latter subjected to a Reformatsky reaction with zinc and ethyl bromo-acetate, thus effecting condensation, partial dehydration and lactonization simultaneously. The lactone[1] melts at 167–168° (corr.) and reacts positively towards Legal's and Tollens' reagents. It shows the following analytical figures: Calculated for $C_{23}H_{34}O_2$: C, 80.6; H, 10.0. Found: C, 80.4; H, 10.1.

A detailed description of this and other lactones will appear in *The Journal of Organic Chemistry.*

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## THE MAGNETIC PROPERTIES OF CATALASE

RECENTLY a modification of Gouy's method of measuring magnetic susceptibilities has been elaborated in this laboratory primarily for the quantitative determination of free radicals of organic dyestuffs during the process of reduction. The result is an increased sensitivity over existing methods. The method will be described in a paper now in press and may be outlined very briefly as follows.

A long cylindrical vessel with a septum in the middle, dividing it into an upper and a lower compartment, quite similar to one first used by Freed and Casper,[1] and later especially by Pauling and Coryell,[2] is suspended between the pole pieces of an electromagnet. The upper end of the suspending wire is attached to the one pan of a semi-micro balance, which is magnetically damped, very nearly critically. The pointer of the balance is equipped with a scale of 200 divisions readable through a microscope, each line corresponding to about one hundredth of a milligram. The upper compartment of the vessel is filled with a solution, or suspension, of the substance to be measured. The lower compartment is filled with the pure solvent. After switching on the magnetizing current only the maximum deflection on the microscope scale is observed, which is reached in 15 seconds. The significance of each line of deflection is previously calibrated in terms of change in magnetic susceptibility. Repeated readings allow an accuracy, according to conditions, within one or a few per cent., even when the experiment is based on a magnetic pull of, say, one fifth of a milligram. This method has been used for the measurement of the susceptibility of crystallized catalase,[3] suspended in a dilute phosphate buffer. Thus far the measurements have been made under conditions not especially favorable for weighing, *i.e.*, warm and humid summer weather, and they may be worth repeating later on under better conditions. Even so, results could be obtained which were scarcely accessible to the method of direct weighing as used by Pauling and Coryell. Since catalase has four times the molecular weight of hemoglobin, yet not more iron in one molecule than the latter, and the concentration at which a suspension—not to speak of a solution—can be obtained, is limited, the increase in sensitivity over previous methods was essential for these experiments. The result obtained so far is that the magnetic moment of catalase, per gram-atom iron, is 4.64 Bohr magnetons. The probable error, under the unfavorable conditions mentioned, is estimated to be $\pm 0.3$. This value would be close to 4.47 as obtained by Coryell and Pauling for ferri-hemoglobin hydroxide (alkaline methemoglobin), and smaller than for ferro-hemoglobin (5.46) or ferri-hemoglobin (5.8). The magnetic experiments on catalase are being continued.

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1 S. Freed and C. Casper. *Physical Rev.*, 36: 1002, 1930.
2 L. Pauling and C. Coryell, *Proc. Nat. Acad. Sci.*, 22: 159 and 210, 1936.
3 J. B. Sumner and A. L. Dounce, *Jour. Biol. Chem.*, 125: 33, 1938; 127: 439, 1939.

## GLASS ELECTRODE FOR DETERMINATION OF HYDROGEN ION ACTIVITY OF SMALL QUANTITIES OF CULTURE MEDIA

INVESTIGATIONS of changes in pH in controlled cultures necessitate means of determining the pH of relatively small quantities of fluid. It was felt that a system whereby three determinations of pH could be made from as little as 1 ml of fluid would be very advantageous. After reviewing the possibilities of several micro vessels for this work, it was decided that a relatively large durable or condenser type of glass electrode, as described by MacInnes and Belcher,[1, 2] could be used, provided it was modified in some respects and a method developed for using the modified instrument. The results have been extremely satisfactory. The instrument is very stable and rugged. It is easily cleaned without being dismantled. Furthermore, the method of sampling and determination of pH precludes errors which might arise from addition or loss of gases such as $CO_2$.

A glass electrode is made (Fig. 1) with the following limitations and modifications:—The Corning No.

1 D. A. MacInnes and D. Belcher, *Industrial and Engineering Chemistry*, Analytical Edition, 5: 199, 1933.
2 D. A. MacInnes and L. G. Longworth, *Transactions of the Electrochemical Society*, 71: 73, 1937.

.015 glass capillary of the glass electrode should be of a length and inside bore to hold approximately 0.3 ml of fluid. The electrodes which we made averaged about four inches in length. The jacket of the electrode is extended to form a funnel 20 mm high and 25 mm across the top. The space at the bottom of the funnel is ground to accommodate a standard ground tip of a 1.0 ml hypodermic syringe. The hypodermic syringe is of the insulin type—long with a solid plunger, modified as follows: a 1 millimeter hole is drilled through the wall of the syringe, 2 mm above the last graduation and a short groove 2 mm long is cut in the lower end of the solid plunger.

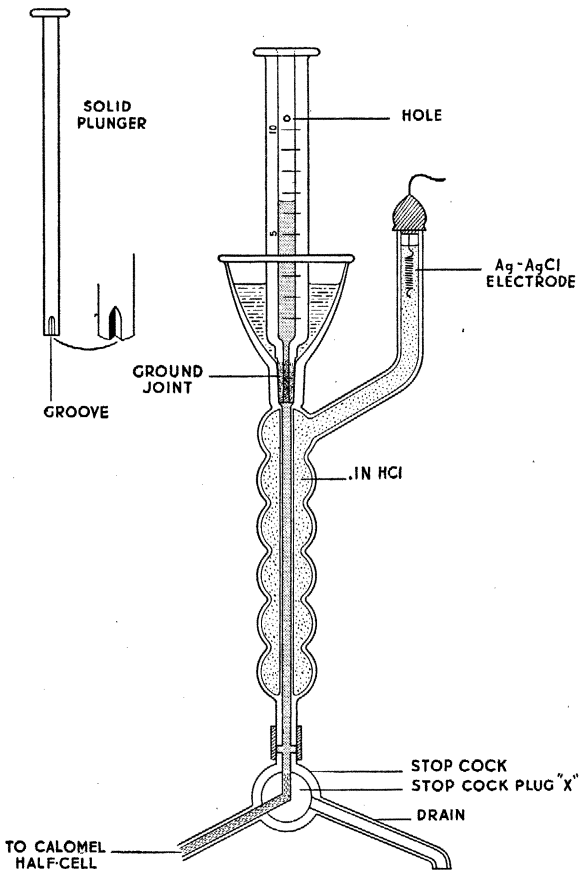


FIG. 1

METHOD OF OPERATION

The glass electrode is set up in an electrically shielded copper box connected by shielded cables to a type #7660 Leeds and Northrup Company pH indicator. After the glass electrode has been calibrated for acetate buffer pH 4.64 it is thoroughly washed out with distilled water. The stop-cock (Fig. 1, X) is then closed so that the distilled water fills the electrode and extends halfway up the funnel.

The material to be studied is introduced into the hypodermic syringe, care being taken to see that the groove in the solid plunger is oriented 180° from the hole in the syringe wall. In the case of pure cultures of protozoa the material is secured through a sterile needle introduced into a vaccine port blown in the side of the culture flask.[3] The needle is removed from the syringe and the syringe tip is introduced through the distilled water in the funnel and seated in the ground joint at the upper end of the glass electrode. This effectively seals off the distilled water in the funnel and places the culture medium in the syringe in direct contact with the column of distilled water in the glass electrode, without the possibility of any air bubbles being formed in the system. The plunger of the hypodermic syringe is now turned 180 degrees until the slot and the hole in the syringe coincide. The plunger may now be withdrawn without exerting pressure on the fluid. By carefully opening the stop-cock (Fig. 1, X) 0.3 ml of the culture medium is allowed to displace the distilled water in the electrode. The amount is determined by following the meniscus on the graduations of the syringe. The stop-cock (Fig. 1, X) is now turned to make a liquid junction and a determination is made. Two more determinations are made with the remaining available fluid. The syringe is then removed and the electrode again thoroughly washed with distilled water.

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[3] G. W. Kidder, *Physiological Zoology*, 14: 209, 1941.

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