ON THE ELECTRICAL PHENOMENA ACCOMPANYING SECRETION IN THE SKIN OF THE FROG.

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History.

Du Bois-Reymond[1] discovered the fundamental fact in 1857. The skin of the frog was found to be the seat of an electromotive force of such a direction that the outside of the skin was negative to the inside; this was shown to be due to the glandular stratum by scraping off the superficial part of the skin, upon which the current vanished. Previously to this, however, du Bois-Reymond[2] had described an experiment which he believed to demonstrate the negative variation of muscle when in a state of voluntary contraction, hence calling it "Willkür-versuch;" but Hermann has since proved the electrical changes manifested here to be due to the activity of the sweat glands. Du Bois-Reymond then was actually the first to detect a secretion current through mistaking its nature.

Currents similar to that described by du Bois-Reymond in the frog's skin were discovered by Rosenthal[3] in the stomach and intestines, by Engelmann[4] in the mucous membrane of the throat, by Hermann and Luchsinger[5] in the tongue, all however in the Frog.

The first investigation on the action of nerve excitation on the skin current was carried on by Roeber[6], under Rosenthal's direction, on the leg of the frog. He saw for the most part a negative variation, but

[1] *Moleschott's Unters.* 1857.
[2] *Unters. über thier. Elect.*
[3] *Arch. f. Anat. u. Phys.* 1865.
[4] *Centralblatt f. d. med. Wiss.* 1868.
[5] *Pflüger's Archiv*, XVII, 1878.
[6] *Archiv f. Anat. u. Phys.* 1869.

also occasionally a positive variation. Engelmann[1] saw on the same object a negative variation, but usually a strong positive after action. Hermann[2] made use at first of the skin of the back of the frog (prepared as described below), and saw almost exclusively a powerful positive variation; even in experiments on the leg he very frequently observed a pure positive variation; in many cases however it was preceded by a much smaller variation of opposite sign, i.e. a negative "Vorschlag;" and extremely rarely a pure negative variation was observed by him.

Hermann and Luchsinger[3] showed that the sweat glands of mammals exhibit a positive variation (the outside of the skin becoming more negative to the inside), and Luchsinger[4] demonstrated the same fact on the snout of the pig as well as on that of the goat, the dog and the cat. Hermann and Luchsinger[5] proved the existence of a secretion current in the tongue of the frog on excitation of the glosso-pharyngeal nerve, consisting of a long positive variation interrupted by a powerful but short negative variation, the complete variation being thus triphasic. In the same paper the authors mention that they attempted to detect the presence of a secretion current in the salivary glands of the dog, but were unsuccessful.

Method.

It is unnecessary to describe minutely our galvanometric circuit since it does not differ materially from that used in nearly all electro-physiological research. The galvanometer used was a Thomson Astatic of high resistance. The potentiometer on Latimer Clark's principle was used as described by Prof. Burdon-Sanderson[6]. Non-polarizable electrodes of the U-form recommended by Prof. Burdon-Sanderson were employed.

Current of Rest.

So far as this is concerned we can entirely confirm the results of previous observers, the outside of the skin being invariably negative to the inside, the amount of the potential difference varying considerably however. To save the continual repetition of a number of figures we

[1] *Pflüger's Archiv*, VI. 1872.
[2] *Pflüger's Archiv*, XVII. 1878.
[3] *Pflüger's Archiv*, XVII. 1878.
[4] *Tageblatt d. 52. deutsch. Naturforschervers. in Baden-Baden*, 1879.
[5] *Pflüger's Archiv*, XVIII. 1878.
[6] *Phil. Trans.* 1882. Part 1.

shall use as unit of electromotive force the fifth volt (volt × 10^{-5}); thus an E.M.F. of ·00024 volt we express as 24 fifth volts (*f.v.*). The E.M.F. of the skin of the frog taken from the middle of the back was usually about 8000 *f.v.*, at times as high as 25000 *f.v.*, and occasionally as low as 3000 *f.v.* It may thus amount to as much as a quarter of that of a Daniell's cell. Du Bois-Reymond[1] gives as the greatest E.M.F. he has observed, ·051 of a Daniell and ·004 as the lowest; these numbers converted into fifth volts become 5610 *f.v.*, and 440 *f.v.* respectively, considerably lower than our numbers. In his first publication on the subject Hermann was inclined to look upon the current of rest as a secretory effect, since he found the effect produced by nerve excitation to be an increase of the current of rest; but, as we shall see, the excitatory change in the skin of most parts of the body is more frequently negative than positive; this view cannot stand therefore, and Hermann[2] himself in a more recent paper attributes the current of rest wholly to the epidermis, that is, he looks upon the cells undergoing horny metamorphosis as being of the nature of dying protoplasm in relation to those of the Malpighian layer, and hence, according to his well-known "Alterations-Theorie," electrically negative to the latter. This view he supports by observations on the skin of fishes, where, in opposition to du Bois-Reymond, who found no current in the fish skin, Hermann asserts the existence of such a current, the outer surface of the skin being negative to the inner surface; the importance of this fact being, as we need hardly say, that there are no glands in the fish skin.

We have repeated Hermann's observations on the skin of the eel, which can be removed from the body without any muscular tissue adhering to it, and we can so far confirm his results that we find a distinct current of rest present and in the same direction as that of the frog but very much weaker. In one experiment the E.M.F. of a piece of skin taken from the dorsal region just behind the fins, one centimetre square, was 569 *f.v.*, while that of a piece of the same size from the back of a frog was 2021 *f.v.*, and this is an exceptionally low figure for the frog.

The opinion therefore to which we incline is that the current of rest, in the case of the frog's skin, as well as that of all animals with cutaneous glands of any kind, is partly due to the epidermis, but partly also to the glands themselves. This opinion is confirmed by the results

[1] *Gesam. Abhand.* II. p. 261.

[2] *Pflüger's Archiv*, XXVII. pp. 280—288.

of the consecutive action of corrosive sublimate and atropin on the frog's skin. It is stated by Hermann that immersion in a saturated solution of corrosive sublimate for 7″—8″ destroys the vitality of the epidermis, while leaving the glands intact, the latter fact being shown by the persistence of the excitatory change. In our experiments a pithed frog was immersed in a saturated solution of corrosive sublimate for 7″, washed, and then a piece of skin removed from the back and led off. In one case its E.M.F. was found to be 1032 *f.v.*, considerably diminished therefore by the action of the corrosive sublimate; a few drops of 1% solution of atropin sulphate were allowed to fall on the preparation, and after seven or eight minutes the E.M.F. had fallen to 344 *f.v.* The excitatory change was present before the action of atropin, absent afterwards. This experiment was repeated several times and always with similar results. We shall have to discuss the action of atropin later on, but in these experiments the excitatory variation was abolished as mentioned. Hence presumably the secretory activity of the gland cells was also abolished, although it may be that only a nervous end organ is attacked by the atropin; if so, however, it is not easy to see why atropin should diminish the E.M.F. of the skin.

Moreover some experiments performed by us in conjunction with Prof. Schäfer seem to demonstrate the fact that atropin acts directly upon the gland cells. These experiments were performed on the submaxillary gland of the dog, a canula being introduced into the duct and the chorda tympani nerve prepared in the usual manner; electrodes made of long slender needles, coated with shellac except at their points, were then introduced into the gland so as to stimulate it directly. Profuse secretion was produced both by stimulating the gland directly and by stimulating the chorda. After a small dose of atropin, i.e. 20—40 mgrms., not a trace of saliva could be caused to issue from the canula either by stimulating the chorda or the gland directly, although the strongest currents at our disposal were employed. We conclude then that atropin in all probability acts on the cells themselves[1].

It might be objected to our experiments on the frog's skin that the water in which the atropin was dissolved might have a deleterious effect on the gland cells, but apart from the fact that we used a 1% solution, which could not have much effect in causing a swelling up, or the reverse, of the tissue, being nearly of normal strength, we tested the

[1] Vide also *Brit. Med. Journal*, June, 1885.

effect of tap-water dropped on the preparation but could detect no diminution whatever in the excitatory effect.

As regards the Current of Rest in other Amphibia, one observation was made upon the green tree frog (Hyla arborea), and here the E.M.F. of the skin of back was found to be 11934 *f.v.* The E.M.F. obtained by leading off the lateral glands of the *Salamander* on one side and the hind leg (which had been amputated and replaced in its position) on the opposite side, was always very great, though of course how much of it was due to the glands it is impossible to say; it amounted to from 20000 *f.v.* to 30000 *f.v.* As will be seen below the excitatory variation in this creature is also very large.

Electrical Excitatory Change.

We have already had occasion to remark that Engelmann asserts that the electrical change occurring in the glands of the frog's skin, on excitation of the nerves supplying it, is of the nature of a negative variation of the current of rest; i.e. the mouths of the glands become less negative to the bases. Hermann on the contrary sees a positive variation either alone or accompanied by a small preceding negative variation, i.e. the so-called negative "Vorschlag." He admits however that he has very occasionally seen a pure negative variation. These contradictory results puzzled us at first, since it was difficult to understand how two such skilled investigators could have arrived at directly opposite results in so simple a matter; but in the course of our experiments we succeeded in clearing up the discrepancy, as will be afterwards seen.

In almost all our experiments the English frog, (Rana temporaria) was used, prepared however in two different ways, both due to Hermann. Hermann, however, used the German frog, i.e. Rana esculenta.

The first preparation consisted of the skin of the back prepared in the following manner. The frog is decapitated, and the spinal cord destroyed. Two parallel incisions are made with scissors just on the dorsal side of the lateral swellings, and a transverse cut joins these two at the posterior extremity. This flap of skin remains in connection with the vertebral column only by the posterior primary branches of the spinal nerves. The vertebral column is now removed from the rest of the body by similar incisions and then removed with the skin and attached nerves to the moist chamber, care being taken not to stretch or otherwise injure the nerves.

The piece of vertebral column is now supported on two wires bent at right angles in a horizontal plane so as to pass into the spinal canal for a short distance. These wires being insulated from one another and connected with the terminals of a du Bois induction apparatus, serve to excite the roots of the nerves. The piece of skin, attached only by its nerves, is carefully spread out, epidermis downwards, on a mound of china clay made into a paste with salt solution, and to this one non-polarizable electrode is connected, thus leading off the outer surface of the skin. The second non-polarizable electrode is arranged to lead off by a fine point the inner surface of the skin (which is uppermost) at any desired point. This preparation, for the sake of brevity, we call No. 1.

The second preparation is more easily made. The brain having been destroyed in a curarised frog (1·5 minims of 0·1 % curare being used) the sacral plexus is exposed from behind and divided on one side. The spinal cord is stimulated by two needle electrodes, one being inserted at each end of the spinal canal, and the two hind legs are led off. Since the skin of one leg only is excited by the above method, the electrode on that leg corresponds to that on the outside of the skin of the back in No. 1 preparation, and can be placed not only on the leg but on any part of the body out of reach of the spreading of the exciting current. This preparation we call No. 2, and it is extremely convenient for testing the effects of drugs, since the circulation remains intact in the skin under experiment. As above remarked, we used almost exclusively the common English frog, but from the results obtained by a few experiments on Rana esculenta we do not believe there is any essential difference between the species as regards the phenomena under consideration.

Our observations began in January, and for some time we obtained results both on preparations 1 and 2 confirming Hermann; but as the breeding season approached pure negative variations became more and more frequent, and during the latter part of February and the first three weeks or so of March it was extremely rare to find a positive variation either alone or with a negative "Vorschlag." After this period the positive variation began to reappear, gradually becoming more frequent, until ultimately it became the usual variation again. It is seen then that during the greater part of the year, Hermann's variation, as we may call it, is the rule, while in the months of February and March Engelmann's variation obtains.

By careful observation of the galvanometric deflections we have found that commonly during the last three months of the year, and not unfrequently in January, there is a third phase to the variation, which is of the same sign as the first, i.e. negative, and compared with the other two is very slow, which probably accounts for its having previously escaped detection. The amount of this third phase varies greatly, sometimes not exceeding that of the "Vorschlag;" i.e. about $\frac{1}{8}$ or $\frac{1}{10}$ of the positive phase, sometimes exceeding the latter. Its duration also seems to vary directly as its extent; for example, the positive phase usually lasts about 5′ or 6″, the third phase reaches its maximum when large at 11″—14″ after the commencement of stimulation, but the spot of light does not return to zero until two minutes have elapsed; when the excursion is small zero is reached after 30″—40″. The excitatory change, therefore, when complete consists of three phases, a small negative "Vorschlag" of about 10—20 scale divisions, a considerable positive phase of 300—400 scale divisions, and a final negative phase which varies immensely in amount.

What is very remarkable is that the relative amounts of these phases may vary within wide limits, and that any one or two phases may be present without the remaining phases or phase, so that it is scarcely possible to speak of a normal excitatory variation, although what has been said above may be regarded as obtaining in by far the greater number of cases. Under these circumstances, to give the E.M.F. of the phases of the variation would be of little value, apart from what has been remarked before, that the effective E.M.F. gives us no measure of that actually developed in the physiological process owing to the short circuiting which occurs; still the numbers in question are of value relatively to one another and as showing to some degree how high the E.M.F. of a certain physiological process may be. The first phase has an E.M.F. varying from 15 to 300 *f.v.*, the second and third may both exceed 2200 *f.v.*, i.e. ·022 volt.

The second phase may be indicated merely by a greater or less return of the spot of light, from its negative deflection, towards zero, or even only by a temporary stoppage or retardation of the onward negative movement.

When the variation is purely negative it is usually large, the spot of light being driven rapidly off the scale, and frequently the needle of the galvanometer comes in contact with the stop.

In the cases where a diphasic or a triphasic variation is present, the negative phase is more readily produced, i.e. when the coils of the induction apparatus are gradually approximated the negative phase appears first, and only under stronger excitation is the positive superadded.

The discrepancy between the results obtained by Hermann and Engelmann is thus satisfactorily cleared up, if it be the case that Engelmann experimented in the early spring months, and Hermann later on in the year. Photographs of the variation with the capillary electrometer have been obtained by us; they only show that the galvanometer gives correct indications of the relative strengths of the phases and does not distort the "Vorschlag" as it does in the case of *Dionaea.*

Reflex Excitation.

Contrary to expectation, we found it very difficult to obtain distinct effects on reflex excitation, and so our attention was not much directed to this part of the subject; one or two experiments however afforded rather interesting results.

The two legs of a frog were led off in the usual way, the sciatic nerve on the left side prepared in the thigh divided, the central end being stimulated a deflection of + 50 was observed, i.e. a small positive variation; on now exciting the cord in the usual way a deflection of − 350 was seen, i.e. a negative variation.

No result was obtained by dipping the toes into sulphuric acid 1°/₀. On another occasion a negative variation was obtained by stimulating electrically the toes of that leg of which the skin was being observed; the variation on cord stimulation was also negative.

Hypotheses.

It will make the subsequent portion of this paper more easily understood if we proceed now to discuss shortly the various hypotheses that have been suggested to account for the facts which we have mentioned above.

Hermann in his first paper suggests that the two directions of the secretory variation are due to the two kinds of glands, which are known to be present in the frog's skin—thanks to the investigations of Leydig, Eberth, and Engelmann; the positive variation having its origin in the small, regularly distributed, mucous glands, the negative variation in the larger, irregularly distributed, granular glands. In the middle portion of the skin of the back of R. esculenta the former are almost exclusively present, whilst in the other parts of the skin the latter are abundantly scattered. Since the secretion of the back is according to Hermann alkaline, while blue or neutral tinted litmus paper placed on other parts of the skin is found spotted with red, it is possible in Hermann's opinion that the direction of the excitatory change stands in intimate relation with the reaction of the secretion afforded by the particular gland in question. Thus glands giving an alkaline secretion would on this view also give a positive variation, those with an acid secretion a negative variation.

Further, it may be that the large acid glands have some connection with the breeding function, perhaps secreting some odoriferous body like the cutaneous glands of the salamander, and hence become more excitable as the breeding season approaches.

With regard to this suggestion of Hermann's we must remember, as Luchsinger[1] has pointed out, that it is not a question only of coarse electro-chemical action at the contact of acid and alkaline substances respectively, with the protoplasm of the cell, but rather that the processes taking place in the gland cell during the elaboration of the two kinds of secretion are chemically and physically of an opposite kind.

We have made a considerable number of experiments in order to discover, if possible, a relation between the reaction of the cutaneous secretion and the direction of the excitatory electrical change, but have been unable to detect any such relation. A powerful negative variation may be present with an alkaline or acid secretion; and similarly with the positive variation. Although it can hardly be said that these results overthrow the hypothesis in question, yet they do undoubtedly considerably weaken it.

In his later paper Hermann[2] has brought forward another hypothesis. Starting from the suppositions that the current of rest is due to the epidermis, and that during rest the glands give no electrical current owing to their closed spheroidal form, he thinks that, when on excitation of the nerve the muscular sheath contracts (as Engelmann has shown that it does), forcing the secretion out at the mouth, there is for a time a cell or cells at the fundus of the gland uncompensated by any opposing cells at the neck, and hence the E. M. F. of this cell or cells makes itself manifest externally. If now the E. M. F. of this cell is less than that of the epidermis, the E. M. F. of the current of rest will diminish, in other words suffer a negative variation, hence the negative "Vorschlag." As soon as the latent period of the gland cells is passed and they enter into secretory activity their E. M. F. will be greater than that of the epidermis, and hence the positive variation. It

[1] Hermann's *Handbuch*, Tom. v.

[2] *Pflüger's Archiv*, XXVII.

is quite conceivable that, owing to various causes, possibly fatigue, amongst others at present unknown, the gland cells may not be excitable and hence the negative variation alone may exist, as we have so frequently found.

At first sight this hypothesis appears somewhat artificial and improbable; it is supported, however, by the results of the action of corrosive sublimate and cauterization, to which we shall presently have to refer.

For our own part we feel neither in the position to accept one of these hypotheses nor to suggest another, the processes going on in secretion being of so complicated a nature that to do so would be premature. In all probability the electrical phenomena connected with osmosis have a share in the excitatory change. We do not think that the muscular sheath of the gland contributes to it, for, in the first place, Hermann has shown that totally tetanized muscle in the living subject is equipotential, and secondly our experiments on the salivary glands, as mentioned in our communication to the Physiological Society and described more fully in a recent communication to the Royal Society, have demonstrated that atropin in small doses totally abolishes the chorda variation in the submaxillary gland of the dog, although, as is well known, it leaves the vascular muscles intact.

Further, the lateral glands of the salamander give a very powerful excitatory change, whereas, according to Leydig[1], they possess no muscular sheath; we are inclined to doubt this statement of Leydig, however, owing to the marked and rapid manner in which the secretion is extruded on stimulation of the spinal cord, but have not yet been able to decide the question by microscopic examination.

Action of Corrosive Sublimate.

Hermann[2] states that immersion of the frog in saturated solution of corrosive sublimate for 6″—8″ destroys the vitality of the epidermis, abolishing thus the current of rest, while leaving the excitatory change intact, thus confirming his hypothesis as to the nature of the current of rest. We are able only partially to confirm this result; we find the current of rest greatly diminished, but not entirely annihilated, as already mentioned. But the most interesting fact about this action is the extraordinary effect of corrosive sublimate on the pure negative

[1] *Histologie Comparée.* Paris, 1866. [2] *Pflüger's Archiv*, XXVII.

variation, which, as we have shown, is the usual one at certain times of the year.

A No. 2 preparation, giving a pure negative variation, is immersed in saturated solution of corrosive sublimate for 7″ in such a way that the legs only are submerged, well washed under the tap, and replaced in the moist chamber exactly as before; it is now found to give invariably a positive variation; usually a pure positive one, but occasionally, possibly owing to greater thickness of the epidermis and hence a feebler action of the reagent on the deeper parts of the skin, there is more or less of the negative variation left, either preceding or following the positive phase. Moreover, if the preparation is giving a positive variation with a negative "Vorschlag," it increases the positive phase, frequently even causing the negative to disappear. These results we have obtained without a single exception; the effect is always to depress the negative phase and to exalt the positive.

Bach and Oehler[1] (pupils of Hermann) have obtained the same result by the action of heat; we can confirm this statement. Immersion in water at 40°C. for from 10″ to 30″ abolishes or greatly depresses the negative phase; and it is interesting to note that Bach and Oehler, more frequently than Hermann, obtained a pure negative variation, which they were thus able to convert into a pure positive or diphasic variation by the action of heat. They do indeed casually mention the action of corrosive sublimate, but appear not to have noted the action of which we speak, simply referring in an indefinite manner to its favouring the positive phase.

Now it is quite possible to account for these phenomena on either of the hypotheses suggested by Hermann, and we were at first inclined to favour the earlier one, having in mind Ringer's theory of the physiological action of acids and alkalies on secretion, i.e. that acids depress acid secretions and excite alkaline secretions, while the effect of alkalies is the reverse in both respects. We proceeded therefore to make various experiments to help in deciding this point. The solution of corrosive sublimate was found to be strongly acid and therefore would be expected to depress the acid glands, which according to Hermann's original hypothesis give the negative variation. Dilute sulphuric acid (1 °/₀ for 10″) produced the same effect, but we found that after carefully neutralizing the corrosive sublimate solution its power was in no way diminished, and a strong solution of zinc chloride

[1] *Pflüger's Archiv*, XXII.

had the same property. The sulphuric acid, however, is not so powerful in this respect as the neutralized corrosive sublimate, for we found it easy by longer or shorter immersion to diminish the negative phase to any extent we pleased. Of course it is quite possible to imagine that the acid glands are more sensitive to these reagents than the alkaline glands, and it may be connected with the fact that the former are more excitable, as mentioned above.

Repeated experiments, however, on the reaction of the cutaneous secretion before and after corrosive sublimate have shown that there is no constant relation whatever between the reaction of the secretion and the sign of the electrical change. Another circumstance which opposes the hypothesis is, that we have been totally unable to find any reagent which has the opposite effect to corrosive sublimate and acids, i.e. to depress the positive phase and excite the negative phase. The constant current (anode and cathode), sodium hydrate, sodium carbonate, camphor, and potassium hydrate have been applied to the skin externally, and lactic acid hypodermically injected, but all had only a depressing effect on both phases.

Hermann's later hypothesis accounts for the phenomena in the following way. It will be remembered that a pure negative variation is the result, according to this hypothesis, of a powerful epidermic current, the gland current even at its highest being inferior to that of the epidermis, and hence when the mouths of the glands are forced open a diminution in the current results. If then the epidermic current is depressed by the corrosive sublimate or acid, the positive phase will appear; and it is also clear why a positive phase once present cannot be replaced by a negative one, for to do so would require a reagent capable of increasing the epidermic current.

On the whole then this hypothesis seems the more acceptable one. For our part nevertheless we are loth to admit that so powerful a negative variation as we have very frequently observed, being far greater than any positive variation with the same strength of exciting current, is the result merely of the preponderance of the epidermic current over the glandular current.

Action of Atropin.

Hermann states that atropin abolishes the electrical excitatory change as well as the secretory effect; we have seen the former greatly diminished by large doses but not entirely abolished, except in No. 1

preparation, when dropping atropin solution on the skin put an end to the excitatory change. In any case, however, large doses are required to abolish the negative phase; the positive is comparatively easily annihilated, 5 mgms. sometimes doing so, while the negative phase remains large. These facts may be explained on Hermann's second hypothesis by bearing in mind, as does Hermann himself, the known paralysing effect of atropin on smooth muscular fibre when given in large doses. The positive phase, according to the hypothesis in question, being dependent on the activity of the gland cells is easily abolished, while the negative, which requires the agency of the muscular coat, will be only abolished by doses which paralyse the latter.

Other Amphibia.

It remains to shortly mention the results we have obtained in other Amphibia. We have obtained excitatory variations in the skin of the toad, newt, salamander and tree frog. In the first three the effect is usually considerable, though very slow in its time course and by no means so regular as in the frog; we have seen pure positive and pure negative variations as well as all kinds of combinations of these; on the whole the pure negative preponderates. The largest variation is given by the lateral glands of the salamander, and is usually a pure negative one.

The actions of corrosive sublimate and atropin appear to be analogous to their actions on the frog but not so constant or so powerful; and owing to the very slow time course of the variation in these creatures it is considerably more difficult to be certain what the effect is, since the current of rest is almost always rapidly varying, either increasing or decreasing; and when the variation happens to be in the same direction as the change of the current of rest, it is frequently impossible to detect it.

We have only made use of one tree frog and therefore cannot generalize in this case, but in this case the excitatory change was the same as in the ordinary frog.