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A Simple Technique for Counting Milligram Samples of Protein Labelled with ^{14}C or ^{35}S

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Radioactive sulphur and carbon have been extensively used in the investigation of protein metabolism. In the study of protein-malnourished children in Jamaica, one of us (J.G.) wished to use ^{35}S as a tracer element and for this purpose a counting apparatus was needed which would fulfil two requirements: (1) It must be capable of measuring ^{35}S activity of the order of 10^{-11}C in very small samples of tissue since, for example, a needle biopsy of liver yields about 5 mg. of tissue of which about 1 mg. is protein. (2) It should be as simple as possible to operate and maintain.

By a combination of a gas-flow counter, a thin sample film technique and a compact scaler—E.H.T.—timer unit, both requirements have been reasonably well satisfied. No part of the technique is essentially original; the merits of a gas-flow counter are well discussed by Tait & Haggis (1949), a thin film technique has been described by Fager (1947), and the scaler unit is based on an A.E.R.E., Harwell, design and was made at the National Institute for Medical Research. A similar scaler, type 1221b, with separate E.H.T. unit is now manufactured by Ericsson Telephones Ltd., Beeston, Nottingham.

The counting gas is helium, which is bubbled through absolute ethanol over sodium sulphate, and then again through ethanol at 0° . The gas passes slowly through the counter at a pressure of about 4 cm. on an alcohol manometer, and then out through a mercury trap. The pulses from the anode of the counter are passed to a probe unit (A.E.R.E., Harwell, type 1014a) which feeds the scaler.

The shape of the counter is shown in Fig. 1. It is made of brass, nickel-plated, and is cylindrical with

a concentric anode wire, a construction which provides a uniform field and a small plateau slope. The cathode diameter is chosen to provide a convenient working voltage, and the anode wire length is just sufficient to give the maximum counting rate from the sample. The anode wire is made of tungsten and is supported at each end, so

Fig. 1. Longitudinal section through gas flow counter.
Scale 1:4.

that a very thin wire can be used and a low starting voltage and long plateau are obtained. The sample is introduced at the side and is arranged within the sensitive volume of the counter. Although sample changing is easier with the end-window Geiger-Müller counter, that type of counter has a lower counting efficiency. In the present counter, large areas of insulating material and polythene planchets have been avoided, as they may give rise to static effects which distort the field.

The biopsy sample is homogenized in saline and the protein is precipitated with 5% (w/v) trichloroacetic acid, washed, extracted successively with ethanol, acetone and ether to remove fat and dried at 37°. The resulting powder is dissolved in N-NaOH to give an approximately 5% (w/v) solution. A circle of lens paper (3 cm.²) is placed on a metal planchette and 20–50 mm.³ of the protein solution are pipetted on to it. The paper is immediately and uniformly wetted and allowed to dry in air at room temperature. When dry it is firmly stuck to the planchette by the sample and is ready for insertion into the counter. Samples prepared in this way give counting rates which are reproducible within 5%. The planchettes are rhodium-plated to facilitate cleaning, and are rimless.

RESULTS

The counter described above had a plateau between 1250 and 1450 v with a slope of 2.4%/100 v. At the working voltage of 1350 the background count rate was 7/min. Using a 1 mg. sample prepared in the manner described above, about 40% of the total disintegrations were counted.

Figures from an animal experiment may illustrate the use of this instrument in comparison with thin end-window and gas counting.

A rabbit weighing 3 kg. was given 50 μ C of ¹⁴C as acid-hydrolysed algal protein. Two weeks afterwards a biopsy of liver was taken, and 1 mg. of the liver protein on the flow counter gave 16 counts/minute, of which 7 were background. A similar sample of protein by gas counting (Bradley, Holloway & McFarlane, 1954) gave 36 counts/minute, of which 18 were background.

It can be shown that the statistical accuracy of counting is improved by increasing the ratio of total counting rate to background counting rate and it is impracticable to work with this ratio much less than two; the counting rate should also be as high as possible, in order that counting can be accomplished within a reasonable time. In the above experiment it can be seen that the ratio of total counting rate to background counting rate is more favourable in the flow counter; the counting rate, however, is greater with the gas counter. Thus the results obtainable by these two methods are approximately the same with 1 mg. samples.

An end-window tube with a window thickness of 1.7 mg./cm.² would not be able to measure accurately the activity in 1 mg. of this protein. If the tube had a background count of 7/minute, it would require about 4 mg. spread as a thin film or 24 mg. as an infinitely thick film to provide the same counting rate as with the flow counter. The value of the flow counter, therefore, lies in its simplicity and sensitivity in counting small samples.

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