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Nervous Control of Mammalian Salivary Glands [and Discussion]

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Nervous control of mammalian salivary glands

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In the production and flow of saliva, sympathetic and parasympathetic nerves generally cooperate, although variations between the different salivary glands are considerable, particularly in the sympathetic innervation. In the submandibular gland of the dog, sympathetic impulses cause secretion via β-adrenoceptors, and since sympathetic motor effects are elicited via α-adrenoceptors it is possible to study separately motor and secretory effects in this gland. Such experiments indicate that myoepithelial contractions serve to accelerate the salivary flow and to support the secreting acinar cells and prevent back-flow of fluid from the luminal system into the glandular tissues. The contractions are elicited reflexly from the oral mucosa together with secretion. A potentiation interaction between sympathetic and parasympathetic nerves occurs in the formation of the primary saliva. In parotid glands of rabbits and rats such an interaction has been demonstrated in the secretion of amylase.

Introduction

Salivary glands have a rich supply of sympathetic and parasympathetic nerves (figure 1). Vasomotor nerves control the flow of blood, and motor nerves contract the myoepithelial cells. Secretory nerves initiate the formation of primary saliva in the acini; they affect the absorption

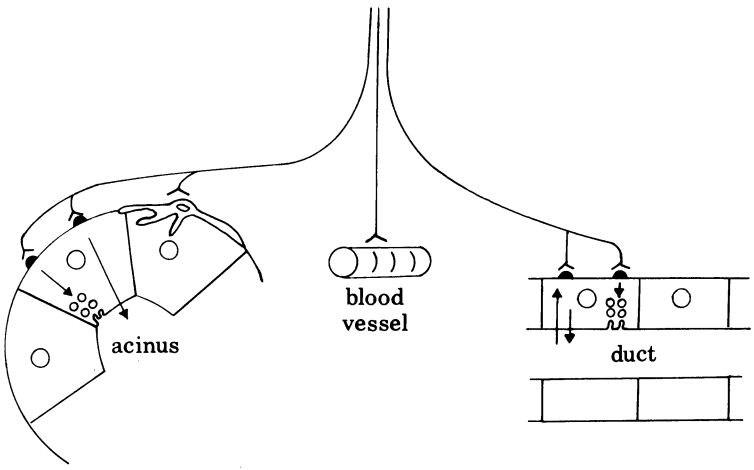

Figure 1. Schematic drawing, showing the distribution of nerves to the different effectors of a salivary gland.

and secretion of certain ions in the ducts, and stimulate exocytosis of granules containing macromolecules such as amylase in the acini or kallikrein in the ducts. In addition, glandular nerves exert some long-term action, the loss of which results in atrophy, lowered maximal secretory capacity and supersensitivity to chemical excitants. Hormonal mechanisms also take part in the control of the glands, for instance in the movement of ions in the ducts, and particularly in the long-term regulation; hypophysectomy causes great atrophy and reduces

the secretory capacity and responsiveness to secretory agents. However, in the production and flow of the saliva the nerves play the main role. They enable the gland to form saliva quickly and empty it into the mouth to fulfil its protective and digestive tasks; conditioned reflexes can be established, so that secretion may begin even before the excitants have reached the mouth; salivation can be closely linked to other centrally regulated events, such as chewing and swallowing in feeding reactions, or to others in vomiting or, in some species, in thermoregulation.

Nerves and receptors in different glands

Variations between salivary glands are considerable, both between corresponding glands in different species, and between the various glands of the same species. The nerves shown schematically in figure 1 may be sympathetic or parasympathetic, and often both nerves act on the same type of effector. The variability in distribution and function of the nerves may be illustrated in those sympathetic secretory nerves that trigger the events leading to the formation of the primary saliva. The composition of this fluid seems to be the same whether acetylcholine

Table 1. Secretion of fluid on sympathetic stimulation

	dog	cat	rabbit	rat	sheep
parotid	−	+	+	++	+
submandibular	++	+++	(+)	++	(+)
sublingual	(+)	++		−	

Table 2. Adrenoceptors for secretion of fluid

	dog	cat	rabbit	rat	sheep
parotid	(β)	β(α)	α(β)	αβ	β
submandibular	β	α(β)	(β)	αβ	
sublingual	?	α(β)			

or noradrenalin starts these processes, but acetylcholine is by far the more effective excitant. The salivary flow caused by electrical stimulation of the sympathetic nerves is generally small and it is very variable (table 1). In the submandibular gland of the cat it is relatively large; from the parotid gland of the dog, or the sublingual gland of the rat, on the other hand, no secretion is obtained when the sympathetic nerve is excited. In the sublingual gland of the rat this can be explained by the fact that there are scarcely any adrenergic axons in contact with its acinar cells; but this does not hold true of the parotid gland of the dog.

Variations in the sympathetic control of the secretion of fluid are striking at the receptor level too (table 2). In submandibular glands, for instance, α-adrenoceptors are mainly or wholly responsible for the secretion in cats and β-adrenoceptors in dogs, whereas both types of receptor contribute in rats. Other receptors for secretion may also vary. Peptides like substance P or physalaemin cause secretion in some species only.

Interactions between the glandular nerves

The classical antagonism between the two divisions of the autonomic nervous system is found in the vascular innervation, but in the control of the formation and flow of the saliva the nerves cooperate, activating secretory and myoepithelial cells. When acetylcholine or noradrenalin

stimulate the production of primary saliva they probably engage the same intracellular mechanisms, and it has been shown that when the parasympathetic nerve is excited at a high frequency, to produce its maximal secretory rate, this rate cannot be further increased by adding stimulation of sympathetic secretory nerves (Emmelin 1955; Ohlin 1965; Emmelin & Holmberg 1967*a*). The situation seems to be analogous for the myoepithelial contraction in glands supplied with motor nerves from both systems (Emmelin *et al.* 1977). The interaction between the two secretory nerves comes more clearly to light when stimuli producing submaximal effects are applied. This can be illustrated by observations made on the phenomenon that Langley called augmented salivary secretion. Such observations also demonstrate the functional relation between motor and secretory mechanisms.

(*a*) *Augmented salivary secretion*

Langley (1889) discovered that the usually rather small secretory responses to sympathetic stimulation became much larger if preceded by a brief period of parasympathetic stimulation. His explanation was that the first stimulation left the secretory cells for some time in a state of heightened excitability. Mathews (1898) offered another hypothesis: the initial parasympathetic stimulation served to fill the luminal system of the gland with saliva, which was then

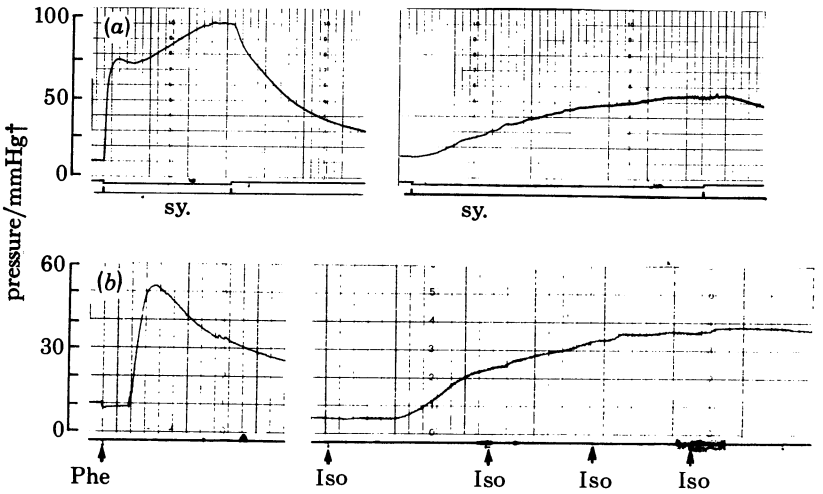

FIGURE 2. Records of the pressure in the submandibular duct of dogs, anaesthetized with chloralose. (*a*) Effects of sympathetic stimulation at 20 Hz (sy.), before (left) and after (right) intravenous injection of dihydroergotamine (0.4 mg/kg) (*b*) Effects in another dog of intravenous injections of phenylephrine (Phe; 20 μg/kg), and isoprenaline (Iso; 20 μg/kg). (Modified from Emmelin & Gjörstrup (1974). † 1 mmHg ≈ 133Pa.

expelled when sympathetic impulses activated some motor mechanism; the myoepithelial cells are generally assumed to be responsible for this extrusion. Babkin (1950) suggested that both secretory and motor components may contribute to the phenomenon of augmented secretion. This view is supported by recent experiments on the submandibular gland of the dog (see Emmelin 1979). The myoepithelial cells of that gland receive sympathetic motor nerves (Emmelin *et al.* 1969*a*; Garrett & Emmelin 1979), which exert their effect via α-adrenoceptors (Emmelin *et al.* 1969*b*). Secretion, on the other hand, is elicited by way of β-receptors (Emmelin & Holmberg 1967*b*). When the sympathetic nerve is stimulated, the ordinary response is a slow flow, which appears after a long latency and is abolished by propranolol; injection of isoprenaline has a similar effect. An α-component of the response can be obtained on condition

that the glandular lumina have been filled in advance, for instance by injection of some saline solution through the duct in retrograde direction. The late, slow flow is then preceded by an early, brief period of rapid outflow, and this phase, also obtainable with injection of phenylephrine, is abolished by dihydroergotamine (Emmelin & Gjörstrup 1974). A biphasic curve is, likewise, produced by sympathetic stimulation when the pressure in the submandibular duct is recorded while the luminal system of the gland is well filled (Emmelin & Gjörstrup 1973). As demonstrated in figure 2 there is an early, steep pressure rise which can be mimicked by injection of phenylephrine, and a later, slow rise. After α-block only a late, slow, rise persists, and isoprenaline gives a similar picture. It is concluded that the initial, short-lived outflow or pressure response is a myoepithelial effect and that the later flow or pressure response is due to secretion. By using the appropriate adrenoceptor agonists or antagonists it is then possible to distinguish between motor and secretory mechanisms and study them separately in this particular gland.

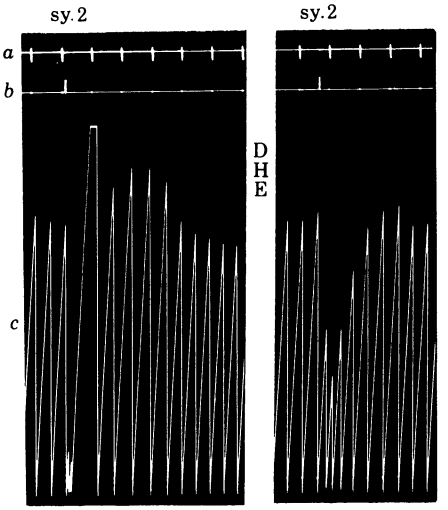

FIGURE 3. Effects of sympathetic stimulation at 10 Hz for 2 s (sy. 2) on a continuous secretion elicited by parasympathetic stimulation at 0.5 Hz throughout the experiment. (*a*) minute marks; (*b*) signal; (*c*) outflow of drops from a bottle in which water was replaced by saliva from the cannulated submandibular duct of an anaesthetized dog. An ordinate recorder wrote the time interval between two drops. DHE, injection of dihydroergotamine (0.5 mg/kg). (Modified from Emmelin & Gjörstrup (1976*a*).)

This was done in the following way. The parasympathetic nerve was stimulated throughout the experiment at a low frequency, usually 0.2–0.5 Hz, producing a slow continuous secretion that kept the luminal system filled. Sympathetic stimulation was superimposed, but only for a few seconds to avoid deleterious effects of vasoconstriction on the secretion. As shown in figure 3 this greatly accelerated the outflow, and when sympathetic stimulation was discontinued the flow ceased for a brief period (Emmelin & Gjörstrup 1976*a*). The inference was that myoepithelial contraction accelerated the outflow of the continuously produced saliva, and afterwards, when the cells relaxed and the capacity of the luminal system increased again, the outflow ceased for a while. Dihydroergotamine changed the picture accordingly. No post-stimulatory deceleration of the outflow occurred, and the acceleration was much diminished; however, it did not disappear completely, as would be expected if it were due entirely to myoepithelial contraction. The remaining acceleration was abolished by propranolol, indicating a secretory component. As a secretory response to sympathetic stimulation for only 2 s it was a remarkably large effect, for in the absence of a parasympathetic activity the latency for the

sympathetic secretion is much longer, even if the luminal system is filled (by injection of fluid through the duct, after α-block). A small parasympathetic activity appears to increase very much the secretory effect of the sympathetic nerve. The sympathetic secretion was in fact sometimes so pronounced and so prolonged that it tended to conceal the decelerating effect of the myoepithelial relaxation; in these cases this component of the myoepithelial response came to light after injection of propranolol. If the term augmented secretion may be applied also to the situation when the two nerves are excited simultaneously and not in succession, then figure 3 illustrates both kinds of augmentation: the expulsion from the lumina of preformed saliva, showing cooperation between secretory and motor mechanisms, and the interaction between the secretory nerves.

(*b*) *Interaction between the nerves in the secretion of fluid*

The secretory interaction, Langley's type of augmentation, is probably the earliest example of potentiation between secretory excitants, which now attracts so much attention in other glands. Figure 4 demonstrates it in an experiment in which the myoepithelial component, and

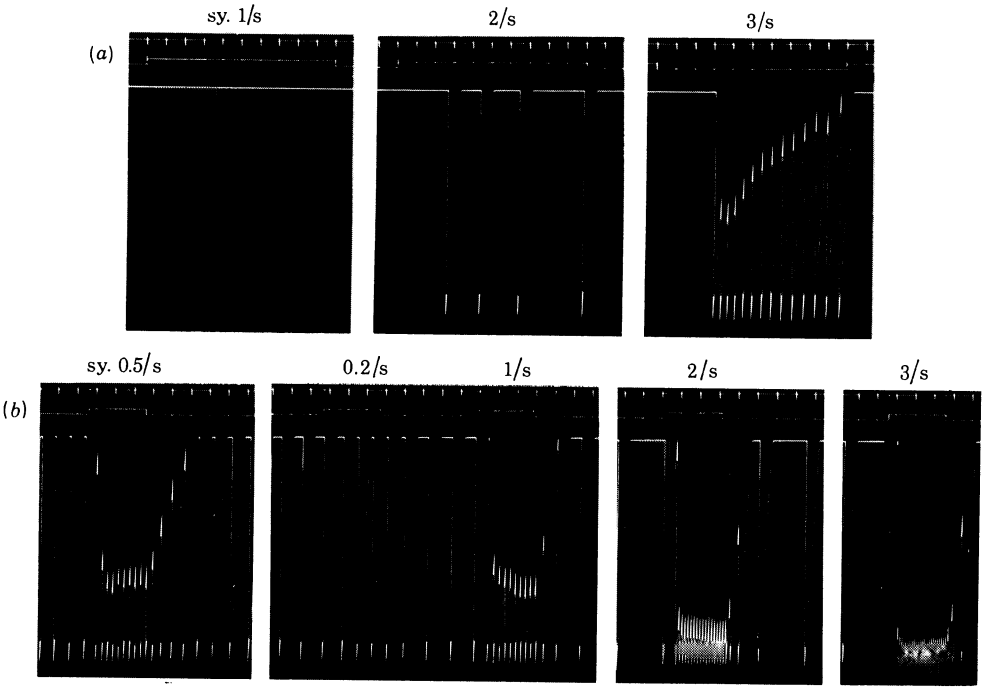

FIGURE 4. Secretory effects in the anaesthetized dog's submandibular gland of sympathetic stimulation (sy.) alone (*a*) and superimposed on parasympathetic stimulation at 0.2 Hz (*b*). Records as in figure 3. Dihydroergotamine (0.5 mg/kg) had been given before the experiment started. (Modified from Emmelin & Gjörstrup (1976*b*).)

vasoconstriction as well, had been prevented by α-block. When only the sympathetic nerve was stimulated, secretion started after a long latency, the flow was slow and decreased during stimulation, and the threshold frequency was high. During sympathetic stimulation against a background of parasympathetic activity, the threshold was lowered about tenfold, the latency was greatly reduced and the flow was maintained throughout the stimulation period. This sympathetic effect was abolished by propranolol. Similar potentiation was obtained when sympathetic stimulation was replaced by isoprenaline, or parasympathetic by pilocarpine (Emmelin & Gjörstrup 1976*b*).

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Pronounced augmentation was obtained when the parasympathetic activity was low; the parasympathetic nerve was excited at frequencies below 1 Hz. The frequency–response curve for the submandibular gland shows that secretion is very slow below 1 Hz when only the parasympathetic nerve is activated, and it reaches a maximum at about 10 Hz (Emmelin & Holmberg 1967*a*). If the effect of each shock applied to the nerve is calculated, the curve shown in figure 5 is obtained (Emmelin & Gjörstrup 1978). The highest efficiency, expressed in volumes of saliva secreted per shock, is found in the frequency range from 1 or 2 Hz up to 7 or 10 Hz. Below 1 Hz the response to each shock is small – but that is where augmentation by sympathetic stimulation is particularly striking.

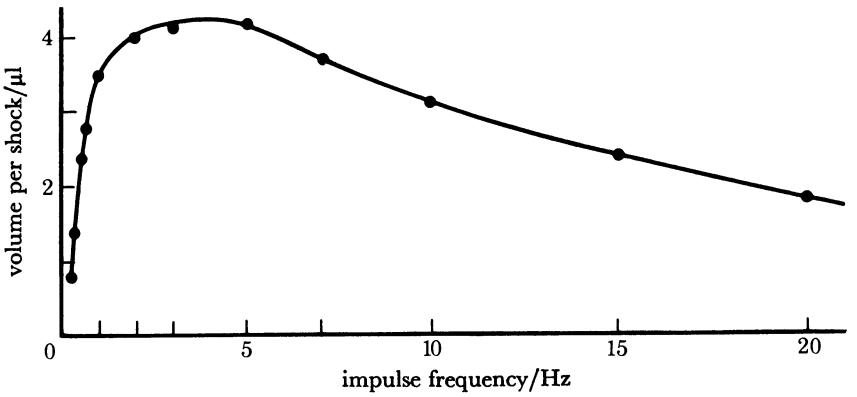

FIGURE 5. Volumes of submandibular saliva secreted per shock when the parasympathetic nerve was stimulated at different frequencies in anaesthetized dogs. (From Emmelin & Gjörstrup (1978).)

In the parotid gland of the dog, sympathetic stimulation causes no secretion (table 1). Only when the glandular lumina have been filled in advance is small short-lived outflow seen; it is abolished by α-block and attributed to myoepithelial contraction. However, when an α-blocking drug has been given and a parasympathetic background activity is supplied, sympathetic impulses are found to have a marked secretory effect, abolished by β-blocking drugs.

In experiments in which only the sympathetic nerve is stimulated, the sympathetic secretory innervation may generally tend to be underestimated. The arrangement shown in figure 4 may reproduce physiological conditions better, in two respects. First, vasoconstriction is avoided, and there is no reason to believe that the vasonconstrictor nerves are engaged in physiological events in which the secretory nerves are activated, for instance during a meal. Secondly, in the waking state there is some small parasympathetic activity, elicited reflexly from the mouth and the throat. The role of the sympathetic secretory nerves in alimentary reflexes has been questioned, because in dogs salivation during feeding ceases after section of the parasympathetic nerves or injection of atropine (see Babkin 1950). But these procedures deprive the glands of a background, in the absence of which sympathetic secretory impulses have only a small effect in the submandibular gland, and none in the parotid.

The rat differs in this respect from the dog. Some secretion can be elicited reflexly from the submandibular gland of the rat even after section of the parasympathetic nerve, and it is abolished by sympathectomy (Ohlin 1968). In this species the sympathetic secretory nerve seems less dependent on a parasympathetic background than in the dog; species differences exist with regard to the augmented secretion of fluid. In dogs and cats the phenomenon is much more pronounced than in rabbits and rats (Emmelin & Gjörstrup 1975, 1976; Gjörstrup 1977; Templeton & Thulin 1978; Templeton 1979, 1980*a*).

(*c*) *Interactions in the secretion of amylase*

Even in rabbits and rats the sympathetic and parasympathetic secretory nerves cooperate closely, as demonstrated in the secretion of amylase from the parotid glands. This secretion is mainly controlled by sympathetic impulses, and fluid transporting the amylase is mainly secreted under the influence of parasympathetic impulses. In rabbits, Gjörstrup (1978, 1979) observed that sympathetic impulses of very low frequency, causing no flow of saliva, could greatly increase the amylase content of parasympathetic saliva. In addition he found that to obtain maximal output of amylase the sympathetic stimulation frequency needed in the presence of a parasympathetic activity was about one-tenth of that needed in its absence. Similar observations have been made in rats (Asking *et al.* 1979; Templeton 1979, 1980*a*). These experiments indicate that the parasympathetic activity may serve not only to transport the amylase but also to augment its secretion. Experiments *in vitro* using drugs to replace nerve stimulation support this opinion (Asking & Gjörstrup 1980; Templeton 1980*b*).

(*d*) *Cooperation between motor and secretory nerves*

In the experiments on dogs, α-block was used to prevent an unphysiological vasoconstriction when the sympathetic nerve was excited. It also abolished, however, sympathetic myoepithelial contraction, which may be thought to participate normally when the gland is activated. This contraction is obviously not necessary for the saliva to be produced and flow from the cannulated duct, as occurs after injection of isoprenaline or sympathetic stimulation during α-block. Nevertheless, the myoepithelial contraction may be important during salivation. It can accelerate transiently the outflow of saliva (figure 3). Furthermore, it seems to support the secretory cells and prevent the back-flow of fluid into the glandular tissues. This is suggested by the pressure-recording experiments. When α-block had abolished myoepithelial contraction in experiments of the type shown in figure 2, sympathetic stimulation could no longer raise the pressure to the high level previously reached; it seemed as if back-flow balanced secretion at a lower pressure when the myoepithelial cells were not contracted. When instead the flow of saliva on sympathetic stimulation was studied and the cannula elevated above the gland, it was found that the outflow continued at a much higher level before than during α-block. If the outflow level was raised while no nerves were stimulated, fluid flowed into the gland. Some of this fluid returned when the cannula was lowered and it was assumed to have filled and distended the glandular lumina; but some was lost into the glandular tissues. Injection of phenylephrine or bradykinin, which both activate the myoepithelial but not the acinar secretory cells, greatly diminished the inflow of fluid into the gland when the cannula was raised, and not only the portion in the lumina but also that flowing into the tissues (Emmelin *et al.* 1977). Such a myoepithelial support may be important when highly viscous saliva flows through narrow channels.

Evidence was recently obtained to show that myoepithelial cells can be activated reflexly together with the secretory cells (Al-Gailani *et al.* 1981). In cats the flow of saliva in the cannulated duct of the zygomatic gland was observed under the dissecting microscope. The saliva of this gland is extremely viscid and is secreted continuously even in the absence of extraneous stimuli. On this spontaneous flow reflexly elicited secretion can easily be superimposed, for instance by pinching the tongue. Parasympathetic impulses cause this secretion, but by stimulating the tongue mildly and very briefly, to avoid a large and dominating secretory response, indications of a motor component could be detected. This is demonstrated schematically

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in figure 6. The meniscus in the cannula, moving slowly at first because of the spontaneous secretion, was on stimulation found to move forward suddenly and rapidly but soon to move backwards, then forward again, gradually slowing down to the speed of the spontaneous secretion. It seems reasonable to attribute the first, quick forward movement to myoepithelial contraction, visible because spontaneous secretion filled the glandular lumina, and the later forward movement to secretion – a biphasic response resembling that seen in dogs but elicited reflexly and not by electrical stimulation of efferent nerves.

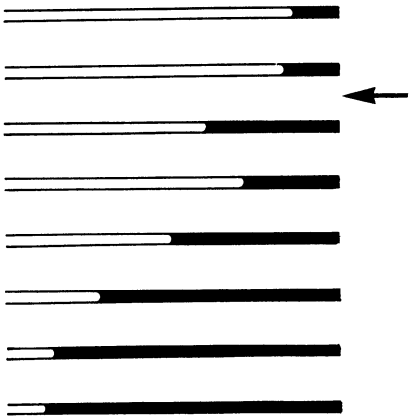

FIGURE 6. Schematic picture of the salivary flow from the zygomatic gland of a cat, anaesthetized with chloralose. The movement of the meniscus of the salivary column in a cannula inserted into the salivary duct was observed under the dissecting microscope. Arrow, pinching of the tongue ipsilaterally.

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Discussion

J. A. Young. Might it not be a possibility that a significant component of the fluid secretion in this preparation results from pressure-driven flow via a paracellular pathway in the endpieces? We have some evidence that this may occur in exocrine glands (Lingard, J. M. & Young, J. A. 1981 Do variations in hydrostatic pressure affect pancreatic fluid output? *Proc. Aust. physiol. pharmac. Soc.* **12**, 85P).

J. R. Garrett (*King's College Hospital Dental School, London, U.K.*). Our studies on the permeability of macromolecules in submandibular glands of dogs, using horseradish peroxidase (HRP) as the marker, have shown that some paracellular movement can occur. The work also supports the idea that myoepithelial contractions may increase the movement of substances between the parenchymal cells. Spaces exist in this species between the myoepithelial cells and the underlying secretory cells and after arterial infusions containing HRP it is taken up into these spaces. It is reasonable to assume that myoepithelial contractions acting on the fluid in such spaces cause an increased filtration pressure at the underlying luminal tight junctions. Such a mechanism may help to explain the increased movement of HRP into saliva that occurs at the onset of each nerve-stimulation period (Garrett 1981; Garrett *et al.* 1981). This suggestion is reminiscent of an idea by Yoshimura *et al.* (1962), though their overall concept was somewhat different.

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