PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Electromotive Properties of the Skin of the Common Eel

E. Waymouth Reid

Phil. Trans. R. Soc. Lond. B 1893 **184**, 335-365
doi: 10.1098/rstb.1893.0004

IV. *The Electromotive Properties of the Skin of the Common Eel.*

By E. Waymouth Reid, *Professor of Physiology in University College, Dundee, St. Andrew's University, N.B.*

Communicated by Professor M. Foster, *Sec.R.S.*

Received November 19,—Read December 15, 1892.

Hermann (1) has investigated the electromotive properties of the skins of Fish. His object in so doing was to attempt to determine, by the employment for experiment of a skin usually credited with being bereft of glands, whether the marked "current of rest" exhibited by the skins of Amphibia is with greater probability to be ascribed to glandular processes in accordance with the opinion of du Bois Reymond (2), or whether the phenomena are not explained with greater simplicity upon the principles of his own 'Alterations-Theorie.' Du Bois failed to obtain evidence of a "current of rest" in the four kinds of Fish with which he worked (Eel, Tench, Pike, and Perch), a fact, which taken in conjunction with the absence of "glands," satisfied him that in the richly glandular Amphibian skin the source of the E.M.F. must lie in the secreting structures. Hermann himself, previous to his examination of the skins of Fish, shared to some extent the opinion of du Bois, for in a paper published in 1878 (3), he inclines to the idea, that preparatory processes of glandular origin are the cause of the E.M.F. of the current of rest in the skin of the Frog, but also advances the supposition of a possible contribution from epithelial action at the surface. Finally, as is well known, Hermann demonstrated the presence of an ingoing current of rest in the skins of some ten genera of Fish, but found that its E.M.F. was far less than that exhibited by the Amphibian skin. After noting that substances, the application of which destroys the current of rest in Amphibian skin, cannot be traced microscopically beyond the upper layers of epidermic cells, and recalling the fact that an electromotive excitatory change was demonstrated by Bach and Oehler (4), in the skin of the Frog, after complete removal of the current of rest, by the action of corrosive sublimate applied to the outer surface, Hermann concludes that the E.M.F. of the current of rest, and that of the current of action are of different origin. In speaking of the origin of the current of rest, he makes the following statement, upon the strength of his demonstration of such a current in the "non-glandular" skins of Fish, "dass nicht, oder nicht in erster Linie, die Drüsen, sondern die Epithelschicht, der Sitz der elektromotorischen Haut-

wirkung ist." Finally he bases his explanation of the source of the E.M.F. of the current of rest of the skins of both Amphibians and Fish upon the axioms of the "Alterations-Theorie." According to this hypothesis, the processes of dying or excitation in the continuity of protoplasm cause the more altered parts to be negative electrically to the less altered, so HERMANN says "Nun haben wir aber zunächst in allen verhornenden Epithelgebilden eine dem Absterben völlig analoge Alteration, welche von aussen nach innen fortschreitet (und durch den Nachwuchs compensirt wird), nämlich die Verhornung." Thus in the case of the Amphibian the keratinized superficial cells of the epidermis are supposed to form a demarcation surface whose electrical sign is negative to that of the deeper less altered portions of the skin tissue.

Analogous to the "keratin-metamorphose" of protoplasm stands in this connection a "mucin-metamorphose," and HERMANN remarks "Am Aal und an der Trüsche kann man direct sehen, wie die äusseren Zellenden unter Mucinbildung zu Grunde gehen."

A protoplasmic alteration, then, on the down-grade side of cell life is the explanation offered of the source of the E.M.F. of the current of rest of the skin of Amphibian and Fish, and the case of the latter is taken to throw light upon the former, since the complication due to the presence of glands is absent.

The explanation for the case of the Fish (mucin-metamorphose) is further supported by a reference to ROSENTHAL'S (5) discovery of an ingoing current in the mucous surface of the stomach and gut of the Frog and Rabbit, and the statement that OEHLER has demonstrated a current with similar direction in the integument of the Snail.

Is it correct to consider the skins of Fish as being non-glandular? If the term "gland" be restricted to collections of secretory cells, in the form of definite organs, such as exist in Amphibian skins, then it is certain that, with the exception of the special case of the dermal glands of Myxinoids, "glands" are absent from the skins of Fish. But if one include under the designation of "gland" any cells capable of forming a secretion—calling such cells, as is usual, "unicellular glands"—then, on the other hand, it must be admitted that the skins of Fish are richly glandular. One has but to turn to the histological works of LEYDIG (6 and 7), F. E. SCHULZE (8), FŒTTINGER (9), LIST (10), and many others, in order to be convinced that unicellular secretory organs not only exist in the skins of Fish, but are in many cases of a high degree of complexity. In the case of the common Eel, ordinary goblet cells, and also the special "kolben" first described by KÖLLIKER (11) for the skins of *Myxine* and *Petromyzon*, compose more than half the structures of the epidermis; and, by comparison of the histology of resting and stimulated skins in this Fish, I have convinced myself that both these structures are engaged in the act of secretion of slime, the goblet cells supplying a mucinous matter, and the "kolben" passing into the fine threads that occur in the slime. The details of this process of secretion must be reserved, however, for another communication. The demonstration, therefore, of a

current of rest in the skins of Fishes, is not of necessity a proof of HERMANN'S explanation, seeing that active secretory processes are at work in such structures. Moreover, HERMANN'S statement that one "kann direct sehen wie die äusseren Zellenden unter Mucinbildung zu Grunde gehen" is not substantiated by microscopic examination of the skin of the Eel. In the metachromatic staining reaction of thionin, as worked out by HOYER (12) one has, I am convinced, a reliable microscopic test for the presence of mucin. If sections of Eel-skin, treated with corrosive sublimate, be stained with this substance, it is the goblet cells alone—and these occur in the deep layers of the epidermis as well as near the surface—that give the characteristic reddish-violet colour, the surface epidermic cells staining bright blue. I propose in the following pages to attempt to demonstrate that the current of rest in the case of the Eel is more probably associated with active preparatory processes in its glandular elements, as HERMANN originally thought to be the case for the Frog, than with any keratinous or mucinous demarcation surface in accordance with his later writing; at the same time it will be shown that both negative and positive variations of the current of rest can be elicited by suitable experimental treatment of the removed skin of the Eel.

The Eel has been chosen for experiment principally on account of the fact that it can be flayed without the removal of any of the subjacent muscular tissue, but also because it is easily obtained with little injury, and lives well in captivity.

METHOD.

Eels, caught in the River Tay without the use of a hook, were transferred as quickly as possible to the laboratory, and kept in running water. Death was effected by transfixion of the medulla, the utmost care being taken not to injure the surface of the skin. Pieces of skin of an area of from 9 to 16 square centims. were removed from the back and sides, and were pinned by means of hedgehog bristles upon a suitable cork frame.

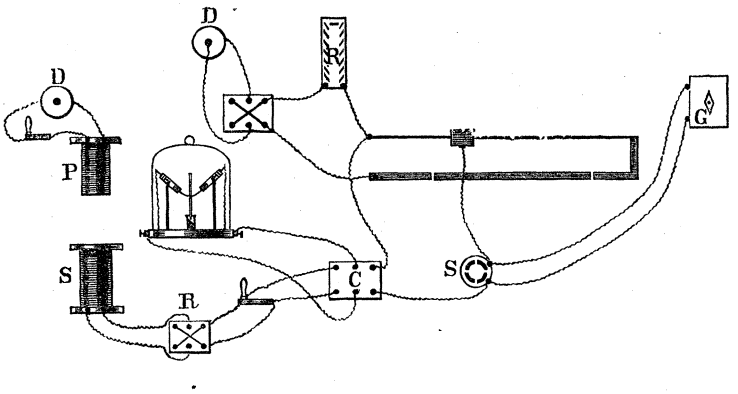
The supported piece of skin was fixed in the special moist chamber devised for the electrical examination of membranes by ENGELMANN (13), and led off by the ordinary non-polarisable electrodes, provided with crochet cotton contacts soaked in physiological salt solution. The galvanometer (ELLIOTT, 20,000 ohms R.) was arranged with the usual compensating circuit supplied with a Daniell cell of very low resistance.

The general plan of circuit is evident from the wood-cut, fig. 1.

By means of a paraffin switch, C, it was possible to stimulate the skin by the current of the secondary coil, S, through the electrodes, with the galvanometer circuit broken, and immediately, by turning over the key, to turn the skin current into the compensating circuit. The reverser, R′, was introduced for purposes of testing for possible polarisation of electrodes, but with the strengths of stimulating current employed this was not found to occur. The moist chamber was furthermore

provided with an inlet and outlet tube, so that gases or vapours could easily be introduced and subsequently removed without any disturbance of the electrical contacts.

Fig. 1.



The Electromotive Force and Direction of the "Current of Rest."

[Hermann quotes ·003 — ·007D for the value of the E.M.F. of the current of rest of the Eel's skin, while Bayliss and Bradford (14) give ·00569 volt for one case. In the course of these experiments I have observed variations from ·00072 — 00936D. As regards the direction of the current, both Hermann and Bayliss and Bradford found it to be ingoing. Though this is the rule, and is always the final result, yet it is not uncommon to find an outgoing current indicative of inner surface negative to outer immediately after setting up the preparation. With time the direction of this abnormal current reverses to the normal. Even if the current is in the normal direction at the first, its E.M.F. generally continues to rise for some time. The conditions that favour the presence of an abnormally directed current of rest are: (i.) the examination of pieces of exsected skin immediately after a process of capture in which the skin secretory mechanism has been greatly tried; (ii.) absence of special care in removal.

As regards the source of the E.M.F. of the normally directed current of rest, it is not in difference of reaction between the two surfaces, for the inner surface is normally more alkaline to litmus than the outer, a condition that would, *per se*, lead to an outgoing current. Moreover, I cannot see that mucin-metamorphosis can be adduced, seeing that microscopic observation shows no evidence of this in the surface scales of the epidermis.

It is more probable, as will be seen in the sequel, that the source of the E.M.F. of the current of rest lies in glandular preparatory processes occurring in the secreting elements of the epidermis.

The amount of the E.M.F. of the normal current is associated with the vigour of the animal—a fact noted for the Frog by du Bois, Budge (15), and Engelmann.

As regards the explanation of the abnormally directed current of rest observed

often immediately after putting up the preparation, and especially in Eels fatigued in the process of capture, it is possible that either or both of two causes may contribute to its production : (i.) injury to the inner surface during removal tending to produce an outgoing current ; (ii.) depression of epidermic activity from fatigue, or "shock" of removal, causing a temporary diminution in the E.M.F. of the normal ingoing current.

Two contacts upon the outer surface will often give evidence of difference of potential, but I have never read more than ·0012D, about one-fifth of the average E.M.F. between inner and outer surface. This difference between two points on the outer surface is to be attributed to an unequal distribution of secreting elements (especially the " kolben ") often observed in microscopic sections.

Mechanical stimulation in the region of one of the externally placed electrodes causes an increase of the negativity of that electrode (see p. 359), a fact much against the mucin-metamorphosis theory of origin of E.M.F.

The fact of proximity of an electrode to a gland mass favouring its negativity is demonstrable on the outer surface of the skin of the Toad, where with two thread electrodes it is found that there is greater difference of potential when one is on a "gland wart" and the other between two, than when both are over glands. Here, however, mechanical stimulation leads to diminution of negativity.

Two points on the inner surface of the skin of the Eel show no appreciable difference of potential.

I am of opinion, therefore, that it is probable that the source of the E.M.F. of the current of rest of the skin of the Eel is in the secretory activity of its unicellular glands.—March 2, 1893.]

Action of Carbonic Acid Gas.

If, as would appear to be probable, the E.M.F. of the current of rest of the skin of the Eel, is the result of secretory processes occurring in its glandular elements instead of a " mucin-metamorphose " of the ordinary epidermic cells, it should be possible by experimental treatment to obtain increments and decrements of the E.M.F. according as the active processes are stimulated or depressed. The first experiments in this direction were made with carbonic acid gas. Engelmann (13) obtained enormous falls of the E.M.F. of the skin of the Frog by the use of this reagent, and also saw that upon replacing the CO_2 by air the original level of potential was rapidly regained. He associated this fall of E.M.F. with the contraction of the skin glands, which he had previously noticed to occur when the skin or membrana nictitans was subjected to the action of this reagent. In the skin of the Eel there are no contractile elements similar to the muscle cells which Engelmann (17) describes as sheathing both varieties of skin glands in the Frog, so that it becomes a rather simpler matter to determine upon what elements of the skin a reagent affecting E.M.F. exerts its action.

The CO_2, prepared from marble by the action of HCl, was washed with water and saturated solution of sodic carbonate, and then passed as required into the gas chamber containing the skin in contact with its electrodes.

As has been already mentioned the outer surface of the skin of the Eel *immediately* after removal may be either negative or positive to the inner surface, though, if time be given, it is the rule that the condition of outer surface negative to inner is that which finally obtains. It is therefore necessary to observe the action of CO_2 upon the E.M.F. of the skin current in both of these conditions.

The following two experiments are illustrative. In Experiment A the gas was applied to a piece of skin in the normal condition, *i.e.*, with outer surface negative to inner, while in Experiment B a case was selected where, at the commencement, the outer surface was positive, though, as is the rule, it was gradually passing towards the normal state.

N.B.—In these and in all subsequent experiments—

A *north* galvanometer deflection (N.) signifies outer surface negative to inner;

A *south* galvanometer deflection (S.) signifies inner surface negative to outer.

Experiment A.

Freshly removed Eel's skin led off from inner and outer surfaces.

Outer surface negative to inner. Deflection of galvanometer N.

The numbers in the right hand column indicate compensator degrees of which 1 = ·000008 D.

11.25 A.M.	250 N.	
11.28	200 N.	
11.30	180 N.	
CO_2 introduced into gas chamber.		
11.30½ A.M.	0	
11.31½	120 S.	Reversal of current.
Air circulated and CO_2 expelled.		
11.34 A.M.	0	
11.39	90 N.	Return to normal direction of current.
11.44	130 N.	
11.49	100 N.	
11.58	70 N.	
CO_2 again introduced.		
12.0 noon.	70 S.	Reversal of current.
Air circulated and CO_2 expelled.		
12. 3 P.M.	90 N.	Return to normal direction of current.
12. 8	40 N.	
12.30	0	

Experiment B.

Freshly removed Eel's skin led off from inner and outer surfaces.
Outer surface positive to inner. Deflection of galvanometer S.
The numbers in the right-hand column have the same unit as in Experiment A.

2.47 P.M.	440 S.
2.51	350 S.
2.55	320 S.
2.56	CO_2 introduced into the gas chamber.
2.57	390 S.
2.58½	260 S.
3. 4	250 S.
Air circulated and CO_2 expelled.	
3.10 P.M.	120 S.
3.11	CO_2 again introduced.
3.13	150 S.
3.15	190 S.
Air circulated and CO_2 expelled.	
3.17 P.M.	120 S.
3.18	50 S.
3.20	25 N. Reversal.
3.25	CO_2 again introduced.
Second reversal occurred. Deflection now again S.	
3.26 P.M.	200 S.
Air circulated and CO_2 expelled.	
3.28 P.M.	150 S.
3.30	100 S.
3.32	50 S.
3.35	10 S.
3.40	100 S.
3.43	150 S.
3.46	170 S.
3.50	200 S.

With reference to Experiment A it will be noticed that the exposure of the skin with normal direction of current to the action of CO_2 leads to a diminution of the E.M.F., and finally to a temporary reversal of the direction of the current. A recovery occurs upon the admission of air, but it is not in this case complete, for, as will be seen by reference to the earlier stages of the experiment, a general fall of E.M.F. is taking place, hastened later on no doubt by the action of the gas. In

fact the precipitations of E.M.F. induced by the CO_2 are seen to be imposed upon a gentle curve of descent.

If, on the other hand, Experiment B be examined, a case where at the commencement the outer surface is positive to the inner, the following points will be evident. In the first place the negativity of the inner surface is diminishing, a fact that has already been alluded to in a previous section of this paper. In the second place the exposure of the skin to the action of the CO_2 leads, in contradistinction to the former normal case of Experiment A, to an augmentation of the E.M.F. of the current in contra-normal direction, but, as before, upon the admission of air the effect is cut out, and the ordinary diminution of negativity of the inner surface continues. In the third place it is seen that though the skin lay for 8 minutes in CO_2, at the first application, recovery from the effect was noticed within $2\frac{1}{2}$ minutes, *while the skin was still subject to the action of the gas*, and the effect was relatively a small one, while in the third application, though the action was only allowed to continue for 1 minute a far greater effect was produced, suggestive of a diminution of vital resistance, though the rapid admission of air excluded any observation as to whether recovery could have still occurred while the gas was still present. Finally it is evident that the repeated exposure to CO_2 has put an end to the general tendency to replacement of negativity of inner surface by negativity of outer, for the state of matters at the end of the experiment is the reverse of that at the beginning, viz., the negativity of the inner surface is now apparently increasing.

It has been suggested that in a piece of freshly removed skin one has two opposing sources of E.M.F. at the two surfaces, the one, due to injury of the inner surface, and tending to diminish with time, the other due to the activity of secretory protoplasm situated nearer the outer surface, finally also tending to diminish, yet capable of increase, either as a result of recovery from shock of removal or on account of excitation, as will be shown later.

Upon which of these sources does CO_2 exert its action, and of what nature is that action ?

It is not probable that CO_2 has any action upon the inner surface, for as far as is known CO_2 is without effect upon the electrical condition of connective tissue fibre. Du Bois (18) has shown that short exposure of muscle to CO_2 has no appreciable effect upon the demarcation current, an experiment that I have often repeated, leading off from the tendon and belly of the gastrocnemius of the Frog, with confirmatory result. In this case, if the current is not affected, there is action upon neither muscle nor tendon, the absence of action upon the former being probably associated with the fact that the muscle tissue is, so to speak, habituated to the presence of this gas. This is not, at any rate to like extent, the case with the protoplasmic structures at the outer surface of the skin, and it is reasonable to suppose that the action of CO_2 is almost exclusively upon this surface.

With the assumption that CO_2 acts principally, if not exclusively, upon the

structures of the epidermis, and that its action is, by lessening vital action, to cause a diminution of the E.M.F. dependent upon such action, the facts arising out of experiment became easily explicable.

In the normal case (Experiment A) the application of CO_2 causes, first a reduction of the E.M.F. of the outer surface to such a degree that it is balanced by that of the inner, so that there is no current, and by a continuance of its depressant action, allows the E.M.F. generated at the inner surface (probably as the result of injury during removal) to gain the upper hand, with the result that a current sets in in the reverse direction to normal. Aëration brings back activity to the protoplasm of the cells of the outer surface, and with renewed action the negativity of injury of the inner surface is again over-compensated, leading to a re-reversal of current back to normal.

Turning to the second case (Experiment B), where, whether from interference with the activity of the cells at the outer surface, or greater injury in removal to the inner, the inner surface is negative to the outer, the same explanation will hold good. The balance is here in favour of the E.M.F. of the inner surface, hence any reagent, such as CO_2, diminishing the activity of the protoplasm of cells at the outer surface that generate an opposing E.M.F., will lead to an increase in the original outgoing current, followed by a decrease when air is readmitted. In this case too a more permanent depression seems to have been produced by the action of the gas, for after the third admission of air, the general recovery that had been progressing throughout the experiment is no longer visible, but, on the other hand, has given place to a condition in which the E.M.F. of the inner surface is apparently gaining the upper hand. It is interesting to note that in the early part of the experiment the cells of the outer surface are far more resistant to the action of the CO_2 than later, for recovery occurs while the skin is actually in the gas.

The explanation offered then of the action of CO_2 upon the current of the skin of the Eel, is that by depressing the activity of protoplasmic structures in the epidermis (presumably the secretory cells) it lessens the E.M.F. constantly being generated therein by vital processes.

Action of the Vapour of Chloroform.

The effect of chloroform vapour upon the E.M.F. of the skin of the Frog was observed by Engelmann (13) to be very similar to that of CO_2. I have repeated Engelmann's experiments, and am able to confirm the results he obtained.

In the experiments with the skin of the Eel the vapour was introduced into the gas chamber containing the skin by means of gentle pressure upon a rubber ball connected with a large vessel containing a sponge soaked in the drug.

The effect of chloroform vapour upon the E.M.F. of the skin of the Eel is of the

same nature as that exerted by CO_2, but, as might be expected, the action of chloroform is the more powerful of the two.

If the outer surface of the skin is negative to the inner (normal condition), chloroform vapour reduces the E.M.F. of the ingoing current till the two surfaces are equipotential, and, if the action be allowed to continue, the ingoing is replaced by an outgoing current.

The replacement of the chloroform vapour by air causes a diminution of the outgoing current, and, if the action of the vapour has been short, the normal condition of an ingoing current is soon again attained.

On the other hand, should the inner surface of the skin be negative to the outer at the commencement of the experiment, then, as with CO_2, the immersion in chloroform vapour leads to an increase in the E.M.F. of the outgoing current.

The following experiment is illustrative of the action of chloroform upon the E.M.F. of a piece of skin with normal direction of current:—

Experiment C.

Freshly removed Eel's skin led off from inner and outer surfaces.

Outer surface negative to inner.—Deflection of galvanometer N.

The numbers in the right-hand column indicate compensator degrees, of which 1 = ·00008 D.

12.32 P.M.	100 N.
12.35	140 N.
12.38	150 N.
12.41	180 N.
12.44	200 N.
12.47	220 N.
12.49	Chloroform vapour passed into gas chamber.
12.50	50 N.
12.50½	Reversed current. Deflection S.
12.51½	170 S.
12.52	Air admitted.
12.53½	0 Skin surfaces equipotential.
12.54	Re-reversal. Deflection N.
12.56½	90 N.
12.58	110 N.
1.0	120 N.

It is to be noticed that, though the E.M.F. of the ingoing current is rising rapidly at the commencement of the experiment, the immersion of the skin in chloroform vapour causes a precipitate fall, leading finally to a complete reversal of current.

Recovery occurs upon the admission of air, but the E.M.F. of the outer surface never attains its original maximum, though the skin was only in the vapour for 3 minutes, and was perfectly fresh.

The rapidity of recovery upon removal of the vapour is as striking in this case as it is with CO_2. By prolonging the action of the vapour, however, the recovery is found to be slower, and may be delayed for some time after the admission of air.

On the other hand, if the action of the vapour is prolonged even further, recovery may actually occur while the skin is still subjected to it.

In the following experiment the skin was left in chloroform vapour for successive periods of 5, 10, and 30 minutes, and it will be seen that, though recovery occurs immediately upon admission of air in the first case, in the second case a period of about 3 minutes elapses, while, in the third case, after the maximum effect has been produced, a period, during which there is no change of potential, occurs, and, finally, recovery commences after some 12 minutes' immersion, while the skin is still subject to the action of the vapour.

Experiment D.

Freshly removed Eel's skin led off from inner and outer surfaces

Outer surface positive to inner. Deflection of galvanometer S.

The numbers in the right hand column indicate compensator degrees of which 1 = ·000008 D.

3.42 P.M.	500 S.	
3.45	150 S.	
3.46	80 S.	
3.49	200 N.	Reversal.
3.51	410 N.	
3.52½	450 N.	
3.54	420 N.	
3.55	Chloroform vapour applied.	
3.57	200 N.	
4.0	50 N.	
Air admitted.		
4.3 P.M.	140 N.	
4.6	130 N.	
4.9	80 N.	
4.10	Chloroform vapour applied.	
4.11	Faint N.	
4.14	50 S.	Reversal.
4.17	120 S.	
4.20	190 S.	
Air admitted.		

4.23 P.M.	220 S.
4.26	180 S.
4.29	150 S.
4·32	145 S.
4.33	Chloroform vapour applied.
4.35	150 S.
4.38	180 S.
4.41	230 S.
4.44	230 S.
4.47	220 S.
5.3	160 S.

If chloroform vapour be applied to a skin whose current is in the contra-normal direction, *i.e.*, outgoing, the effect is similar to that already seen in the case of CO_2. Under these conditions an increase in the outgoing current takes place attributable to a diminution in the E.M.F. of the opposing ingoing current. A case of this is seen in the commencement of Experiment O, p. 362.

If the vapour of chloroform be applied exclusively to the outer or inner surface of the skin, it then becomes evident that it is upon the former almost exclusively that its action is exerted. In the following experiment a stream of chloroform vapour was gently directed upon the surface of the skin in the region of the electrode. A blank experiment with electrodes only showed that there was no appreciable galvanometer deflection from cooling of electrodes.

Experiment E.

Freshly removed Eel's skin led off from inner and outer surfaces.

Outer surface negative to inner. Deflection of galvanometer N.

The numbers in the right hand column indicate compensator degrees, of which 1 = 000008 D.

2.18 P.M.	255 N.
2.20	265 N.
Chloroform vapour applied to *Inner* surface.	
2.21 P.M.	270 N.
2.22	270 N.
Chloroform vapour applied to *Outer* surface.	
22.3 P.M.	250 N.
22.4	270 N.
22.5	268 N.
Chloroform vapour applied to *Inner* surface.	
2.26 P.M.	270 N.
2.27	268 N.

Chloroform vapour applied to *Outer* surface.

2.28 P.M. 225 N.
2.29 270 N.

The results then of the action of chloroform vapour upon the electromotive phenomena of the current of rest of Eel's skin, seem to be capable of the same explanation as that offered in the instance of the action of CO_2, viz., that the reagent leads to a diminution of the activity of the processes going on in the secretory structures of the epidermis, with a concomitant fall in E.M.F.

It must here be mentioned that chloroform vapour may act in quite an opposite manner upon the skin of an *intact Eel* to that in which it works upon a piece of *removed skin*. A reflex copious secretory action occurs when a pithed Eel is subjected to the vapour, and the histological features of the skin of such an animal give clear indications of the process; these features are absent when the chloroform is applied to exsected skin.

It seemed of interest in connection with the action of chloroform vapour upon the skin to test the effect produced upon the demarcation current of muscle, for here, unlike the case of CO_2, which is hardly a foreign substance to muscle, one would expect to get a marked result.

The results lend some support perhaps to the theory of the action of chloroform upon the skin of the Eel just stated, for it is found that an increase in the positivity or decrease of the negativity of a contact upon the surface of a muscle occurs as the result of exposure to chloroform vapour.

Three cases are quoted below in illustration. In the first, a muscular surface made negative by section became positive when the muscle was surrounded with chloroform vapour. In the second, the contact on the belly of a removed muscle which was positive to that on the tendon, became more so from treatment with chloroform vapour, but recovered completely later. In the third case, an instance is taken where the belly of the muscle was weakly negative to the tendon, and in this case a reversal and development of a strong opposing current with belly positive to tendon resulted from the action of the vapour.

These results were obtained by the application of a vapour weak in chloroform. If, on the other hand strong vapour is pumped directly on to the muscle, the results are not constant, for injury currents from coagulation of the muscle substance are then originated.

(i.) *Gastrocnemius of Frog led off from artificial section and tendinous end.*

10.38 A.M. E.M.F. of Demarcation current ·03032 D. Cut end negative. Deflection N.

Chloroform vapour passed into the gas chamber.

10.39 A.M. *Current reversed. Cut end positive.* Deflection S.

Air circulated,

10.42 A.M. E.M.F. of reversed current ·0356 D.
10.55 ,, ,, ·0312 D.

(ii.) *Gastrocnemius of Frog led off from longitudinal surface and tendinous end.*

11.5 A.M. E.M.F. of Demarcation current ·0184 D. Muscle surface positive. Deflection S.
11.6 E.M.F. of Demarcation current ·0168 D.

Chloroform vapour passed into the gas chamber,

11.7 A.M. E.M.F. of Demarcation current ·0280 D. Deflection S.
11.8 ,, ,, ·0192 D.
11.9 ,, ,, ·0168 D.

(iii.) *Gastrocnemius of Frog led off from longitudinal surface and tendinous end.*

11.20 A.M. E.M.F. of Demarcation current ·004 D. Muscle surface negative. Deflection N.

Chloroform vapour passed into gas chamber,

11.21 A.M. *Current reversed. Muscle becomes positive.* Deflection S.
E.M.F. ·0368 D.
11.21½ ,, ·0312 D. Deflection S.
11.22 ,, ·0296 D. ,,
11.27 ,, ·0232 D. ,,

It is evident that in all the above cases the action of the chloroform vapour was to cause diminution of negativity or increase of positivity of the muscular substance, *i.e.*, the same effect as that produced upon the outer surface of the Eel's skin.

Excitation.

In the above pages, the experiments quoted have dealt solely with depressions of the value of the E.M.F., generated at the outer surface of the skin. It is now necessary to turn to the question whether it may not be possible to obtain increments of E.M.F. as a result of excitation.

Excitatory electrical variations in glandular structures have long been known. Valentin (19), in 1861, and Roeber (20), in 1869, demonstrated the fact for the skin of the Frog. Hermann and Luchsinger (21), in 1878, did the same in the case of the tongue glands of the Frog and the sweat glands of the paw of the Cat, Luchsinger (22), in 1880, in the glands of the snout of the Pig, Goat, and Cat, and Bayliss and Bradford (16), in 1887, in the salivary glands of the Cat and Dog.

The case of the skin of the Frog has been more frequently examined than the others, but the results have proved most conflicting at the hands of the several observers

The conditions here which appear to affect the nature of the electrical variation upon excitation of the cutaneous nerves are numerous. They are given as follows by the different authors. The magnitude of the original current of rest (Roeber, Hermann, Bach, and Oehler). The state of humidity of the surface of the skin (Roeber and Engelmann). The strength of the stimulus (Hermann, Engelmann, Bach, and Oehler, and Bayliss and Bradford). The temperature of the skin (Bach and Oehler). The time that has elapsed since the commencement of the experiment (Bach and Oehler). Finally, the condition of the animal, with special reference to that obtaining during the breeding season (Bayliss and Bradford).

On the whole, perhaps, the mass of the evidence seems to point in favour of a diphasic variation as a result of excitation of the skin of the Frog, the first phase being negative, and the second positive. Of these two phases, the second, or positive, appears to be the more marked, the first being often spoken of as a "Vorschlag," though under various conditions of experiment the reverse is the case, so that Bayliss and Bradford (14, p. 223) observe, "It is scarcely possible to speak of a normal excitatory variation." My own experiments upon the excitatory variation of the current of the Frog's skin are as yet too incomplete to justify any entrance upon a discussion of the nature of the variation in this paper; it appears, however, that the chief difficulty in predicting the variation which will follow a stimulus is associated with the great variability in the states of contraction and expansion of the skin glands, as demonstrated by Engelmann (17).

In the lingual glands of the Frog, Hermann and Luchsinger noted, upon stimulation of both the glosso-pharyngeal and hypoglossal nerves, a strong positive electrical variation of the rest current, interrupted by a negative phase so short in duration that the positive phase outlived it, and was still able to make itself subsequently evident.

In the Cat's paw, the same observers saw that a current directed from the outer to the inner surface of the skin (corresponding to a "positive variation") was developed, as a result of excitation of the sciatic nerve.

In the glands of the snout of the Pig, Goat, Dog, and Cat, Luchsinger also observed a positive variation of the current of rest.

The observations of Bayliss and Bradford upon the variations of the current of rest of the submaxillary glands of the Dog and Cat led them to the conclusion that "the sign of the electrical disturbance varies with the nature of the secretion, as measured by its amount and its viscidity," so that the excitation of a nerve trunk in which "secretory" fibres predominate leads to a positive variation of the rest current, whereas the stimulation of one containing an excess of "trophic" fibres leads to a negative variation.

In the glands, then, of the paw of the Cat and the snout of the Pig, Goat, Dog, and Cat, the excitatory variation observed has been purely positive, while in the Frog's tongue it has been mainly positive, but interrupted by an interpolated negative phase.

In the case of the skin of the Frog the evidence is not perfectly clear, but of the

two phases of the variation the positive appears to be the more marked. Finally, in the Mammalian salivary gland, there would appear to be a definite connection between the nature of the nerve fibres stimulated and that of the electrical change.

In experiments upon excitation of the skin of the Eel it is unfortunately impossible to make use of indirect stimulation by cutaneous nerves. This fact is occasioned by the absence of a large subcutaneous lymph space, such as exists in the case of the Frog and Toad, so that a "nerve-skin preparation" is not feasible. It is, therefore, necessary to resort to direct stimulation, which may be electrical, thermic, or mechanical.

In the circuit represented in fig. 1, p. 338, it is evident that, by means of the switch C, induction shocks can be applied to the skin through the leading-off electrodes, and the current of the skin subsequently passed into the previously compensated galvanometer circuit.

With carefully made "non-polarisable" electrodes, and by reversals of the direction of the stimulating current, it is possible to assure oneself of freedom from error due to polarisation.

Whatever method of stimulation is employed, it is found, firstly, that the skin of the Eel is excitable; and, secondly, that the electrical variation is one indicative of increase in the negativity of the outer surface, *i.e.*, a positive variation, if the resting current is in the normal direction.

Stimulation with Single Induction Shocks.

When a single induction shock is passed through the skin and the electrodes subsequently connected with the galvanometer, the following points are observed. Firstly, an electrical variation, indicative of increased negativity of the outer surface, occurs; secondly, the excitatory state rapidly reaches its maximum, falls quickly at first, and then very slowly. Thirdly, the amount of the primary variation is dependent upon the strength of the stimulus.

These points are illustrated by the following example:—

Experiment F.

Freshly removed Eel's skin, led off from outer and inner surfaces.

Outer surface negative.—North deflection of galvanometer.

Stimulation with break induction shock. Coil 15 centims. Positive excitatory variation. N. deflection.

Reversal of direction of stimulating current did not affect variation.

Make shock at 15 centims. coil: no effect.

Stimulation with coil at 10 centims.—Break shock: larger deflection than before. Make shock: no effect.

Coil at 5 centims.—Make shock: effective.

STIMULATION with Coil at 5 centims.

Make shock.		Break shock.	
Seconds after stimulus.	Galvanometer reading.	Seconds after stimulus.	Galvanometer reading.
	°		°
0	0	0	0
5	45	5	230
10	30	20	120
20	20	30	60
30	15	40	50
40	12	50	40
50	10	60	35
60	8	70	30
70	8	90	22
90	7	100	20
110	6	110	17
130	3	130	15
150	2	150	11
200	0	180	8
		230	5
		260	3
		300	0

The rapid arrival of the E.M.F. of the excitatory variation at its maximum is remarkable, and, as far as my own experience goes, considerably more marked than in the case of the Frog's skin directly excited. The slowness, however, of the subsequent decline agrees closely with the phenomena of the variation of the Frog's skin, as already noted by HERMANN and BAYLISS and BRADFORD. In the case of the submaxillary gland, BAYLISS and BRADFORD mention the occurrence of a quick maximum and slow decline of potential as a result of chorda stimulation.

As regards the length of the latent period, I have as yet been unable to make any exact measurements; all that can be here stated is that it is shortened by increasing the strength of the stimulus—a fact already noticed by ENGELMANN in the case of the skin of the Frog.

The relation, too, between the magnitude of the variation and the strength of the stimulus evident in Experiment F. (where the break shock gave a deflection of 230° and the make only 45° with coil at 5 centims., while at 10 the make shock was ineffectual), has been observed by both ROEBER and ENGELMANN in the case of the Frog's skin, the former using indirect stimulation by means of the cutaneous nerves, the latter stimulating directly through the leading-off electrodes.

Stimulation by Faradisation.

The excitatory effect following faradisation of the skin is, as would be expected, far more marked than that produced by the application of a single induction shock.

The increase of E.M.F. upon stimulation, as measured by the method I have used, is, of course, no criterion of that actually developed in the skin, on account of the short circuiting that occurs in the skin structures themselves, but the percentage value in terms of that of the current of rest is of interest.

Some observations of the increase of the E.M.F. upon faradic stimulation of the skin of the Eel will be found in Table I.

Table I.—Increase of E.M.F. of the Skin of the Eel as a Result of Faradic Excitation.

I.	II.	III.	IV.	V.	VI.
Number.	E.M.F. of current of rest.	Increase of E.M.F. following excitation.	Percentage increase of E.M.F. in terms of that of current of rest.	Stimulus.	Remarks.
1	·00128 D	·00032 D	25·0	Coil 15 for 5″	Same Eel.
2	·00320 D	·00032 D	10·0	,,	
3	00212 D	·00040 D	18·8	,,	
4	·00248 D	·00044 D	17·7	,,	
5	·00276 D	·00036 D	13·0	,,	
6	·00264 D	·00040 D	15·1	,,	Same Eel.
7	·00280 D	·00048 D	17·1	,,	
8	·00288 D	·00068 D	23·6	,,	
9	·00292 D	·00056 D	19·1	Coil 15 for 15″	
10	·00116 D	·00112 D	96·5	Coil 20 for 5″	
11	·00112 D	·00060 D	53·5	,,	Same Eel.
12	·00076 D	·00144 D	189·4	Coil 15 for 5″	
13	·00120 D	·00036 D	30·0	Coil 20 for 5″	Same Eel.
14	·00116 D	·00120 D	103·4	,,	

The method employed in obtaining these measurements, viz., compensation as rapidly as possible after stimulation, must give a value considerably below the maximum, for, as has been already noted, the rise of E.M.F. on stimulation is sudden and at first declines rather rapidly; but as will be seen in Case 12 the E.M.F. of the action current may almost double that of the current of rest; this is, however, very exceptional.

Engelmann observed a variation of 25 per cent. to 30 per cent. in the E.M.F. of the Frog's skin upon stimulation with a single induction shock and a variation of 40 per cent. to 50 per cent. with repeated stimuli; these variations were, however, of the nature of falls of the E.M.F. of the current of rest.

Bayliss and Bradford state that the E.M.F. of the positive phase of the variation of the Frog's skin current may exceed ·022 volt, but since they give no relative values for the E.M.F. of the current of rest this number is of little value, though taking ·1 volt as an average E.M.F. for the current of rest, an increase of 22 per cent. is a possibility.

In the case of injured muscle, Sanderson and Gotch (23) have demonstrated that the E.M.F. of the current of action may exceed considerably that of the demarcation current. In the gastrocnemius of vigorous Frogs they observed that the E.M.F. of the action current might be more than double that of the current of rest (·084 D as against ·04 D); in the sartorius, however, though the E.M.F. of the action current undoubtedly exceeds that of the demarcation current, the ratio is not so large as in the case of the gastrocnemius. These results, however, are not comparable with those stated in Table I., for not only were they gained with a single induction shock as stimulus, but the method of measurement was far more accurate than that which I have employed.

If stimulation be applied to a piece of Eel's skin, which is in the condition of outer surface positive to inner, the variation of the current of rest is negative instead of positive, for, as has been stated above, excitation always results in an increase in the negativity of the outer surface.

An instance of this is given in the following experiment.

Experiment G.

Freshly removed Eel's skin, led off from inner and outer surfaces.

Outer surface positive to inner. Deflection of galvanometer S.

The numbers in the right-hand column indicate compensator degrees, of which 1 = ·000008 D.

8.50 P.M.	350 S.
8.51	305 S.
8.52	280 S.
8.53	255 S.
8.57	195 S.
Stimulation, coil 15 for 5″.	N. variation, *i.e.*, negative.
8.58 P.M.	165 S.
9.0	135 S.
Stimulation, coil 15 for 5″.	N. variation, *i.e.*, negative.
9.1½ P.M.	95 S.
9.2	106 S.
9.3	95 S.
9.4½	85 S.
9.5	78 S.

Stimulation, coil 15 for 5″.*	N. variation, *i.e.*, negative.
9.6 P.M.	49 S.
9.7	55 S.
9.8	49 S.
9.9	35 S.

The reductions of E.M.F. which follow excitation in this experiment are obviously the result of the excitatory increase of the E.M.F. of the ingoing current, which at the time the experiment was commenced was below that of the outgoing current, though evidently gradually gaining the upper hand, as is the general rule.

When, on the other hand, the skin is in the normal condition, *i.e.*, with outer surface negative to inner, the variation upon faradisation is invariably, as far as I have seen, of a positive nature.

The following experiment is typical of the normal variation upon faradisation.

Experiment H.

Freshly removed Eel's skin led off from inner and outer surfaces.

Outer surface negative to inner. Deflection of galvanometer N.

The numbers in the right-hand column indicate compensator degrees, of which 1 = ·000008 D.

3.0 P.M.	145 N.
Stimulation, coil 20 for 15″.	N. variation, *i.e.*, positive.
3.1 P.M.	285 N.
3.4	195 N.
3.7	195 N.
3.10	185 N.
3.13	170 N.
3.16	140 N.
Stimulation, coil 20 for 5″.	N. variation, *i.e.*, positive.
3.17 P.M.	215 N.
3.17½	185 N.
3.20½	155 N.
3.23½	95 N.
Stimulation, coil 15 for 5″.*	N. variation, *i.e.*, positive.
3.24½ P.M.	275 N.
3.25	225 N.
3.28	210 N.
3.31	185 N.

* Indicates reversal of direction of stimulating current.

The quick rise of E.M.F. upon excitation and the slow return to the previous value are clearly seen in this experiment, and also the dependence of the magnitude of increase of the E.M.F. upon the strength of the stimulus.

Since ROEBER had noticed the occurrence of fatigue phenomena in the case of the excitatory variation of the skin of the Frog, I deemed it of interest to search for evidence of the fact in the case of the Eel. Pieces of skin, the E.M.F. of whose current of rest was increasing with time, were chosen for experiment, since, if any evidence of fatigue were forthcoming as a result of repeated excitation, it would obviously be more convincing in such instances.

The following experiment is an instance of an attempt to produce a condition of fatigue.

Experiment K.

Freshly removed Eel's skin led off from inner and outer surfaces.

Outer surface negative to inner. Deflection of galvanometer N.

The numbers in the right-hand column indicate compensator degrees of which 1 = ·000008 D.

1.35 P.M.	125 N.
1.37	135 N.
1.39	155 N.
1.41	180 N.
1.43	190 N.
1.45	200 N.
1.47	215 N.
1.49	225 N.
1.51	245 N.
1.53	255 N.
1.55	265 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
1.55½ P.M.	315 N.
1.57	295 N.
1.58	300 N.
1.59	295 N.
2.1	310 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.1½ P.M.	365 N.
2.3	350 N.
2.5	345 N.
2.7	345 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.7½ P.M.	390 N.

2.9 P.M.	375 N.
2.11	370 N.
Electrodes moistened.	
2.13 P.M.	330 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.13½ P.M.	380 N.
2.14	360 N.
2.15	350 N.
2.17	350 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.17½ P.M.	410 N.
2.19	375 N.
2.21	360 N.
2.23	360 N
2.25	360 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.25½ P.M.	445 N.
2.26	405 N.
2.27	375 N.
2.29	370 N.
2.31	370 N.
2.32	365 N.
2.35	365 N.
2.37	365 N.
Stimulation, coil 15 for 15″.	N variation, *i.e.*, positive.
2.37½ P.M.	435 N.
2.39	410 N.
2.41	400 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.41½ P.M.	435 N.
2.43	410 N.
2.45	405 N.
Stimulation, coil 15 for 5″.	N variation, *i.e.*, positive.
2.45½ P.M.	445 N.
2.47	425 N.
2.49	415 N.
Stimulation, coil 15 for 60″.	N variation, *i.e.*, positive.
2.51 P.M.	470 N.
2.53	440 N.
Stimulation, coil 15 for 60″.	N variation, *i.e.*, positive.
2.54½ P.M.	490 N.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2.55 P.M. 475 N.
Coil now put to 0, and stimulation continued for 60″.
E.M.F. of current of rest 500 N.
Stimulation, coil 0 for 10″. N variation, *i.e.*, positive.
½ minute later 510 N.

It is noticeable in this experiment that in spite of the repeated stimulation, the E.M.F. of the current of rest continues to rise, and during the early period of the experiment, at any rate, there is no evidence of any diminution in the amount of the positive response to excitation. The amount of the variation is a little reduced in the case of the eighth and ninth excitations, in spite of the longer period of stimulation; but it is not till the skin has been subjected to stimulation for a period of sixty seconds, with the coil at zero, that any marked diminution in the excitatory variation becomes evident, though even then no fall in the E.M.F. of the current of rest occurs.

It would appear then, that the skin of the Eel is remarkably resistant as regards fatigue.

Whether the continuation in the rise of E.M.F. throughout this experiment is in part due to some beneficial effect of the stimulus, somewhat of the nature of the rise in the E.M.F. of the rest current of the submaxillary gland noted by BAYLISS and BRADFORD after stimulation of the sympathetic, it is difficult to decide, for in this case the E.M.F. of the current of rest was rising before the stimulation was commenced. I have attempted to convert a case of falling E.M.F. of current of rest into one of rising E.M.F., by repeated excitation, but so far without success. Any attempt to explain the continuation of increase of the negativity of the outer surface upon a hypothesis of increase in alkalinity as a result of excitation is negatived by the fact that long faradisation of pieces of skin leads to the opposite effect, *i.e.*, diminution of alkalinity of the outer surface.

THERMIC STIMULATION.

ENGELMANN obtained a negative electrical variation upon the application of heat to the skin of the Frog, using a platinum wire traversed by the current from two GROVE's cells. This is, as far as I am aware, the only case recorded of the effect of heat upon the electrical phenomena of a glandular structure.

In the case of the skin of the Eel, I applied heat by bringing a small heated copper spatula into the neighbourhood of the skin. Such a position of the electrodes was always selected that the thermo-electric variation due to heating of the electrode nearer the spatula was against any electrical variation arising in the skin itself.

I found, as in the case of electrical excitation, a pure positive variation indicative of increase in the negativity of the outer surface, in all cases where the outer surface of the skin was heated above the temperature of the inner.

The following experiment is given in illustration.

Experiment L.

Electrodes placed in contact with one another lie east and west.
Heating *west* electrode. S deflection of galvanometer.
,, *east* ,, N ,, ,,
Freshly removed Eel's skin now led off by the electrodes.
West electrode in contact with *outer* surface.
West electrode negative. *Deflection N*, E.M.F. ·00128 D.
Heated spatula held 3 to 4 centims. from *outer* surface.
N deflection, 20° of scale.
Return to zero in 2 minutes.
Heated spatula held near *inner* surface.
Faint N deflection (thermo-electric current from east electrode ?).
Repeated many times with similar result.

The positive excitatory variation (N deflection) in the case of heating of the outer surface, was evidently sufficiently strong to swamp any thermo-electric current (S deflection) due to heating of the electrode in contact with the skin.

Mechanical Stimulation.

Engelmann has noticed that the current of rest of the skin of the Frog is subject to a negative variation upon mechanical stimulation, and I have already mentioned that the same holds good for the skin of the Toad. Indeed, the skin of the Toad is even more sensitive to this kind of stimulus than that of the Frog, the slightest pressure with a glass pointer, and without any possibility of shifting of the thread electrodes, leading to a diminution of the negativity of the electrode in the neighbourhood of the stimulated spot.

Two electrodes on the outer surface of the skin of a Toad spread on glass.

	Electrode A.	Electrode B.
First reading	+	−
1′ later, spontaneous change to	−	+
Fall of potential, marked by irregularities, to	0	
4″ later, reversal to	+	−
Pressure near B change to	−	+
,, ,, A ,, back to.	+	−

The above is an extreme case, and is not only noticeable for the complete reversal

of sign upon irritation, but also for the spontaneous reversal and re-reversal at the commencement of the experiment. With the glands of the "ear swellings" I have obtained similar results.

With the skin of the Eel, I have exclusively obtained a variation indicative of increased negativity of the spot excited upon mechanical stimulation. It is, of course, impossible to apply mechanical stimulation with safety, when the skin is on the cork frame and led off from the inner and outer surfaces, on account of the danger of shifting the electrodes. It is necessary to spread the skin upon a glass plate with the outer surface uppermost, and then stimulate by pressure with a fine glass pointer with knobbed extremity in proximity to one electrode.

The following experiment is an instance of mechanical excitation :—

Experiment M.

Freshly removed Eel's skin led from two points on the outer surface. E.M.F. of current of rest ·000768 D. Deflection S.

Electrode A.	Electrode B.
+	−

Slight pressure near B. S deflection of 23°, *i.e.*, increased negativity of B.
Return to zero not complete.
Rest current recompensated.
Slight pressure near A. N deflection 20°, *i.e.*, increased negativity of A.

The variation, therefore, upon mechanical stimulation corresponds to that obtained when excitation is electric or thermic.

Any discussion as to the reason of the opposite nature of the variations in the Batrachian skin on the one hand, and in the Fish skin on the other, in the cases of both direct electrical and mechanical excitation, is out of place in this paper; it must, however, be here noted, that though the excitatory variation in the skin of the Eel is invariably such as to indicate increased negativity of the outer surface, pure and simple, *i.e.*, a positive variation with normal direction of the current of rest, the variation upon direct excitation of the skin of the Frog and Toad is often not purely negative, but presents a second positive phase, preceded by a negative "Vorschlag" similar to that obtained upon nerve excitation, and already noted by Hermann, Bach and Oehler, and Bayliss and Bradford.

Action of Atropine.

The Eel appears to be extraordinarily resistant to intoxication with atropine. It is, of course, impossible to employ subcutaneous injection, for the animal cannot be handled for the purpose if regard is to be had for the integrity of the epidermis.

Atropinisation has therefore to be effected by adding the drug to the water in which the animal is placed. TIEGEL (24) has used this method with success for the purpose of intoxication of Eels with strychnia, obtaining evidence of absorption of the drug in one and a-half hours when six milligrammes of strychnia were added to the litre and a-half of water in which the Fish was swimming. I have kept Eels for two days in ·15 per cent. solution of atropine sulphate aërated by a Bunsen pump, with apparently little effect as regards general vital phenomena. The animals were lively at the end of this period, and though the pupil was slightly dilated it reacted to light, yet chemical tests applied to proteid free watery extracts of the livers showed that atropine had been absorbed.

The removed skins of such atropinised Eels are found to give either a very feeble ingoing current of rest or outgoing currents which do not reverse with time. This fact is perhaps best explained upon the hypothesis that atropine diminishes the activity of the glandular preparatory processes, and is of interest in connection with the statement by BAYLISS and BRADFORD that the E.M.F. of the current of rest of the skin of the Frog is reduced by treatment with atropine.

When the skin of an atropinised Eel is subjected to stimulation by means of the break induction shock, it is found that the normal excitatory variation can no longer be obtained, provided the atropinisation is sufficient.

The following experiment is illustrative of this fact.

Experiment N.

Eel allowed to live in ·15 per cent. solution of atropine sulphate for 48 hours. Pupil slightly dilated but reacted to light. Skin removed immediately after pithing and led off from outer and inner surfaces. *Outer Surface negative to inner.* Deflection N. The figures in the right hand column indicate compensator degrees of which 1 = ·000008 D.

5.10	130 N.
5.13	113 N.
5.15	85 N.
5.16	Stimulation, break induction shock, coil 15. No effect.
5.17	75 N.
5.18	60 N.
5.19	Stimulation, break induction shock, coil 10. No effect.
5.20	60 N.
5.21	Stimulation, break induction shock, coil 5. No effect.
5.24	35 N.
5.25	20 N.
5.26	Stimulation, break induction shock, coil 0. No effect.
5.42	Current reversed. Outer surface +. S. deflection. 55.
	Stimulation, break induction shock, coil 0. No effect.

Liver and skin extracted with water for 17 hours at 40° C. Extract freed from proteid by saturation with ammonium sulphate, evaporated with HNO_3 to dryness and alcoholic soda added, reddish-violet reaction, presence of atropine.

The abolition of the excitatory variation by atropine lends considerable support to the glandular hypothesis of origin of its E.M.F.

Exclusion of the Excitatory Variation by Narcotisation.

It has been seen that by means of CO_2 and also by the vapour of chloroform, the negativity of the outer surface of the skin of the Eel may be diminished; while, by electrical, thermic, and mechanical stimulation it may be increased, so that, according to the sign of the outer surface of the skin at the moment that it is the subject of experiment, a variation is induced which, in the normal state of outer surface negative to inner, is negative with the depressants and positive with excitation; while, should the inner surface be negative to the outer, the reverse is the case.

Thus :—

	Sign of variation.		
	CO_2.	Chloroform.	Excitation.
(*a*.) Outer surface of skin negative to inner surface (normal) . . .	−	−	+
(*b*.) Outer surface of skin positive to inner surface	+	+	−

The fact that in skin with normal direction of rest current, the E.M.F. may be brought down to zero by chloroform, and by continued action a reversal of current occasioned, so that the inner surface is negative to the outer, but that recovery to the normal is possible upon readmission of air (*vide* Experiment C, and Experiment O) is very strongly in favour of the source of the E.M.F. of the normal current of rest being in some vital processes going on near the outer surface whose activity can be temporarily reduced by narcotisation.

That it is one and the same set of vital processes that is acted upon, in the direction of augmentation by excitation, and diminution by the presence of a narcotic, is shown by the interesting fact that it is possible to narcotise the skin with chloroform vapour, with accompanying diminution in the negativity of the outer surface, to such a degree that it absolutely fails to give a positive response to stimulation by electricity during the continuance of the narcotisation, though removal of the vapour allows of complete recovery.

The following experiment is illustrative of this point in particular :—

Experiment O.

Freshly removed Eel's skin led off from inner and outer surfaces.

Outer surface positive to inner.—Deflection of galvanometer S.

The figures in the right-hand column indicate compensator degrees, of which 1 = ·000008 D.

3.47 P.M.	415 S.
3.49	295 S.
3.51	195 S.
3.52	175 S.

Stimulation, coil 15 for 5″ N deflection, *i.e.*, negative variation.

3.53 P.M.	125 S.
3.55	85 S.
3.56	75 S.

Chloroform vapour passed into gas chamber.

3.57 P.M.	205 S.

Air admitted.

3.58 P.M.	150 N, *i.e.*, reversal to outer surface negative.
4.1	130 N.

Chloroform vapour passed into gas chamber.

Reversal so that outer surface now again positive.

4.3 P.M.	75 S.

Air admitted.

Reversal again to condition of outer surface negative.

4.5 P.M.	195 N.
4.7	170 N.

Stimulation, coil 15 for 5″. N deflection, *i.e.*, positive variation.

4.7½ P.M.	215 N.
4.9	185 N.

Chloroform vapour passed into gas chamber.

4.11 P.M.	15 N.

Stimulation, coil 15 for 5″ while in chloroform vapour. No effect.

Air admitted.

4.13 P.M.	35 N.
4.17	95 N.
4.19	225 N.
4.24	255 N.

CO_2 passed into gas chamber.

Reversal to outer surface positive.

4.26 P.M.	40 S.

Stimulation, coil 15 for 5″ in atmosphere of CO_2. N deflection.

4.26½ P.M.	25 S.
Air admitted.	
Reversal to outer surface negative.	
4.30 P.M.	65 N.
4.32	155 N.
4.35	185 N.

The early part of this experiment illustrates points already referred to, viz., the action of electrical stimulation and of chloroform vapour upon the skin in the condition of outer surface positive to inner. The main point of interest is seen between the times of 4.7 and 4.24. It will be noted that the same stimulus which at 4.7 liberates a positive variation of the rest current while the skin is in the normal condition, at 4.11, when the skin is under the influence of chloroform vapour, *is unable to produce any effect.* This is not due to any permanent depression of vitality, for it will be noticed that by 4.24 the E.M.F. of the current of rest is considerably above the level at which it stood at 4.7. It is also to be noted that with CO_2 the same degree of narcotisation is not produced, for, in this case (4.26), the skin is still able to respond to the stimulus, though not to such good effect as before.

Conclusion.

In the foregoing pages it has been demonstrated that an electromotive force is developed as a result of excitation of the skin of the Eel, which manifests itself as an increase of the negativity of the normally negative outer surface. Is this E.M.F. to be rightly considered as due to an increase in the activity of the glandular preparatory processes, to whose charge that of the normal rest current has been laid, or are the E.M.F. of the current of rest and that of the current of action of different origin?

Hermann, in his explanation of the negative "Vorschlag" and positive after variation of the Frog skin current as a result of indirect excitation, has assumed, mainly upon the ground of his results with the Fish skin, that the E.M.F. of the current of rest is mainly epidermic in origin, while that of the action current is glandular.

It has been seen that an epidermic origin for the E.M.F. of the rest current of the Eel's skin upon the theory of mucin-metamorphosis will not hold good, and that it is far more probable that the explanation that the source of the E.M.F. of the current of rest is in the preparatory processes going on in glandular structures, is the correct one. If such be the case, the most simple explanation of the origin of the E.M.F. of the current of action in the Eel's skin is that it is simply due to an increase in the activity of processes whose molecular changes give rise to that of the current of rest. Most striking support is given to this explanation by the fact, evident in Experi-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ment O, that, when the E.M.F. of the rest current is reduced nearly to zero by the action of chloroform vapour, the normal excitatory development of E.M.F. is absent.

It remains now to recapitulate a few of the more important points with reference to the electromotive properties of the skin of the Eel dealt with in this paper:

1. The assumption that the E.M.F. of the current of rest of the skin of the Fish is entirely due to epidermic mucin-metamorphosis, and that it is not possible to attribute it to the presence of glandular elements, is negatived, in the case of the Eel, by the absence of any such mucinous change in the superficial epidermic cells, and by the presence of abundance of secretory cells throughout the structure.

2. The existence of considerable differences of potential between two contacts upon the outer surface of the skin, and the fact that such electromotive force is capable of excitatory increase upon mechanical stimulation, coincides with the assumption that the E.M.F. of the current of rest is the outcome of glandular processes of variable activity, and is not compatible with the theory of origin of the E.M.F. in mucin-metamorphosis.

3. The reductions in the E.M.F. of the normal rest current following exposure of the skin to carbonic acid gas and to the vapour of chloroform, and the subsequent recovery upon admission of air, are strong evidence that the origin of the E.M.F. is in some active vital processes taking place in the skin, and it is reasonable to assume that these occur in its glandular elements.

4. The demonstration that the E.M.F. of the skin of the Eel undergoes an excitatory variation as a result of electrical, thermic, and mechanical stimulation, is in accordance with what is known to occur in other glandular structures, and the fact that such excitatory change manifests itself as a positive variation of the current of rest agrees in the main with the phenomena observed in other cases.

5. The fact that chloroform narcosis excludes the possibility of the excitatory variation upon stimulation at the same time as it reduces the E.M.F. of the normal current of rest to zero, supports the assumption that the E.M.F. of the current of rest and that of the current of action originate in one and the same source.

6. Finally, the reduction of the E.M.F. of the normally directed current of rest by atropinisation, and the complete absence of any excitatory variation under such conditions, are facts strongly in favour of the hypothesis that both the E.M.F. of the current of rest and that of the current of action are from a glandular source.

Bibliography.

1. Hermann. Neue Untersuchungen über Hautströme. Pflüger's Archiv, vol. 27, 1882, p. 280.
2. Du Bois-Reymond. Untersuchungen über thierische Elektricität, vol. 2, Abth. 2, p. 9–20.
3. Hermann. Über die Secretionsströme und die Secretreaction der Haut bei Fröschen. Pflüger's Archiv, vol. 17, 1878, p. 291.

4. Bach und Oehler. Beiträge zur Lehre von den Hautströmen. Pflüger's Archiv, vol. 22, 1880, p. 30.
5. Rosenthal. Über das elektromotorische Verhalten der Froschhaut. Reichert und Du Bois-Reymond's Archiv, 1865, p. 301.
6. Leydig. Über die Haut einiger Süsswasserfische. Zeitsch. f. wissensch. Zoologie, vol. 3, 1851, p. 1.
7. Leydig. Hautdecke und Hautsinnesorgane der Fische. Halle, 1879.
8. F. E. Schulze. Epithel- und Drüsen-Zellen. Arch. f. mikr. Anat., vol. 3, 1867, p. 137.
9. Foettinger. Recherches sur la structure de l'épiderme des Cyclostomes, etc. Bulletin de l'Académie royale de Belgique, 2me série, vol. 41, 1876, p. 599.
10. List. Zoolog. Anzeiger, Jahrg. 8, 1885, p. 388.
Biologisches Centralblatt, vol. 5, 1885–6, p. 698.
Archiv f. mikr. Anat., vol. 27, 1886, p. 481.
11. Kölliker. Würzburger naturwissensch. Zeitschrift, Hft. 1, 1860.
12. Hoyer. Über den Nachweis des Mucins in Geweben mittelst der Färbemethode. Archiv f. mikr. Anat., vol. 36, 1890, p. 310.
13. Engelmann. Die Hautdrüsen des Frosches. Pflüger's Archiv, vol. 6, 1872, p. 97.
14. Bayliss and Bradford. On Electrical Phenomena accompanying Secretion in the Skin of the Frog. Journal of Physiology, vol. 7, p. 217.
15. Budge. Über den galvanischen Strom, welcher sich in der Haut des Frosches zu erkennen giebt. Poggendorff's Annalen, 1860, p. 537.
16. Bayliss and Bradford. The Electrical Phenomena accompanying Secretion. Internat. Monatsschrift für Anat. u. Physiol., vol. 4.
17. Engelmann. Die Hautdrüsen des Frosches. Pflüger's Archiv, vol. 5, 1872, p. 498.
18. Du Bois-Reymond. Untersuchungen, vol. 2, Abth. 1, p. 187.
19. Valentin. Zeitsch. f. rat. Med., vol. 15, 1861, p. 208.
20. Roeber. Über das elektromotorische Verhalten der Froschhaut bei Reizung ihrer Nerven. Reichert und Du Bois-Reymond's Archiv, 1869, p. 633.
21. Hermann und Luchsinger. Über die Secretionsströme der Haut bei der Katze. Pflüger's Archiv, vol. 17, 1878, p. 310.
Hermann und Luchsinger. Über Secretionsströme an der Zunge des Frosches. Pflüger's Archiv, vol. 18, p. 460.
22. Luchsinger. Neue Beiträge zur Physiologie der Schweiss-secretion. Pflüger's Archiv, vol. 22, 1880, p. 152.
23. Burdon Sanderson and Gotch. Journal of Physiology, vol. 12, No. 4.
Proceedings of Physiological Society. Oxford, June 20, 1891.
24. Tiegel. Vom Rückenmark der Schlangen und der Aale. Pflüger's Archiv, vol. 17, 1878, p. 594.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES