OBSERVATIONS ON THE INJURIOUS EFFECTS OF HIGH TEMPERATURES ON FROG SKIN.

By JOHN W. KLOPP.

(From the Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole.)

(Accepted for publication, June 22, 1924.)

It has been shown by Osterhout (1) that the electrical conductance of living tissues may be used as a general criterion of their vitality, and may in particular be employed in quantitative studies of injuries inflicted upon them. It has been found by this investigator that such injuries may give rise to one of three results, namely (*a*) progressive change leading to death, (*b*) partial recovery, or (*c*) complete recovery. These studies of Osterhout suggest the possibility of using in a similar way some other electrical property of tissues in attempting to define such indefinite terms as vitality, injury, and death. Thus, for example, it has been known since the early work of Du Bois-Reymond (2) that there is a difference of electrical potential between the inner and outer surfaces of the skin of the frog; and this potential difference has been the subject of much investigation since that time.¹ The question naturally arises how far the results of Osterhout might be paralleled by those obtained by studies on it; and the observations here reported were made with the object of determining this point.

The method employed was as follows: The skin, having been removed from various parts of the pithed animal with a minimum of trauma, was cut into squares of sufficient size to fit without undue stretching over the flanged end of a short glass cylinder of about the caliber of an ordinary test-tube, and was held in place there by a rubber band. The tube was then filled with Ringer's solution, and its skin-covered end immersed in a dish of the same solution. Non-polarizable Zn-ZnSO₄ electrodes served as leads from each of these two solutions, and the

¹ More extensive references to the literature on this and certain other points will be given when the complete data are published in a later paper.

difference of potential between them was measured by means of an accurate potentiometer. Precautions were taken to see that the two electrodes themselves were at the same potential at the beginning and during the course of the experiments.

Measurements made in the manner described have confirmed the observations of others that when Ringer's solution is used on both sides of the skin, the external surface is always electronegative to the internal, and none of the procedures used in these experiments sufficed to reverse this polarity. The skin of the large bullfrog (*Rana catesbiana*) obtained at Woods Hole showed considerably higher P.D. than those recorded by most previous observers who have usually obtained values from 30 to 90 mv. The present observations on freshly captured bullfrogs gave a range of from 55 to 141 mv., though values of the usual magnitude were obtained with ordinary laboratory frogs in Philadelphia.

That there is some correlation between the vitality of the skin and its observed P.D. is indicated in a general way by the fact that sickly frogs and those held captive a long time usually give much lower values than do healthy ones recently captured. Similarly, it is easy to show that various noxious agents which are generally admitted to damage the skin (*e.g.* drying, formalin, alcohol, strongly hypertonic salt solutions, etc.) cause a progressive drop in the P.D., leading, finally, to a zero potential. While it is thus possible to use for purposes of study a variety of injurious agents, high temperatures alone were employed in these experiments because of the ease with which they can be controlled and measured.

The nature of the effects produced by continuous exposures to high temperatures was first studied, and the results from one typical experiment are reproduced in Fig. 1. For purposes of more ready comparison the readings have been changed from millivolts to percentages of their value at the beginning of the experiment. This device was utilized because it usually is not possible to obtain, even from the same animal, a number of pieces of skin the P.D. of which are exactly the same. It will be noted that the control curve for room temperature follows a somewhat erratic course (see also Fig. 2). This is typical; and the most common result is a more or less regular fall in the P.D. toward zero. Usually, with the skin on the tubes in

Ringer's solution, this point has not been reached in 24 hours; and the skin may be kept for this length of time on the dead animal without entirely losing its P.D. if precautions are taken to keep it moist.

It will be observed that with the exception of the one representing the lowest temperature, where there was a short characteristic preliminary rise, the curves all show a progressive fall, rapid at first, but becoming slower and slower as the zero point is approached.

FIG. 1. Course of the P.D. of frog skin during continuous exposure to injurious temperatures. Each curve represents a single experiment.

In certain cases a good straight line relation may be obtained by plotting the logarithm of the P.D. (measured in per cent of its original observed value in millivolts) against time; but the author does not wish to draw the conclusion that a monomolecular reaction is necessarily involved, since many purely physical processes are known to follow the same law. Brooks (3) and others have warned against the mistaken resemblance of the time curves of a number of destruc-

tive biological processes to that for monomolecular reactions, pointing out that the apparent logarithmic straight lines are in reality S-shaped curves. Some of the logarithmic curves obtained from the present experiments show this latter relation. A number of attempts have been made to determine in an exact manner a temperature coefficient for the injurious effects of heat by comparing the times required for different temperatures to depress the P.D. to a given level (*e.g.* 50 per cent of the original); but these attempts were abandoned because of the great variability of the material. It was found, however, that such temperature coefficients as could be obtained were invariably much higher than those characteristic of ordinary chemical reactions, and were of the same general order of magnitude as those found, for example, by Chick and Martin (4) for the coagulation of proteins, and by various observers for the destruction of living cells (5).

Having determined the effects of continuous exposures to high temperatures, experiments were then performed to study the after-effects produced by short exposures of various lengths. The general procedure was to measure the P.D. of the skin as usual in Ringer's solution at room temperature for 30 to 40 minutes, to establish the normality of the specimen, subsequently immersing it in a hot Ringer bath at the desired temperature. As soon as this temporary exposure was completed the skin was replaced in the original Ringer's solution, and measured at room temperature from that time on. From these experiments a few typical ones are selected to illustrate the nature of the effects produced (Fig. 2). It will be seen that by varying the intensity of the injury it is possible to obtain all three types of response described by Osterhout, namely (*a*) progressive decline to death, (*b*) partial recovery, and (*c*) complete recovery. It is impossible in a figure of this size to continue the curves beyond a point representing 200 minutes from the beginning of the experiment, but the author has followed such experiments for many hours without obtaining further changes of significance. In addition to the method thus described another has been used in some experiments, namely varying the time of the exposure with the temperature constant, instead of using the temperature as the variable. The results, while somewhat less regular than those already described, nevertheless have been of the same nature.

The results obtained, therefore, parallel in a general way those of Osterhout. The P.D. of frog skin may to a certain extent be used as a criterion of injury, and from measurements of its magnitude at intervals curves of recovery and death may be constructed. It must be admitted, however, that for purposes of quantitative study involving the exact nature of the processes of death and recovery the method is

FIG. 2. Showing the after-effects of temporary exposure to injurious temperatures for 10 minutes. (Heat applied from the 40th to 50th minutes.) Each curve represents a single experiment.

inferior, at least in the hands of the author, to the electrical conductance method of Osterhout. By reason of its more limited applicability, and because of the greater complexity and variability of the material, the method herein described yields results which are not easily reproducible; though it may perhaps lay some claim to value as an independent and supplementary means of testing the conclusions of previous workers.

SUMMARY.

1. The P.D. between the two surfaces of the skin of the frog, when measured under standardized conditions, may be used as a means of studying the phenomena of injury, recovery, and death. Injurious conditions decrease the P.D.

2. According to the severity of the injury produced by temporary exposure to high temperatures, frog skin may show, as judged by this criterion (a) complete recovery, (b) partial recovery, or (c) a progressive change leading to death.

It is the pleasant duty of the author to acknowledge his great indebtedness to Professor M. H. Jacobs for the help he so generously extended in the preparation of this paper.

BIBLIOGRAPHY.

1. Osterhout, W. J. V., Injury, recovery, and death in relation to conductivity and permeability, Monographs on experimental biology, Philadelphia and London, 1922.
2. Du Bois-Reymond, E., Untersuchungen über thierische Elektrizität, Berlin, 1848-1860.
3. Brooks, S. C., *J. Gen. Physiol.*, 1918-19, i, 61.
4. Chick, H., and Martin, C. J., *J. Physiol.*, 1910, xl, 404.
5. Kanitz, A., Temperatur und Lebensvorgänge, Berlin, 1915.