

the animals made an uneventful recovery, whereas in others a generalized peritonitis resulted.

This is a preliminary report.

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<sup>1</sup> Eisberg, H. B., *Ann. Surg.*, 1921, lxxiv, No. 5.

<sup>2</sup> Dragstedt, L. R., Moorehead, J. J., Bureky, F. W., *J. Exp. Med.*, 1917, xxv, 421.

## 3518

### A Simple Respirometer for Microbial Respiration.

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Since the comprehensive work of Novy, Roehm and Soule<sup>1</sup> on microbial respiration appeared in 1925, an effort has been made to devise a simple apparatus for determining CO<sub>2</sub> production in cultures which would be simple and available for ordinary laboratory use. It is doubtful if any device known at present will give the fine results which Novy, Roehm and Soule are able to obtain with the compensation manometer.

Bronfenbrenner<sup>2</sup> described a simple micro-respirometer in 1926 using as a test object a strain of bacteriophage. He was unable to demonstrate that the bacteriophage respired. In a more recent report this author has studied the respiration of the herpes virus and of rabies virus<sup>3</sup>. In this study in order to differentiate between the respiration of tissue and that of the filterable virus he made use of the Warburg<sup>4</sup> respirometer to determine the oxygen uptake and of his closed cell respirometer for the respiration proper.

Bertrand<sup>5</sup> was perhaps the first to make use of an alkali trap for the absorption of respired CO<sub>2</sub>. In searching for some simple method to demonstrate microbial respiration to students in bacteriology we decided to make use of this simple procedure. From ordinary culture tubes containing smaller tubes within, which served for a trap, a more elaborate apparatus has been devised by which it is possible to make quantitative tests for respiration as well as qualitative tests.

Figure 1 shows such an apparatus in the form of a flask carrying a side chamber which serves as the trap. The flask E is of 100 cc. capacity. It is entered through stop-cock B and can be exhausted

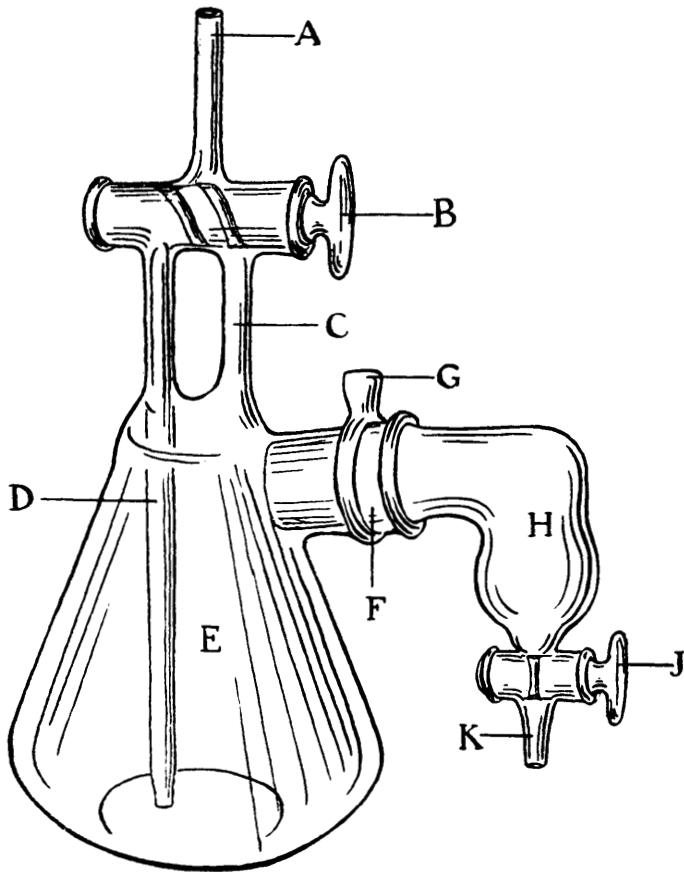


FIG. 1.

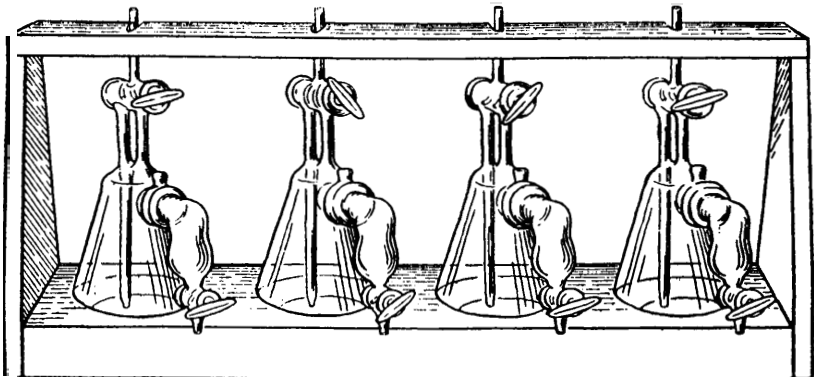


FIG. 2.

through C. Substances can be introduced and withdrawn through inlet tube D. The side chamber H is fitted into flask E through a ground joint F and this joint is further protected by a mercury seal G. The side chamber has an outlet through stopcock J through the nipple K. Figure 2 shows a series of the apparatuses in a special rack for control work.

The broth culture is first placed in flask E (20 cc. of culture material usually being employed) and 5 cc. of distilled water containing an indicator (usually phenol red) and a known quantity of carbonate free alkali is placed in the side chamber H. After a given

TABLE I.

Sample	CO <sub>2</sub> Determination in Duplicate				Average 1st series	Average 2nd series	1st series corrected for broth	2nd series corrected for broth
	Vol. per cent CO <sub>2</sub> First Series		Vol. per cent CO <sub>2</sub> Second Series					
Broth	4.4	4.4	4.2	4.0	14.65	14.7	10.25	10.6
Flask 1	14.7	14.6	14.9	14.5	14.7	14.85	10.3	10.75
Flask 2	14.7	14.7	15.0	14.7	9.8	8.85	5.4	4.75
Flask 3	9.8	9.8	8.9	8.8	10.8	9.1	6.4	5.0
Flask 4	10.9	10.7	9.2	9.0	10.8	10.4	—	—
Trap 1	9.6	9.6	10.2	10.7	9.6	10.2	—	—
Trap 2	9.5	9.6	9.9	10.5	9.55	10.2	—	—
Trap 3	9.2	9.2	8.3	8.0	9.2	8.15	—	—
Trap 4	9.1	9.0	7.9	7.8	9.05	7.85	—	—

period of incubation the contents of E and of H or a mixture of the two can be examined for CO<sub>2</sub> content by the Van Slyke CO<sub>2</sub> apparatus.

A typical protocol in which no additional oxygen is added to the apparatus follows:

CO<sub>2</sub> production was determined upon a culture of Coli "D," which is sensitive to a strain of bacteriophage. An equal amount of a young broth culture of B. coli "D" was added to the 20 cc. of beef extract broth pH 7.6 in flasks E, Figure 2. To flasks one and two .5 cc. of B. coli "D" bacteriophage was also added. In side chamber H, 1, 2, 3 and 4 5 cc. of distilled water containing 5 drops of a .2 per cent phenol red solution and 10 drops of a carbonate free N/20 NaOH, were introduced. F was sealed at G with mercury. The set-up was placed in the incubator for 24 hours and CO<sub>2</sub> determinations were made at the end of that period upon the culture fluid and upon the contents of the alkali trap with the Van Slyke CO<sub>2</sub> apparatus.

It will be noted in Table I that the reading obtained with the uninoculated broth in the Van Slyke apparatus, when corrected, gives 4.4 cc. volume per cent of CO<sub>2</sub>. The culture material in apparatus 1 and 2 gave readings consistently higher than the culture material in apparatus 3 and 4. Apparatus 1 and 2 contained bacteriophage, while 3 and 4 did not. Again the determination of CO<sub>2</sub> in the trap showed that the trap in apparatus 1 and 2 contained more CO<sub>2</sub> than did the trap in apparatus 3 and 4. When these readings are corrected for the CO<sub>2</sub> content of uninoculated broth it is found that there is about the same quantity of CO<sub>2</sub> bound by the culture fluids by the alkali in the trap. Much of this CO<sub>2</sub> can be released by introducing a strong acid through tube D, but it is obvious that the method has its limitations.

The excess of CO<sub>2</sub> production in apparatus 1 and 2 which contained bacteriophage is not interpreted to indicate that the bacteriophage respire, as it is more likely due to the stimulus to growth imparted to the sensitive strain of the test organism. Attention has been drawn to this phenomenon elsewhere.<sup>6</sup>

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<sup>1</sup> Novy, F. G., Roehm, H. R., and Soule, M. H., *J. Inf. Dis.*, xxxvi, Nos. 2, 3, 4.

<sup>2</sup> Bronfenbrenner, J. J., *Science*, 1926, lxiii, 51.

<sup>3</sup> Bronfenbrenner, J. J., *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiv, 176.

<sup>4</sup> Warburg, O., *Biochem. Z.*, 1923, cxlii, 317.

<sup>5</sup> Bertrand, G., *Annal. de l'Inst. Pasteur*, xxvi, 852-857.

<sup>6</sup> McKinley, E. B., and Holden, M., *J. Inf. Dis.*, xxxviii, No. 6, 451-456.