A RAPID METHOD OF DETERMINING $^{35}SO_4$ IN PLASMA AND URINE

I. H. Koven, D. Leask, A. Sarson and A. Pollard

The New Mount Sinai Hospital, Toronto, Ontario, Canada

Radioactive sulfate, $^{35}SO_4$, a weak beta emitter, is probably the best means of measuring the extracellular fluid space in man and animals. Its use was first described by Walser *et al* (*1*) in 1953 using gas-flow counting. Liquid-scintillation counting gives higher efficiency, letting one use smaller doses of radioactivity, but there still exist the problems of extracting the radioactive isotope from tissue fluid quickly and easily and of minimizing "quenching."

Jeffay *et al* (*2*) described a method in which any ^{35}S as well as pre-existing $^{35}SO_4$ is estimated by oxidation with a modified Pirie's reagent prepared from concentrated nitric acid and 60% perchloric acid followed by solution in glycerol and dimethyl formamide for counting. Although the recovery rate is good, the procedure takes 3 hr and is hazardous. Albert *et al* (*3*) used a specially designed flow-through anthracene crystal coupled to a photomultiplier. While the tedious preparation of samples for liquid-scintillation detection is eliminated, there is a 15–20 min delay between samples to permit washout of the crystal and reduction of background count. Recently Newton *et al* (*4*) precipitated $^{35}SO_4$ as a barium sulfate salt and suspended the precipitate in a scintillating solvent converted to a gel state by using a thixotropic powder. One must remove the sulfate from the rest of the serum or urine constituents to minimize "quenching." In using this method we found there was a loss of the precipitate in transfer from the centrifuge tube to the counting vial.

We wish to describe a method in which the entire procedure is carried out within the scintillation vial. This prevents loss of the precipitate and permits rapid and accurate determinations. The rapidity is advantageous if one wishes to use this method for repeat determinations in a clinical setting.

APPARATUS AND MATERIALS

We used a Nuclear-Chicago scintillation spectrometer (Model 6801) coupled to a three-channel beta pulse-height analyzer. The scintillating liquid contained 2,5 diphenyloxazole (PPO) (4 g) and 1,4-bis-2-5 phenyloxazole benzene (POPOP) (50 mg) per liter of reagent-grade toluene. The precipitate was resuspended with N,N-dimethyl formamide. The thixotropic powder used was Cab-o-sil (Cabot Co., Boston, Mass.) a silicone powder.

METHOD

To a glass counting vial containing 0.25 ml of plasma or urine, exactly 0.4 ml of a molar Na_2SO_4 solution and 3.0 ml of a molar $BaCl_2$ solution was added. The resulting barium sulfate suspension was centrifuged at 2,000 rpm for 10 min in a centrifuge with a horizontal head of 14.0 cm radius, and the supernatant fluid was aspirated with a water pump. Five milliliters of N, N-dimethyl formamide were added and the precipitate resuspended using a vortex mixer (Scientific Instruments, Inc.). The cap was removed from the vial and enough Cab-o-sil powder was added to fill the counting vial to the very top (the amount used is not critical). Ten milliliters of the scintillating solution were added and the vial capped. The preparation was vigorously shaken again on the vortex mixer for 1 min.

We have had no breakage of glass counting vials during centrifugation and the use of nylon vials is also satisfactory. Opaque polyethylene counting vials do not permit accurate aspiration of the supernatant. Forming the gel within the counting vial eliminates the messiness of preformed gels. The time and rate of centrifugation is critical. However, absolute accuracy in the volumes of dimethyl formamide or the scintillating solution is not critical to the method.

RESULTS

Table 1 represents the results of experiments to determine the recovery from plasma.

Stock isotope solutions were prepared in quin-

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For reprints contact: I. H. Koven, The New Mt. Sinai Hospital, 550 University Ave., Toronto 2, Ontario, Canada.

TABLE 1. RECOVERY OF RADIOACTIVITY FROM PLASMA

	0.25 ml	0.50 ml	0.75 ml	1.00 ml
1. 0.9% saline, cpm (± s.d.)	4,788(72.8)	9,403(184.8)	14,563(166.7)	18,701(203.5)
2. Plasma, cpm (± s.d.)	4,430(33.7)	8,195(260.3)	12,046(161.7)	14,367(674.6)
3. % recovery (corrected for plasma water)	99	94	89	83
4. Additional, cpm (± s.d.)	4,670(34.1)	4,060(46.3)	3,786(30.2)	3,341(33.4)

tuplicate. Series 1 contained 0.25, 0.50, 0.75 and 1.00 ml of the saline solution and Series 2 the same volumes of plasma. All the vials were then prepared and counted by the method described. After counting 0.25 ml of the saline $^{35}SO_4$ solution was added and the vials vigorously shaken and recounted.

In Table 1, lines 1–3 show that the recovery of radioactivity is effectively complete using 0.25 ml of plasma and falls off moderately as the amount of plasma analyzed is increased from 0.25 to 1.00 ml.

That this loss is due to quenching by some component of plasma being carried down with the $BaSO_4$ is shown by the results with the addition of the internal standard (line 4).

The internal standard is not ideal because it is not in the same chemical form as the sample and has been added to a preformed gel. Nevertheless, it serves to confirm that all the radioactivity in the plasma is being precipitated.

To determine the linearity of counting, a series of different solutions of $^{35}SO_4$ in plasma were prepared. From these dilutions 0.25 ml was processed by the described method and counted in quintuplicate. Figure 1 shows a clear linear relationship between the observed counts and the dilutions of $^{35}SO_4$.

These experiments were repeated using urine instead of plasma, and similar results were obtained.

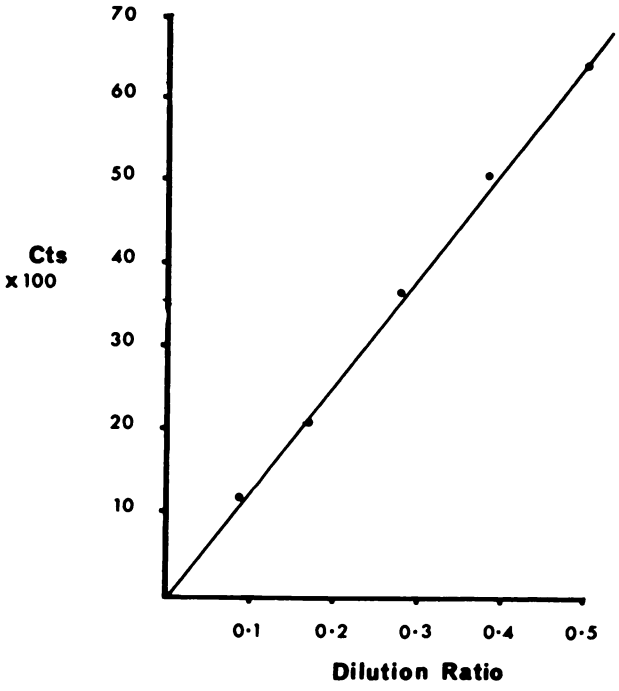

FIG. 1. Shows linear relationship between observed counting rate and dilutions of $^{35}SO_4$.

EFFICIENCY

Using a standard of ^{35}S as sodium sulfate, calculated to give 31,000 dpm and 0.25 ml of plasma, a sample was prepared by the method described. The observed counting rate was 21,945 cpm at a background of 42 cpm or 71% of the total radioactivity was detected.

SUMMARY

This method is rapid and simple and lends itself to measurements of large numbers of samples over a short period of time. The results are accurate and reproducible. All manipulations are performed within the counting vial.

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