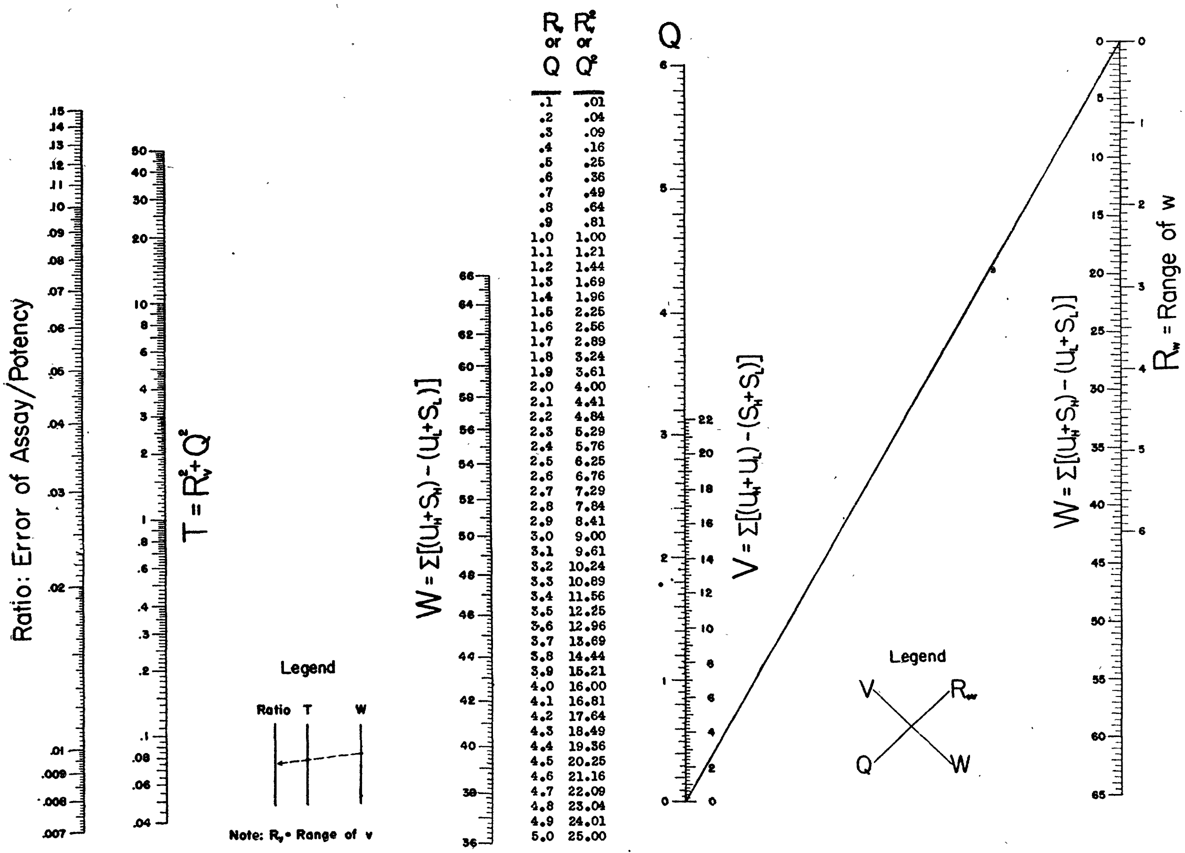

FIG. 2. Penicillin assay nomographs for estimating the error of the assay from the formula

$$\frac{\text{Error of Assay}}{\text{Potency}} = \frac{k}{W}\sqrt{R_v^2 + \frac{R_w^2 V^2}{W^2}}$$

V,W,R_v, and R_w are calculated from the diameters of the zones of inhibition on the two dose, four plate, penicillin assay in which the ratio of doses is 4: 1. A more complete table of squares such as Barlow's "Tables" should be substituted whenever possible for the brief table of squares given between the nomographs. Here k = 1.35, this being ln 4 times the square root of the number of plates all divided by the average number of standard deviations in the range.

variation within the assay. One can not state on the basis of this calculated error of assay that the lot of penicillin was, say, 650 ± 20 units/mg unless the method was so standardized and the results so kept in "statistical control" that any assayists at various laboratories can check each others' results as closely as one assayist can check himself.

The charts and methods can be used in similarly-designed assays of other drugs, *e.g.*, in the Vitamin A assay involving two-dose comparisons of standard and unknown, with four male rats from each litter.

LILA F. KNUDSEN

FOOD AND DRUG ADMINISTRATION,
WASHINGTON, D. C.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MANOMETRIC APPARATUS FOR RESPIRATORY STUDIES OF SMALL ANIMALS

THE equipment described below was designed to aid in an investigation of chronic cyanide poisoning in the rat. It is presented because the method has certain advantages not found in other techniques and is adaptable to use in a variety of biological research problems.

The apparatus is essentially a constant volume manometer with a small pump to continuously circulate the gas (Fig. 1). It is simple and inexpensive and except for the air pump may be easily assembled from ordinary laboratory materials. The experimental animal is placed on a screen in a museum jar of about six liters capacity with the groundglass edge sealed with petrolatum to a piece of plate glass. A hole in the top plate contains a rubber stopper through which pass three glass tubes: a short one just enters the jar, another one reaches about half way down, and the third goes to the bottom of the chamber. Air is circulated through the system by a simple diaphragm pump with glass valves which is operated by a vari-

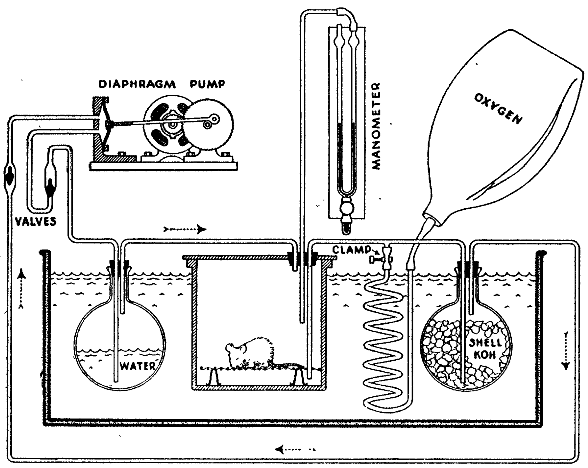

FIG. 1. Diagrammatic sketch of manometric respiration apparatus.

able speed electric motor adjusted to deliver approximately a liter of gas per minute. The air bubbles through a flask of water, enters the top of the animal chamber through the short tube, and is taken out at the bottom by the longest tube. This removal of the bottom layer of gas prevents CO_2 from accumulating in the animal compartment. Carbon dioxide is removed from the air as it passes through the flask containing shell potassium hydroxide, and the dried, CO_2-free gas is then returned to the pump and recirculated. The third tube from the animal compartment leads to a Warburg manometer containing Brodie solution.[1] Changes in the height of this fluid column indicate pressure variations in the system. A T connection to the gas line leads through a coil to a rubber anesthesia bag which contains oxygen for refilling the system. The animal compartment and the flasks are nearly submerged in a constant temperature water bath.

Since the manometer has one end open to the air it is necessary to correct the readings of the fluid column for changes in barometric pressure. This is done with a second manometer, not shown in the sketch, which is connected to a second chamber of approximately the same volume as the total gas space in the first system. This vessel is immersed in the water bath and therefore will compensate for slight temperature changes also.

Before making an oxygen consumption determination, the apparatus is checked for leaks by observing whether it will maintain a positive or negative pressure. The animal is then placed in the chamber in the water bath at 28° C., connections with the air flow line and manometer are made with rubber tubing, and the pump is started. During an initial ten-minute equilibration period the manometer stopcock is closed and the clamp on the line leading to the oxygen bag is left open; since there is a slight positive pressure due to the elasticity of the bag, there is a flow of gas to compensate for any oxygen consumption by the animal. This clamp is closed before manometer readings are started. When the manometer fluid falls to the bottom of the scale more oxygen is admitted by opening the clamp and the fluid is releveled to the 150 mm mark. The system may then be closed and read again within one minute. Since the oxygen is thus quantitatively replaced there is never a significant deficit.

Readings and thermobarometric corrections are made as in the Warburg type of manometer,[1] and a constant for the apparatus may be calculated in the same way if the volume of the system is measured. A simple multiplication of the corrected reading by the constant then gives the volume of oxygen adjusted to normal temperature and pressure.

Since the sensitivity of the system depends upon the gas volume, it is possible to adjust it by altering the size of the animal chamber. The device as illustrated with a volume of about seven liters is sufficiently sensitive to permit readings each minute with an adult rat.

W. A. ROBBIE
P. J. LEINFELDER
DEPARTMENT OF OPHTHALMOLOGY,
COLLEGE OF MEDICINE,
STATE UNIVERSITY OF IOWA

[1] M. Dixon, "Manometric Methods." Cambridge. 1934.

A MAGNETIC STIRRER FOR CONTINUOUS GAS-FLOW APPARATUS

IN the course of growth studies on the alga *Chlorella,* it was found desirable to compare growth in continuously stirred cultures with that in intermittently stirred cultures. In the latter, 5 per cent. CO_2 in air was bubbled through eight flasks in series. To maintain analogous gas conditions, a simple magnetic stirrer was devised which permitted the same stream of gas to flow through the series of cultures. The accompanying diagram (Fig. 1) shows the essential features of the apparatus.

A constant-temperature bath having a shoulder with a glass inset bottom (A) is employed. In the space below the shoulder is a bank of Mazda lamps (B) that may be adjusted in elevation to vary the intensity of illumination. The lamps are cooled by a fan; and the bath temperature is regulated by a thermostatically controlled heater and refrigerator coil (not shown).