Physiology. — *The electrogram of the submaxillar salivary gland.* By A. VAN HARREVELD. (Communicated by Prof. G. VAN RIJNBERK.)

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Since the investigations of BAYLISS and BRADFORD dating from about 1885 it is known that stimulation of the chorda tympani and of the sympathetic in the neck is accompanied by changes of potential in the submaxillar gland. Other investigators studied those action currents also, but they did not obtain corresponding results. The differences in the electric phenomena described are partly due to the use of different methods. It is important that some workers made use of instruments with a large inertia for the registration of the potential differences; BAYLISS and BRADFORD using a THOMSON galvanometer, CANNON and MC KEEN CATTEL and also GESELL using a galvanometer after the type of DEPREZ—D'ARSONVAL, while others, BECK and ZBYSZEWSKI, RABL and also GAYDA used the string-galvanometer.

In the experiments described below the action current of the gland was registered with the latter type of galvanometer. In each photogram the secretion was registered with a continuous method as well as the change of volume of the gland. BUNCH stated, that the change in volume of the submaxillar saliva gland after stimulation of the chorda is determined by two factors of opposite influence; the secretion causes the volume of the gland to decrease, and the vascular dilatation, also caused by the stimulation of the chorda tympani, results in an increase of volume. If the venous flow is slightly impaired the change in volume caused by the vascular dilatation should be decisive. In the experiments described below the venous flow certainly was not wholly free, since a hollow rubber cuff was placed round the gland with slight over-pressure. The changes in volume as registered, therefore will be chiefly caused by the changes in vascular lumen.

The action current of the submaxillar saliva gland in dog showed a multitude of forms. Not only that stimulation of chorda and sympathetic nerve yielded electrograms of different aspect, but stimulation of one of the nerves showed large individual variations.

Faradic stimulation of the chorda during about 10 seconds resulted after a latent period of an average of 0.3 sec. in electrical changes in the gland, which caused, at least shortly after starting of the stimulus, the surface to become negative in regard to the hilus. The electrogram usually showed several peaks in the first seconds, sometimes preceded by a small peak in opposite direction, the surface being temporarely positive in regard to the hilus. Often the galvanometer remained in rest during the later period of stimulation, sometimes the surface of the gland became more and more negative during further stimulation, and in other cases the variation in potential changed its direction, the surface of the gland turning positive. In the cases in which the string showed clear deflections in the later period of stimulation, the action current was followed by an after-effect.

The electrical phenomenon resulting from a 10 second faradic stimulation of the sympathetic nerve also showed individual variations. Usually after a latent period averaging 0.8 sec. the surface of the gland turned positive in regard to the hilus. Those changes in potential had a much slower course than those resulting from chorda stimulation. Usually after about 2 seconds the positivity of the surface reached a maximum, and decreased afterwards, sometimes even excessively, causing the string to cross the zero-line, and the surface becoming negative in regard to the hilus. Sometimes the positivity of the surface of the gland increased during the entire time of stimulation. In a few cases the surface immediately became negative. The latent period however, then was longer, and amounted to about 2 sec. No after-effect was ever seen when stimulation of the sympathetic was stopped.

Since the electrical resistance of a tissue is to a certain extent looked on as a measure of the permeability of the walls of the cells, this value becomes of special importance for a gland that absorbs substances during the process of secretion from the blood and also secretes the same, changed or unaltered. It must be born in mind that the ionic permeability only determines the resistance of an organ. BRONK and GESELL registered the changes in resistance with an inert galvanometer. As indicator current an alternating current of 1000 Herz was used. Stimulation of the chorda resulted in an increase in resistance after a latent period of 3 sec. Stimulation of the sympathetic also increased the resistance of the gland somewhat. PESERICO registering the changes in resistance with a string galvanometer, found an initial decrease in resistance stimulating the chorda, when using direct as well as alternating currents of 100.000 up till 1.000.000 Herz. Those changes occurred after a latent period of 0.25 sec. After this initial decrease, the resistance increased again, causing the string to cross the zero-line, and the resistance of the gland becoming larger than in rest. The same course of the changes in resistance during stimulation was seen by me. Stimulation of the sympathetic resulted after a latent period of several seconds in an immediate increase of resistance of the gland.

When the tracings from the action current and from the changes in resistance caused by stimulation of the chorda tympani are compared, a certain analogy in those electrograms after the third or fourth second of stimulation becomes evident. This analogy gave rise to the question: is it possible that a change in resistance in the gland manifests itself in the action current?

The system of gland and string may be compared with a source of current shunted through two circuits, one consisting in electrodes and galvanometer, the other in the tissue of the gland itself. In the gland a rest-current was seen of various intensity and index. If this change in potential be imagined to take place in a certain level of the gland, this level can be compared with a sieve. The holes in the sieve are represented by the saliva ducts, the substance by the total amount of cells. The substance of the sieve being the seat of the potential difference, this is short-circuited by a conductor consisting in the saliva ducts and partly in the glandular tissue. The resistances of electrodes and string do not change when the chorda is stimulated ; but the second shunt, consisting a.o. in the glandular cells does change. This change in resistance is even considerable ; the total resistance against direct currents being after PESERICO 600 Ohm, I noted in several animals during stimulation of the chorda an increase of 500 Ohm. Since the resistance of the shunt, which short-circuits the rest-potential, becomes about twice its value by stimulation of the chorda tympani, the E.M.F. in the place of the electrodes will increase, therefore changes in resistance will show as a change of the rest-current. Even after compensating the rest-potential, which certainly is less than the true difference in potential by which it is caused, the change in resistance will cause a deviation of the rest-current.

In the cases which showed a clear and constant rest-current this appeared to be analogous with the action current during the last period of stimulation. When no clear rest-potential was apparent, the string remained in rest during this period. Furthermore, if this opinion on this part of the electrogram be true, it must be possible to reproduce the various forms of action currents in the later period of stimulation by sending a constant current through the gland, that will be changed by the variations in resistance caused by chorda stimulation. This turned out to be the case. The variations in resistance being much less pronounced by stimulation of the sympathetic, no influence of change in resistance could be seen in this electrogram.

One of the most marked changes shown by the electrogram during chorda stimulation is caused by the injection of a certain dose of atropin. The discoverers of the gland action current, BAYLISS and BRADFORD, noticed already the change of direction of the current under the influence of this alcaloid. The electrograms of Fig. 1 and 2 represent the action

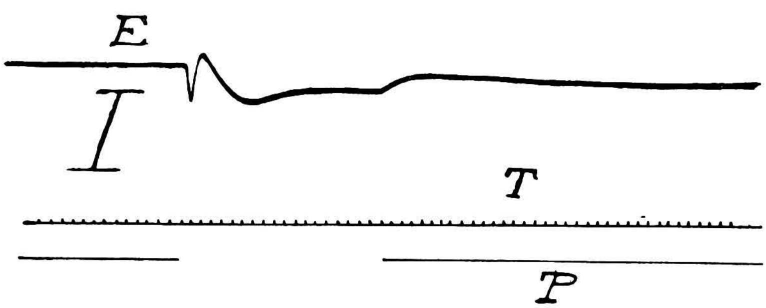

Fig. 1.

currents before and after intravenous injection of ¾ mgrs. of atropin sulfate. As is shown by the absence of deflection during the later period

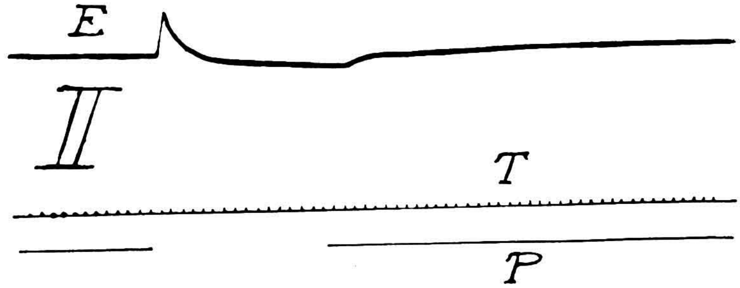

Fig. 2.

of stimulation, the change in resistance of the glandular tissue during chorda stimulation was not apparent in this electrogram. A comparison of both electrograms shows that the atropin has caused a change of direction of the action current and an increase of latent period from $^{1}/_{3}$ to ½ second. This change in the electrogram shows the probability of the action current being a compound of two electric phenomena of opposite direction. Usually the change in potential causing the surface of the gland to become negative should be the larger. The atropin causes the disappearance of the latter part of the action current thus enabling the current of opposite direction to become apparent.

We will call the part of the action current that causes the surface of the gland to become negative in regard to the hilus during chorda stimulation, the "negative" phase, and the part that causes the surface to become positive in regard to the hilus the "positive" phase.

The positive phase can directly be registered from the gland, after the disappearance of the negative phase by the use of a certain dose of atropin, but the aspect of the negative phase cannot be determined in this simple way. But if the conception of the electrogram during chorda stimulation as the compound of two electrical phenomena of opposite direction be true, it must be possible to get an impression of the negative phase by combining the electrogram before the injection of atropin and the negative of the electrogram after the injection. This construction is carried out in Fig. 3. The result is a line of simple form. The action current during stimulation of the chorda therefore appears to be composed of two electrical phenomena of simple

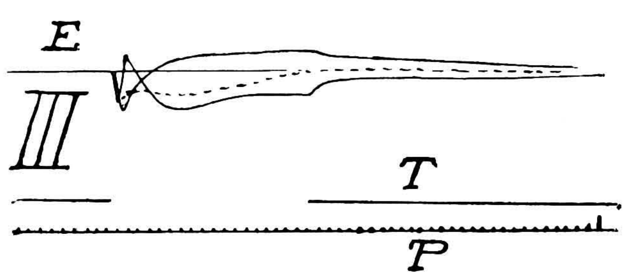

Fig. 3.

course and this can be regarded as an argument in favour of the hypothesis that the electrical disturbances are due to two single processes in the gland.

In most cases the latent period of the negative phase was shorter than the same of the positive phase, the initial deflection therefore showing negativety of the gland surface, but some cases occurred in which the latent period of the positive phase was the shorter. Repeated stimulation of the chorda in one and the same dog changed the latent period of negative as well as of positive phase. In one dog it was noticed that the latent period of the positive phase was originally the shorter, but increased during the experiment, the latent period of the negative phase on the other hand became shorter ; this resulted finally in a change of direction of the initial deflection of the electrogram.

The aspect of the change in resistance during chorda stimulation changed too, when a certain dose of atropine was injected. Comparing two resistance curves, one before, the other after the injection of $\frac{3}{4}$ mgrs. of atropin, it appears that the latent period of the change in resistance has changed under the influence of the alcaloid from $^{1}/_{3}$ to $\frac{1}{2}$ sec. In the curve of change in resistance obtained before the injection of atropin, the initial decrease of resistance is followed by a marked increase, but after injection this increase remains absent (Fig. 4 and 5). If the

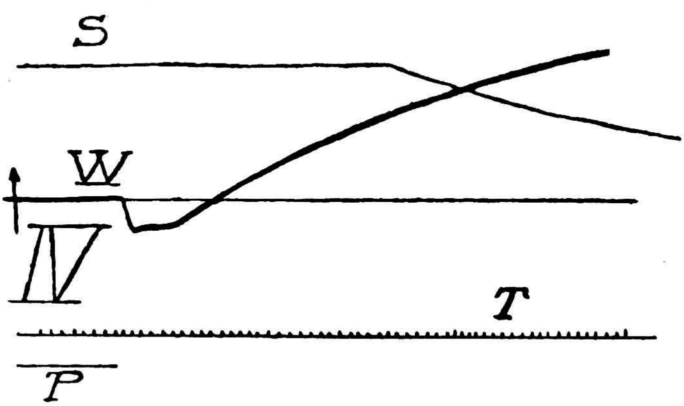

Fig. 4.

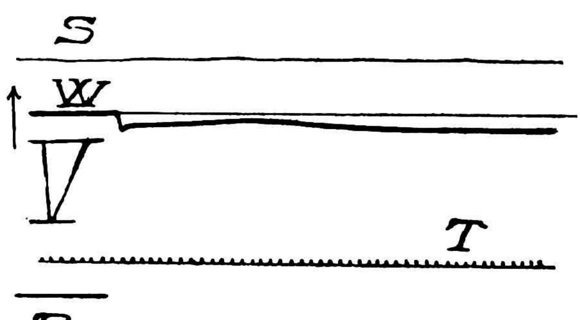

Fig. 5.

attempt is made to trace the parallel existing between action current and change in resistance still further, and to construct a compound curve of the change in resistance before the injection of atropin and its negative after the injection, in order to get an impression of the part destroyed by the alcaloid, a curve is obtained showing a course as irregular as shown by the resistance curve before atropin was administered (Fig. 6).

A priori it seems probable to suppose that, when an electrical phenomenon

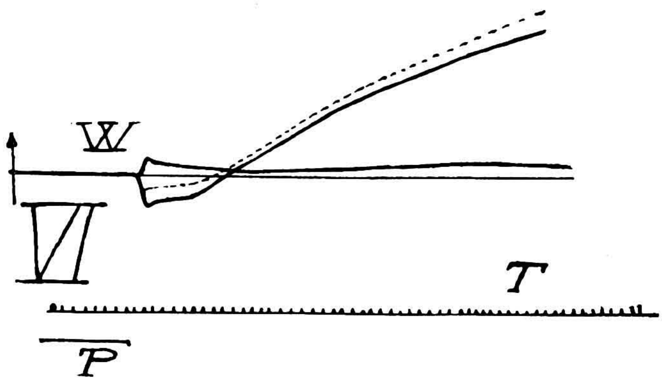

Fig. 6.

is caused by one single process, the curve representing this phenomenon will have a simple course. If this supposition be true, we must assume that the compound curve mentioned above representing the part of the change in resistance that can be destroyed by a certain dose of atropin, cannot be ascribed to a single process in the gland, or that the part of the change in resistance, remaining after atropin and which was supposed not to be altered by the alcaloid, has been altered by the influence of the atropin injected. It will be shown presently that the positive phase and the change in resistance remaining after the injection of atropin do agree in so many aspects, that it is probable to suppose both phenomena to be caused by the same process. Figures 1, 2, 4 and 5 show the effect of an equal dose of atropin on action current and change in resistance during chorda stimulation of the same gland. Since the compound curve of the action current before the injection of $\frac{3}{4}$ mgrs. atropin and the negative of the same after the injection appears as a line of simple form, the positive phase cannot have been altered greatly by the injection. Since, the positive phase and the part of the change in resistance remaining after injection of atropin are probably results of the same process, we must assume that the latter part of the change in resistance also is uninfluenced by the injection. We therefore reach the conclusion that the initial decrease in resistance of the non-poisoned gland is the sum of two decreases in resistance. The increase in latent period of resistance variation after injection of atropin supports this conclusion. In the curve of the action current shown in Fig. 1 we see $\frac{1}{2}$ sec. after the beginning of stimulation the influence of the positive phase becoming apparent, by a decrease of the negativity of the gland surface. This shows that also in a gland unaltered by atropin the positive phase has a latent period of $\frac{1}{2}$ sec. Now, since, as said above, the positive phase and the decrease in resistance remaining after injection of atropin both are supposed to be due to the same process in the gland, the decrease in resistance in the non-poisoned gland appearing after a latent period of $^1/_3$ sec. cannot be caused alone by the variation of resistance due to the same process as the positive phase. The initial decrease of resistance during chorda stimulation therefore must be caused by two decreases of resistance with a different latent period. The resistance variation during chorda stimulation which disappeared by injection of a certain dose of atropin consisted, as is shown in the compound curve Fig. 6, of an initial decrease of resistance followed by a larger or smaller increase. Since the increase was often very slight in the beginning of the experiment or could not be noted at all, and on the other hand attained often very high values after repeated stimulation, it is probable that those two kinds of variations are to a certain extent independent from each other. We therefore believe that the typical course of the resistance variation that disappears under the influence of a small dose of atropine is caused by a compound of two decreases in resistance reaching its maximum in a few seconds, and of an increase which reaches its apex much later, sometimes even after 40 sec. This latter variation was in most cases much larger than the former. This explains why during the first seconds of chorda stimulation the decrease dominates the increase which grows but slowly, during the latter seconds however the second variation being the larger.

The analysis of action current and change in resistance during chorda stimulation succeeded by reason of the different sensibility of its components for atropin, but the lack of such a substance made it impossible to do the same with the electrogram resulting from stimulation of the sympathetic. The diphasic form of the action current, that was commonest after stimulation of the sympathetic raised the question whether this phenomenon might be caused by several electrical changes in the gland, as is the case with the action current after chorda stimulation. If the monophasic action currents that appear after stimulation of the sympathetic but which are much rarer, can be looked on as consisting of one of the components of the diphasic current, it is possible to obtain the complicated electrogram by summating both the simple curves. The course of the initial deflection is determined by the electrogram with the shortest initial period, this being the change which turns the surface positive in regard to the hilus. In the diphasic electrogram during sympathetic stimulation usually two seconds after starting the stimulus the initial deflection decreases. Two seconds is also exactly the latent period of the monophasic electrogram which turns the surface negative in regard to the hilus. After this opinion the diphasic electrical phenomenon which is usually the result of stimulation of the sympathetic consists of two potential variations of different latent period and different direction. By some reason unknown to us, sometimes one of those phenomena may fail to appear, which results in the occurrence of the other two types of action current during sympathetic stimulation.

We failed to demonstrate relations between action current and vascular changes as a result from stimulation of the gland-nerves, as neither did BAYLISS and BRADFORD, CANNON and MC KEEN CATTEL. Neither a direct relation between secretion and action current could be demonstrated. We could confirm PESERICO's observation, that there exists no relation between variation in resistance and changes in vascular lumen during indirect stimulation of the gland. No relation could be shown to exist between secretion and change in resistance.

We spoke already of relation between electrogram and change in resistance of the gland. We ascertained that the electrogram registered without further precaution, must be looked on as the compound of the curves of action current and change in resistance in the gland.

It is also possible that for instance one of the resistance variations is related with one of the components of the action current, both electrical phenomena being due to one and the same process in the gland. If this possibility is investigated, it becomes apparent that in the dog which yielded the photograms shown in Figs. 1, 2, 4 and 5, the latent period of the negative phase amounted to about $^1/_3$ sec., and of the positive phase to $\frac{1}{2}$ sec., both decreases in resistance occurring also after $^1/_3$ resp. $\frac{1}{2}$ sec. Furthermore, after injection of atropin in a dose of $\frac{3}{4}$ mgrs. the negative phase disappeared as well as the decrease of resistance with the latent period of $^1/_3$ sec., the positive phase and the decrease in resistance with $\frac{1}{2}$ sec. latent period remaining. After injection of another dose of atropine the electrical phenomena last mentioned disappeared together. This makes it probable that action current and both the variations in resistance during chorda stimulation are caused by not more than two processes in the gland.

PESERICO found that the changes in resistance measured with alternating currents from 100.000 up till 1.000.000 Herz showed the same course, but were less intense than measured with direct current as an indicator. He therefore drew the conclusion that variations in the concentration of electrolytes were not the cause of those changes in resistance, but changes in the mobility of the ions. In each cell membranes are present which are but little permeable for ions. The passing of direct current should cause an accumulation of ions at both sides of such a membrane, representing a potential difference. The thousands of membranes between the electrodes together might be the cause of the potential difference that results in the discrepancy between direct and alternating current resistance as it is measured. From the variations in resistance of the submaxillar gland during stimulation of its nerves we must draw the conclusion, that a change in permeability of its cellmembranes has occurred.

In the arrangement of TARCHANOFF, for studying the psychogalvanic reflex, without an indicator current, an action current is demonstrated. In the arrangement of VERAGUTH in which an indicator current goes through the skin, a decrease in resistance occurs under the same circumstances, that give rise to an action current in TARCHANOFF's experiment. GILDEMEISTER demonstrated that this decrease in skin resistance must be regarded as an increase of permeability of the cells. PESERICO demonstrated the same to be true in the case of variations in resistance in the submaxillar gland. As in the skin a close relation must be assumed to exist between decreasing polarisation of certain parts of the cell and action current. It may even be supposed that the action current is the direct result of this change of polarisation of the gland cells.

EXPLANATION OF THE FIGURES.

Figs. 1, 2, 4 and 5 show the influence of a small dose of atropin on the action current and resistance variation in the same submaxillar saliva gland during chorda stimulation. Fig. 1 represents the electrogram before, Fig. 2 after injection of the alcaloid. Figs. 4 and 5 represent the resistance variation before and after atropin injection. Figs. 3 and 6 show the compound curves of electrical phenomenon resp. resistance variation before injection, and the negative of those values after injection.

The letters in the figures have the following meaning: The line indicated with "*E*" represents the action current (A deflection of the string under the zero-line represents an electric phenomenon which turns the surface of the gland negative in regard to the hilus) "*T*" is the time in seconds, "*P*" stimulation of the nerve (the interruption represents duration of stimulus) and finally "*W*" represents variations in resistance (if the galvanometer deflects in the direction of the arrow drawn through this line, the gland resistance increases).