Pipette for Use with the Van Slyke-Neill Manometric Apparatus

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In the use of the Van Slyke-Neill manometric apparatus, the accurate measurement of reagents maintained free from dissolved air is much facilitated by use of the special pipette illustrated (Fig. 1). This pipette allows 50 ml. reagent to be kept out of contact with air for an indefinite period and for any required quantity to be accurately measured into the apparatus without any possibility of coming into contact with air.

Fig. 1. Pipette for use with the Van Slyke-Neill manometric apparatus.

Construction

The pipette is built round two 3-way stopcocks *X* and *Y*, with branches at 120° and with an angle of 120° in the bore so that any two branches may be connected. Stopcock *Y* should have capillary bore connexions. Bulb *E* and cup *A* should have about 50 ml. capacity. *B* is a calibrated tube; for most purposes a capacity of 2 ml. $\times \frac{1}{20}$ is convenient since it is unusual to measure greater volumes of reagent into the chamber of the Van Slyke apparatus, but, if required, the capacity of this tube may be greater. The bottom of cup *A* should be approximately level

with the zero mark on *B* and the top of *E* should be approximately level with the lowest graduation on *B*. Tubes *D*, *F* and *C* should be of capillary bore throughout. In those models of Van Slyke's apparatus with a standard ground socket at the top of the reaction chamber, *F* should be fitted with a corresponding standard cone; otherwise the tip of *F* is fitted with a piece of rubber as usual in the use of Ostwald pipettes. The pipette should be built as compactly as possible, otherwise the weight of mercury in *A* and *E* will be liable to fracture the tubing. If it is so built the apparatus is reasonably sturdy, but a stand with three clips to support the pipette when not in use is an advantage, especially if several pipettes are required.

Operation

Filling pipette. (a) *Filling with mercury.* To fill the pipette open *X* to connect *A* to *E*, and *Y* to connect *C* to *F*. Pour mercury in through *A* until *E* is full and mercury flows from *F*. Close *X* and *Y* and add about 15 ml. more mercury to *A*. Open *X* to connect *A* to *B* until mercury reaches the zero mark in *B*, then close *X*. Pour mercury into *D*, open *Y* to connect *D* to *F* until mercury flows from *F*.

(b) *Introduction of reagent.* Free the reagent from air in the Van Slyke apparatus in the usual manner. Place a small quantity of mercury in the cup above chamber *G*, insert the tip of *F*. Raise the levelling bulb of the Van Slyke apparatus to the upper ring, open *Z* to connect *G* to *F*, open *Y* to connect *F* to *C* and *X* to connect *E* to *A*. The solution will flow into *E* displacing mercury into *A*. When all the reagent is in the pipette or the mercury is almost entirely driven from *E*, close *X*, *Y* and *Z*, remove the pipette, open *Y* to connect *D* to *F* so that mercury fills the bore of *F*.

Addition of measured amount of reagent to the Van Slyke apparatus. Place the tip of *F* in the cup above chamber *G* of the Van Slyke apparatus (the cup should contain a few drops of mercury). Open *X* to connect *B* to *E* and *Y* to connect *C* to *F*. Turn *Z* slowly until the mercury in *B* indicates that the required volume of reagent has entered chamber *G*. Close *X*, open *Y* to connect *D* to *F* until mercury flows into *G*; close *Y* and *Z*.

To set the pipette for use again, open *X* to connect *A* and *B* and allow mercury to rise to the zero mark in *B*.

It is not usually necessary to empty the pipette. If a different reagent is to be put into it, the aqueous contents may be removed by placing the pipette on the Van Slyke apparatus, opening *X* to connect *A* to *E* and *Y* to connect *C* to *F* and drawing them down into *G*. The fluid is then expelled from *G* to waste, and *G* refilled with water which is passed up into the pipette, this process being repeated until the pipette is clean. Should it become necessary to

empty mercury out of the pipette this is most conveniently done by holding the pipette face downwards over a suitable wide container and withdrawing the plug of cock *X*.

SUMMARY

A special pipette is described for use with the Van Slyke manometric apparatus. It allows storage of reagents out of contact with air for indefinite periods and measurement of any required quantity of reagent into the manometric apparatus without its coming into contact with air.

Synthesis of Glutamic Acid in Animal Tissues*

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In earlier experiments (Krebs & Henseleit, 1932) it was noted that slices of rat liver, suspended in a saline medium containing ammonium salts, often removed more ammonia from the medium than could be accounted for by the formation of urea. The present work arose from a study of the reactions other than the synthesis of urea, which cause a disappearance of ammonia in liver tissue.

EXPERIMENTAL

Analytical methods. Urea was determined manometrically with urease (Krebs & Henseleit, 1932; Krebs, 1942); NH_3 was steam-distilled *in vacuo* according to Parnas and Heller, as described by Krebs (1935), and measured photoelectrically after nesslerization. Amino N was determined with Van Slyke's (1929) manometric nitrous acid method, after deproteinization with trichloroacetic acid and removal of NH_3 . Glutamic acid was determined according to Gale (1945) with the decarboxylase of *Clostridium welchii*, strain S.R. 12. Succinic and α -ketoglutaric acids were determined by the succinic dehydrogenase method (Krebs & Eggleston, 1948a), acetoacetate with aniline citrate (Edson, 1935), and oxaloacetate with $\text{Al}_2(\text{SO}_4)_3$ (Krebs & Eggleston, 1945).

Tissue preparations. Homogenates were made in the glass apparatus of Potter & Elvehjem (1936), and in a similar instrument made from stainless steel. The medium used for the dilution of the tissue was 'phosphate saline without Ca' (Krebs & Eggleston, 1940) or phosphate bicarbonate saline without Ca. Lower rates of reaction were found when Ca was present in physiological concentrations. A medium simulating the ionic composition of liver cells (containing 6 ml. of 0.1 M-CaCl₂, 18 ml. of 0.1 M-MgCl₂, 15 ml. of 0.1 M-K₂HPO₄, 10 ml. of 0.1 M-KH₂PO₄, 10 ml. of 0.155 M-KCl

and 41 ml. of 0.155 M-NaCl) gave rates of about 25% and, when Ca was omitted, of about 90% of those in 'phosphate saline without Ca'. The homogenates were used as freshly as possible. On storage their activity, as measured by their capacity to form glutamic acid, fell fairly rapidly, e.g. by c. 50% when kept in the refrigerator for 24 hr.

Slices were suspended in phosphate saline or phosphate bicarbonate saline.

Adenosine triphosphate (ATP). Ba-ATP (64.7 mg.) was dissolved in 3.3 ml. of 0.1 N-HCl. Ba was precipitated by the addition of 0.1 ml. of M-Na₂SO₄ and the solution was neutralized with 0.15 ml. of 2N-NaOH. Water was added to 5 ml. The precipitate was removed by filtration or centrifugation. The solution contained 0.02 M-ATP.

General. The material was incubated in conical Warburg vessels. The substrates were placed in the side arm and added to the tissue preparation at zero experimental time, usually when the manometers were attached to the water bath. The temperature was 40°. All substrate concentrations mentioned in the tables refer to the final concentrations in the medium. In anaerobic experiments yellow P was placed in the centre well. Quantities of metabolites are expressed in μl . and rates of metabolic processes in the usual *Q* values. When homogenates were used the dry weight for calculating the *Q* values was assumed to be 30% of the fresh weight in the case of liver and 20% in the case of brain cortex, kidney cortex or muscle.

To stop the metabolic processes in homogenates after a specified time the flasks were detached from their manometers and 1 ml. of 2N-HCl/4 ml. suspension was added as quickly as possible. If the analyses could not be carried out immediately the solutions were stored in the refrigerator. Of the acidified suspension usually 1 ml. was used for the determination of glutamic acid, 1 ml. for NH_3 , 3 ml. for succinic acid. If a larger amount of material was required for further analyses, duplicate Warburg cups were set up and the contents were combined after completion of the incubation.

* Preliminary account, *Nature, Lond.*, 159, 808 (1947).