

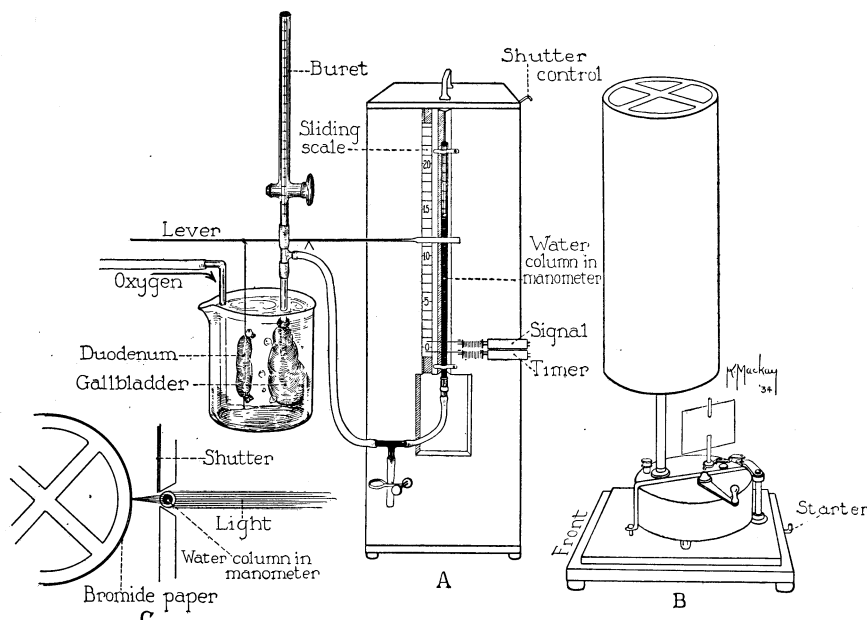


FIG. 1. The recording device in use. The significance of the initials is given in the text.

record of volume and pressure changes in any given system.

As early as 1883, E. A. Schäfer² utilized the photographic method to record changes in volume, whereas Baldes³ and Corbeille, in 1929, utilized the method in recording plethysmograms.

Records were made on photographic bromide paper fastened to the drum of a "Harvard" kymograph (Fig. 1, B), which is enclosed in a light-tight box (Fig. 1, A). A vertical slit, 2 mm wide, opened or closed by means of a shutter, was made along the front elevation of this box and a water manometer was placed immediately in front of this slit. The source of light was an ordinary projection lamp, which was placed about 30 feet (9 m) from the recorder, so that the light rays were directed against the manometer. To secure optimal results it is essential that the effective light rays, the long axis of the manometer, the midline of the slit in the box and the axis of the revolving drum should be properly aligned (Fig. 1, C). The manometer, containing a liquid, functioned as a lens, so that light passing through the liquid was focused on the light-sensitive paper fixed to the revolving drum of the kymograph. It is apparent that changes in the level of the liquid were recorded on the paper by the marked contrast between the intensity of exposure above and below the level of the meniscus. Likewise, any movements of levers, time signals or markers may be recorded simultaneously with the changes in the level of the meniscus.

A manometer tube, of the type employed clinically

for measuring spinal fluid pressures, was found convenient in our experiments. These tubes are of particular value for records of this sort, since they are accurately calibrated and the photograph of these lines on the record is of value in computing changes in the level of the meniscus. These tubes contain 0.02 cc of fluid per centimeter of length and thus permit the recording of changes in pressure without appreciable changes in the volume. A satisfactory type of contrast bromide paper, 70 feet (21.3 m) in length and either 6 or 12 inches (15.2 or 30.4 cm in width), is obtainable in rolls. A developer which gives the maximum of contrast must be used.

Reproductions of records made with the apparatus that has been described above accompany an article by Deissler and Higgins, entitled "The Extrahepatic Biliary Tract during Anaphylaxis"; this article has been accepted by *The American Journal of Physiology*.

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A MICRO-METHOD FOR DETERMINING THE UTILIZATION OF CARBOHYDRATES AND POLYHYDRIC ALCOHOLS BY MICROORGANISMS

It is sometimes desirable to know whether a single colony of bacteria, without further sub-culture, is composed of organisms capable of utilizing one or more particular carbohydrates. This can be easily and quickly accomplished by the following micro-technique.

² E. A. Schäfer, *Jour. Physiol.*, 5: 127-129, 1883-1884.

³ E. J. Baldes and Catherine Corbeille, *Proc. Soc. Exper. Biol. and Med.*, 26: 711-715, May, 1929.

The colony is removed from the surface of the agar by a platinum inoculating loop and mixed with several loopfuls of a M/750 phosphate buffer at a pH of 7.3, containing 0.04 per cent. phenol red in a depression slide. By means of the loop (or a smaller one, if several substrates are to be studied) droplets are removed from the depression to the flat surface of the slide. A loopful of distilled water is mixed with one droplet as a control, and a loopful of a 1.0 per cent. distilled water solution of a given carbohydrate is mixed with another droplet. The suspensions of cells prepared in this manner are then taken up in capillary tubes having an inner diameter of 0.35 mm and observed for change in color of the indicator. If the substrate is oxidized the solution will become acid, due either to the liberation of fixed acid or to an excess CO_2 production, while the control will remain unchanged, since the CO_2 production is too low to affect the buffer. In the case of a 2 mm colony of *Escherichia coli* treated in this manner, the change from orange-red to yellow occurred within fifteen minutes, with glucose as substrate, while the control underwent no change over a period of several days in the incubator.

The same method has been fully tested on a slightly larger scale, using capillary tubes of 1 to 2 mm inner diameter, with the washed cells of luminous bacteria (*Vibrio phosphorescens* and *Achromobacter fischeri*) as well as *Escherichia coli* and yeast. Thirty substrates, including pentoses, hexoses, heptoses, polysaccharides and two-, three- and six-carbon polyhydric alcohols, have been tested with each of the above species. The method works equally well in small test-tubes, 10×75 mm, which insure partial anaerobiosis by virtue of the fact that oxygen diffuses in sufficiently rapidly only for the cells in the uppermost layer of the suspension to remain aerobic. Washing the cells with the aid of the centrifuge is desirable in order to get the results of the "resting" metabolism, uncomplicated

and unobscured by extensive products and processes of vigorous growth. Except with the most slowly fermented substrates, such as dulcitol, the results are obtained within a few minutes, or at most a few hours. The rate depends, of course, on the concentration of cells, the concentration of buffer, and the rate and products of decomposition of the substrate, but in general, satisfactory results are obtained when washed cells from a heavily inoculated 18-hour nutrient agar Petri plate are resuspended in 15 to 20 cc of M/150 buffer. The suspension is aerated by a stream of air for about fifteen minutes, and 1 cc portions diluted with an equal volume of substrate solution in small test-tubes.

This method has given results entirely consistent with those found in the case of the aerobic oxidation of substrates by the "resting" cells of luminous bacteria in Warburg respirometers, except for glycerine which is easily oxidized aerobically, but apparently not in a limited oxygen supply. While only the oxidations occurring at an initial pH of 7.3 have been studied, it should be possible, with appropriate indicators, to study different pH ranges.

Gas formation could be detected in the case of yeast by large bubbles in the capillaries, but not very well with *Escherichia coli*. With the latter, gas formation could be detected by means of very small glass tubes sealed at one end, and filled by a micro-pipette, and inverted in the small test-tube containing the suspension. In this case, however, gas was not formed from certain substrates apparently because acid accumulated so fast that the cells were precipitated in a few minutes. The accumulation of gas required incubation over night. No further change occurred after several days, and the controls never indicated any decided acid production or gave any indication of gas formation.

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SPECIAL ARTICLES

THE CULTURE OF WHOLE ORGANS

THE method to be described consists of the transplantation of an organ or of any part of the body into a sterile chamber, and of its artificial feeding with a nutrient fluid through the arteries. It is not in any way a substitute for the method of tissue culture. Its techniques, as well as its purposes, are quite different. As is well known, tissues and blood cells grow like bacteria in flasks containing appropriate media. The techniques for the cultivation of tissues are somewhat analogous to bacteriological techniques, although far more delicate. But it is through the employment of complex mechanical and surgical procedures that

organs are enabled to live isolated from the body. Tissue culture deals with cells as units of bodily structures; the new method, with cellular societies as organic wholes. Its ultimate purposes are the manufacture *in vitro* of the secretions of endocrine glands, the isolation of the substances essential to the growth, differentiation and functional activity of those glands, the discovery of the laws of the association of organs, the production *in vitro* and the treatment of organic and arterial diseases, etc.

The idea of maintaining alive a portion of the body in order to study its functions is not new. In 1812, the physiologist Le Gallois¹ wrote that, "if one could sub