AN IMPROVED ANAEROBE JAR.

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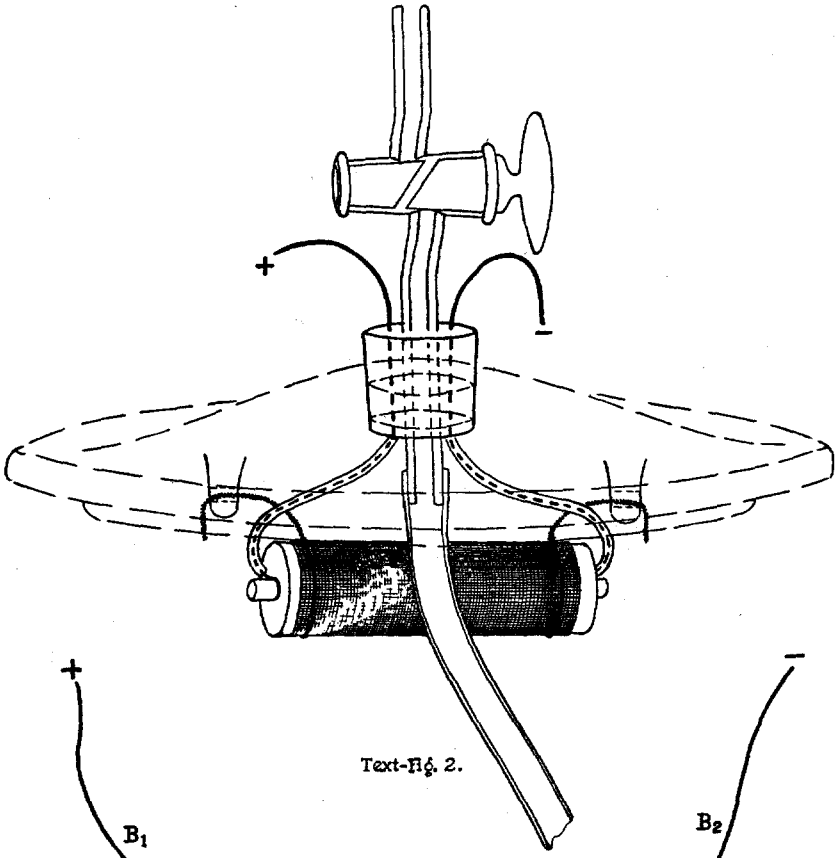
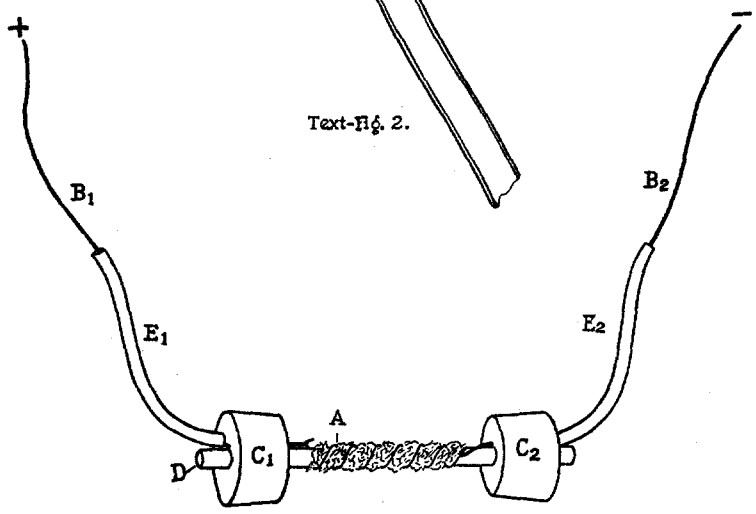
Laidlaw (1) introduced the use of platinized carbon as a catalyzer for the combustion of oxygen and hydrogen to produce conditions favorable to the growth of anaerobic bacteria. His method was adapted for use with single tube cultures only. McIntosh and Fildes (2, 3) utilized the principle for the growth of anaerobes in jars. Their catalyzer consists of a small amount of platinized or palladinized asbestos wrapped in a piece of wire gauze and suspended from the lid of the jar. Hydrogen is introduced into the jar through a stop-cock in the lid. The method of McIntosh and Fildes was modified by W. G. Smillie (4) who enclosed the platinized asbestos in a small perforated glass bulb attached to the end of the tube through which the hydrogen is introduced into the jar, the hydrogen thus entering the jar through the asbestos. Fildes (5) has published a very useful review of these and other methods for the growth of anaerobic bacteria.

In the above mentioned methods the catalyzer must be heated in a flame, the tube or jar quickly closed, and the hydrogen introduced before the catalyzer has cooled, or else combustion does not occur. When the reaction has once started, however, the heat of combustion is sufficient to carry it to completion. There may, however, remain in the recesses of the jar or tubes or within the media traces of oxygen which diffuse out into the jar very slowly after the catalyzer has cooled and combustion has ceased. It is to be noted that Smillie's jar provided for intimate contact of the hydrogen but not of the oxygen with the catalyzer. He may have experienced some difficulty in this regard for in describing another form of apparatus he says: "The following method was devised to remove all the oxygen." The platinized asbestos was wrapped in a coil of fine nichrome wire the ends of which were joined to larger wires which passed upward through a rubber stopper. Hydrogen was introduced into a tube of inoculated solid medium and the rubber stopper inserted. The catalyzer was heated by passing an electric current through the coil surrounding it. Smillie points out that the tube may be set aside for a while and the asbestos reheated to ignite any residual oxygen. Apparently he got the most perfect anaerobic conditions with this apparatus. He did not utilize an electrically heated coil in jars in which many cultures could be enclosed, probably because of the danger of explosion when larger volumes of hydrogen and oxygen are used.



Text-Fig. 2.



Text-Fig. 1.

TEXT-FIG. 1. Showing the construction of the catalyzer coil. *A*, the fine nichrome wire coiled about the platinized or palladinized asbestos. B_1 and B_2, larger copper wires joined to the ends of the nichrome wire. C_1 and C_2, rubber stoppers. *D*, the core of glass tubing. E_1 and E_2, small rubber tubing serving as insulation.

TEXT-FIG. 2. Showing the coil enclosed by fine copper wire gauze and in position beneath the lid of the anaerobe jar.

In the apparatus to be described the danger of explosion is eliminated by completely enclosing the asbestos and coil of nichrome wire in a copper wire screen which does not come in contact with the coil or asbestos at any point. This introduces the principle of the Davy safety lamp. The apparatus is illustrated in Text-figs. 1 and 2, and is made as follows:

A piece of fine nichrome wire (B. and S. gauge No. 28) (Text-fig. 1, *A*) is joined at each end with pieces of larger copper wire (B_1 and B_2). One of the copper wires (B_1) is inserted through a No. 1 one-hole rubber stopper (C_1) and beside the wire is inserted also the end of a short piece of small glass tubing (*D*) holding the wire in the position shown in Text-fig. 1. Some palladinized asbestos is spread out onto a square of lens paper. This is then wrapped about the center of the glass tube and held in place by coiling the nichrome wire around it. The other copper wire (B_2) is then passed through another rubber stopper (C_2) which is placed over the other end of the glass tube. Pieces of small rubber tubing provide insulation for the copper wires at E_1 and E_2. A piece of fine copper wire gauze is rolled around the entire core and held in place by wires twisted about the stoppers at each end of the gauze. The twisted ends of these wires serve to fasten the cell to the lid of the jar as shown in Text-fig. 2. An ordinary round museum specimen jar is used. A one-hole rubber stopper carrying a glass stop-cock is inserted into a hole bored in the lid of the jar. The two copper wires coming from the coil are run up through the rubber stopper on either side of the stop-cock. This is easily done by sticking a large hypodermic needle down through the stopper, running the copper wire up through the bore of the needle and then withdrawing the needle, leaving the wire in place. In time leaks are likely to appear around the stopper, the wires, or the stop-cock, so that cement or sealing wax should be placed over and around the rubber stopper. From the lower end of the stop-cock a rubber tube leads to the bottom of the jar to insure a good mixture of the oxygen with the hydrogen entering the jar.

In use, the lid is clamped down onto the jar of cultures over a gasket of "plasticine" modeling clay. An electric current is connected with the two protruding copper wires and hydrogen is run into the jar through the stop-cock under pressure of about 5 pounds.

Combustion is soon manifested by the collection of moisture inside the jar and by the lid becoming quite warm. This is allowed to continue until the flow of hydrogen ceases, as may be detected by observing no more bubbles in the wash bottles of the hydrogen apparatus. 20 or 30 minutes are usually sufficient, after which the stop-cock is closed, the electric current disconnected, and the jar incubated. At any time during incubation the electric current may again be passed through the coil to consume any residual oxygen. Sufficient hydrogen for this purpose will remain in the jar. A tube of gelatin tinted with methylene blue and decolorized in boiling water just before being sealed within the jar serves as an indicator of the presence or absence of oxygen.

An electric light current of 110 volts reduced by passage through a 60 watt Mazda lamp has been used for heating the coil. Very little hydrogen is required since none is passed into and out of the jar as is the case with the Novy jar.

A number of coils and jars as described have been in use for several months. There have been no accidents due to explosion such as have been known to occur with other jars employing the combustion principle. Whenever perfect anaerobic conditions have not been attained the failure has been due to small leaks in the stopper or stop-cock. The coils have shown no deterioration with use or age. The bit of lens paper used to hold the asbestos in place when the nichrome wire is being coiled about it is burned off with the first passage of the electric current. Similar cells in which the hydrogen was introduced through the asbestos into the cell were made but found to possess no advantage over the one described.

SUMMARY.

There has been described a modification of the anaerobe jars of McIntosh and Fildes and of Smillie in which the oxygen is consumed by combustion with hydrogen under the catalytic action of platinized or palladinized asbestos.

The special advantages of the apparatus described reside in its greater safety and in the fact that the catalyzer is heated electrically after the jar is closed and may be reheated at any time during incubation without opening the jar.

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