THE EFFECT OF CARBON MONOXIDE AND OF HYDROGEN SULPHIDE ON NERVE IRRITABILITY

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Indirect evidence has been accumulating which indicates that the conduction of the impulse in nerve depends in some way upon phenomena of oxidation. The purpose of the experiments to be reported in this preliminary paper is to determine, if possible, whether the iron-containing respiratory enzyme of Warburg is required for the activation of the oxygen used in the production and propagation of the nerve impulse. For this purpose we have adopted the procedure of treating the tissue with substances which unite with the respiratory enzyme and hence act as poisoners of this oxidative system in the cell. Warburg (1927) has shown that carbon monoxide may inhibit cellular respiration very completely in the absence of light and that this inhibition is reversible to a considerable extent by illumination. Similar effects are produced by hydrogen sulphide although the complex formed by the union of hydrogen sulphide with the respiratory enzyme is not sensitive to light (Negelein, 1925).

EXPERIMENTAL

Nerve irritability was followed by determining the threshold shock required to produce a muscle twitch. Nerve-muscle preparations from green frogs were used, the customary arrangement being as shown in Fig. 1. The nerve passed through two chambers, *A* and *B*, each containing a pair of platinum stimulating electrodes and an inlet and an outlet tube for the passage of gases. It was also possible to immerse the nerve in different solutions in each chamber if so desired. Condenser discharges were used for stimulation. Fig. 2 illustrates the electrical arrangement employed for this purpose. As shown in the diagram, when the key was up the condenser was being charged to a potential indicated on the voltmeter. Depression of the key discharged the condenser through the nerve. A selector switch facilitated shifting from one condenser to another. The choice of the condenser value was determined by the relative irritability of the preparation at the beginning of the experiment. In most cases a 0.005 mfd. condenser was used and the threshold at the outset was

usually less than one volt. Having once determined upon a condenser value to be used in any particular experiment, this value was used throughout the experiment.

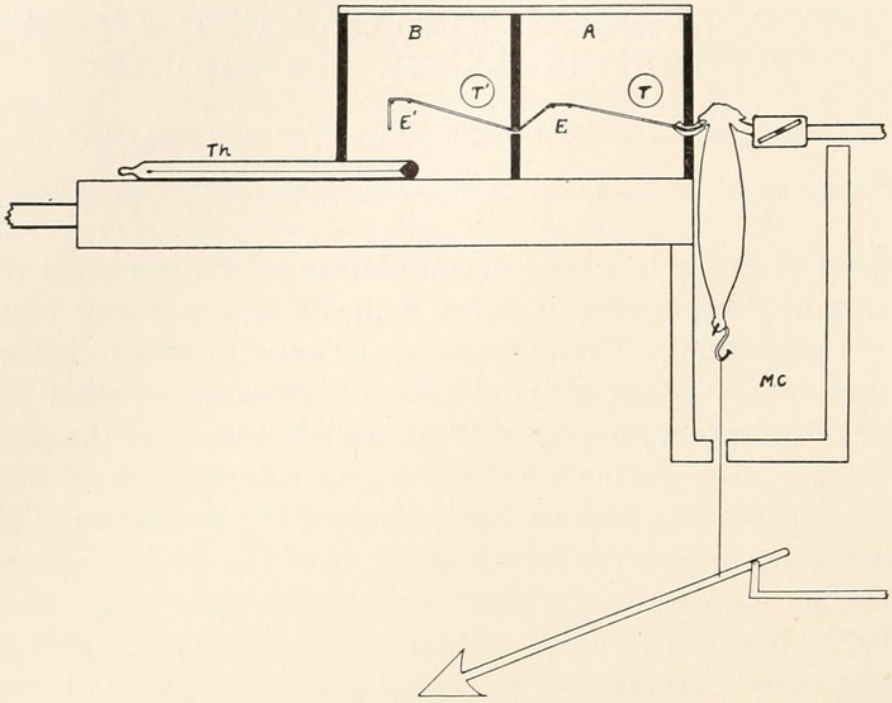

FIG. 1. Sketch of nerve-muscle preparation in place. Nerve lies over pair of electrodes E in chamber A, and E' in chamber B. T and T', inlet tubes for passage of gas; Th, thermometer; MC, moist chamber for muscle.

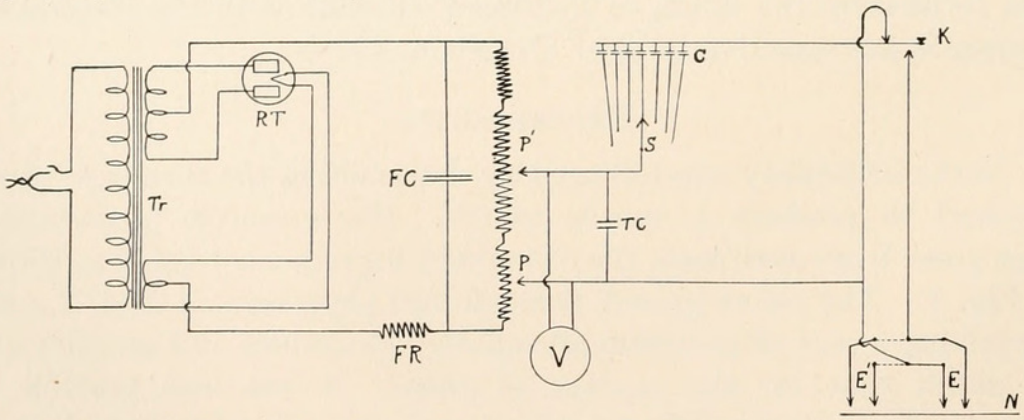

FIG. 2. Electrical diagram for stimulating device designed to operate on alternating current. C represents a series of condensers varying in capacity from 0.001 mfd. to 0.1 mfd. E and E' are pairs of platinum electrodes in chambers A and B, respectively. FC, 2 mfd. filter condenser; FR, filter resistance; K, key; N, nerve; P, potentiometer, 1000 ohms; P', potentiometer, 10,000 ohms; RT, rectifier tube UX 280; S, selector switch; Tr, transformer; TC, 2 mfd. tank condenser; V, multiscale voltmeter.

THE EFFECT OF CARBON MONOXIDE ON NERVE IRRITABILITY

The carbon monoxide was generated by dropping concentrated formic acid into hot sulphuric acid. In experiments in which pure

carbon monoxide was used, the gas was passed from the generator over glowing copper through wash bottles to the preparation through sealed tubes. When the carbon monoxide was to be diluted with oxygen it was passed from the generator into large calibrated bottles and the proper dilution with oxygen made. After allowing some time for diffusion of the gases, and having analyzed samples, the gas mixture was passed through wash bottles to the preparation. Some difficulty was encountered in preventing gas leaks from the outside into chamber *A*, and from chamber *A* into chamber *B*. To prevent this, a very slow stream of moist gas was passed through each chamber for the entire duration of the experiment. In experiments in which carbon monoxide was passed through one chamber and nitrogen through the other, the pressure of the carbon monoxide was maintained at a somewhat higher level than that of the nitrogen. An arc light served as a source of illumination and the rays were passed through copper sulphate to filter out the long wave lengths. A thermometer lying next to the nerve in the chamber indicated the temperature.

It was found that nerves lose their irritability fairly rapidly in mixtures of carbon monoxide and oxygen in which the concentration ratio is 20 to 1 or greater, although failure in pure carbon monoxide does not appear to be more rapid than in pure nitrogen. While it was possible to determine irritability quite accurately by the present method, it would perhaps have been more satisfactory to have used the action potential as the criterion of the reactivity of the nerve.[1] Under the conditions of these experiments it was possible for all but a few of the nerve fibers to have failed completely without greatly affecting the threshold readings. Consequently the usual result was that treatment of the nerve with carbon monoxide had little effect on the threshold readings until the last fibers began to fail, at which time the threshold rose rapidly and reached infinity in a short time. This greatly restricts the period of time during which one might expect to obtain an effect with illumination. Nevertheless, in a number of experiments it was found that illumination of nerves during the period of failure in mixtures of carbon monoxide and oxygen caused a definite decrease in the threshold (see Fig. 3). The threshold usually continued to decrease as long as the nerve was illuminated. Numerous instances were recorded in which illumination caused a return of irritability in nerves which had become completely non-irritable in carbon monoxide mixtures; that is, the threshold returned from infinity to definitely readable values (100–200 volts with a 0.005 mfd.

[1] This has now been accomplished by means of the cathode ray oscillograph and will be published shortly by one of us.

condenser). In some cases a definite after-effect of the light was noted which lasted for some minutes, but, owing to the limitations of the threshold method, investigation of this effect must be deferred.

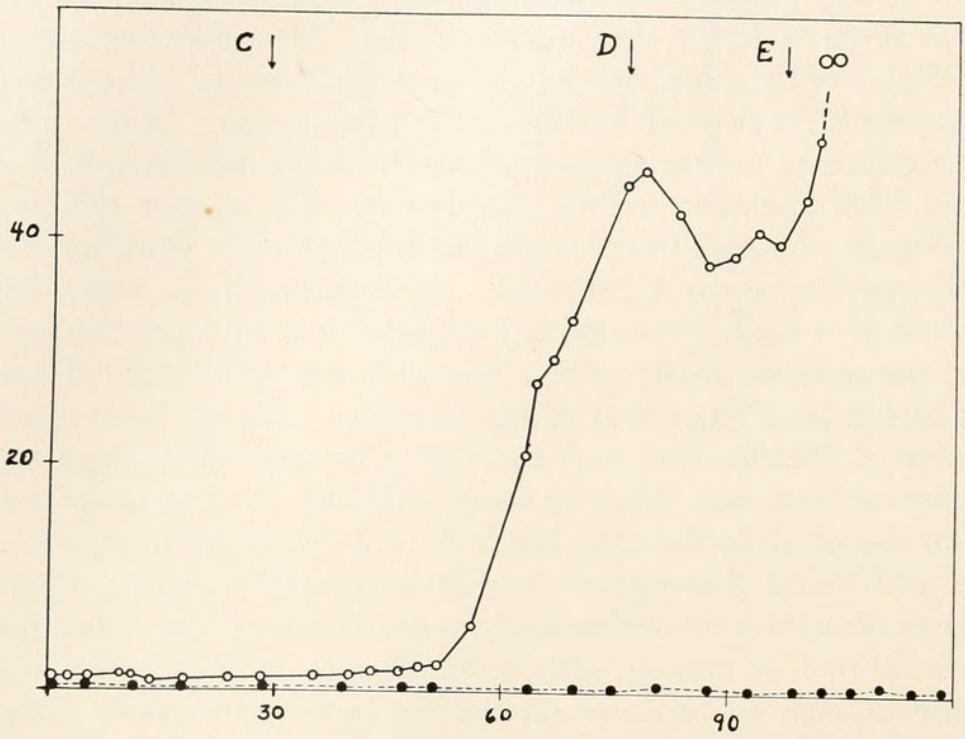

FIG. 3. Effect of carbon monoxide on nerve irritability. Ordinates, threshold in volts (with a 0.001 mfd. condenser); abscissae, time in minutes. Circles represent thresholds of portion of nerve in chamber *B*; points, in chamber *A*. At *C*, a mixture containing about 99.5 per cent carbon monoxide and 0.5 per cent oxygen was passed into chamber *B*, air being passed through chamber *A*. At *D*, nerve in chambers *A* and *B* was illuminated; at *E*, illumination was discontinued.

Although the most striking light effects were observed in nerves which were failing in carbon monoxide-oxygen mixtures, similar results were also obtained when carbon monoxide carefully freed of oxygen was used. It seems probable that these results are best explained on the assumption that traces of oxygen leaked into the chamber through the tube which conveyed the nerve and that this small amount of oxygen sufficed to unite with the iron catalyst under the influence of light, causing a temporary partial return of the irritability.

THE EFFECT OF HYDROGEN SULPHIDE ON NERVE IRRITABILITY

To test the effect of hydrogen sulphide on nerve function, two types of experiments were performed. In the first type the nerve was freely suspended over the electrodes in both chambers and hydrogen sulphide from a generator was passed into chamber *B*, air being circulated through chamber *A*. In the second type of experi-

ment the nerve was made to dip into a solution containing hydrogen sulphide in chamber *B*, but was freely suspended over the electrodes in chamber *A*. In the latter case the solution was made by dissolving sodium sulphide in Ringer solution and adjusting the pH to 7.6 by the addition of hydrochloric acid. It was possible by this method to replenish the sulphide solution frequently, thus preventing escape of gas and corresponding dilution of the solution. Fig. 4 illustrates

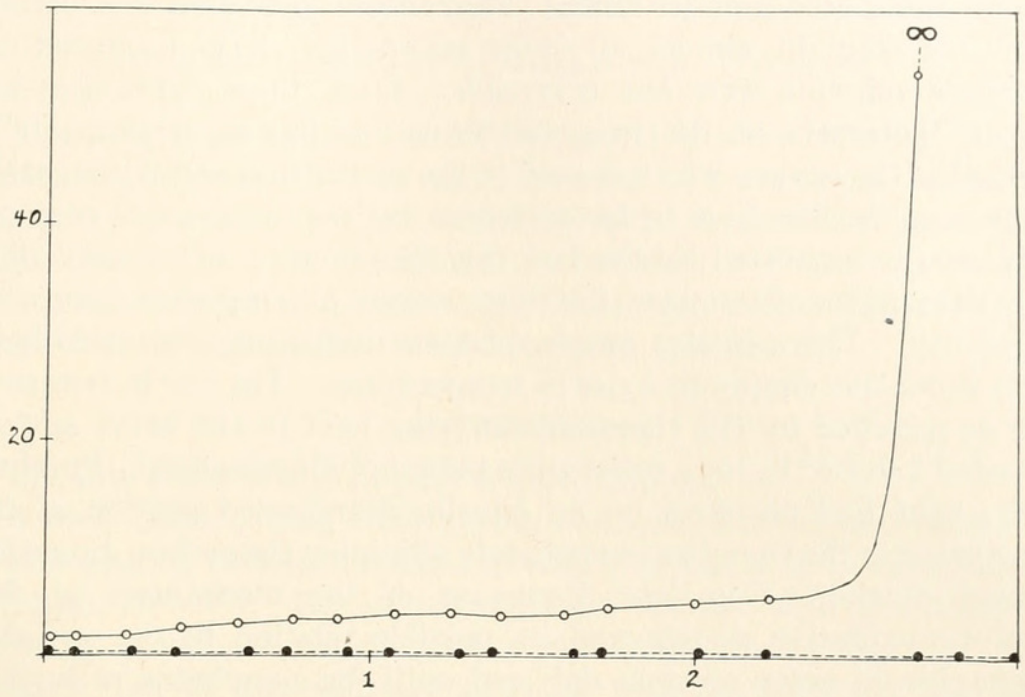

FIG. 4. Effect of M/500 solution of sodium sulphide (adjusted to pH 7.6) in Ringer solution on nerve threshold. Ordinates, threshold in volts (with a 0.005 mfd. condenser); abscissae, time in hours. Circles represent thresholds of the portion of the nerve dipping in sulphide solution in chamber *B*; points represent thresholds of control stretch of nerve in chamber *A*.

typical results obtained in these experiments. The threshold of the portion of the nerve treated with hydrogen sulphide was found to rise slowly for a period of time and then to rise very rapidly. The threshold of the control stretch of nerve in air remained unaffected. Recovery in Ringer solution was usually incomplete. Replacing the sulphide-containing solution with Ringer solution produced scarcely any recovery of the nerve; it was usually necessary to withdraw the nerve from the chamber and bathe the whole preparation in Ringer solution for a time. This was doubtless due to the fact that during the period of poisoning, hydrogen sulphide diffused into the nerve a considerable distance beyond the point of immersion in the solution. Subsequent replacement of this solution with Ringer solution had little effect on the portion of the nerve poisoned by the diffused hydrogen

sulphide. Hence to obtain recovery it was necessary to remove the preparation and bathe it in Ringer solution to remove this remaining sulphide.

Discussion

Although the present experiments are subject to certain limitations inherent in the threshold method, as pointed out above, the data demonstrate that both carbon monoxide and hydrogen sulphide may render the nerve non-irritable. The effects produced by carbon monoxide were in almost all cases reversible, those produced by hydrogen sulphide were less reversible. Since these substances are efficient poisoners of the iron-containing respiratory enzyme, it is likely that the oxygen which is used in the production and propagation of the impulse has first to be activated by the respiratory enzyme. This view is supported by the fact that illumination of nerves failing in carbon monoxide-oxygen mixtures causes a temporary return of irritability. There is also ample evidence indicating that this light effect is not due simply to a rise in temperature. The rise in temperature as recorded by the thermometer lying next to the nerve seldom exceeded 1.0–1.5° C. for a ten minute period of illumination. Furthermore, light had no effect on an equally illuminated portion of the same nerve in the chamber immediately adjoining the carbon monoxide treated portion. Complete discussion of the mechanism of this catalytic oxidative system and its possible relation to the irritable mechanism in nerve must be deferred until the completion of further experiments now in progress, in which the action potential has been measured by means of the cathode ray oscillograph.

Summary

1. Both carbon monoxide and hydrogen sulphide, which presumably inhibit cellular oxidations by combining with the iron-containing respiratory enzyme, render nerve non-irritable. The effects of carbon monoxide appear to be completely reversible, those of hydrogen sulphide, less so.

2. Illumination of nerves failing in mixtures of carbon monoxide and oxygen causes a temporary partial return of irritability and there is some evidence that there is an after effect of the illumination which may last some minutes.

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