THE ELECTRICAL PHENOMENA ACCOMPANYING THE EXCITATION OF SO-CALLED SECRETORY AND TROPHIC NERVE FIBRES, IN THE SALIVARY GLANDS OF THE DOG AND CAT. BY J. ROSE BRADFORD, B.Sc., M.R.C.S.

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IN the *Proceedings of the Royal Society* for February 1886, I published, in conjunction with my friend Mr Bayliss, the results of an investigation, showing that the process of secretion in the salivary glands of the dog and cat was accompanied by definite electrical variations.

The following communication contains the results of work carried on during the year 1886, on the parotid of the dog and cat, and the submaxillary of the latter.

The results communicated to the Royal Society and elsewhere[1], summed up shortly, were as follows: In the dog, excitation of the chorda caused a large variation, indicating the surface of the gland to become negative to the hilus; and excitation of the sympathetic caused the outer surface to become positive to the hilus, this variation being neither so rapid nor so great as the chorda effect. In the cat excitation of the chorda, or of the sympathetic caused a diphasic variation. Finally in the parotid of the dog, excitation of the tympanic plexus, caused the surface of the gland to become negative to the hilus, and excitation of the sympathetic caused the surface to become positive to the hilus.

It seemed desirable, as these results were very constant and definite, to continue the work, and try to determine if possible, which of the many changes that occur during the activity of these glands, are concerned in the production of the electrical phenomena.

[1] Bayliss and Bradford, *Internat. Monatsschrift für Anat. u. Physiologie*, 1887.

The experiments described below were performed with this object; and the results obtained seem to point to the conclusion, that the electrical phenomena produced by the changes in the gland, that follow excitation of so-called secretory fibres, are opposite in character, to those produced on excitation of so-called trophic fibres. This conclusion was hazarded by us in the paper mentioned above; but only provisionally, as the facts obtained then were not sufficient to establish it definitely, since they referred almost exclusively to the submaxillary gland of the cat.

It may be as well to mention here, that the word "trophic" is used in this paper in Heidenhain's sense only, i.e. a trophic fibre is a fibre, the excitation of which causes the elaboration of the organic constituents of the saliva. This is of course not the true sense of the word trophic, and there are no doubt true trophic or anabolic fibres, in the gland nerves, but with these we are not at present concerned. Some observers are inclined to the belief, that the real trophic or anabolic fibres are also secretory, but it seems not improbable that they are altogether distinct from these, as I trust to show in a future communication.

On excitation of such a nerve as the chorda of the dog the following changes in the gland probably occur, besides others possibly as yet unknown.

1. Vasomotor changes.
2. Chemical changes in the gland cells, connected with the elaboration of the organic, and possibly of the inorganic constituents of the saliva.
3. Changes presumably physical, owing to which water is secreted, i.e. is enabled to pass through the basement membrane and gland cells, through which prior to the excitation it was unable to pass.
4. Movement of fluid through the cells, ducts etc.

It may not seem necessary to separate the last two statements; but it is obvious that before the water can pass from the blood-vessels or lymphatics, through the gland cells into the lumen, some change must have occurred in the gland cells; since before the nerve excitation they were practically impervious to fluid, and then as a result of the excitation, they suddenly allow fluid to pass through in abundance. The nature of this change is not known, since Stricker and Spina's[1] view of the sponge-like action of the gland cell has not been very generally

[1] Stricker and Spina, *Sitzungsberichte der Wiener Akad.* LXXX. 1879.

accepted, but whether the change is chemical, or partly, as Stricker and Spina argued, mechanical, it is at any rate a rapid one.

The phenomena, as observed by Stricker and Spina in the nictitating membrane of the frog, are very striking and easily seen; but the apparent great swelling up of the cells may be due to the contraction of the longitudinal gland muscle outside the cells, and not to a distension of the cell with fluid. Hence at present we can only conclude that this change, whatever its exact nature may be, is a rapid one, and therefore, *à priori*, is likely to be accompanied by differences of potential. It is of course as yet, scarcely possible to attempt to differentiate between the preparation of the cell for the passage, and the actual passage of the fluid. The most that we can do at present, is to attempt to show that this process is accompanied by electrical changes. Histologists have paid most attention to the changes in the cells connected with the elaboration of the constituents of the secretion; so possibly it is for this reason, that we know so little concerning the passage of the fluid. Besides the above changes, the nerve excitation may cause a growth of the protoplasm of the gland cell, and possibly other changes; but with these we are not at present concerned, and an attempt in the present paper will be made to connect the electrical phenomena observed, with one or more of the series of changes enumerated above.

I. *Vasomotor changes.*

The electrical phenomena are in all probability not due to vasomotor changes, for the following reasons.

The electrical variations accompanying the excitation of the chorda and sympathetic nerves of the cat, are very readily abolished by small doses of atropin, this is also true for the chorda of the dog, and it is only in the case of the sympathetic of the dog, that the variation is abolished with difficulty. It is however precisely with this nerve, that the secretory activity is abolished with difficulty. Further excitation of the dog's sympathetic always causes the same vasomotor change in the gland vessels; but it does not always cause the same secretory effect either in the dog or cat (as will be described more fully below), and coincidently with this, we find that the electrical variation is not always the same.

The fact, that the same nerve in the same animal, will at different times give very different results as regards the nature and amount of secretion, seems to be an important one not only with regard to the matter under discussion, but also with regard to the much larger question, as to the nature of gland nerves, and as to the manner in which they are connected to the gland cell.

Thus to consider first the dog. Excitation of the sympathetic causes, as a rule, a scanty and viscid secretion of submaxillary saliva; but in different animals and in the same animal, according to the functional activity of the gland, there are considerable differences as to both the amount of the saliva obtained, and as to its viscidity. In the cat, these differences are if anything more marked. Here as a rule the sympathetic saliva is distinctly less viscid than the chorda-saliva, that is to say that at first sight, we appear to have the opposite to what obtains in the dog.

A little consideration, however, will show, I think, that in the cat, it is rather that the chorda and sympathetic secretions are very much alike, and that the difference in viscidity is very variable. At times, there is scarcely any difference to be detected between the saliva obtained from the two nerves; and occasionally, but not by any means rarely the sympathetic saliva of the cat's submaxillary is distinctly more viscid than the chorda saliva. In other words, in the cat, we do not find that marked difference between the chorda and sympathetic saliva, that is present in the dog; and such difference as exists, is not only less, but is usually in favour of the chorda saliva being the more viscid. However, as mentioned above, this is not constant. Finally in the parotid of the dog, sympathetic excitation usually yields no secretion, but occasionally a few drops are obtained. In the cat, the sympathetic gives a copious secretion of parotid saliva. It will be seen from these examples that within certain limits, there are considerable differences in the effects produced in the glands, by the excitation of this nerve, as measured by the amount and character of the resulting secretion. Regarded as a vasomotor nerve, no such great differences are known to result on its stimulation. Hence we find differences in the actual secretion, on stimulation of the same nerve at different times, and along with these differences there are differences in the electrical phenomena; the same nerve giving at one time a single variation, at other times a diphasic variation, or even a variation of opposite sign to the initial one. Further, as will be shown below, the differences in the electrical phenoména are clearly linked to differences in the character of the secretion. All the above facts point strongly against the conclusion, that the vasomotor effects are concerned in the production of the electrical variations. Against the above view it might be urged, that what has been called the first phase, i.e. outer surface of gland negative to hilus, is not vasomotor, owing to the ease with which it is abolished by atropin; but that the second phase, i.e. outer surface of gland positive to hilus, is due to these vasomotor changes. At first sight, there are a certain number of facts, apparently in favour of the view that this second phase is vasomotor. Thus this is the phase that occurs most often on sympathetic excitation in the dog, and this is the phase that is most refractory to atropin, requiring as much as 100 mgrms. sometimes to abolish it. Further in the dog, after 5—10 mgrms. of atropin have abolished the usual chorda variation, i.e. outer surface negative to hilus, a small second phase is seen, and it might be concluded that both here and on sympathetic excitation, this second phase was due to vasomotor effects. But a fact strongly militating against this conclusion, is found in the cat. In this animal this second phase, which is seen both on chorda and sympathetic excitation, is readily abolished by doses of atropin, only slightly larger than those necessary to abolish the chorda first phase in the dog; 20—40 mgrms. of atropin being sufficient in the cat, to completely abolish both first and second phase, in the case both of the chorda and of the sympathetic. Hence if we were to assume that the second phase was due to vasomotor effects, we should have to suppose that these are more readily abolished by atropin in the cat, than they are in the dog: for which hypothesis there is no proof, whereas it is well known that atropin abolishes the secretion in the cat in smaller doses, than those required to do the same in the dog. In fact it is only in the case of the sympathetic of the dog, that it is at all probable, that the electrical phenomena are due to vasomotor changes. However, when we consider that a variation of similar sign is much more readily abolished in the cat than in the dog; that the secretory activity of this nerve in the dog, requires as much as 100 mgrms. of atropin to abolish it, and that this dose also abolishes the electrical phenomena; we can scarcely draw any other conclusion, than that the electrical phenomena observed are not due to vasomotor changes.

We will now proceed to discuss the question, as to whether any of the electrical phenomena are due to phenomena grouped under heads 3 and 4; that is to say, to the chemical or physical changes in the gland cells, that necessarily precede the flow of liquid, or to the flow of liquid itself.

There is no doubt that some of the electrical phenomena are closely connected with the flow of liquid, as was tentatively advanced in previous communications. Then the evidence was derived almost exclusively from the submaxillary of the cat, now however this view is confirmed from an examination of the parotid, and from certain striking results obtained in the dog. It is however very difficult to decide whether the electrical changes are connected with the actual flow of liquid, or whether they are connected with the changes in the gland cells, that precede this flow.

In the first place, it is well known, as shown by observations of Quincke[1], that the movement of fluid through fine tubes, is capable of causing differences of potential. Again in Dionæa[2], the movement of fluid such as the imbibition of the water by cells, is no doubt largely concerned in producing the electrical phenomena observed.

The relation mentioned above between the electrical variation and the flow of secretion, is so close, that the observer at the galvanometer, can frequently from the inspection of the deflection say whether the excitation is producing a secretion or not. "In all cases, the excitation of a gland nerve, that produces an actual secretion of saliva, also causes the outer surface of the gland to become negative to the hilus, when the two surfaces are led off."

That this statement is correct is shown by the following facts. This effect is produced in the dog on excitation of the chorda, and of the tympanic plexus, in the case of the submaxillary and parotid glands respectively. In the cat, not only is it produced by stimulation of the same nerves, but also when the sympathetic is the nerve excited; since in this animal this nerve causes a free secretion, both from the submaxillary and parotid glands. Hence it is only in the case of the sympathetic of the dog, that the nerve excitation is not followed by the above variation; and this nerve, as is well known, gives as a rule no secretion from the parotid, and but very little from the submaxillary.

When the excitation of a gland nerve causes an actual secretion of saliva, and also causes the surface of the gland to become negative to the hilus, there may or may not be a second phase of opposite sign (the importance of which will be discussed below), but the first phase is always present. Finally, this first phase is readily abolished by atropin, and so is the secretion that accompanies it.

[1] Quincke, *Pogg. Ann.* 107, 1859 and 1860.

[2] Burdon Sanderson, *Phil. Trans.* 1882. Kunkel, *Pflüger's Archiv* xxv.

The following table illustrates the above facts, and gives the actual galvanometer deflections observed[1].

Animal	Gland	Nerve excited	Electrical variation		Saliva	Coil		Anaesthetic
			Sign	Amount		Strength	Duration	
Dog	sub-maxillary	chorda	–	off scale	abundant	50 mm.	10″	chloroform
Dog	parotid	tympanic plexus	–	95	4 drops	10 mm.	10″	chloroform
Cat	sub-maxillary	chorda	–	360	abundant	80 mm.	10″	chloroform
Cat	parotid	tympanic plexus	–	100	2 drops	50 mm.	10″	chloroform
”	”	sympathetic	–	40	scanty	80 mm.	10″	chloroform
Dog	parotid	tympanic plexus	–	120	3 drops	10 mm.	10″	chloroform and morphia
”	”	sympathetic	+	40	none	50 mm.	10″	

As was mentioned above, the sympathetic of the dog yields as a rule a scanty and very viscid secretion of sub-maxillary saliva; the secretion being accompanied by the surface of the gland becoming positive to the hilus, but under certain circumstances, these are not the results that follow its excitation. This was particularly well observed in two cases, in two different dogs. In the first case the sympathetic was being stimulated, and was yielding as usual a scanty viscid secretion, accompanied by a variation, indicating the surface of the gland to become positive to the hilus. This variation, as is usual, was a slow one and was rather small; suddenly the galvanometer indicated a large first phase, and coincidently with this, a rapid and copious secretion of watery saliva occurred.

What the circumstances are that lead to this remarkable result, are not known; but many observers have noted the fact, that occasionally this nerve yields, on excitation, a copious watery secretion instead of the usual scanty viscid secretion.

The following table gives the results of the experiment quoted above.

[1] In the table the sign – means the surface of the gland negative to the hilus and the sign + the surface positive to the hilus.

Dog. Chloroform and curare.

Time	Gland	Nerve	Saliva	Electrical variation		Coil	
				Sign	Amount	Strength	Duration
10.50	parotid	tympanic plexus	6 drops	–	100	10 mm.	10″
11.15	sub-maxillary	chorda	copious	–	off scale	80 mm.	10″
11.20	,,	sympathetic	1–2 drops viscid	+	50	80 mm.	10″
11.25	,,	,,	2 drops	+	80	50 mm.	10″
11.30	,,	,,	12 drops } after long latent period	+ –	+ 80 – off scale	50 mm.	10″
11.35	,,	,,	14 drops }	+ –	+ 50 – off scale	,,	,,

In another experiment, the chorda tympani was excited at intervals of five minutes for an hour and a half, with the usual result, i.e. a copious secretion, and a variation indicating the outer surface to become negative to the hilus. After this prolonged stimulation of the chorda, the sympathetic gave also, on excitation, a copious watery secretion; in some instances as much as fifteen drops. This secretion only appeared after a very long latent period. During the latent period, an electrical effect of the usual sign was observed, i.e. outer surface positive to hilus, but immediately the secretion began a large deflection of the opposite sign was obtained. After allowing the gland to rest for some time, excitation of the sympathetic failed to produce a watery secretion, and then the usual second phase was obtained.

The results of these two experiments are particularly striking and conclusive with regard to the question, as to the relation existing between the character of the secretion and the sign of the variation, since the nerve in question usually gives neither a copious secretion nor a large first phase; but in these two instances both these results were obtained, thus showing that if one is not the actual cause of the other, yet they must be very closely connected. Further the same fact is more often seen in a less marked form; that is to say the sympathetic yields rather more secretion at some times than at others, and if this secretion be not only more abundant, but also less viscid than normal, a diphasic variation accompanies it, instead of one consisting of a second phase only.

In the parotid of the dog similar phenomena, to these just described for the submaxillary of the same animal, are to be observed. Excitation of the sympathetic causes as a rule no actual secretion of parotid saliva, although it produces profound morphological changes in the gland cells. Occasionally however a slight secretion is obtained, as has been noticed by previous observers, particularly Langley[1]. This observer further noticed that it was only when an actual secretion was produced, that a clear outer zone was developed; this point is of importance with regard to the question, as to whether anabolic changes in the gland cells are accompanied by electrical variations.

It will be seen from the following table, that when an actual secretion is produced on sympathetic excitation, the surface of the gland becomes negative to the hilus.

Dog. Parotid. Chloroform and morphia. Curare.

Time	Nerve	Electrical Variation Sign and Amount	Saliva	Coil	
				Strength	Duration
12.30	tympanic plexus	– 120	3 drops	10 mm.	10″
12.35	sympathetic	+ 40	none	50 mm.	10″
12.40	tympanic plexus	– 120	3 drops	10 mm.	10″
12.41	tympanic plexus	– 20 + 20 – 60	1 drop	10 mm.	10″
12.43	tympanic plexus	– 10 + 10 – 70	1 drop	10 mm.	10″
12.46	sympathetic	– 60 after long latent period	1 drop	40 mm.	10″
12.49	sympathetic	– 70 after long latent period	1 drop	50 mm.	10″
12.50	tympanic plexus	– 120	2 drops	10 mm.	10″

[1] Langley. This *Journal*, Vol. VI. Page 71.

The tympanic plexus was excited by electrodes thrust into the tympanum, through the bulla, and so the gland nerve was really only excited by the spreading of the current; hence the excitation was not really so strong, as it would seem from mere inspection of the table. The conditions are doubtful, which determine that excitation of the sympathetic, should cause a watery secretion from the sub-maxillary gland of the dog. This effect is apparently most readily obtained in an exhausted gland, as is well seen in the second experiment quoted above. In this case when the gland had been allowed to rest for some time, this watery secretion was no longer obtained. In the case of the parotid also, it was only after repeated excitation of the tympanic plexus, that stimulation of the sympathetic caused the scanty secretion described above; so here too it is apparently a question of exhaustion.

The fact, that in the sub-maxillary it was only after prolonged excitation of the chorda, that this effect was obtained on sympathetic excitation, seems to indicate that the effect is due rather to exhaustion of the gland cells, or of the peripheral nervous structures, than of the nerve fibres. It is difficult however to understand, how excitation of such a nerve as the sympathetic of the dog, supposing it to contain principally so-called trophic fibres, should produce such a copious secretion as fifteen drops. If it were merely a question of exhaustion of the gland cells, one would I think expect on excitation of such a nerve, either less secretion, or the same amount as previously obtained but more watery in character. That is to say, that there should be a diminution either in the fluid portion, or in the solid of the secretion. On the contrary what is observed, is an actual increase in the amount of the secretion, although there is a diminution in the amount of solids it contains.

Further, the effect is probably not due to exhaustion of the so-called trophic fibres present in the nerve, since it was the chorda or the tympanic plexus, that was stimulated previously, and thus if the effect is due to exhaustion, it is evidently not the fibres of the nerve stimulated that are exhausted. A fact that possibly may have an important bearing on the explanation, is that in all cases where the sympathetic produces this anomalous result, it is only after a very long latent period, much longer than the usual one.

It might be urged, that the effect was simply due to exhaustion of the organic constituents of the saliva, and to the sympathetic containing secretory fibres, and so the effect would be due to the stimulation of these fibres. It seems to me however that this explanation will not meet the case for many reasons. If the sympathetic contains these secretory fibres, there is no reason why the amount of the secretion should be so enormously increased as it is, and this explanation will not hold for the parotid; since normally no secretion is produced in this gland, on excitation of the sympathetic; further this will not account for the great length of the latent period. We cannot assume that the sympathetic of the dog contains two different sets of nerve fibres, because opposite effects are produced on stimulation of the same nerve, first in a resting gland, and secondly in a gland after prolonged stimulation of its cerebral nerve. We might assume this if the observed effects were seen after prolonged stimulation of the sympathetic, but as just mentioned, it is after prolonged stimulation of the cerebral nerve; that is to say, of the nerve that is considered to contain mostly so-called secretory fibres. It is possible that the differences expressed by the terms secretory and trophic nerve fibres, are differences in the peripheral connection of the nerve fibres with the gland structures, rather than actual differences in the nerve fibres; on this view different results might follow the excitation of the same nerve, owing to the effect of exhaustion, etc. on this peripheral mechanism. Be the explanation what it may, the fact remains, that if the sympathetic yields a copious secretion, this is accompanied by the variation, consisting of a first phase.

In all the experiments performed by Mr Bayliss and myself, and in those subsequently performed by myself, there was only one exception to the proposition mentioned above, i.e. that a copious secretion is accompanied by a variation, showing the surface of the gland to become negative to the hilus. In this case the submaxillary of the cat on chorda excitation, yielded a free secretion, but the outer surface became positive to the hilus. The usual effect in the cat is a diphasic variation of which the second phase is the larger; and the only explanation I can suggest, is, that in this case, this second phase being very large obscured the first phase. The converse of the above proposition is also true: that if on nerve excitation there is no actual secretion, the phase of the electrical variation indicating the surface of the gland to become negative to the hilus, is either absent or is extremely small, i.e. 10—20 divisions of the galvanometer scale, and it is replaced by a second phase of opposite sign. When an extremely small first phase is present, it is probable that there is a slight amount of secretion, but too little to be detected by the eye.

Apparently from the above facts, we may conclude that a flow of secretion is intimately connected with a certain definite electrical variation; and it remains to attempt to determine, whether the former is the cause of the latter, or whether they are both results of some other and antecedent process.

This first phase is probably due to one or more of the following three causes.

A. The actual movement of the fluid through the cells and ducts.

B. The change in the gland cells, which presumably precedes the passage of fluid through them.

C. The anabolic changes that are supposed by some observers to follow the excitation of secretory fibres.

With regard to these anabolic changes, it is undoubted that they follow the stimulation of certain of the gland nerves; and as mentioned above, Langley has seen a clear outer zone produced in the parotid by sympathetic stimulation, when this nerve caused an actual secretion from this gland; that is to say he has seen this zone produced in precisely those cases, where the excitation would have produced a variation consisting of a first phase. There is however a considerable mass of evidence tending to show, that the anabolic nerve fibres are distinct from the secretory fibres of Heidenhain. Further, there is another much more important fact bearing upon this question, and this is, that it is only after prolonged excitation of these gland nerves, that these anabolic changes occur, whereas the electrical phenomena are at their maximum, at the beginning of an experiment, and gradually diminish pari passu with the exhaustion of the gland from prolonged excitation. Again, anabolic changes presumably proceed much more slowly than such katabolic phenomena, as the actual secretion of water; and this first phase is very rapid in its course, its latent period being short. It is of course very difficult to demonstrate, that these anabolic changes are not the cause of the observed electrical phenomena, as we know so little of these anabolic changes; but the intimate manner in which these electrical changes are connected with the flow of liquid, and further the fact, that the anabolic nerve fibres are probably distinct from secretory nerve fibres, seems to point decidedly against the view that the anabolic changes are the cause of the first phase. Because, as will be mentioned below, the second phase is probably due to the katabolic changes leading to the elaboration of the constituents of the saliva, it by no means follows that the first phase, because it is of opposite sign, is due to anabolic changes. No doubt both the flow of liquid and the formation of mucin, etc. are to be classed as katabolic changes; yet it does not follow that if they are both accompanied by electrical phenomena, these should necessarily be of the same sign. Hence I think we may conclude that at present, there is not sufficient evidence to show that the first phase is due to these anabolic changes; whereas there is a very considerable mass of facts to show that it is connected with the flow of liquid.

Finally with regard to the question, as to whether it accompanies or precedes this flow, there is much the same uncertainty, owing to the difficulty of estimating the latent period of the actual secretion. However if the sub-maxillary duct is clamped, and the chorda excited, a large electrical effect is observed as usual, although the flow must be somewhat impeded; and on removing the clamp after the excitation has ceased, although the saliva flows freely, no electrical change is observed. Again by making simultaneous observations of the rapidity of the flow of the saliva, and of the rapidity of the galvanometer deflection, it is found that the maximum of the galvanometer deflection is reached before the maximum of the salivary flow. It is obvious that neither of the above methods is above criticism; but as far as they go, they serve to show that at any rate, the passage of the fluid through the ducts is not the cause of the electrical effects. Hence the first phase is due probably, either to the passage of the fluid part of the secretion through the walls of the alveoli, or to the changes in the gland structures, that follow the excitation of a secretory nerve and precede the actual flow.

The second phase remains to be accounted for. In the earlier part of this paper, it was shown that the vasomotor changes were in all probability not the cause of the second phase. This second phase, as will be remembered, is best seen in the sympathetic of the dog, and least well seen in the chorda of the dog. In this latter case, it is generally only seen after the first phase has been abolished by atropin. In the cat it is generally present, both in the case of the sub-maxillary and the parotid. This second phase varies with the viscidity of the saliva, just as the first phase does with the amount; this is particularly well seen in the submaxillary of the cat. It is more refractory to atropin than the first phase, requiring much the same doses to abolish it, as the so-called trophic fibres require to abolish their activity. It is the usual variation seen in the parotid of the dog on sympathetic excitation; so that from the above facts, we may conclude that it is due to the changes in the gland cells, leading to the elaboration of the organic constituents of the saliva.