·5 to 9·7 ml. $min^{-1}.g^{-1}$. By contrast, in duct-ligated glands *PS* for [^{57}Co]cyanocobalamin was independent of the perfusion flow. At the lowest flows no difference between the two groups of data appears to exist, whereas at higher flows the *PS* values in duct-ligated glands were only about 60 % those of the control values. The same comparison was made with a larger test molecule, [^{125}I]insulin, and as shown in Table 3, *PS* in duct ligated glands was reduced to about one half of the control values.

The effect of parasympathetic nerve stimulation on *PS* in duct-ligated glands was investigated in five experiments in three cats. The mean *PS* for [^{57}Co]cyanocobalamin in resting duct-ligated glands was $2·09 \pm 0·12$ ml. $min^{-1}.g^{-1}$ and $1·91 \pm 0·20$ ml. $min^{-1}.g^{-1}$ during parasympathetic nerve stimulation. The capillary extraction

TABLE 3. Effect of duct ligation on fractional capillary extraction (E_o) and permeability-surface area products (PS ml. min^{-1}.g^{-1}). Data was obtained in resting, non-stimulated glands in the flow range from 4 to 7 ml. min^{-1}.g^{-1}. Values are mean ± S.E., n is the number of experimental runs and the number of animals is given in parentheses

Experiment	Resistance (mmHg/ml. min^{-1}.g^{-1})	[^{51}Cr]EDTA		[^{57}Co]cyanocobalamin		[^{125}I]insulin	
		E_o	PS	E_o	PS	E_o	PS
Control	9·7 ± 0·6	0·57 ± 0·07	5·26 ± 0·60	0·43 ± 0·01	3·22 ± 0·12	0·24 ± 0·01	1·52 ± 0·07
n (animals)	55 (18)	9 (4)		48 (17)		39 (12)	
Duct-ligated	12·5 ± 0·4	0·51 ± 0·02	4·20 ± 0·12	0·32 ± 0·01	2·02 ± 0·08	0·13 ± 0·04	0·72 ± 0·23
n (animals)	20 (4)	4 (1)		15 (4)		11 (3)	
P	< 0·01	< 0·30	< 0·30	< 0·001	< 0·001	< 0·001	< 0·001

(E_0) of [^{125}I]insulin in resting duct-ligated glands was very low (Table 3) and significant changes were not observed on stimulation. The mean transit time for [^{131}I] or [^{125}I]albumin in the duct-ligated glands did not differ significantly from the controls and in contrast to the normal gland, was not affected by parasympathetic stimulation.

DISCUSSION

Parasympathetic stimulation in glands perfused at constant flow

Parasympathetic stimulation significantly reduced transcapillary exchange in the fully dilated gland perfused at constant flow (Table 1). The submandibular gland consists of an acinar and ductal circulation perfused in parallel (Fraser & Smaje, 1977), and hence the present indicator dilution technique only measures an overall diffusion capacity for the organ. The changes in *PS* on stimulation may reflect a shifting pattern of flow, which is known to occur during the first 15 sec of stimulation in the rabbit submandibular gland (Fraser & Smaje, 1977) or they could represent a direct reduction in permeability induced by parasympathetic stimulation.

In the present experiments, glands were stimulated for 2 min before an isotope injection by which time, if the cat is similar to the rabbit, relative blood flows in the acinar and ductal circulations would not differ from the resting state. However, in our study a significant increase in the mean transit time for [^{131}I] or [^{125}I]albumin was observed during parasympathetic stimulation suggesting distribution into a larger vascular volume. The residual effects on vascular resistance (Table 1) may also indicate such a change. It is interesting to note that Kowalevsky (1885), Flint (1902) and Spanner (1937) all found that the capillary density around the acini was low compared with the rich vascularization of the ducts, and Fraser & Smaje (1977) confirmed that on a weight basis the ducts received a greater flow than the acini. A relative increase in flow to a denser microvascular network around the ducts would be consistent with the increase in albumin mean transit time, but its permeability would need to be less than that of the acinar vessels as *PS* was reduced over-all.

Although stimulation led to a decrease in *PS* for [^{51}Cr]EDTA and [^{57}Co]cyanocobalamin the *PS* ratios for EDTA/cyanocobalamin and cyanocobalamin/insulin did not change significantly. If the dimensions of the exchange pathways had become smaller, one would have expected these *PS* ratios to increase (see Mann, 1978; Mann *et al.* 1979). It would appear that the number rather than the size of the 'pores' is reduced by parasympathetic stimulation. If the endo-endothelial layer were the main barrier to solute exchange (Luft, 1966), then a reduction in the number of fenestrae without a change in the nature of the layer could explain most of our results. Michel (1978) has explained the increasing permeability encountered at the venous ends of frog mesenteric capillaries by a similar mechanism. He found no change in the reflexion coefficient along the length of a capillary and suggested that this was due to the endo-endothelial layer being the main barrier to exchange. The increased permeability towards the venous end was attributed to the increased number of intercellular junctions and hence the effective 'pore' area.

There is no morphological evidence to support our speculation that the ductal microcirculation is less permeable than the acinar circulation, and until this is

forthcoming an alternative explanation for our findings is that parasympathetic stimulation may reduce the number of fenestrae and incidentally leads to an increase in vascular transit time.

Ineffectiveness of bradykinin on PS

The failure of bradykinin to alter transcapillary solute exchange is not as surprising as it appears at first sight. The increase in capillary filtration capacity observed by Eliasson *et al.* (1973) can probably be explained by an increase in surface area due to the vasodilator activity of the peptide (Lundvall & Holmberg, 1978). Transcapillary protein flux has been shown to increase in the presence of bradykinin (Renkin, Carter & Joyner, 1974), but an increase in fluid flux has been less easy to detect in preparations perfused at constant flow (Diana, Colantino & Haddy, 1967; Haddy, 1970). Even in mammalian mesenteric venules, where bradykinin has been shown to increase the filtration coefficient, the increase is a factor of only 2 or 3 despite a demonstrable protein efflux (Verrinder, Fraser & Smaje, 1974). The accepted mechanism for the initial action of bradykinin appears to be an opening of the inter-endothelial cell junctions (Majno, Shea & Levanthal, 1969) particularly at points of contact between three endothelial cells. Such points represent a relatively small proportion of the total exchange area of continuous capillaries and venules, assuming that endothelial cell junctions are the point at which small lipid-insoluble solutes and water cross the endothelium. In fenestrated vessels they would represent an even smaller proportion of the exchange area. If we assume a constant permeability for intercellular junctions in all capillaries, then the greater solute permeability of the salivary gland can be ascribed to the fenestrae and an opening of the intercellular junctions, even if it occurred, would not be detected in our PS measurements. Although [^{125}I] and [^{131}I]albumin recoveries in paired control and bradykinin-infused experiments were similar, a small change in protein flux could have escaped detection and it should be emphasized that we were primarily concerned with small solute exchange.

Effects of chronic duct ligation on PS

Chronic ligation of the salivary duct is known to lead to several striking changes in salivary gland function. There are variable changes in gland morphology partly depending on the duration of ligation (Harrison & Garrett, 1976) and a variety of changes in enzyme concentration and output. There is a fall in amylase and protease output, and in particular a dramatic fall in the glandular and salivary kallikrein concentration (Darke & Smaje, 1973). In the cat, kallikrein is now known to be confined to the ducts (Hojima, Maranda, Moriwaki & Schachter, 1977) while amylase is acinar in origin, and in keeping with these changes there is an increase in both acinar and ductal lysosomal activity (Harrison & Garrett, 1976). The ductal Na transport is also reduced and there is a concomitant increase in the number of goblet cells (Bond, Fraser & Smaje, 1979). A reduction in transcapillary exchange of lipid-insoluble solutes may now be added to this list.

The experiments in duct-ligated glands virtually ensured a study of capillary permeability in the absence of endogenous kallikrein. Furthermore, as the perfusate contained no plasma substrate for any remaining kallikrein and bradykinin itself

had no effect on *PS*, it seems very unlikely that a decreased kallikrein concentration could account for the fall in *PS*.

If our hypothesis that the ductal vessels are less permeable than acinar vessels is true, then the lowered *PS* following duct ligation could be ascribed to a proportionately increased ductal flow relative to the acinar flow. The absence of a change in albumin mean transit time during parasympathetic nerve stimulation in duct-ligated glands would lend support to this hypothesis. A relative decrease in acinar activity is suggested by some histological reports (Standish & Shafer, 1957), and by our observation of a substantially reduced fluid secretion. The striking flow-independence of the *PS* curve for [^{57}Co]cyanocobalamin in duct-ligated glands (Fig. 4), supports the conclusion that a capillary bed with a low permeability is being perfused (Mann *et al.* 1979; Alvarez & Yudilevich, 1969). The reduced *PS* could also be produced by a reduction in the capillary surface area but measurements of capillary surface area are not available for either control or duct-ligated glands. However, a change in surface area seems unlikely in view of the similar albumin mean transit times measured in resting normal and duct-ligated glands.

It is interesting that differing permeability in acinar and ductal capillaries, combined with changes in relative flow, could explain the fall in *PS* both on parasympathetic stimulation and following duct ligation. If true, this has important implications for water and electrolyte flux in the secreting gland.

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