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# Anthracene Cell for Counting Weak-Beta-Emitting Nuclides (Sulfur-35) in Liquid Samples[1,2]

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In an attempt to simplify the technique for measuring extracellular fluid volume with sulfur-35, a weak beta-emitting radioisotope, an anthracene cell was devised with constant volume and geometry. Anthracene crystals emit a blue flourescent light when exposed to ionizing radiation. Different forms of anthracene cells have been devised. All consist of packed anthracene crystals in a container exposed to a photomultiplier tube system. (1, 2, 3, 4, 5) The liquid containing the radioactive isotope to be analyzed is intimately mixed with the anthracene. The problems encountered with the anthracene cells are variations in geometry, distribution of liquid in the cell, and decontamination of the anthracene.

This anthracene cell consists of a disc of transparent acrylic resin[3] machined to form a shallow unidirectional channel. The channel bed is covered with a thin layer of anthracene crystals which is permanently and securely imbedded on the surface of the acrylic resin. White reflector acrylic resin, with appropriate inlets, is sealed on the surface of the channeled transparent acrylic resin producing a unidirectional liquid-tight tunnel. The cell is fixed with optical silicone paste to a photomultiplier tube which is attached to a high voltage supply and counting system.

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[1]Project supported in part by U.S. Atomic Energy Commission Research Grant, Contract AT(30-1)-3687 and assistance of E. R. Squibb & Sons and Picker-Nuclear Corp.

[2]Anesthesiology Research Laboratory, Washington Hospital Center, Washington, D.C.

[3]Lucite or Plexiglass—trade names.

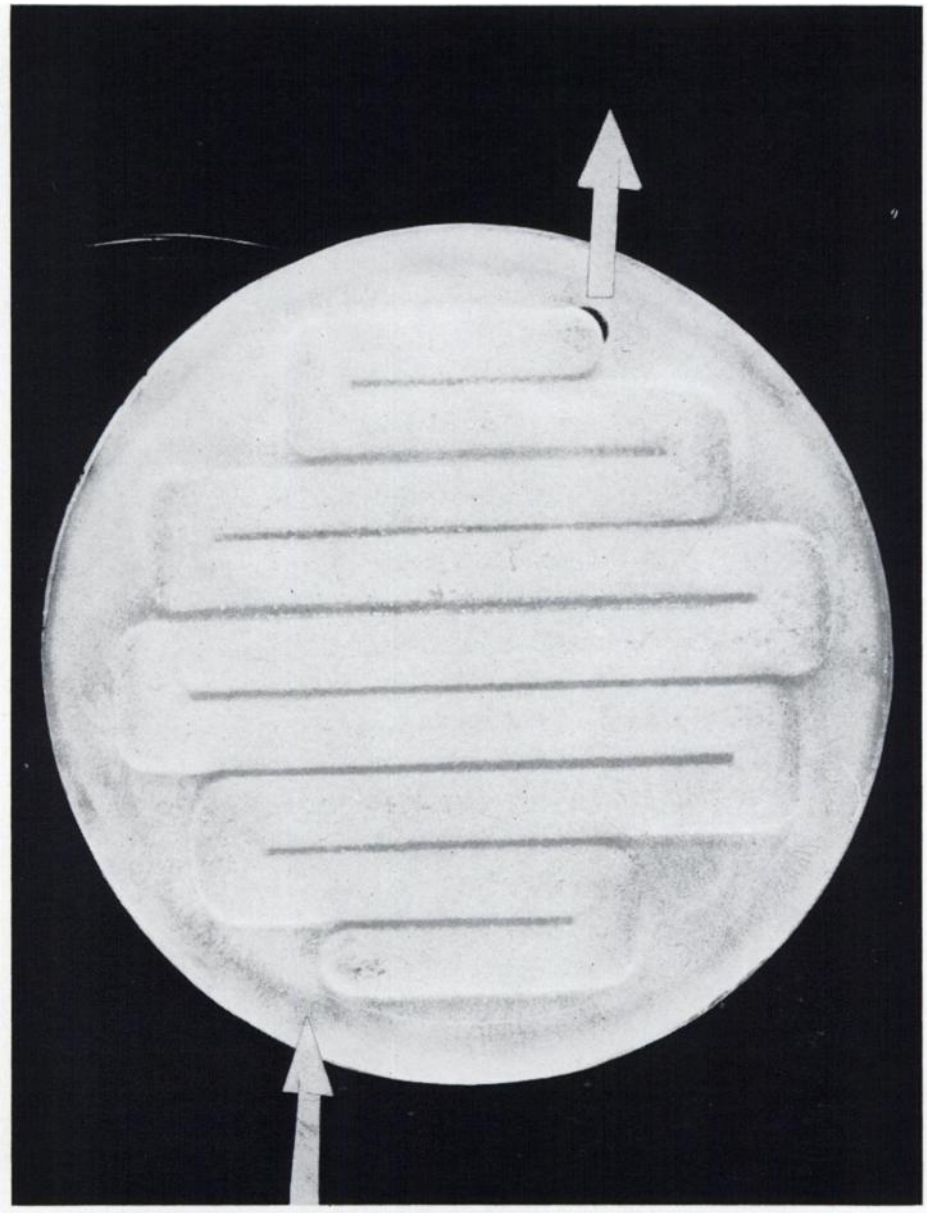

**Fig. 1. Anthracene Cell, for counting sulfur-35 in liquid samples. Front view-clear lucite-facing photomultiplier tube. Anthracene crystals are imbedded in channel base with lucite solvent. (Designed by S. N. Albert, M.D., constructed by R. MacK. Timby.)**

*Construction of the Anthracene Cell:*

A circle 7 cm in diameter is marked on a clear transparent acrylic resin disc 8 cm in diameter and 5 to 10 mm. thick. A zig-zag unidirectional channel is milled in the surface of the plastic within the 7 cm diameter limit with a round-tipped tool. The channel is approximately 4 to 6 mm. wide, 2 mm. deep, with a 1 mm. island separating each limb of the channel. The channel is cleaned and polished with fine pumice powder. The continuous groove is filled with scintillation-grade anthracene crystals[1] and acrylic resin solvent[2] is gently applied drop by drop all along the anthracene surface with a syringe and fine needle until the trough is full. The cell is allowed to dry for 10 minutes at room temperature. The free anthracene crystals are removed by tapping the disc and gently brushing with a fine soft brush. The disc is then placed under a stream of water to dislodge further any unbound loose crystals.

A round white reflector acrylic resin disc 8 cm in diameter is prepared with 2 portholes to coincide over each end of the channel. Solvent is applied over the 1 mm islands and 0.5 cm outer margin of the transparent acrylic resin disc as well as over the whole surface of the white reflector disc. The 2 discs are joined and placed in a press for 2 hours. Inlet and outlet connectors are glued into the portholes of the reflector disc. Lengths of black ("spaghetti") tubing are connected to the inlet and outlet nozzles (Figures 1 and 2). Optical cement is applied to the clear plastic face of the cell which is placed in apposition to a photomultiplier tube by using clamps. This assembly is then enclosed in a light-tight chamber shielded by 1-inch lead.

Liquid samples for analysis should be clear in order to avoid quenching of luminescence. Samples for assay are injected through one arm of the cell until it is filled and all of the air is removed and the liquid overflows from the other end. The volume necessary to fill the cell and tubing is approximately 4-5 ml. Decontamination of the anthracene is effected by washing the cell with a soap solution[3] followed by flushing with water. Geometry and volume of liquid samples analyzed remain constant.

*Adjusting the High Voltage of the Photomultiplier Tube*

In order to increase the efficiency of the anthracene cell, a 3-inch photomultiplier tube is suggested. The tube is placed in a light-proof chamber shielded with half-inch lead. The cell is placed in contact with the photomultiplier tube and is filled with a solution containing sulfur-35, 5 microcuries/liter. The high voltage is raised until a count rate of this sample registers 600-800 counts per minute. This count rate is usually six to eight times above background count rate. Background rate with the cell in position should remain between 50-100 counts per minute.

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[1] Nuclear Enterprises, Ltd., Winnipeg, Canada.

[2] Methylene chloride.

[3] 1 part neutral liquid detergent
1 part antifoam solution (silicone emulsion)
5,000 parts tap water

In the event a pulse height analyzer is incorporated in the system, the high voltage is adjusted with the gain at maximum, the lower or base level at 150 and the window in the out position. The average high voltage level is between 1150 and 1250 volts.

*Calibration of the Cell*

The cell is calibrated at the termination of a series of sample assays. The curve is established by plotting the count rate, corrected for background, versus known concentrations of sulfur-35 ranging from 1 to 8 microcuries per liter, prepared in distilled water.

The points are plotted on linear graph paper and the locus of the points is a straight line (Figure 3). Counting time is limited to 10 or 15 minutes. The reference curve seems to be stable and reproducible. Nevertheless, the cell should be calibrated with at least 2 known dilutions following every 20 to 30 measurements. With our equipment we have been able to obtain a count rate of 1,500 counts per 10 minutes (approximately 1.5 times above background) with a concentration of 1.0 microcurie per liter.

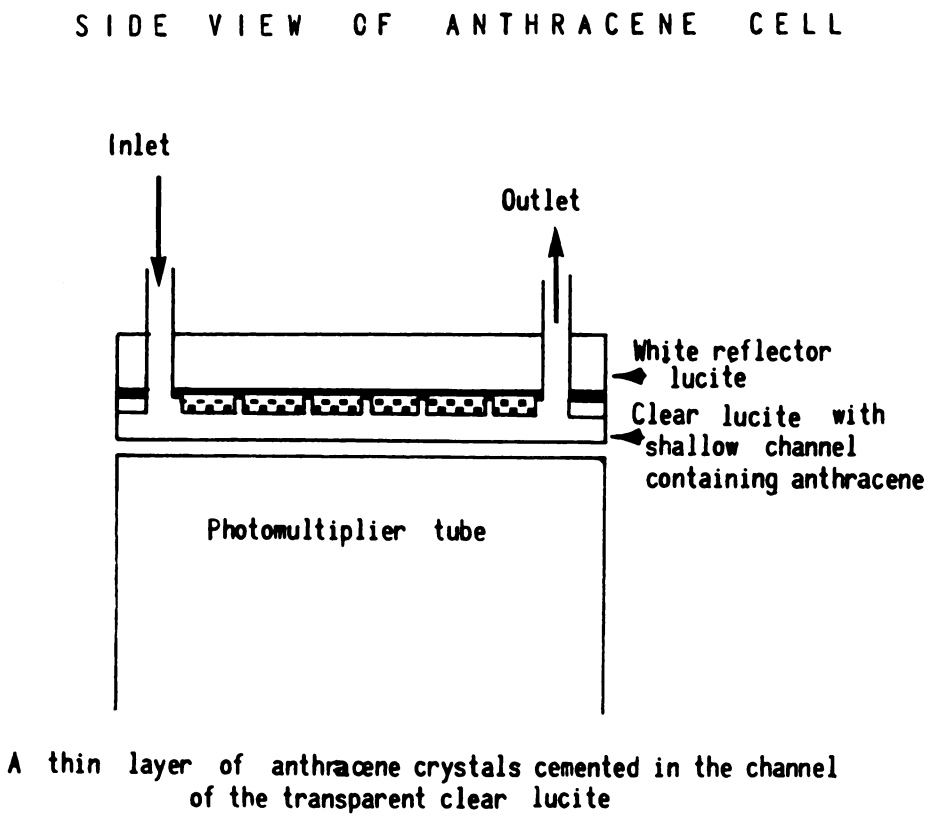


Fig. 2. Side view of anthracene cell.

*Assaying Samples*

The cell is emptied by suction following the assay of each sample then washed with the soap solution followed by flushing with water. Before introducing the next sample the excess water is removed by a stream of air blown through the cell. Background should be checked occasionally, and this should be performed when the cell is filled with water.

*Determining the Concentration of Sulfur-35 in Samples*

The concentration of sulfur-35 in the samples analyzed, expressed in microcuries per liter, is obtained from the reference curve and the count rate of the sample.

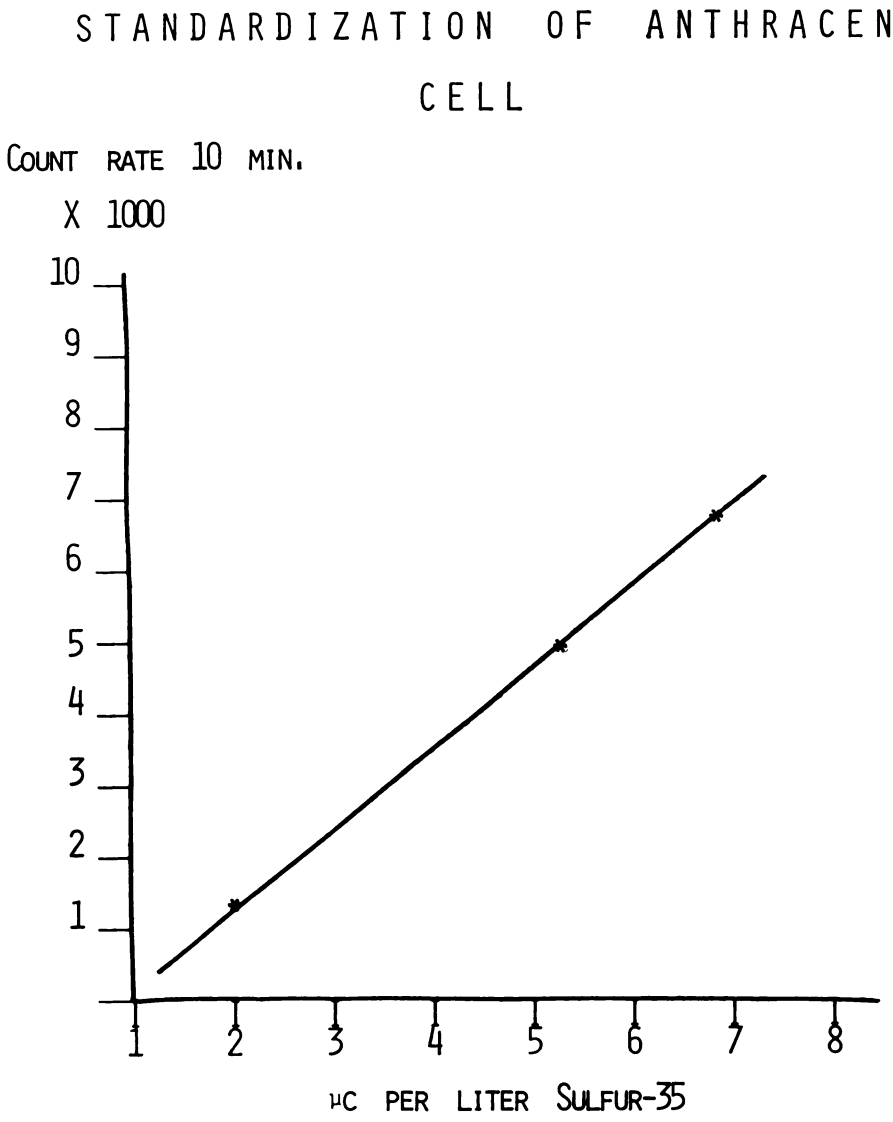


**Fig. 3. Standardization of the anthracene cell for counting sulfur-35. Count rate curve established with known dilutions of sulfur-35 prepared from the standard administered. Concentrations of sulfur-35 in sample established from the dilution curve in microcuries per liter.**

*Comments on Utilization of the Anthracene Cell*

There is some retention of fluorescence in the anthracene cell. When a sample is introduced, fluorescence reaches a plateau and the count rate is stable within 15 minutes (Figure 4). For this purpose, the following procedure for counting and determining the concentration of sulfur-35 in samples should be followed.

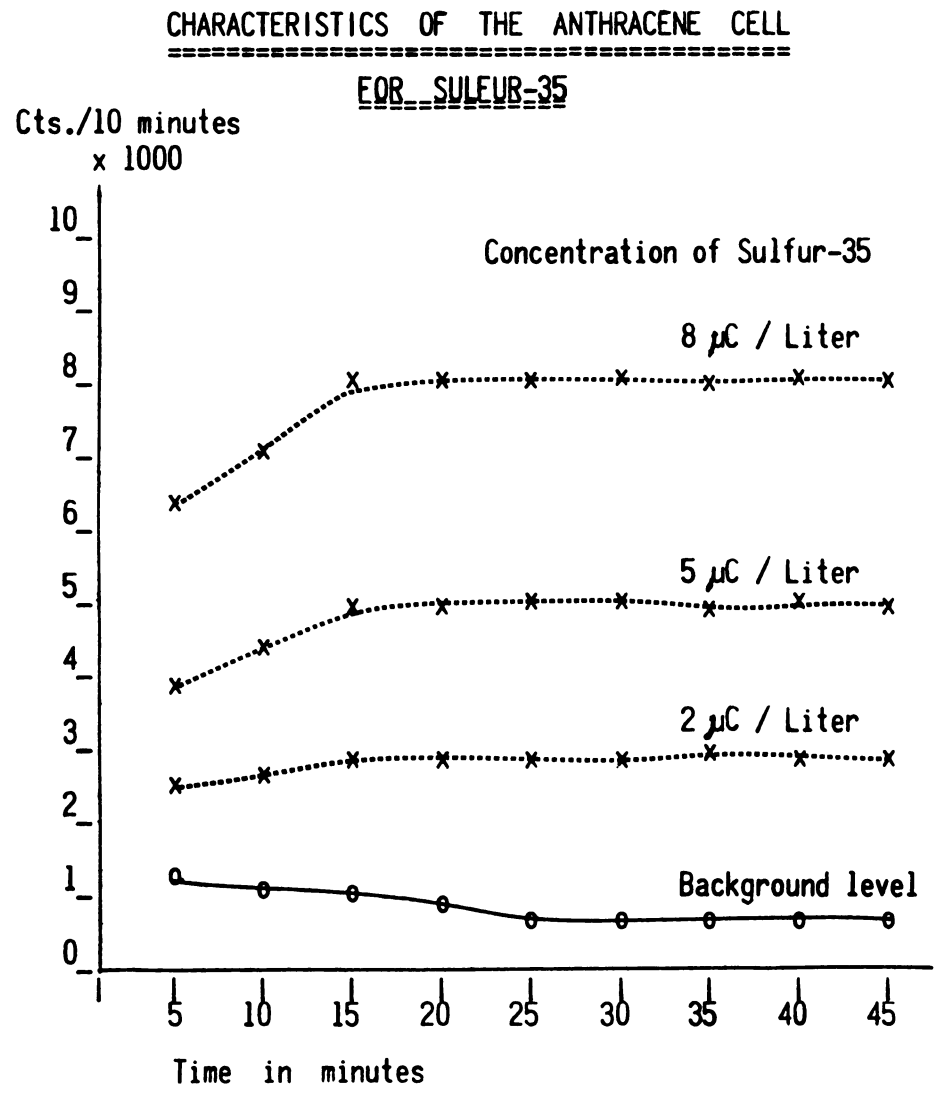


**Fig. 4. Peak fluorescence of the anthracene is reached within 10 to 15 minutes and remains stable thereafter. Samples introduced into the cell for radioanalysis should remain in contact with the anthracene for a period of 15 to 20 minutes before taking the count rate. Immediately following washing the background level falls and within 10 to 20 minutes reaches atmospheric baseline level. It is not necessary to wait for background to return to baseline level before introducing samples.**

1) *Establishing Atmospheric Background.*

The washed anthracene cell is left filled with water overnight. The cell is washed again in the morning and refilled with water before counting background. Background count is obtained by averaging three or four repeated measurements.

2) *Establishing the Standard Curve.*

The standard curves is obtained by analysis of dilutions 2, 5, and 8 microcuries per liter of sulfur-35. The sample of 2 microcuries per liter is introduced first for analysis and is allowed to remain in contact with the anthracene, for a period of 20 minutes, before taking the 10-minute count rate. The cell is then washed several times with soap solution and flushed with water. A sample of 5 microcuries per liter is next introduced and after a 20-minute waiting period the 10-minute count rate is taken. The same procedure is followed to establish the count rate for the sample with 8 microcuries per liter concentration. The atmospheric background established earlier is subtracted from the count rates obtained and the net counts of each are plotted on graph paper as counts versus concentration in microcuries per liter.

## CONCLUSION

With this simplified system for counting sulfur-35 in clear liquid samples we have been able to measure extracellular fluid volume utilizing 1 microcurie per kg body weight of sulfur-35 as sulfate. Duplicate and repeated studies were accurately reproduced. The system obviates the tedious and time-consuming processing of blood samples for analysis by either gas flow or liquid scintillation techniques. The equipment is simple and the cost is comparatively small.

## REFERENCES

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