(which has been encountered with many RNA's) was the clean separation from polysaccharides, and, although it is possible to reduce glycogen contamination of liver preparations by starving the animals for 24 hr. before the experiments, the RNA's could be separated by the 2-methoxyethanol technique without either perfusing the liver or starving the animals. This particular two-phase system may not always be successful, and an example of this is given in the attempted isolation of RNA from Rous tumour, but there is little doubt that other effective systems could be found. The extraction of hyaluronic acid into the organic layer suggests that similar two-phase systems may be of value in fractionating polysaccharides.

The separation of RNA's from mammalian tissues by treatment with phenol indicates that in the main these poly-acids cannot be attached to the proteins electrostatically. It seems reasonable to assume that hydrogen bonds exist between the bases of the RNA's and the proteins concerned, and that these bonds are broken by the phenol. No doubt the solvent properties of phenol are important in the separation, as a considerable amount of protein can be precipitated from the clarified phenol layer by addition of sodium acetate and ethanol.

SUMMARY

1. Ribonucleic acids have been prepared from mammalian tissues by extraction with phenol and water at room temperature.

2. Deoxyribonucleic acids remain completely insoluble under the conditions used.

3. Ribonucleic acids have been separated from polysaccharides by extraction with 2-methoxyethanol from potassium phosphate solution.

4. Pancreatic ribonuclease was inactivated by the same phenol treatment.

The author thanks Professor A. Haddow for his interest, Dr R. J. C. Harris for supplying the Rous tumour and Miss G. E. Adams for technical assistance.

This investigation has been supported by grants to the Royal Cancer Hospital and the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund, and the National Cancer Institute of Health, United States Public Health Service.

REFERENCES

Beale, R. N., Harris, R. J. C. & Roe, E. F. M. (1952). *J. chem. Soc.* p. 1034.

Grinnan, E. L. & Mosher, W. A. (1951). *J. biol. Chem.* **191**, 719.

Jeanloz, R. W. & Forchielli, E. (1951). *J. biol. Chem.* **190**, 537.

Kay, E. R. M. & Dounce, A. L. (1953). *J. Amer. chem. Soc.* **75**, 4041.

Kirby, K. S. (1955). *Biochim. biophys. Acta*, **18**, 575.

Lea, C. H., Hannan, R. S. & Rhodes, D. N. (1951). *Biochim. biophys. Acta*, **7**, 377.

Magasanik, B. (1955). In *The Nucleic Acids*, vol. 1, p. 373. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.

Meyer, K. H., Fellig, J. & Fischer, E. H. (1951). *Helv. chim. acta*, **34**, 939.

Volkin, E. & Carter, C. E. (1951). *J. Amer. chem. Soc.* **73**, 1516.

Westphal, O., Lüderitz, O. & Bister, F. (1952). *Z. Naturf.* B, **7**, 148.

A Windowless Flow-type Geiger Counter for the Assay of Solid Materials Containing Soft β-Emitting Isotopes

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(*Received* 22 *April* 1956)

Although gas-counting and scintillation-counting methods are more efficient than solid-sample counting methods for isotopes emitting only soft β-particles, there are many occasions when measurement of the radioactivity of a solid sample, with a suitable Geiger or proportional counter, constitutes a perfectly satisfactory and sufficiently sensitive method of assay. The method also has much to recommend it from the point of view of speed, simplicity, and the ability to recover the sample unchanged after assay.

The thinnest mica windows on Geiger counters commercially available in this country absorb over 50% of the β-particles from ^{35}S or ^{14}C, and do not permit the passage of ^{3}H β-particles at all. By the use of a windowless counter the sensitivity with which ^{14}C or ^{35}S may be detected and measured in solid samples can, therefore, be at least doubled, and the method is theoretically also applicable to the measurement of ^{3}H.

Windowless counters for solid samples may be of two main types: demountable, and continuous gas

flow. In the first type the sample is introduced into the counter, which is then reassembled and refilled with a suitable gas mixture. In 1942 we carried out experiments with this type of counter, for measuring ^{35}S, but decided that the increased sensitivity obtained was more than counterbalanced by the loss of time involved in refilling the tube before each successive sample could be assayed, and the variations in the characteristics of the tube produced by small changes in the composition and pressure of the gas filling. The method was therefore abandoned.

In the second type, the filling gas is kept continuously flowing through the counter tube at a pressure slightly above atmospheric, and successive samples are introduced by a mechanism involving the minimum disturbance of the tube. Any air admitted with the sample will alter the characteristics of the tube, and must be flushed out by the filling gas before counting can be commenced.

For the successful operation of a flow-type counter it is essential that the composition of the gas mixture (usually helium and a suitable quenching vapour) shall not vary. This may be achieved by the use of a cylinder of premixed gas, but since such gas mixtures have not until now been readily available in this country, quenching vapour is usually incorporated by bubbling the helium through a liquid of suitable vapour pressure before passing it into the counter tube.

We have used this method with a variety of quenching agents, including water, methanol, ethanol and ethyl formate. The water-vapour pressure was reduced to about 1 cm. by using 50 % (v/v) sulphuric acid in the bubbler, and the organic vapours were obtained at suitable pressures by cooling the bubbler in a Dewar flask containing water and crushed ice. Satisfactory operation was achieved with any of these quenching agents, but the best and most readily reproducible voltage characteristics were obtained with cooled ethanol, with a depth of liquid in the bubbler of not less than 6 in.

Gas-flow counters based on this general principle have been constructed by a number of workers (e.g. Eidinoff & Knoll, 1950; Garrow & Piper, 1955) and have proved exceedingly useful where a somewhat higher sensitivity than that obtainable with a thin-mica-window counter was desirable, without going to the trouble of gas counting. However, these have all suffered in greater or lesser degree from the failing that some time must be allowed to elapse between starting the gas flow and commencing the first assay, and between assaying successive samples, to ensure complete elimination of all the air originally present in the tube or introduced with the samples. For this reason we decided to incorporate into our counter a 'preflushing' device similar to that used in the Tracerlab SC16 tube, whereby each sample is preflushed with the filling gas in a separate chamber before being transferred to the counter, and this transfer is effected without any significant admission of air to the tube. By this means, the initial waiting period has been reduced to about 20 min., and the waiting period between samples to 30 sec. Assays can therefore be made as quickly as on a conventional thin-window counter. In the authors' opinion this makes the increased complexity of the construction well worth while. This counter has been in use for several years, and a brief description of an earlier model was given by Blow & Francis (1953). Since then a number of requests have been received for more detailed information, and an improved model has been designed. In earlier models the passage of gas was uninterrupted during the transfer of samples from the preflushing position to the counting position, but this entailed a less positive seal between the open end of the counter and the base plate, with a consequent risk of slight air leaks into the tube. The new design allows temporary interruption of the gas flow during the transfer, which results in a slight increase of pressure within the tube during this process and helps to prevent influx of air.

CONSTRUCTION

Basically, the apparatus (Fig. 1) consists of two circular plates, *C*, mounted face to face; the upper plate carries the counting chamber, *D*, and the lower one three planchet holders, *E*. By rotating the lower plate each planchet holder in turn is brought under the counter.

The upper plate is of stainless steel (6 in. $\times \frac{5}{8}$ in.) and the lower of brass (5 in. $\times \frac{1}{8}$ in.); the two are held together under slight pressure by three spring-loaded steel balls, *F*, which positively locate each station by means of dimples in the lower surface of the lower plate. The plates are mounted horizontally on a cast base, the upper plate being fixed and the lower one free to rotate about the vertical axle, *G*. The planchet holders are mounted in the upper face of the lower plate in three recesses which are equi-spaced on a pitch circle of diameter 2·5 in. Each holder carries an O-ring seal pressing firmly against the lower face of the upper plate when the holders are raised by the screw-lift mechanism, *J*, which operates by a nut on the centre screw (8 threads/in., square form) turned by the lever, *K*; the screw is restrained from turning by a small keyway in the upper plate. The stem of each planchet holder passes through a gasket, *L*. The upper plate is drilled with two holes, in positions corresponding to two of the planchet holders. The counting chamber is mounted over one hole and hermetically sealed in position by O-ring gaskets, *M*, and the other gives access to a second planchet holder. The upper plate also carries a small glass bubbler, *N*, containing a silicone vacuum-pump oil.

The counting chamber is of the bell type, made of stainless steel, bored out to $\frac{3}{4}$ in. diameter and polished, open at the lower end where it fits into the mounting, and closed at the upper end by a Distrene (polystyrene) insulator, *O*, firmly pressed into the tube. A brass boss, *P*, is sealed into the

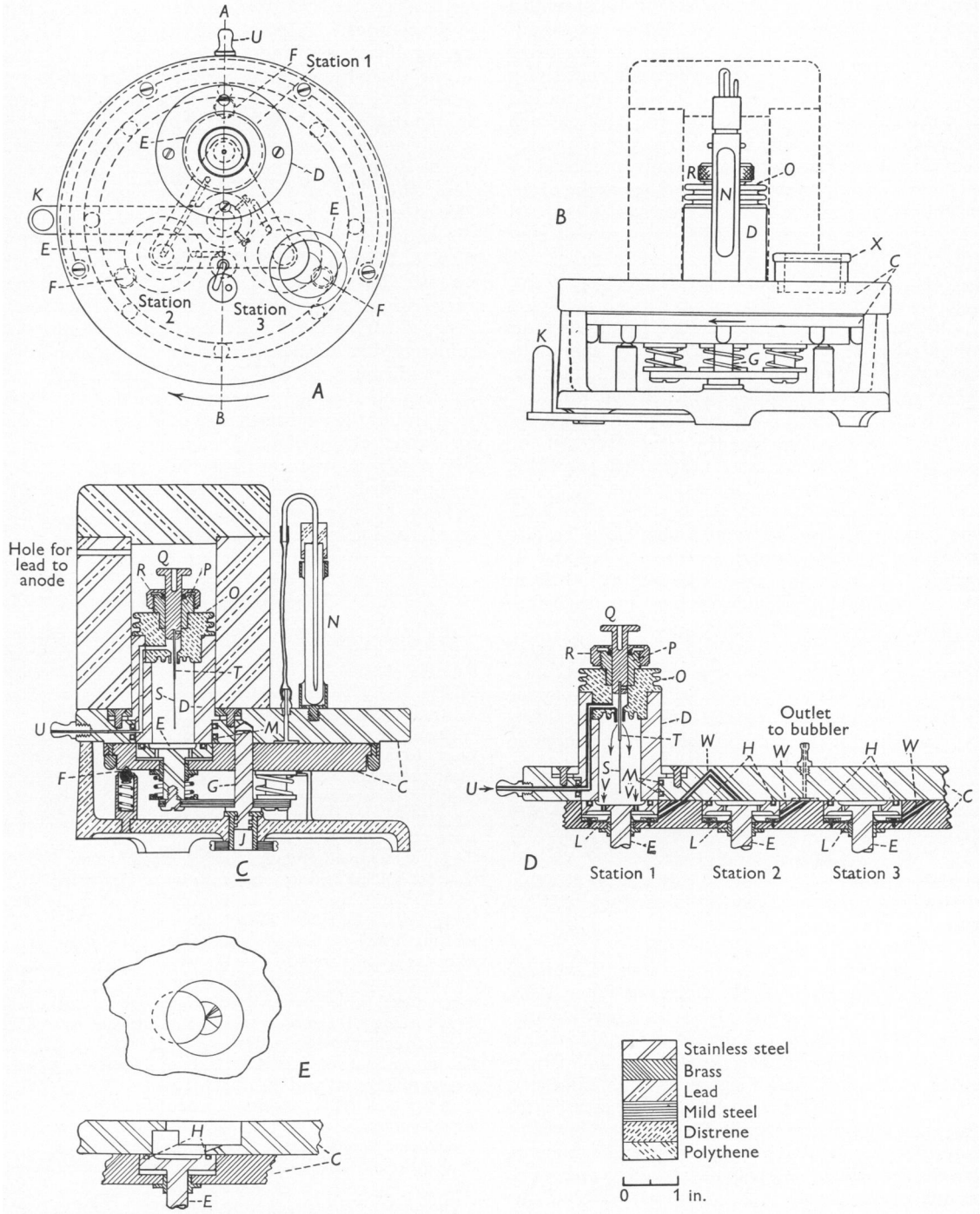

Fig. 1. Windowless flow-type Geiger counter. *A*, Plan view; *B*, general elevation; *C*, section through *A*–*B* of plan view (*A*); *D*, section between stations to show the path of gas and the rubber seals on the shafts of the planchet holders; *E*, loading aperture seen in plan and sectional views. Reference letters are identified in the text.

insulator, and this in turn carries a $\frac{5}{16}$ in. brass rod, *Q*, fixed and sealed in position by a screw cap, *R*, and O-ring gasket. The tungsten-anode wire, *S* (0·1 mm. diameter), is sheathed by a copper tube, *T* (diameter $\frac{1}{16}$ in.) to which it is soldered at its upper end. This is held in the rod, *Q*, by a small grub-screw. By this means the whole anode assembly is easily taken apart for cleaning and changing. The anode is terminated at its lower end, $\frac{3}{8}$ in. above the planchet holder, by a glass bead 0·02 in. in diameter.

The helium gas, after passing through dry ethanol, as described above, is admitted to the counting chamber via a channel within its wall (at *U*) and the insulator carrying the mounted anode. The gas escapes around the planchet through twin orifices, *V*, in the base of the planchet holder and thence via a series of drill holes, *W*, in the plates, as shown in the diagram of the section between stations. The opening into this channel is sealed by the planchet holder in the lowered position during the transfer of samples. The gas channels lead the gas issuing from the counting chamber over and around the second planchet holder (the preflushing position) and finally out through the bubbler. This bubbler serves the triple purpose of maintaining a positive seal on the outlet of the gas system, maintaining a pressure slightly above atmospheric within the system and enabling the rate of flow of gas to be observed.

OPERATION

The helium gas is turned on and adjusted to give a flow of about 4 bubbles/sec. The first sample is loaded into the planchet holder facing the aperture in the top plate. The lower plate is rotated through 120° and the second sample loaded into the second holder, which is now opposite the opening. The turntable is again rotated; a third sample is loaded in the same way, the lever is operated to raise the planchet holders and complete the sealing of the chamber and the loading aperture is closed with a Perspex cover (*X*).

After approximately 20 min. the gas flow may be reduced to 1 bubble/sec. and counting commenced. To change samples, the lever is operated to lower the planchets and the turntable is again rotated through 120°. This brings the second (preflushed) sample under the counting chamber, the third into the preflushing position and the first into the loading position, from which it may be removed and replaced by a fourth. The lever is again operated to raise the planchet holders and seal the base of the counter; provided that the second sample has been preflushed for not less than 1 min., counting may be recommenced immediately.

RESULTS

The counter has a threshold of 1150 v, and a plateau of 200–500 v, according to the length of time for which the gas has been flowing through the counter. The average slope of the plateau is usually about 2 %/100 v. The pulses may be fed directly into any suitable conventional recording unit; the results quoted have actually been obtained with a Panax 100C combined EHT and scaler unit.

No absolute measurements have been made of the efficiency for ^{14}C or ^{35}S, but samples containing either of these isotopes, mounted on nickel planchets (G.E.C. Ltd.) of 1·76 cm.^{2} area, give count rates three to four times that when the samples were counted under a commercial mica-window Geiger counter (G.E.C. type EHM2S, or 20th-Century Electronics type EW3H, window thickness about 2 mg./cm.^{2}). The use of the windowless counter for determination of tritium is discussed, and an estimate of its efficiency given, by Banks, Crawhall & Smyth (1956).

The background count rate, when the chamber is surrounded by 1 in. of lead, is 4–6/min.

SUMMARY

1. The construction of a windowless Geiger counter for the assay of soft β-emitting isotopes is described.

2. The counting gas mixture is arranged to flush air from a second sample while the first is being counted.

3. The counter has a low background count rate; for ^{14}C or ^{35}S its efficiency is three to four times that of commercial thin-window Geiger counters, and it may also be used for measuring ^3H.

REFERENCES

Banks, T. E., Crawhall, J. C. & Smyth, D. G. (1956). *Biochem. J.* **64**, 411.
Blow, L. W. & Francis, G. E. (1953). *Biochem. J.* **53**, xxii.
Eidinoff, M. L. & Knoll, J. E. (1950). *Science*, **112**, 250.
Garrow, J. & Piper, E. A. (1955). *Biochem. J.* **60**, 527.

Some Techniques in the Assay of Tritium

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(*Received* 20 *March* 1956)

Schoenheimer & Rittenberg (1935) discussed the possibility of using an uncommon isotopic form of an element as a tracer in biochemical research, and introduced methods for using the stable isotope of hydrogen, deuterium, as a label, which they applied in the study of fatty acid metabolism. These workers, and many others, have since applied the technique of deuterium labelling to many problems of metabolism in a wide variety of fields. Recently a radioactive isotope of hydrogen, tritium, has become readily available. Radioactive isotopes are in general more valuable than stable isotopes as biochemical labels, as the methods for assay of radioactive isotopes are more sensitive than those