tions by the chronometric method of Miller & Dawson (1941), showed that wheat germ contains no measurable amount of tyrosinase. Peroxidase, on the other hand, was present and qualitative reactions with guaiac and with benzidine, remained positive even after wheat-germ suspensions had undergone 13 min. treatment in the Waring blender. This behaviour of wheat peroxidase is in keeping with the high resistance of peroxidases from various sources to conditions which are injurious to other enzymes, e.g. high temperature. The fact that wheat peroxidase survives treatment in the Waring blender would seem to indicate that the darkening effect is not due to peroxidase action since failure to darken after treatment is not connected with destruction of peroxidase. It is to be assumed, however, that peroxidase is only one member of a more complex enzyme mechanism which may provide H_2O_2 necessary for peroxidase action. For this reason failure to darken might be due to the breakdown of some other part of this enzyme mechanism. An investigation of the darkening effect is in progress.

DISCUSSION

Potter & Elvehjem (1936) described a device for grinding tissue material, and reported that enzyme inactivation occurred on dilution of the ground material. According to their explanation, dilution causes the coenzyme concentration to drop below the level necessary to maintain enzyme activity. This explanation does not apply to the enzyme inactivations reported in the present paper. While

Potter & Elvehjem compare suspensions of different concentrations the present paper deals with suspensions of equal concentration, but obtained by different means, viz. by grinding with sand in one case and by treatment in the Waring blender in the other.

The Waring blender, with its speed of about 10,000 rev./min. is far more likely to cause protein denaturation than the grinder used by Potter & Elvehjem (1936), with its much lower speed. The present authors suggest that, while in their experiments oxidation of activating thiol groups may have been responsible for the inactivation of certain enzyme systems, the main damage caused by the Waring blender was perhaps enzyme destruction due to protein denaturation.

The experiences reported above make it justifiable to sound a note of caution with regard to the Waring blender and similar homogenizers, and to recommend restricting their use to applications which do not involve inactivation of enzymes or in which such inactivation does not matter.

SUMMARY

Treatment of mill stream suspensions in the Waring blender caused oxidation of thiol groups and inactivation of enzyme systems.

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A Mercury Gasometer for the Preparation of Gas Mixtures

By E. F. HARTREE AND C. H. HARPLEY, *Molteno Institute, University of Cambridge*

(Received 23 November 1948)

The need for a means of preparing gas mixtures containing accurately known proportions of carbon dioxide arose during manometric studies of tissue metabolism. The measurement of glycolysis in terms of CO_2 liberated from a bicarbonate buffer requires an atmosphere containing a small proportion of CO_2 , and cylinders of nitrogen and oxygen containing 5% of CO_2 are commercially available. When, however, different concentrations of CO_2 or mixtures of CO_2 with more than one other gas are required, one of the following methods must be employed:

- (1) A continuous-flow method in which flowmeters measure the rate of delivery of the individual gases.
- (2) Collection of the gases, in a graduated Mariotte bottle or other container, over a saturated salt solution in which gases are generally less soluble.
- (3) Preparation of the gas mixture in a pressure vessel fitted with a gauge.
- (4) Collection of the gases in a graduated vessel over mercury.

The flowmeter method is unreliable since the proportions in the mixture will vary widely with changes both in individual gas pressures and in resistance to

flow. The drawbacks in the use of saturated salt solutions are crystallization in tubes and taps, and the 'creep' of the salt. In any case the solubilities of

great a pressure will be applied to the apparatus, and furthermore some gases (e.g. CO), are not generally available under pressure for filling the vessel. On the

Fig. 1.

different gases in such solutions are still high enough to preclude the precise preparation of gas mixtures. With a pressure vessel there is always a risk that too

other hand, a graduated mercury gasometer permits gas mixtures to be made up with accuracy and to be delivered under a controlled pressure.

APPARATUS

The mercury gasometer (Fig. 1) consists of two identical graduated Pyrex glass mixing cylinders of 1500 ml. capacity each connected by reinforced rubber tubing, through a 2-way glass tap, to two arms of a glass T-piece. The third arm of the T-piece is connected to a glass reservoir of just over 1500 ml. capacity. At the top of each cylinder is a 3-way tail-pattern tap and the upper half of the cylinder, which is 4.3 cm. in external diameter, is graduated from 0 (at the tap) to 300 ml. at intervals of 15 ml. The lower half is somewhat wider (7.5 cm. diameter) and 100 ml. graduations are continued down this section to the 1500 ml. mark which is at the upper end of the neck to which the reinforced rubber tubing is attached. The distance between the 1500 ml. graduation and the top is 63 cm. The neck rests within a fibre ring which fits into a hole in the lower steel plate of the cylinder carrier. The reservoir is similarly supported in a fibre ring (*FR*).

The framework within which the glass parts are supported was manufactured in mild steel and consists of a base plate (*BP*), $30.5 \times 40.5 \times 1.0$ cm., to which are bolted five perpendicular 20 mm. diameter rods (R_1 , R_2). The upper ends of these rods are bolted to a top plate (*TP*) which is cut away so as to permit the upper parts of the glass vessels to pass through. The distance between top and base plates is 107 cm. To the top plate are bolted two pulley supports (*PS*) each carrying a pair of grooved pulleys. Over each pair of pulleys passes a stranded steel cable (2 mm. in diameter), one end of which is attached to a steel carrier in which glass parts are supported while the other end winds around an 8 cm. diameter flanged pulley (*Pu*). These flanged pulleys are mounted on an extension to the pinion, or driven, shaft of two tennis-net winders (*Te*) bolted to the base plate. In order to reduce the strain on the winder bearings, the shaft extensions are supported in extra bearings (*Be*). The two glass mixing cylinders are supported on the fibre rings within a carrier consisting of two horizontal, 5 mm. thick, steel plates (*P*) which are separated by three 26 cm. lengths of steel tube about 22 mm. internal diameter. The rods R_1 pass through these tubes and act as guides when the carrier is raised or lowered. In the upper plate are two holes to take the cylinders and in the lower plate are two smaller holes into which fit the fibre rings. The steel cable passes down through a small hole in the upper plate and is retained by means of a terminal blob of hard solder. The other end of the cable is retained in the same way on the flanged pulley. The carrier for the reservoir is similarly built up on two 23 cm. lengths of steel tube moving along the rods R_2 . It incorporates a stirrup above the upper plate consisting of two 13 cm. threaded rods to which is screwed by means of milled nuts a horizontal $\perp$ section bar (*Ba*). This bar, to which the steel cable is attached, has a hole at one end for the threaded rod and a slot at the other so that it may be swung aside when it is necessary to add mercury or to remove the reservoir. Each of the five steel rods (R_1 , R_2) carries two collars (*Co*) with set screws which are fixed so as to limit the travel of the carriers.

If these are raised too far when rubber tubing is attached to the tail-pattern taps there is a risk of breakage should the tubing become strained as it passes through the top plate. On the other hand, if the carriers are allowed to go too low a strain is thrown on the mercury-containing rubber tubes attached to the lower outlets of the glass vessels.

The connexions between the three vessels are made with canvas interlined rubber tubing of 10 mm. internal diameter. This gives a tight fit over the necks of the glass vessels which are drawn out to 10 mm. diameter with a bulge to 11 mm. The glass T-piece (*T*) and pressure taps (*PT*) are screwed down by means of saddles to strips of wood bolted to the base plate. These strips are thick enough (2.5 cm.) to accommodate, when suitably drilled, the projecting spring-loaded barrels of the taps of the type used in the Van Slyke apparatus. By means of these taps it is possible to isolate one or both of the mixing cylinders from the reservoir. This layout was adopted since it appeared impracticable to have two reservoirs and a double charge of mercury. The present design requires 22 kg. mercury.

The rubber-to-glass joints are bound with insulating tape and copper wire; this procedure eliminates the risk of splits in the rubber tubing. Only after 12 years was it found necessary to replace the tubing on account of its decreasing pliability. The only other 'servicing' that the apparatus requires is lubrication of the bearings and of the steel cables which should be occasionally rubbed with soft paraffin ('Vaseline').

The gasometer is mounted on a wheeled angle-iron trolley by means of bolts passing through the base plate and through a similar plate, 65 cm. from the ground, which forms the top of the trolley. A drain plug (*DP*) facilitates the collection of spilled mercury.

USES OF THE GASOMETER

In addition to its value for the preparation of mixtures of water-soluble gases, the apparatus serves well for making up gas mixtures whenever a higher accuracy is required than can be obtained over water where a certain gas exchange between the two phases is inevitable. It is thus of great advantage in preparing, by serial dilutions, a mixture containing a very small proportion of one constituent (e.g. CO in O₂ for haemoglobin studies). The gasometer is also useful for the storage of highly purified gases or of gases, such as nitric oxide, which react with water or with oxygen.

SUMMARY

A description is given of a mercury gasometer in which two gas mixtures of up to 1500 ml. in volume can be accurately made up.