ANESTHESIA IN ACID AND ALKALINE SOLUTIONS

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We have found[1] that anesthesia is produced in *Nitella* by treatment with distilled water. Our experiments show that the action of distilled water is hastened by adding acid or alkali and retarded by adding calcium.

We can secure the same effects by exposure to 0.0001 M HCl for an hour or to 0.001 M NaOH for 2 or 3 hours as by an exposure of 2 or 3 days to distilled water (all of these effects are fully reversible).

This may be illustrated by describing some typical experiments (all the details are as in the first paper[1] unless otherwise stated[2]).

Effects of Alkali

A group of cells which had been kept for 3 weeks in Solution *A*[1] was transferred to paraffin blocks (Fig. 1) and tested, and all of them responded to electrical stimulation of 160 mv. (*A* responded on break). Solution *A* in Cups *A* and *E* was now replaced by 0.001 M NaOH, and the cells were tested at short intervals. After 75 minutes of extraction with 0.001 M NaOH at *A* and *E*, the cells were tested with 300 mv., with the following results. Of 12 cells

None	responded	at *A*
12	"	" *D* (control spot)
3	"	" *E*

[1] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

[2] Preliminary experiments showed that cells placed in 0.0001 M HCl (pH 4) and 0.001 M NaOH (pH 11) lived 24 hours or longer (the pH value in NaOH fell during the exposure). In applying or removing acid or alkaline solutions there is an effect on P. D. due to the diffusion potential in water of the acid or alkali: the amount of this can be estimated from experiments on dead cells.

The temperature was 23–25°C.

To test whether the effects of NaOH could be reversed by neutralization of the NaOH, Cups *A* and *E* were filled with 0.0001 M HCl and tested by applying 300 mv. a few minutes later. Only one cell responded at *A*. Tested 1 hour later, none responded, and in 5 hours all were dead. Obviously something more than removal of the NaOH is necessary to restore irritability.

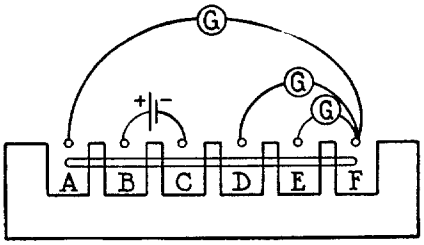

FIG. 1. Diagram of a series of paraffin cups, *A*, *B*, *C*, etc., with a single cell of *Nitella* passing through all of them (in each partition the *Nitella* cell is sealed in with vaseline). Stimulation is applied in the circuit between *B* and *C*. We lead off from *A* to *F*, *D* to *F*, and *E* to *F* through a string galvanometer (*G*) in circuit with a vacuum tube. The whole is covered with a glass plate to prevent evaporation.

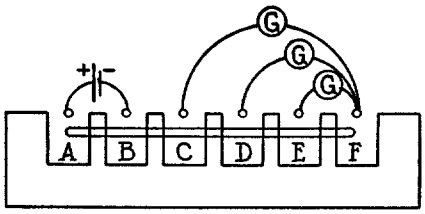

FIG. 2. As in Fig. 1 with a different arrangement of leads.

A second group of 12 cells which had been kept in Solution *A* for 3 weeks was tested and all responded to stimulation by 160 mv. Solution *A* at one point (*C*, Fig. 2) was then replaced with 0.001 M NaOH, which was left in the cups for 3 hours, until no cell responded to stimulation by 300 mv. The 0.001 M NaOH was then replaced by 0.01 M $CaCl_2$, which produced no immediate effect. Left overnight with $CaCl_2$ at *C*, 7 cells out of 12 responded at *C* to stimulation in the circuit between *A* and *B* (200 mv.). The average magnitude of

the response was 36 mv., about one-half of the normal value. Partial recovery from the effects of NaOH was thus induced by 0.01 M $CaCl_2$.

A third group of 12 cells, with 0.001 M NaOH at *C*, was stimulated in the circuit between *A* and *B* with 200 mv. at short intervals for 3 hours until irritability disappeared. The 0.001 M NaOH in Cup *C* was then replaced by Solution *A*. Left overnight, 5 cells out of 12 responded at *C* (2 responded to 300 mv., and 3 required 500 mv. for stimulation).

To determine the value of the outwardly directed potential in cells which had lost their irritability in 0.001 M NaOH, a group of 15 cells was treated by immersion in a bath of the alkali for 2 hours. The potential was then measured, using saturated calomel electrodes, with 0.001 M NaOH on one end of the cell, and 0.001 M NaOH saturated with chloroform at the other. The outwardly directed potential at the living end had an average value of 112 mv. 15 control cells in Solution *A* exhibited an average potential of 76 mv. (when one end was killed with Solution *A* saturated with chloroform). It would seem that the P.D. across the protoplasm had been somewhat increased by the treatment, as is the case with distilled water.[1]

Effects of Acid

We had observed in earlier unreported experiments that *Chara* kept for 48 hours in pond water nearly saturated with CO_2 lost its irritability, and had an outwardly directed potential about 15 per cent above the value for cells kept in normal pond water. The control cells in normal pond water responded to electrical stimulation. Another group of cells kept for 48 hours in a mixture of 0.01 M $NaHCO_3$ and CO_2 at pH 6.0 lost their irritability, and retained the normal P.D. across the protoplasm.

A group of 12 *Nitella* cells which had been kept in Solution *A* for 3 weeks was tested with Solution *A* at all contacts, and responded to stimulation of 160 mv. Solution *A* in Cups *A* and *E*, (Fig. 1) was now replaced with 0.0001 M HCl, and the cells were tested after 1 hour with 300 mv. Every cell responded at *D*, which had been in contact with Solution *A* throughout. Four responded slightly at *A*, and none at *E*. The 0.0001 M HCl at *A* was now replaced by 0.001 M $CaCl_2$ and the 0.0001 M HCl at *E* was replaced by Solution *A*. $1\frac{1}{2}$ hours later the

cells were tested by stimulation with 200 mv. at *B*. Every cell responded at *D*, 10 responded at *A*, and 10 responded at *E*. Thus Solution *A* and 0.001 M $CaCl_2$ seem to be equally efficacious in restoring irritability which has been removed by leaching with 0.0001 M HCl.

In another experiment Solution *A* in Cup *D* was replaced by 0.0001 M HCl. The other cups contained Solution *A*, in which the cells had been kept for 3 weeks. The cell was stimulated (160 mv.) just before putting 0.0001 M HCl at *D*, then immediately after the change, and thereafter at intervals of about 2 minutes for 30 minutes until irritability disappeared (*i.e.*, no response to 400 mv.). The magnitude of the response gradually fell off, the reduction being most marked in the second peak. The second peak disappeared in about 5 minutes, and response failed altogether after 30 minutes.

To determine the value of the outwardly directed potential of a spot which has lost its irritability in 0.0001 M HCl, Solution *A* in Cups *D* and *E* (Fig. 2) was replaced by 0.0001 M HCl. 2 hours later *F* was killed with chloroform. The average value of 10 cells in contact with Solution *A* at *C* was then 66 mv., and the average value of 20 spots in contact with 0.0001 M HCl was 82.5 mv. There is thus a rise in the P.D. across the protoplasm when the cells are leached with 0.0001 M HCl, similar to that when they are leached with distilled water[1] and with alkali.

DISCUSSION

It thus appears that the action of distilled water is hastened by adding acid or alkali. It has been shown previously[1] that it is retarded by calcium. These may all be effects of leaching substances out of the cell. It is of interest to note that Magistris and Schäfer[3] found that the action of distilled water in leaching substances out of plant cells was similarly hastened by the addition of acid and alkali and retarded by calcium.

Alternative explanations are direct action of acid or alkali and the absence of calcium (which has been previously[1] discussed) but

[3] *Cf.* Magistris, H., and Schäfer, P., *Biochem. Z.*, Berlin, 1929, **214**, 440. They regard these substances as phosphatides but this identification is not confirmed by Steward (Steward, F. C., *Biochem. J.*, London, 1928, **22**, 268; *Brit. J. Exp. Biol.*, 1928–29, **6**, 32).

if lack of calcium alone were the cause acid should not increase the effect.

It is possible that some of the reported cases of narcosis in various organisms induced by acid or alkali are due to leaching substances out of the cell.

SUMMARY

The action of distilled water in producing anesthesia (loss of response to electrical stimulation) in *Nitella* is hastened by the addition of acid and alkali and retarded by the addition of calcium. The loss of irritability is fully reversible.