GAS PROPORTIONAL COUNTING COMPARED WITH LIQUID SCINTILLATION COUNTING FOR ASSAYING TRITIUM IN BIOLOGICAL MATERIALS

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Gas proportional counting is a simple and accurate method for determining tritium in biological materials and is suitable for clinical and experimental purposes. Samples of tissue, blood, feces, and urine may be analyzed without pretreatment, and the counting procedure is independent of the consistency and composition of the materials. Amounts from 0.001 mg to 75 mg may be counted with equal efficiency (40%). Good agreement was found between results of gas proportional counting and liquid scintillation counting in determining tritium activity in urine. On counting nonhomogeneous biological materials the precision of gas proportional counting is thought to be better than the precision of liquid scintillation counting because radioactivity may be wasted during complicated pretreatment procedures.

Difficulties are often encountered with determining tritium activity in biological materials by liquid scintillation counting. Factors like quenching, chemiluminescence, phosphorescence, and lack of solubility of the material studied in the scintillator may disturb counter efficiency from one assay to another and demand inconvenient corrections. Pretreatment of material with strong base until complete disintegration, and distilling of tritium water from the material may be necessary to gain reproducible counts.

None of these disadvantages are present with gas proportional counting, and this method may be preferable for counting tritium activity in nonhomogeneous biological matter especially if only small amounts of material are available for assay.

METHOD

The technique was introduced by Wilzbach, et al (*1*) as a zinc hydrogen cracking process in which tritium in organic matters is converted to a gas consisting mainly of hydrogen and methane. The gas mixture is expelled into an evacuated ionic chamber and the activity counted. Hasan (*2*) found that admixture of nitrogen, sulphur, halogens, and unreacted water from the cracking process interfered with operation of the counter but satisfactory results were obtained by addition of sodium carbonate into the reaction mixture.

The gas proportional counting procedure. An aliquot of biological material is weighed or pipetted into an ampoule (200 × 9 mm) of supremax glass (Jena) which is closed at the bottom with an easily breakable tip. After drying the material, 2 gm of zinc powder and 0.5 gm of sodium carbonate are added. The ampoule is closed by fusing and heated for 3 hr at 640°C.

After cooling, the ampoule is placed in a glass apparatus (Fig. 1) suitable for transferring the tritium gas. The tip is broken by rotation of the introduction chamber (K), and the gas is allowed to expand into the previously evacuated counter tube which is connected afterwards to the electronic equipment used for counting the radioactivity.

RESULTS

A plateau (Fig. 2) on the gas proportional counter (FZ 50 P, Frieseke and Hoepffner) is obtained at 2,900–4,300 volts with a slope of 0.7%/100 volts. The precision of the gas proportional counter was tested by counting an aqueous solution of tritium-labeled folic acid with an activity of 1 nCi (2,200 dpm)/ml. The mean value of 12 determinations was found to be 882 ± 32 s.d. Efficiency of the counter was calculated as (822/2,200) × 100 = 40%. No significant differences in precision were observed between counts on different days. Neither the volume nor the presence of urea up to 75 mg per assay

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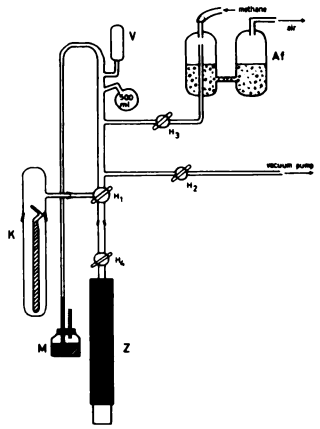

FIG. 1. Schematic of apparatus for transfer of tritium gas. K is chamber for introduction of ampoule, M and V are manometers, Z is counter tube, Af is double chamber with paraffin oil for methane inlet, and H_1, H_2, H_3, and H_4 are inlet valves.

changed the precision of the gas proportional counter (Table 1).

On counting 1 nCi of tritium (^{3}H-folic acid) mixed with samples of urine, feces, plasma, and pieces of intestine, counts were alike and not different from counts of a reference of tritium in water (Table 2). On examination of larger quantities of biological materials (plasma, intestine), however, precision deteriorated.

Counting of tritiated oubain and mannitol in aqueous solution and dissolved in blood plasma gave results identical to those obtained on tritiated folic acid in water and in blood plasma (Table 3). A comparison was made between gas proportional counting and liquid scintillation counting to determine the activity of tritium added to urine. The liquid scintillation counter used was a Beckmann LS 233, and a mixture of dioxane and naphthalene with "PPO" was used as the scintillator. Correction for the quenching effect was carried out by recounting all samples after adding tritiated water as an internal standard. On counting tritium activity in samples of urine with both gas proportional counting and liquid scintillation counting, a correlation coefficiency of 0.96 was found between the two methods (Fig. 3).

DISCUSSION

In contrast to liquid scintillation counting, tritium activity in nonhomogeneous biological material may be determined by gas proportional counting without time-wasting correction for nonuniform efficiency because the efficiency of the gas proportional counter is independent of the composition and the consistency of the test material. Tritium activity on electrophoretic strips has also been successfully analyzed by gas proportional counting. Moreover, gas proportional counting has a higher efficiency (40%) than liquid scintillation counting, which when counting organic materials may display an efficiency of only 10–15%.

The efficiency of gas proportional counting depends on the dimensions of the counter tube. With a counter tube of a smaller volume (FZ 35 P) an efficiency of 32% was found. Probably efficiency may be enhanced by increasing the volume of the counter.

In contrast to the liquid scintillation counting method no tritium activity is wasted during complicated pretreatment procedures. Once a sample is placed and heated in the ampoule no further pretreatment of the material is necessary to gain reproducible counts.

A fairly stable counting condition is insured during gas proportional counting because the counter has a long plateau with a low slope. The composition of the gas mixture is not known in detail. Liberated nitrogen, sulphur, halogens, and unreacted

$$\text{plateau slope} = \frac{14671 - 13180}{4300 - 2900/100} \times \frac{100}{14671 + 13180/2} \text{ \%/100} = \underline{0{,}7 \text{ \%/100 V}}$$

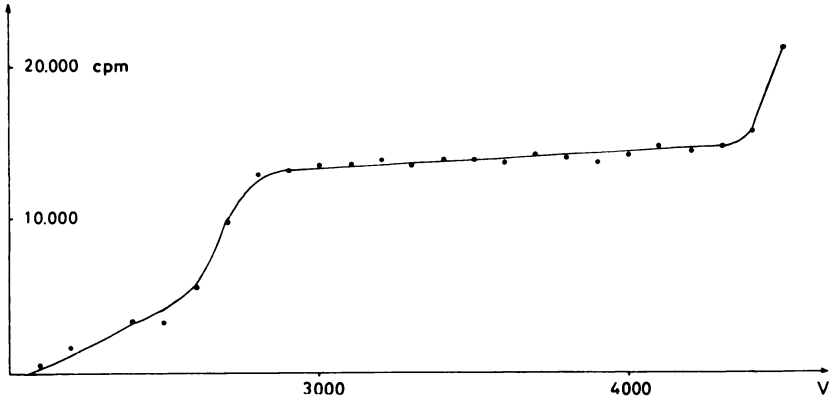

FIG. 2. Characteristic curve of gas proportional counter tube.

TABLE 1. GAS PROPORTIONAL COUNTING OF 1 nCi ^{3}H-FOLIC ACID IN SOLUTIONS OF UREA OF VARIOUS CONCENTRATIONS

Volume (ml)	Urea (mg/ml)			
	0	1	5	25
½	963	994	976	1006
1	970	996	952	994
2	992	957	966	1049
3	943	908	995	1061

cpm corrected for background.

TABLE 2. GAS PROPORTIONAL COUNTING OF 1 nCi ^{3}H-FOLIC ACID IN VARIOUS BIOLOGICAL MATERIALS

Material	Wet sample weight (mg)	Dry sample weight (mg)	^{3}H activity in % of reference	s.d.
Urine	1,013	18	98.4	2.8
Urine with albumin (1%)	1,015	23	100.1	2.5
Feces	300	45	95.4	2.8
Plasma	511	21	102.8	1.7
Plasma	1,022	42	103.5	2.6
Rat intestine	50	10	95.8	4.5
Rat intestine	150	30	99.4	13.4

Reference: one nCi ^{3}H-folic acid in water.

TABLE 3. EFFICIENCY OF GAS PROPORTIONAL COUNTER FOR DETERMINING TRITIUM ACTIVITY IN VARIOUS TRITIATED MATERIALS DISSOLVED IN WATER AND BLOOD PLASMA

Tritiated material	Aqueous solution		Blood plasma	
	Efficiency (%)	Coeff. of variance	Efficiency (%)	Coeff. of variance
Folic acid	47	0.04	48	0.03
Mannitol	36	0.02	36	0.01
Oubain	39	0.03	39	0.05
Mean	41	0.03	41	0.03

water from the cracking process are bound to sodium carbonate in the reaction mixture but when present in larger amounts these impurities may diminish the precision of the counter. This is probably due to ionization of the gases. In such cases a smell of sulfur is sometimes noted from the ampoules. Erroneously low counts are noted when the amount of organic material in aqueous solution is less than 0.001 mg per assay. However, reliable results are obtained by supplying the reaction mixture with 1 or 2 mg urea.

Using a counter tube similar to the one used above,

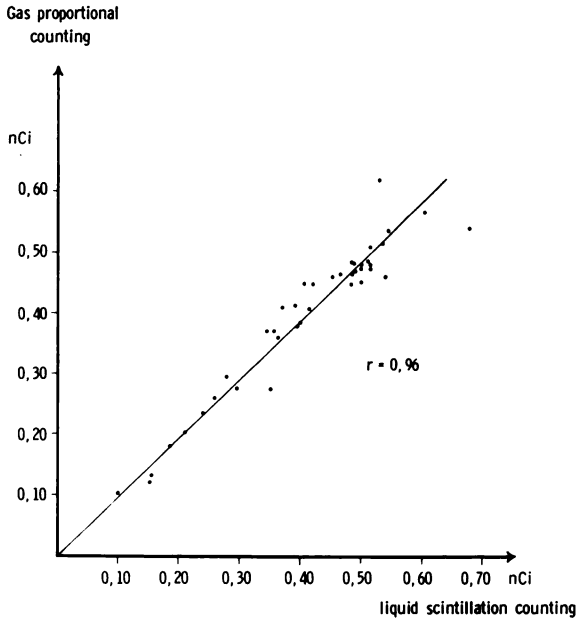

FIG. 3. Correlation between gas proportional counting and liquid scintillation counting in determining various activities of ^{3}H-folic acid in urine.

Wilzbach, et al (*1*) found that the maximal amount of organic matter per assay was 15 mg. In the present study urea (dry weight) in amounts up to five times this quantity and feces (dry weight) in amounts up to three times this quantity have been analyzed for tritium activity with satisfactory results. Precision was better, however, when determining tritium activity in 10-mg dry intestine than in 30-mg dry intestine. Presumably, it is not the dry weight itself but the amount of impurities admixed to the gas that limits the quantity of organic material per assay. Comparison between gas proportional counting and liquid scintillation counting showed that a fairly good correlation exists between the two methods when counting a relatively simply composed material like urine. It is evident that the precision of gas proportional counting is at least as good as in liquid scintillation counting and may even be better than the latter on counting nonhomogeneous biological materials requiring complicated procedures to solubilize tritium into the scintillator. Under these circumstances the efficiency of liquid scintillation counting may also be tested by gas proportional counting, the efficiency of which is independent of the consistency of the material studied.

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