Volume Calibration of Apparatus with Standard Ground Joints, Especially Warburg Manometers

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It is now current practice for Warburg manometers to be supplied with interchangeable ground joints between the manometer and its vessel. Consequently it is possible to use any vessel with any manometer. Spare vessels can be kept in stock to replace breakages. But to make proper use of these advantages, the method of calibration must enable the volume of any such combination of vessel and manometer to be at once available. The following method, which has been in use in our laboratory for several years, has proved most practical and useful for this purpose. It is more versatile and convenient than other recently described methods (Schales, 1944; Somis, 1949; Maxwell, 1949). It also has advantages over the method of Allen (1948), which only recently came to our notice.

METHOD

The extra apparatus required consists of a small conical flask (*B*, Fig. 1) of about 10 ml. capacity which is fitted with a ground stopper (*A*) of the standard taper and size used for the manometers. The stopper is provided with a short capillary as shown in Fig. 1, so that with flask *B* it constitutes in effect a small specific gravity bottle.

Mercury is normally used for calibration. The volume (*c* ml.) of the flask closed by its capillary stopper is determined once and for all by weighing it first empty and then filled completely with mercury. The vessels and manometers to be used are then calibrated separately in conjunction with *A* and *B* respectively. For this purpose the manometer vessel (*D*, Fig. 1) is first filled with mercury and stopper *A* is inserted so that its capillary is filled to the top. The volume of mercury so contained is denoted by *v* ml. Now the contribution to the total volume made by the manometer (*C*) is determined. The small flask *B* is filled with mercury and pressed on to the ground joint of the manometer, which should be lightly greased as usual. This action causes the mercury to rise in the manometer capillary side arm until it fills this as far as the T-junction with the U-tube of the manometer, any excess mercury being allowed to flow away down the U-tube. In the usual way (see, for example, Schales, 1944), the main limb of the manometer is filled with mercury from the top stopcock down to the zero mark at which the manometer fluid will normally be levelled in use. We

customarily use the 150 mm. centre calibration on the manometer scale. This total quantity of mercury is then run into a tared beaker and weighed, giving the volume, *m* ml., of the manometer in conjunction with the calibration flask.

Fig. 1. Calibration bottle and a manometer with its vessel (diagrammatic). *A*, stopper of calibration bottle; *B*, flask of calibration bottle; *C*, Warburg manometer; *D*, Warburg manometer vessel. (For other symbols see text.)

The factor for calculation of volume from weight of mercury at the temperature of the respective measurements is taken from one of the published tables.

Calculation

The volume (*V* ml.) of the vessel and of its manometer capillary to the usual zero mark on the manometer limb is given by simple arithmetic

$$V = m - c + v.$$

It is convenient to write on each manometer back the value of (*m* - *c*), expressed in ml., and to keep in the note-book a list of values of *v* (ml.) for the various manometer vessels. The volume of any combination of vessel and manometer can then be worked out instantly.

Theory

In Fig. 1 the broken reference line, *RR*, is drawn at an arbitrary level such that it passes through cross-sections of equal diameter of each of the ground joints shown in Fig. 1. This diameter does not need to be known, but drawing *RR* makes the explanation

clearer. (I wish to thank Dr C. T. Beer for this suggestion.) Reckoning from this reference line, the volumes of mercury (ml.) contained in the shaded areas of Fig. 1 are denoted by w, x, y, z , as shown by the lettering on Fig. 1. The volume of glass below the reference line for the stopper A is g_A , and for the manometer C is g_C . The total volume of the vessel plus manometer capillary is then evidently

$$V = z + (y - g_C).$$

But

$$c = x + w - g_A,$$

and

$$m = x + y - g_C;$$

also

$$v = w + z - g_A.$$

Hence

$$m - c = y - w - g_C + g_A,$$

and by addition $m - c + v = z + (y - g_C) = V$.

Check of accuracy

A set of thirty-six vessels and fourteen manometers has been calibrated both by the usual method

and by the new method described. All estimations agreed within 0.02 ml. except in one instance where a repeat showed that there had been an error due to leakage in the calibration by the older method. This was due to the very large weight of mercury dangling on the end of the manometer which is inevitable in the older method. The repeat agreed exactly with the calibration by the method described above.

SUMMARY

1. A method is described for the volume calibration of vessels provided with standard ground connexions.

2. It is particularly suitable for the calibration of Warburg manometers, which can be used interchangeably with any vessels also calibrated by the method described.

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Studies on the Metabolism of Semen

7. CYTOCHROME IN HUMAN SPERMATOZOA

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It was not for lack of endeavour that the spectroscopic evidence for the occurrence of cytochrome in human spermatozoa remained negative for so long. The repeated failures to demonstrate cytochrome led to much speculation as to the nature of the mechanism involved in the respiration of human semen, the more so since it has been stated that the oxygen consumption of the semen is associated predominantly with the seminal plasma and not with the spermatozoa as such (MacLeod, 1941, 1943; Ross, Miller & Kurzrok, 1941; Zeller, 1941). In contrast to these findings, the results of our studies on ram and bull semen (Mann, 1945*a, b*) have shown that (1) the oxygen uptake of bull and ram semen is due almost entirely to the spermatozoa themselves; (2) bull and ram spermatozoa show, on direct spectroscopic examination, a complete spectrum of cytochrome, with all three cytochromes, a , b and c , clearly discernible; (3) the cytochrome components of spermatozoa undergo oxidation and reduction according to prevailing conditions of aerobiosis or anaerobiosis; (4) spermatozoa contain cytochrome

a_3 which reacts with carbon monoxide to form a compound identical with the carbon monoxide compound of cytochrome oxidase or cytochrome a_3 originally described by Keilin & Hartree (1939) in heart-muscle preparations.

The direct spectroscopic technique, as used for the detection of cytochrome in bull and ram spermatozoa, was found to be of no avail for human semen, mainly, as we suspected, because of the low sperm density of human semen: specimens of normal human semen seldom exceed a density of 100,000 sperm/ μ l., whereas in bull semen the sperm concentration is of the order of 1,000,000 cells/ μ l., and in ram semen it may reach 5,000,000 cells/ μ l. (Mann, 1949). However, a re-examination of the problem concerning the occurrence of cytochrome in human semen was made possible recently through the new spectroscopic technique of Keilin & Hartree (1949, 1950), who discovered that freezing of biological material to the temperature of liquid air sharpens and intensifies 20-fold the absorption spectra of certain haemoproteins including cytochrome. The