tubing of 10 mm. internal diameter. The glass tubing was bent in ∽ shapes and was placed on three sides of the bath. The thermostat head was of the usual design. Fig. 1 illustrates the thermostat diagrammatically. The thermostat was operated with a Sunvic electronic relay and Proportioning Head. The temperature of the bath can be maintained for months within $\pm 1{\cdot}5 \times 10^{-3}°$ with this regulator at 27°. The *heating element* was bare Nicrome wire wound on glass tubing and distributed on three sides of the bath at the bottom; the total resistance of the heating element was 4 Ω draining 3 amp. at 12 V. No trouble was encountered on account of lack of insulation of the heating element at this low voltage. Ordinary distilled water was used in the bath.

*The stirrer* was a centrifugal type driven by a small 24 V. motor (see Fig. 2). This stirrer was found very efficient without causing troublesome vibrations. A drop of permanganate placed under the funnel of the stirrer appears through the top jets within a few seconds and stains the water of the bath uniformly in about 10 sec. No difference in temperature at the top and bottom of the bath could be detected with a Beckmann thermometer.

*Maintenance of bath.* In the last instance this bath has been in constant operation day and night for 1 year without change of water. No preservative was added to the water, but a layer (about 1 cm. thick) of liquid paraffin was poured over the surface. The anaerobic condition suppressed completely the growth of moulds, etc. Before the use of liquid paraffin the water had to be changed about every month in spite of various preservatives added.

It was found that a layer of benzene (free of S compounds) between the Hg column and contact needle prevented oxidation of the surface of Hg and ensured a clean contact and smooth running of the thermostat. For the continuous replacement of this benzene the cup of the thermostat head was connected through a small side arm with a reservoir.

*The micropipette* used for the delivery of the water drops was designed by Dr B. W. Robinson (see Fig. 3). It consisted of a calibrated micrometer screw which moved a piston in a snugly fitting barrel filled with mercury. A Pyrex capillary (1·5 mm. bore) bent in ∩ shape and drawn out at the end into a fine tip was fixed air tight to the metal parts with an expandable rubber washer. The metal parts were made of stainless steel.

When the tip of the Pyrex capillary has the correct shape shown in Fig. 4 the water drops delivered under the surface of the *o*-fluorotoluene hang from the tip, otherwise the drops creep up on the outside of the capillary and lead to inaccuracy of delivery. The pipette assembly was clamped in a rack and pinion.

While the construction of this pipette requires precision machining it has great advantages over the one described by Keston, Rittenberg & Schoenheimer (1937–8) as no suction is required when filling. It can be filled with water sufficient for the delivery of at least three drops and can easily be rinsed between successive determinations with 2–3 cu.mm. of the new water sample. The accuracy of delivery is better than 1% in the region of 10 cu.mm. The water drops are delivered under the surface of the *o*-fluorotoluene and are detached by the surface tension of the latter on raising the pipette.

## REFERENCE

Keston, A. S., Rittenberg, D. & Schoenheimer, R. (1937–8). *J. biol. Chem.* **122**, 227.

## APPENDIX 2

# Preparation of Solid Samples for Assay of $^{14}C$

By G. POPJÁK

All radioactivity measurements reported in the previous investigation were carried out on solid samples (cholesterol, cholesterol-digitonide, fatty acids, Pb salts and Na salts of fatty acids). 'Infinite thickness' samples, i.e. 25 mg. of material/sq.cm. were prepared on disks of identical geometry. The measurements were carried out with a He-filled bell-shaped Geiger-Müller counter which had a thin mica window. Under our conditions of counting a solid sample which contained $10^{-3}$ μc. of $^{14}C$/mg. of substance gave approximately 1800 counts/min. in an 'infinite thickness' sample of 2 sq.cm. area. The background of our apparatus was 8–10 counts/min. The calibration of the counter was carried out with a sample containing known amounts of $^{14}C$.

When sufficient material is available, as in the investigation described, assay of $^{14}C$ in 'infinite thickness' samples has the great advantage that the radioactive counts obtained on the various samples are linearly proportional to the specific activities and hence the $^{14}C$ contents may be expressed without self-absorption corrections. Further, the assay of $^{14}C$ directly on organic compounds of high C content avoids the undesirable dilution when the organic compounds are first combusted and the $CO_2$ obtained is collected as $BaCO_3$. The assay of $^{14}C$ on organic materials has the added advantage that the samples may be stored in common atmosphere, whereas with $Ba^{14}CO_3$ special measures are required to avoid exchange with atmospheric $CO_2$.

Several methods have been described for the preparation of solid samples for the assay of $^{14}C$ (Calvin, Heidelberger, Reid, Tolbert & Yankwich, 1949) to suit individual requirements. The technique to be described has been used in this Institute for the assay of $^{14}C$ in a variety of crystalline and amorphous compounds (apart from those named above) and found very satisfactory.

*Mounting material.* Disks made of plastic material (Perspex or Polythene) and moulded to the shape shown in Fig. 1*a* were used. Two types were employed: (i) with solid, and (ii) with perforated, bottom.

*Preparation of samples on solid disks* ('pellet' technique). The solid disks were used for preparation of 'infinite thick-

ness' samples from solid substances (amorphous and crystalline) when plenty of material was available. Somewhat more than the weight required to give 'infinite thickness' of the finely powdered substance was first spread evenly over the area of the disk with a spatula, then the material was compressed into a pellet with a stainless steel press (Fig. 1*b*), which just fitted into the sample area of the disk. The edge of the disk was wiped clean while the press was still in position. When samples of sticky material (e.g. cholesterol, cholesteryl acetate and some Na salts of fatty acids) are being prepared, the press is gently rotated before lifting off the disk; this prevents the pellet getting stuck on to the metal. With other substances, like $BaCO_3$, cholesterol-digitonide, haemin, choline derivatives, Pb salts of fatty acids, the above precaution is not necessary. Samples of free fatty acids (solids) may be prepared by first smearing the material with a spatula as evenly as possible over the entire surface of the disk, and then levelling off any unevenness by the mere touch of a microflame (from a glass capillary jet) without melting the whole sample. Disks of two sizes were used: (1) with a sample area of 2·0 sq.cm.; and (2) with a sample area of 1·2 sq.cm. The ratio of counts obtained with these two disks was exactly 2:1·2. These sample areas are much smaller than those used by many American workers, who also employ counters with windows of 5–7·5 cm. diameter. Our counter had a window diameter of only 2·33 cm. The low background count (8–10 counts/min.) of such a counter is a good recommendation for its use.

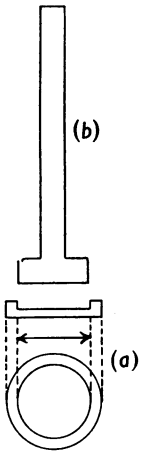


Fig. 1.

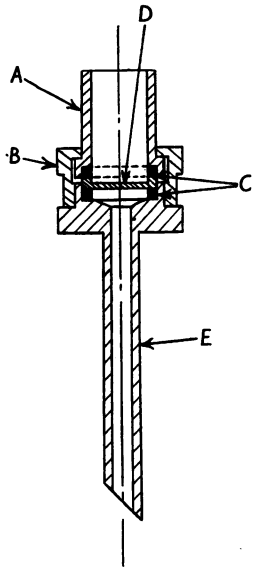


Fig. 2.

Fig. 1. (*a*) Shape of plastic disks used for preparation of samples for assay of $^{14}C$. (*b*) Stainless steel press used for 'pellet' technique.

Fig. 2. Small demountable Büchner funnel made of stainless steel for collection of precipitates for assay of $^{14}C$. *A*, funnel; *B*, ring for clamping funnel to lower portion; *C*, neoprene washers; *D*, perforated plastic filter disk fitted with filter paper; *E*, tube to fit into filter flask.

*Preparation of samples on perforated disks.* The perforated disks, which had the same dimensions as the solid ones, were used with a filter assembly shown in Fig. 2. Filter paper, punched slightly larger than the sample area of the disk, was first pressed into the disk with the tool shown in Fig. 1*b*. The disk, thus prepared, after being dried and weighed was clamped into the filter. The whole assembly is equivalent to a small demountable Büchner funnel from which the filter pad with the assay material can be taken out and placed under the counter. The substance to be assayed (e.g. cholesterol-digitonide, $BaCO_3$, etc.) was first ground in a test tube with a glass rod to a fine powder and suspended in a suitable medium (ethanol, ethanol-ether, methanol, or any other medium in which the compound and sample holder were insoluble). The suspension was then transferred into the filter and first allowed to settle for 1–2 min., then suction was applied. When the suspension medium was filtered through, the funnel of the filter assembly was removed and the suction continued under an infrared lamp until the material became dry. The disk then could be removed and the $^{14}C$ assayed. The counts obtained on duplicate samples usually agreed within 2–3%, and with the 'pellet' technique within the statistical error of counting.

## REFERENCE

Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. & Yankwich, P. E. (1949). *Isotopic Carbon. Techniques in its Measurement and Chemical Manipulation.* New York and London: John Wiley & Son; Chapman & Hall.