Changes in the Permeability of the Salivary Gland Caused by Sympathetic Stimulation and by Catecholamines

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ABSTRACT The permeability of the submaxillary gland of cats and dogs has been tested by determining the rates at which non-electrolytes penetrate from the plasma into the saliva. Electrical stimulation of the cervical sympathetic trunk or administration of epinephrine or norepinephrine increases the permeability of the gland enabling glucose (molecular radius, MR = 3.5 Å), sucrose (MR = 4.4 Å), raffinose (MR = 5.6 Å), polyglycol 1000 (MR = 7.2 Å), and polyglycol 1540 (MR = 8.1 Å) to penetrate into the saliva from which they are otherwise excluded. Inulin (MR = 14.7 Å) does not enter the saliva under these circumstances. Analysis of the transfer rates suggests that the molecules diffuse through a pore structure permitting free diffusion for molecules with a radius less than 5.7 Å. Close intraarterial injection of C^{14}-glucose demonstrates that at least part of this permeability is located in the duct system of the gland. Since epinephrine does not enable sucrose to enter the cells of the gland, it appears that penetration from the extracellular space into the saliva occurs by diffusion through intercellular gaps. The characteristics of the permeability allow conclusions as to the localisation and geometry of the ultrastructural change produced.

INTRODUCTION

It was first shown by Hebb and Stavraky (1) that in addition to its ability to elicit secretion from the cat's submaxillary gland, epinephrine can also cause very persistent changes in the composition of the saliva secreted by subsequent parasympathetic stimulation. Whereas before epinephrine, submaxillary saliva contained only the barest traces of reducing sugars, after the drug was administered reducing substance (presumed to be glucose) was found in a relatively high concentration; its concentration was maximal immediately after epinephrine was given and fell off slowly over a period of

hours. Endogenous epinephrine released from the adrenal medulla by cold stress (immersion in ice water) caused a similar response. Subsequently it was found (2) that the concentration of sodium, chloride, and non-protein nitrogen in the saliva was also increased. These authors were, however, unable to demonstrate any reducing sugar in the saliva after stimulating the sympathetic supply to the gland although they did find changes in the concentration of electrolytes.

No further studies have been reported on this effect which is of interest both because of its prolonged duration and because of the apparent distinction between the effects of epinephrine injection and those of stimulation of the sympathetic nerves.

In the present study we have examined the nature of this effect, using methods based on those previously worked out for a study of the effects of the parasympathetic nerves on the secretion of non-electrolytes by the gland (3).

METHODS

Cats (1.5 to 3 kg) were anesthetised with chloralose 65 mg/kg intravenously after induction with ethyl chloride and ether. Smooth induction was necessary to avoid endogenous catecholamine release. Dogs (7 to 17 kg) were anesthetised by intravenous injection of 6 ml/kg of a chloralose-urethane mixture (1 gm chloralose and 12 gm urethane in 100 ml of 0.5 per cent saline).

Dissection of the submaxillary and parotid glands was carried out in the usual way. Stimulation of the nerves was by rectangular pulses of 1 to 2 msec. duration through stout platinum wire electrodes. Care was taken that the nerves did not become dry and at intervals both electrodes and nerves were rinsed with 0.9 per cent saline.

When exogenous substances were administered both renal pedicles were ligated and the substance administered in a single dose. A period long enough for equilibration was allowed before the saliva collections were started. Satisfactory blood levels were attained by administration of 400 mg/kg creatinine, 1 gm/kg sucrose, 1 gm/kg raffinose, 2 to 2.5 gm/kg polyglycol 1000,[1] 2.5 gm/kg polyglycol 1540,[1] 1 gm/kg inulin, 1 gm/kg urea, 100 μc/kg $S^{35}O_4$. The amino acids (alanine, valine, glutamate, arginine, histidine, proline) were infused continuously at a rate of 10 mg/kg min. In collecting saliva samples for steady state measurements the first 8 to 10 drops of saliva were discarded.

In the experiments on the site of transfer, rapid injections were made into the central end of the lingual artery and saliva drops collected on a filter paper strip or in scintillation counting vials as described by Burgen and Terroux (4). Glucose U-C^{14} 20 μc and THO 150 μc were used as the test substances. In the experiments with $S^{35}O_4$, 10 μl of saliva and plasma were placed on aluminum planchets and counted with a thin end window G-M counter.

[1] Linear ethylene glycol polymers supplied by Carbide and Carbon Chemicals as carbowax 1000 and carbowax 1540.

Analytical Methods

Creatinine was determined by Burgen's modification of the Jaffe alkaline picrate method (3).

Glucose was determined by a highly specific enzymatic method using glucose oxidase, peroxidase, and dianisidine (glucostat, Worthington Biochemical Co., Freehold, New Jersey). The colour was read at 400 mμ in a Beckman DU spectrophotometer. With 0.2 ml samples of saliva accurate determinations were possible down to 100 mg/liter.

Sucrose, Raffinose, and Inulin were determined by the method of Deane (5).

Polyglycols were determined by forming complexes with phosphomolybdate and colorimetric measurement of the molybdenum blue as described by Shafer and Critchfield (6).

Urea was determined by the method of King and Wootton (7).

Amino acids were determined on equilibrium dialysates of plasma and saliva by the semiquantitative paper chromatographic method of McMenamy *et al.* (8).

Chromatographic Separation of Glycerol and Sugars

10 μl samples of saliva or plasma were applied to a sheet of Whatman No. 11 paper, dried, and then descending chromatograms developed in an *n*-butanol–acetic acid–water (40:10:22) system. The separation was improved by repeating the development two to three times. The dried papers were sprayed with alkaline silver nitrate (9) and the amounts of glycerol, glucose, sucrose, and raffinose determined semiquantitatively by visual comparison with simultaneously run standards.

Sucrose and Inulin Spaces in the Glands

Sucrose or inulin was administered as described previously and 3 hours allowed for equilibrium; at the end of this time one submaxillary gland was removed as a control; following epinephrine administration the second gland was removed. The minced glands were deproteinized with $ZnSO_4$ and $Ba(OH)_2$ and the filtrate used for the sucrose and inulin determinations.

RESULTS

When tested by the specific glucose oxidase method only traces (<30 mg/liter) of glucose were present in the submaxillary or parotid saliva of the cat or dog when the parasympathetic nerve was stimulated. This is consistent with the results previously obtained with mannitol ((3); S/P 0.01 to 0.03), a molecule comparable in size and lipid solubility to glucose. When epinephrine was infused intravenously in the cat in a dose sufficient to cause a flow of saliva from the submaxillary gland (5 to 20 μg/kg min. for 10 minutes), the concentration of glucose rose to 200 to 1500 mg/liter (Fig. 1). In the dog a similar dose was effective but the rise in glucose concentration in the sub-

maxillary saliva was less impressive. In parotid saliva of the dog, the increase in glucose concentration was even smaller.

It can be shown that the effect of epinephrine on the submaxillary gland is a local one, because much smaller doses (0.5 to 1.0 μg/kg) are effective when injected directly into the arterial supply. Norepinephrine was approximately as effective as epinephrine by either intravenous or intraarterial route (Fig. 1).

Stimulation of the cervical sympathetic trunk in the cat at 10 CPS was effective in most cats in raising the concentration of glucose in the saliva, but

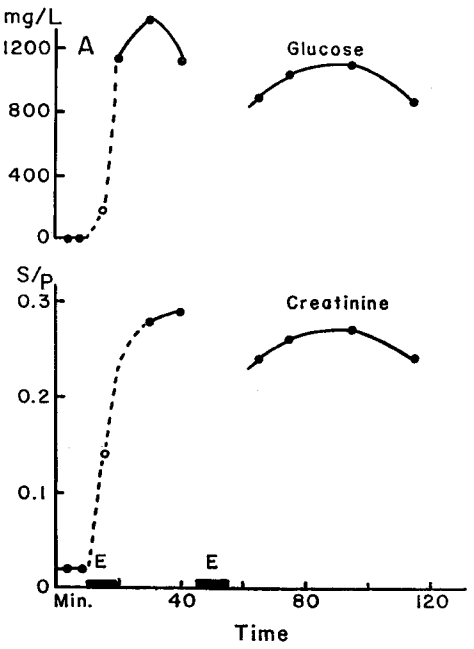

FIG. 1A

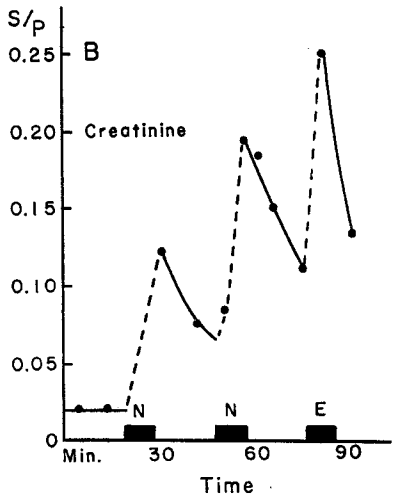

FIG. 1B

FIGURE 1A. Changes in the concentrations of glucose and creatinine in the submaxillary saliva of the cat following administration of epinephrine. The first two samples were obtained by parasympathetic stimulation at 7.7 CPS. An intravenous infusion of epinephrine of 15 μg/kg min. was then given for a 10 min. period indicated by the block marked *E*. Some secretion resulted (sample O). Thereafter saliva flow was elicited by 10 CPS stimulation of the parasympathetic. A further infusion of epinephrine did not increase the effect. Creatinine concentration is expressed as a fraction of the plasma concentration (*S/P*). The plasma glucose concentration before infusion was 1000 mg/liter and at 90 min. 3830 mg/liter.

FIGURE 1B. Infusion of norepinephrine (15 μg/kg min. indicated by the block marked *N*) increased the concentration of creatinine in cat submaxillary saliva, and a further increase in effect was produced by a second infusion of 30 μg/kg min. A similar effect was produced by 15 μg/kg min. of epinephrine.

the maximum increase in concentration found was only about one-half of that found with epinephrine (Fig. 2). This response to sympathetic stimulation was the usual finding and contrasts with the negative result of Langstroth *et al.* (2). It seems possible that the difference is due to the use of an induction coil by these authors, with the higher frequency of stimulation leading to the rapid development of synaptic block. It is known that at high frequencies of sympathetic stimulation it is not possible to produce maintained effects on the salivary gland (10).

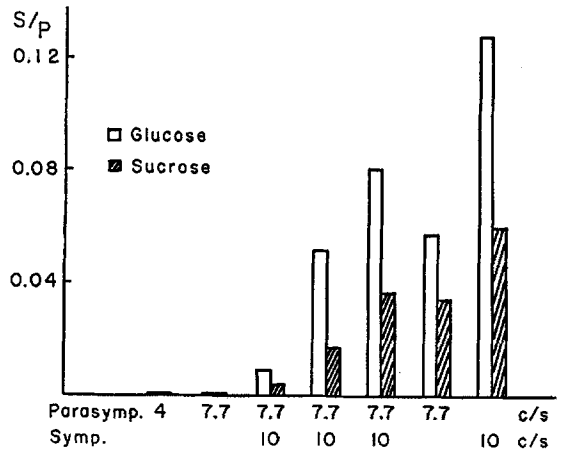

FIGURE 2. With parasympathetic stimulation alone, no detectable amounts of glucose or sucrose enter the submaxillary saliva of the cat. When the sympathetic nerve was stimulated at 10 CPS together with the parasympathetic both glucose and sucrose appeared in the saliva. With successive periods of stimulation the effect grew but it also persisted even when the parasympathetic nerve was stimulated alone. Note that the intensities of the effects on glucose and sucrose concentration run parallel.

An analysis of the changes in gland permeability based mainly on a study of glucose secretion might suffer from the perturbations due to its important metabolic role; after all, epinephrine administration leads to large changes in plasma glucose and quite possibly to changes in the metabolism of glucose by the gland. We therefore sought other test molecules free of this disadvantage. We have found that the permeability of the gland to sucrose parallels that to glucose very closely (Fig. 2). It is not detectable in parasympathetically evoked saliva (saliva/plasma ratio, $S/P < 0.005$), but following a short intravenous infusion of epinephrine at a rate of 15 μg/kg min. in the cat, the S/P rose to 0.10 to 0.15 and returned towards the control value with a half-time of the order of 1 hour. We have also used creatinine extensively, both because of the sensitivity and accuracy of the method of analysis used and also because in experiments in which other sugars were to be used, sucrose

was an undesirable reference substance. In this case the S/P before epinephrine was 0.02 to 0.04 and rose after epinephrine to 0.15 to 0.25 (Fig. 1).

With repeated doses of epinephrine the permeability may stay elevated for some hours, allowing a relatively prolonged period for collecting samples. This is particularly the case with the dog submaxillary gland and for this reason the dog has been used when a more stable increase in permeability

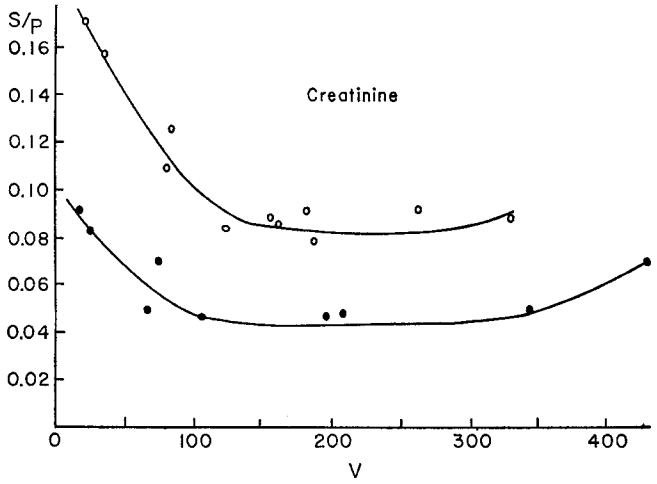

FIGURE 3. The concentration of creatinine in the submaxillary saliva of the dog at flow rates elicited by different frequencies of parasympathetic nerve stimulation. The lower curve (solid circles) is the control, the upper curve (open circles) was obtained after intravenous infusion of epinephrine 20 μg/kg min. for 10 min. The abscissa shows the rate of saliva secretion (V) in microliters per gram gland per minute.

was needed. On the other hand the effect of sympathetic stimulation is frequently short lived and may outlast the period of stimulation by no more than a minute or two; in general, the longer the period of sympathetic stimulation the more persistent is the poststimulation increase in permeability, but the duration does not approach that obtainable by systemic administration of epinephrine.

Effect of Rate of Saliva Flow

It is known that the concentration of non-electrolytes in saliva produced by parasympathetic stimulation is dependent on the rate of saliva secretion (3). In Fig. 3 it can be seen that the increase in the concentration of creatinine in the saliva produced by epinephrine is present at all saliva flow rates so that the basic relationship between flow rate and concentration is not much changed. The significance of this finding will be considered later.

Range of Neutral Molecules Affected

As shown in Table I, molecules up to and including polyglycol 1540 (molecular weight 1450) entered the saliva after epinephrine. Inulin with a molecular weight of 4500 was not found to do so. No molecules suitable for studying the intermediate molecular weight range were available. Quantitative studies of

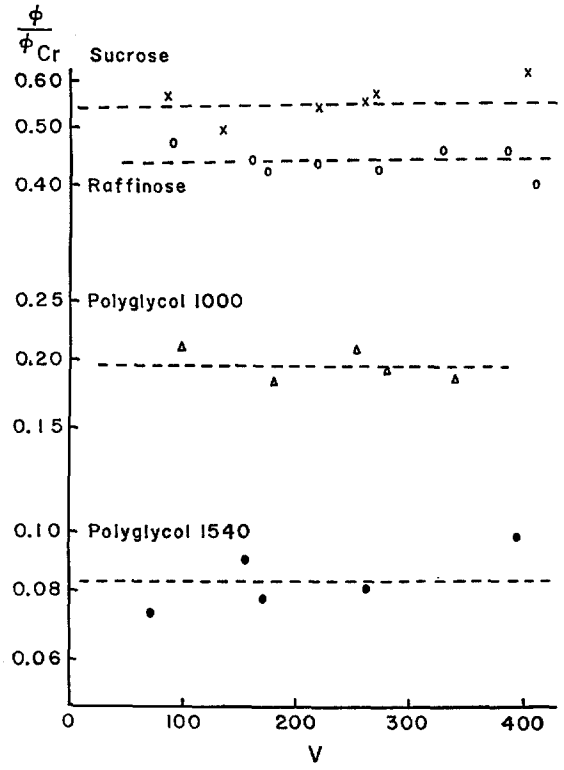

FIGURE 4. The permeability (ϕ) to sucrose, raffinose, polyglycol 1000, and polyglycol 1540 expressed as a fraction of the simultaneously determined permeability to creatinine (ϕ_{cr}) in the cat submaxillary gland after epinephrine infusion. The abscissa records the flow rate of saliva (microliters per gram minute) for each sample. Each of the four curves was obtained from a separate cat.

permeation are made difficult because of the practical problems in obtaining a constant permeability over more than a relatively short period in the cat, as well as the effect of variation in saliva secretion rate on the permeability. The more constant effects in the dog did not entirely overcome this because the smaller permeability increase in the dog led to lower saliva concentrations of some of the more impermeable molecules and hence to difficulties in analysis. A solution to this dilemma was found by always comparing the effect of epinephrine on at least two substances, one of which was creatinine.

The comparison could be made most accurately by calculating the ratio of the permeabilities of the two substances by the expression used by Burgen (3).

$$\phi = \frac{VS/P}{1 - S/P} \therefore \frac{\phi_x}{\phi_{cr}} = \frac{(S/P)_x}{(S/P)_{cr}} \cdot \frac{1 - (S/P)_{cr}}{1 - (S/P)_x}$$

The application of this relationship reveals the remarkable (and very useful) fact that if epinephrine leads to permeation of a substance, the ratio of the permeability of the gland for this substance to that for creatinine is constant whatever the intensity of the epinephrine effect. This ratio is also inde-

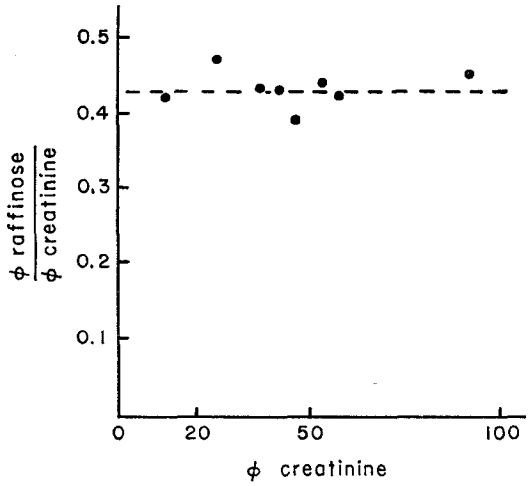

FIGURE 5. The ratio of permeability for raffinose to that for creatinine after epinephrine infusion expressed as a function of the absolute permeability to creatinine. The latter was varied by changing the rate of parasympathetic stimulation. The units for ø creatinine are milliliters × 10^{-3}/gm gland/min.

pendent of the saliva secretion rate and varies very little in different animals (Figs. 4 and 5). The values of this ratio are shown in the last column of Table I; it can be seen that this manipulation has the effect of converting S/P values of limited quantitative value (because of their variability) into a rather precise measurement of the permeability of the gland.

While one implication to be drawn from Figs. 4 and 5 is that the change in permeability affects all the molecules proportionately whatever the intensity of the effect, this seemed such an important finding that it was tested independently by another method. Glycerol, sucrose, and raffinose were infused intravenously and saliva was collected first in response to parasympathetic stimulation and then with combined parasympathetic and sympathetic stimulation. Samples of saliva and plasma (diluted 1:10) were chromatographed in a butanol–acetic acid–water system and the location of the

glycerol, glucose, sucrose, and raffinose spots revealed by spraying with an alkaline silver reagent. While the method is only semiquantitative it showed quite clearly that the concentrations of the four molecules varied completely in parallel at different intensities of stimulation. In other experiments, single drops of saliva were collected successively after starting sympathetic stimulation. The concentrations of these four molecules increased in parallel in successive drops. There was no indication at all that the glycerol concentration increased first, followed successively by the concentrations of the larger molecules. The evidence from these experiments points quite clearly to a permeability process that is graded in intensity but that permits the passage of all the molecules listed in Table I (except inulin) in a fixed proportion.

TABLE I

	Molecular weight	*S/P* before epinephrine	*S/P* after epinephrine	ϕ/ϕ_{cr}
Creatinine	113	0.02–0.04	0.10–0.31	1.00
Glucose	180	<0.02	0.08–0.25	0.79±0.030*
Sucrose	342	<0.005	0.06–0.19	0.57±0.007
Raffinose	504	<0.005	0.04–0.13	0.44±0.008
Polyglycol 1000	1000‡	<0.01	0.03–0.07	0.22±0.010
Polyglycol 1540	1450§	<0.01	0.02–0.08	0.12±0.005
Inulin	4500	<0.002	<0.002	<0.006

* SE.
‡ Molecular weight range 950 to 1050.
§ Molecular weight range 1300 to 1600.

The Site of the Increased Permeability

It is possible to obtain some information about the site at which a substance enters the saliva by following the pattern of outflow in the saliva, when substances are injected into the artery supplying the gland while secretion is in progress (4, 11). If a substance enters the saliva exclusively through the acini there will be a considerable latency before it appears in the secreted saliva. This latency corresponds to the volume of the lumina of the salivary ducts. If, however, the substance can enter the saliva across the duct epithelium, the latency will be shorter.

Before administration of epinephrine, no radioactivity was detected in the saliva following intraarterial injection of C^{14}-glucose, but after epinephrine administration a similar injection (Fig. 6) led to the appearance of radioactivity with a gross latency of 30 μl/gm (this includes the dead space of the cannula).

Paper chromatography of the saliva (in the butanol–acetic acid–water system used above) showed that all this radioactivity was due to C^{14}-glucose. When tritiated water was injected intraarterially in a similar experiment its

latency was 23 μl/gm. It is known that tritiated water is able to enter the saliva along practically the whole length of the duct (4); the small difference in latency between tritiated water and glucose makes it evident that after epinephrine glucose can cross the ducts. Unfortunately, this kind of experiment gives no useful information about what is happening in the acini; it is quite possible that glucose can permeate through the acini as well as through the ducts, but we have no information on this point.

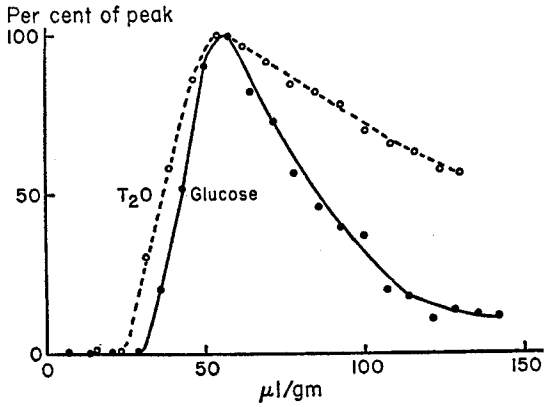

FIGURE 6. Rapid intraarterial injection of glucose-C^{14} and T_2O into the central end of the lingual artery of the cat. Submaxillary saliva was flowing continuously due to parasympathetic stimulation. The abscissa represents the cumulative volume of saliva secreted from the moment of injection. The ordinate is the concentration of radioactivity in the saliva as a percentage of the maximum. The permeability of the gland had previously been increased by epinephrine infusion.

Permeation of Urea

The salivary glands are permeable to urea even with parasympathetic stimulation alone, but after epinephrine administration the permeability increases approximately in proportion to the creatinine and sucrose permeability. The results are less regular than in the case of the substances listed in Table I. In some instances the urea permeability is much greater than would be expected from these changes; it was never less than expected from the changes in creatinine permeability.

Permeation of Electrolytes

Langstroth *et al.* (2) showed that both epinephrine administration and sympathetic stimulation increased the concentration of sodium and chloride in parasympathetic saliva; this was readily confirmed in the present series of experiments but will not be examined further here. However, epinephrine also permits the entry of some electrolytes into the saliva which do not appear

with parasympathetic stimulation alone. For instance in the experiment illustrated in Fig. 7 when radiosulfate was injected intravenously in a cat, only traces of radioactivity were found in submaxillary saliva ($S/P \sim 0.003$) when the parasympathetic nerve was stimulated alone; when stimulation of the sympathetic nerve was added, the concentration rose after a latency and eventually reached an S/P of 0.092; the latter is similar in magnitude to the S/P found for sucrose.

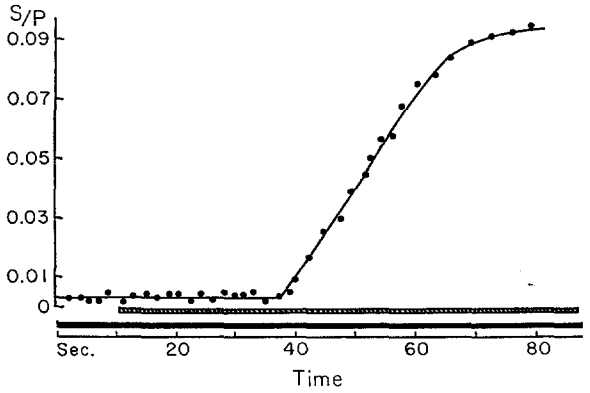

FIGURE 7. Radiosulfate in the submaxillary saliva of the cat. The animal had received 150 μc/kg of $S^{35}O_4$ intravenously an hour previously. When the parasympathetic was stimulated alone (heavy solid bar) only traces of radioactivity were found in the saliva ($S/P \sim 0.003$) but when sympathetic stimulation was added (cross-hatched bar) the concentration of radioactivity rose and eventually reached $S/P = 0.092$.

The anionic dyes, eosin and phenol red, do not usually appear in saliva, but do so after epinephrine. Free amino acids are usually found in saliva only in low concentrations ($S/P \sim 0.05$ for valine, histidine, arginine, glutamate), and the concentration of these acids is also greatly increased by epinephrine.

Penetration of Solutes into the Cell Water

In the case of substances that penetrate into the saliva in the absence of epinephrine or sympathetic stimulation, the concentration in the cell water is known to be close to that in the plasma (3). This suggests that these substances penetrate the basal surface of the gland cells readily. Sucrose, however, does not appear to enter the cell water since its concentration in the gland is consistent with it being confined to the extracellular space; its distribution accords closely with that of inulin (Table II).

After epinephrine administration the weight of the gland and the total gland water usually increased by a variable amount and with this the sucrose and inulin spaces increased, but to the same degree. However, if the space

from which sucrose and inulin are excluded ("cell water") is calculated, this shows no significant change in either case. It appears that the same space in the gland is available to these two saccharides, one of which is excreted in the saliva whereas the other is not. The results suggest that epinephrine produces an increase in the extracellular fluid of the gland but little or no change in the intracellular fluid.

DISCUSSION

The change in permeability produced by epinephrine is unusual in that it affects the permeation of a wide variety of substances in a qualitatively similar degree. While it is difficult to envisage a transcellular process having so unselective a character, these are exactly the properties that would be expected for permeation through uncharged extracellular pores.

TABLE II

WATER CONTENT AND SUCROSE AND INULIN SPACES IN THE CAT SUBMAXILLARY GLAND

	Before epinephrine	After epinephrine
	ml/kg	*ml/kg*
Total water	757±4.4*	806±28.1*
Sucrose space	259±5.4	310±58.5
Inulin space	238±8.4	333±66.7
"Cell" water (Total water − sucrose space)	498±8.3	496±16.2

* SE.

It was shown previously (3) that the permeation of non-electrolytes through the parotid gland (excited by parasympathetic stimulation) depended both on the lipid solubility and the molecular radius of the molecules. Substances with a low solubility in lipids and with a molecular radius greater than 3.4 Å were severely restricted in their diffusion into the saliva. This restriction due to molecular size suggested that the permeation by lipid-insoluble substances occurred through pores whose radius was a little greater than 3.4 Å.

For substances diffusing from the blood into the saliva the one dimensional Fick equation may be used for quantitative evaluation of the process, provided that the system is in a steady state and that the transfer process is purely diffusional. It is very difficult to obtain proof that specific transfer processes are not occurring in the present experiments but the fact that the change in the transfer of a number of heterogeneous molecules after epinephrine occurs so much in parallel suggests this is the case. The further examination of the process will be posited on this assumption.

The general form of the equation is

$$\frac{dn}{dt} = D \cdot \frac{A}{x} \cdot \Delta C$$

and taking into account the quantities determined in a salivary experiment this can be rewritten

$$\frac{A}{x} \cdot D = \frac{V \cdot S/P}{1 - S/P}$$

The right hand part of this equation has been used previously to determine the permeability constant ϕ.

TABLE III

	Molecular radius	D_{tr}	ϕ/ϕ_{cr}	$A/x/(A/x)_{cr}$
	Å	$cm^2/sec. \times 10^5$		
Creatinine	3.20*	1.23‡	1.00	1.00
Glucose	3.57§	0.91§	0.79±0.030‖	1.06±0.04‖
Sucrose	4.40§	0.74§	0.57±0.007	0.95±0.012
Raffinose	5.6§	0.56§	0.44±0.008	0.96±0.027
Polyglycol 1000	7.2*	0.43‡	0.22±0.010	0.615±0.029
Polyglycol 1540	8.1*	0.32‡	0.12±0.005	0.464±0.018
Inulin	14.8§	0.21¶	<0.006	<0.03

* Calculated from Stokes-Einstein equation.
‡ Interpolated using relationship that $D\alpha\sqrt{\text{molecular weight}}$ for small molecules.
§ Renkin (13).
‖ SE.
¶ Pappenheimer (12).

In the case, therefore, in which transfer is diffusional we have

$$\phi = \frac{A}{x} \cdot D$$

D is the free diffusion coefficient of the substance and A/x a geometrical factor composed of the diffusional area divided by the length of the diffusional path. This is the same geometrical factor used by Pappenheimer (12) in his analysis of vascular permeability.

We can now examine the relative diffusional paths of our test substances by dividing the ratio ϕ_x/ϕ_{cr} shown in Table I by D_x/D_{cr}. This is shown in Table III and Fig. 8 together with the radii of the molecules concerned. It can be seen that the diffusional path is reasonably constant for creatinine, glucose, sucrose, and raffinose, but becomes reduced for polyglycol 1000 and polyglycol 1540 while that for inulin is less than 3 per cent of that for creatinine.

This relationship suggests that diffusion occurs through pores with a maximum radius of ~12 Å. The constancy of the diffusional path for creatinine, glucose, sucrose, and raffinose provides support for the assumption made above that the transfer does follow Fick kinetics.

The absolute value A/x varies both with the intensity of the epinephrine effect and also with rate of secretion. The latter point can be illustrated if the data from Fig. 3 are calculated as diffusional areas (*i.e.* taking advantage of the relative stability of the epinephrine effect in the dog). The value of A/x increases with flow rate and indeed preserves the general relationship found for creatinine before epinephrine (Fig. 9). In the cat submaxillary gland,

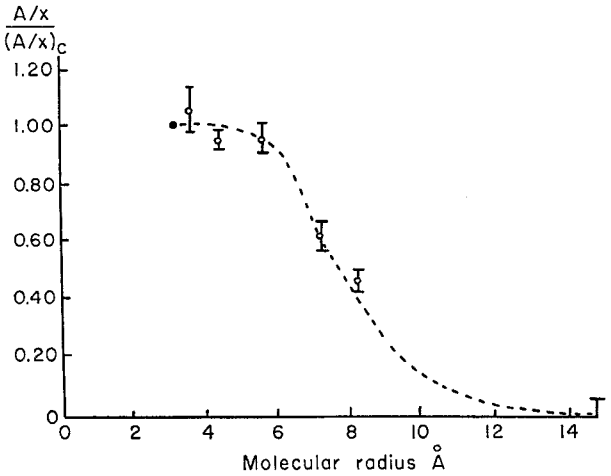

FIGURE 8. The relative diffusional paths (A/x) after epinephrine as a function of the molecular radius. The diffusional paths are expressed as a fraction of that of creatinine. Creatinine itself is indicated by a solid circle. Horizontal bars indicate the limits of two standard errors away from the mean.

the maximal permeability produced by a large dose of epinephrine when tested by stimulation of the parasympathetic nerve at 20 CPS leads to maximum diffusional path (A/x) averaging 94 cm/gm gland. In order to make the magnitude of this quantity clearer let us assume for the moment that the diffusional path opened up by epinephrine is the whole of a space between two cells, *i.e.* the length of the path (x) is equal to the cell height (15 to 20 μ), the area involved (A) would therefore be 0.15 to 0.2 cm^2/gm. Since the total basal surface of the gland cells is about 500 cm^2/gm (not allowing for interdigitations), the area available for diffusion is only a small fraction of this.

If this diffusional path were distributed evenly throughout the intercellular spaces of the gland the mean width of the spaces would be 40 Å. To completely exclude the diffusion of inulin the mean spacing must be less than 30 Å

and indeed for it to exert considerable restriction on polyglycol 1540 (molecular diameter 16.2 Å) a figure of 20 to 25 Å is more probable. If only a proportion of the cell junctions in the gland is involved, the mean intercellular spacing will be correspondingly increased and it certainly seems unlikely that epinephrine would affect *all* intercellular boundaries. It seems clear that if the diffusional pattern affects the whole of an intercellular space uniformly

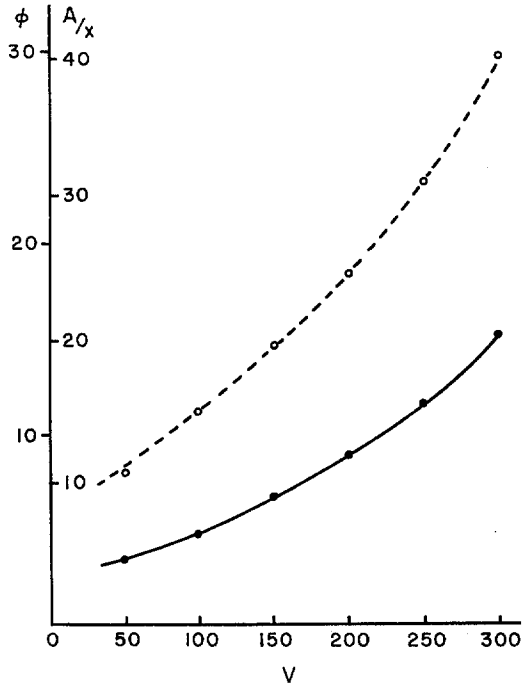

FIGURE 9. The diffusional path for creatinine in the dog submaxillary gland before (solid circles) and after (open circles) epinephrine infusion. Data of Fig. 3. Ordinate, diffusional path (A/x) in centimeters per gram gland and ø(ml × 10^{-3}/gm min.) Abscissa, saliva secretion rate in microliters per gram gland per minute.

it is not possible to account for both a sieving effect for molecules larger than 25 Å diameter and the large diffusional area. There is an alternative, however, which does account for these facts satisfactorily. The intercellular junction in the salivary glands consists in part of ordinary apposed cell membranes, which may also open up partly to form intercellular canaliculi, and also of tight junctions, which may be identified with terminal bars and desmosomes (14, 15). We may suppose then that the barrier to diffusion across the intercellular junction consists in part of diffusion in the "loose" cell junctions which exerts no sieving effect on molecules with diameters < 25 Å and a tight junctional region upon which the selective permeability depends. This

arrangement is shown schematically in Fig. 10. The diffusional equation appropriate to this configuration contains the geometrical relationship

$$\frac{A}{x} = \frac{2n \cdot l \cdot f}{\frac{h_1}{d_1}\frac{h_2}{d_2}}$$

where h_1 and h_2 are the heights and d_1 and d_2 the width of the loose and tight junctions; l is the length of the cell edges seen from surface, n the number of cells in the gland, and f the fraction of cell boundaries involved in the perme-

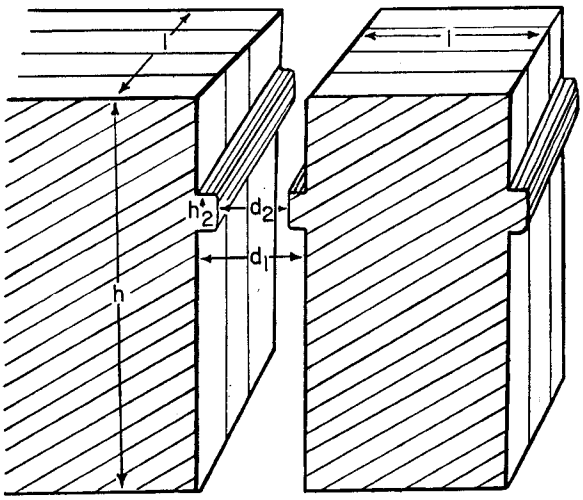

FIGURE 10. A schema of the intercellular spaces in the gland. The total cell height is h and the apical surface of the cell has a side of length l. The intercellular space is comprised of narrow section width d_2 and height h_2 and a wider section of width d_1 and length $h - h_2(h_1)$.

ability change. We can insert into this equation the total length of the cells $(h_1 + h_2)$, taken as 20 μ, and l, also taken as 20 μ. According to Rutberg (15) the mean length of the tight junctions in the mouse salivary gland is 0.4 μ, and we will assume that this value applies to the cat gland. The value of d_2 is determined at 25 Å by the requirement that this dimension should explain the sieving properties of the junction. We can therefore compute the remaining unknown; *i.e.*, the width of the loose intercellular boundary (d_1) in terms of the fraction of cell junctions involved. This is plotted in Fig. 11. It can be seen at once that a minimum of 3 per cent of cell boundaries is a limiting value, and indeed if less than 3.6 per cent of cell boundaries are involved d_1 would have to exceed 1 μ. It is probable that at least the part of the cell boundary at the basal part of the cells is narrower than this, so that if we take the upper limit for d_1 as 0.1 – 1 μ this corresponds to ~4 to 7 per cent of

intercellular junctions. We can set an upper limit to the fraction of boundaries involved from the following consideration. When the gland secretes at slow rates in response to a low frequency stimulation of the parasympathetic, no selective restriction of larger molecules, for instance polyglycol 1540, compared with smaller molecules, occurs despite the fact that A/x may decrease to approximately one-tenth of its maximum value. If the permeability change produced by the parasympathetic nerves resides in the loose intercellular junction this means that when A/x = 9.4 cm/gm, d_1 may not fall below ~25 Å, and therefore when A/x is maximal d_1 must be > 250 A, which corresponds to the involvement of 19 per cent of cell boundaries.

The theory presented so far suggests that the tight junction does not normally allow the molecules considered in Table I to penetrate, but that after

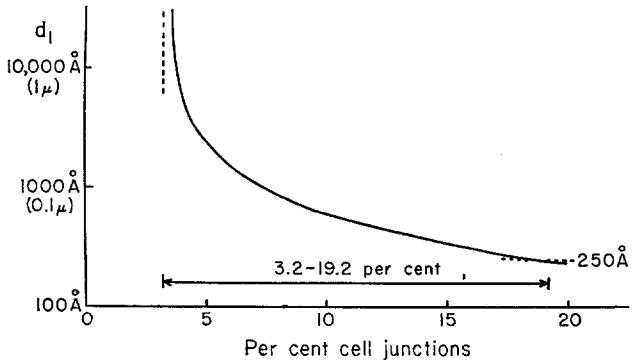

FIGURE 11. The relationship between the width of the loose intercellular spacing d_1 and the percentage of cell junctions involved in the epinephrine effect.

epinephrine administration these junctions undergo a change which permits molecules up to 25 Å diameter to penetrate. We may ask then whether the tight junctions are normally permeable to small molecules (*e.g.* urea) and whether the permeation of these molecules can be mostly intercellular as well. The maximal diffusional path for urea in the normal gland in the cat is about 40 cm/gm, which is quite compatible with diffusion through the same intercellular junction involved in the epinephrine effect but with some restriction such as might be expected for the diffusion of molecules with a diameter of 5.4 Å through pores ~7 Å in diameter. After epinephrine administration urea permeability in some experiments increased up to the same value found for sucrose, *i.e.* to a maximum of ~100 cm/gm which would be compatible with the same pathway being utilised but with the restriction due to pore size removed; in other experiments, however, it increased to ~200 cm/gm which suggested that additional paths became available for urea that are not available for the larger molecules. It is perhaps of some

interest to recall that in the dog submaxillary gland about two-thirds of the diffusional area for urea has been shown to be in the duct system (16).

As a corollary of the "series resistance" hypothesis, it is required that the width of at least part of the loose intercellular space should vary with the intensity of parasympathetic stimulation. This change in spacing could be identical in the normal gland and after epinephrine.

A variant of the above theory is one that presupposes that epinephrine increases the permeability of the tight junctions to such a degree that they are no longer restrictive at all for the molecules studied and that the sieving structure is the basement membrane. In any case the basement membrane cannot be ignored as a permeability barrier between the extracellular space and the saliva. It appears to be a continuous structure in the salivary glands with a mean thickness of 500 Å (15). If the basement membrane is supposed to have pores of 25 Å, a calculation similar to that for the tight junctions can be made but in this case the fraction of spaces affected need be only 2 to 16 per cent. These two hypotheses seem to be ones that could reasonably be tested by electron microscopy. If either is valid it seems likely that perceptible changes in the intercellular junctions should be found after epinephrine treatment.

Up to this point it may seem that we have discussed rather summarily the possibility of transcellular movement of the test substances. There are three pieces of evidence that seem to speak against the possibility of explaining our results by transcellular movement. The first is that sucrose still fails to penetrate an appreciable number of cells after epinephrine. The second is that if the transcellular movement were not due to simple permeation through the cell membrane but to vesicular transport it seems odd that the rates of permeation of the molecules creatinine, glucose, sucrose, and raffinose should agree so well with their rates of free diffusion; this is not a circumstance to be expected for any kind of bulk transfer. Third, if the process involved were vesicular transport, a considerable transfer of fluid should occur. Indeed if the S/P value were 0.25, then this would require that if the transferred fluid contained the same concentration of the solutes as that in the extracellular fluid, then 25 per cent of the secreted fluid must pass by this route. This seems improbable because (*a*) epinephrine or sympathetic stimulation never increases the saliva secretion rate in the face of maximal parasympathetic stimulation (17), and (*b*) the epinephrine effect is completely dissociable from any effect on water secretion; *i.e.*, it long outlasts any secretory effect.

One possible objection to our diffusional treatment of non-electrolyte movement is that Fick's equation only applies if solvent flow through the same pores is absent (18). If we consider water flow through a diffusion path of 100 cm/gm occurring along the osmotic gradient ($\sim$50 to 200 mOsM) between saliva and extracellular fluid it is found to amount to <1 μl/gm

min. This bulk flow is so small that the use of the classical Fick equation does not introduce appreciable error.

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