An Apparatus for the Micro-electrolysis of Water

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In tracer work using D and ^{18}O it is often desirable to be able to decompose small amounts of water. This is particularly the case with ^{18}O , where rather limited amounts of ^{18}O are available as H_2^{18}O . In a study of the mechanism of the action of notatin (Bentley & Neuberger, 1949) the isotope was required as oxygen gas; an apparatus was devised in which quantities of the order of 1.5 ml. of water may be satisfactorily electrolysed and the oxygen collected in a vacuum system.

each of a roll (1 sq.in.) of platinum foil spot-welded via a short length of platinum to the tungsten wires *A* (1 mm. diam.) sealed into each arm of the U-tube. In operation, degassed H_2^{18}O (about 1.5 ml.) containing a little Na_2SO_4 was placed in the U-tube, and the apparatus evacuated with stopcocks *B* and *C* closed. (All stopcocks were greased with Apiezon vacuum grease, grade L.) By cautious manipulation of stopcock *B* the water was allowed to rise just to the barrel. Stopcock *B* was then closed, and approximately 200 V. d.c. applied to the electrodes, so that the current flowing was about 0.1 amp. Hydrogen produced in the

Fig. 1. An apparatus for the micro-electrolysis of water.

EXPERIMENTAL

The apparatus (Fig. 1) was constructed in pyrex glass: 6 mm. tubing was used for the cell, and 3 mm. capillary for the rest of the vacuum system. The electrodes consisted

cathode limb was allowed to escape, or could be collected separately depending on the position of the 3-way stopcock *D*. The oxygen evolved was collected under the barrel of stopcock *B*, and as the level of water in this limb diminished, stopcock *B* was cautiously opened so that the oxygen

escaped into the vacuum system and the water level was restored. It was necessary to cool the cell during electrolysis and for this purpose it was wound with heavy-gauge copper wire, the ends of which dipped into an ethanol-CO₂ bath. As this cooling was still insufficient, the cold ethanol was splashed over the cell with a small brush. With a current of 0.11 amp., 1.6 ml. of ¹⁸O₂ at N.T.P. were evolved in 5 min.

When electrolysis was complete, the oxygen was expanded into the Toepler pump (*H*) with stopcocks *E* and *F* opened. With stopcock *E* closed it was then pumped into a previously evacuated apparatus, attached to the vacuum line through the standard joint *G* (B10). With water of 0.575 atom % excess ¹⁸O in the cell (determined by equili-

bration with CO₂ and carbonic anhydrase as described by Bentley, 1948), oxygen gas of 0.579 atom % excess ¹⁸O was obtained.

SUMMARY

An apparatus is described in which quantities of the order of 1.5 ml. of water may be electrolysed, and the evolved oxygen (or hydrogen) collected in a vacuum system.

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Growth Factors for *Corynebacterium Diphtheriae*

4. PREPARATION FROM YEAST

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In an earlier paper, Chattaway, Happold & Sandford (1944) showed that certain gravis (and intermedius) strains of *Corynebacterium diphtheriae* required an unidentified growth factor when cultivated on a medium of known composition containing pimelic, nicotinic and pantothenic acids (Mueller, 1937*a, b*; Evans, Handley & Happold, 1939*a, b*). This factor, present in liver, was shown to be dialysable and resistant to proteolytic enzymes. Its properties were consistent with the presence in its molecule of both acid and basic groups probably in the form of an α -amino-acid component, since the activity was destroyed by ninhydrin.

The factor appeared to have no direct connexion with any of the unidentified growth factors for *Lactobacillus casei*, cf. Dolby, Happold & Sandford (1944), but showed some similarity to streptogenin, which was prepared by Woolley (1941) from various purified proteins, and is required by certain strains of *Streptococcus haemolyticus*. The present paper reports the separation and concentration from yeast of a number of factors similar in many respects to the liver factor of Chattaway *et al.* (1944).

EXPERIMENTAL

Method of microbiological assay

Organism. The strain of *C. diphtheriae* used for assessing the potency of preparations was a gravis strain, subtype Dundee (Barton). In all cases the growth was observed

in $6 \times \frac{5}{8}$ in. test tubes containing the medium outlined below.

Inocula. The strain was maintained on Löffler slopes in the refrigerator and subcultures were made on blood-agar plates. A sufficient amount of an 18 hr. subculture was introduced into 10 ml. of sterile water to produce a faint turbidity at the surface. This suspension was dispersed by shaking and one drop (approx. 0.03 ml.) used for inoculation.

Basal medium. The basal medium employed, shown in Table 1, differed from that described by Chattaway *et al.* (1944). Certain amino-acids hitherto omitted were included in order to bring its composition more nearly into line with a casein-hydrolysate medium on which the organism grows profusely. In the early experiments it was found that test cultures occasionally grew in the basal medium. This was traced to an impurity in the aspartic acid, which was then omitted without alteration in the response of the organism to the factor. Pimelic acid was also omitted since the organism was provided with preformed biotin.

Determination of growth. The organism used grows exclusively as a pellicle under the conditions of test, a fact which enables a visual assay of growth to be made, and which precludes all other methods with the exception of a determination of the N₂ content of a whole culture. This latter method was discarded since an adequate expression of growth could be made in terms of arbitrary degrees of pellicle formation. Ten degrees of growth were recognized, ranging from one given a numerical value of 4, corresponding to a heavy pellicle over the surface of the medium, through 3, 2, 1.5 and 1, at which value the whole surface of the medium was covered with a thin film of growth, down to degrees of growth given the numerical values 0.5, 0.2, 0.1, 0.05 and 0.02, the last being a just discernible trace. Growth was determined after 72 hr. incubation at 37°. This method