IN VITRO NUCLEAR MEDICINE

Liquid Scintillation Vial for Radiometric Assay of Lymphocyte Carbohydrate Metabolism in Response to Mitogens

Ngo Tran and Henry N. Wagner, Jr.

The Johns Hopkins Medical Institutions, Baltimore, Maryland

We have demonstrated that mitogens—i.e., PHA and Con.A—stimulate lymphocyte carbohydrate metabolism using a liquid-scintillation vial with conventional liquid-scintillation detectors. The results showed that this enclosed system can be useful for development of rapid in vitro tests of lymphocytes immune responsiveness, as well as for radiometric detection of bacterial growth in various gaseous atmospheres.

J Nucl Med 19: 61–63, 1978

For the metabolic study of cell-mediated immunity, we have recently demonstrated a semi-automated ionization-chamber system to provide rapid quantification of lymphocyte carbohydrate metabolism in response to mitogens, namely, phytohemagglutinin (PHA) and concanvalin A (Con. A) (*1,2*). In the present study, a modification of a culture-scintillation vial designed by Buddemeyer (*3*) is adapted for use with conventional liquid solutions.

MATERIALS AND METHODS

The assembled unit consists of a capped 5-cc vial placed within a scintillation vial containing 2 cc of scintillation liquid mixed with hyamine, as shown in Fig. 1. Isolation of lymphocytes was performed by the Ficoll-Hypaque method described previously (*4*). For each study, 2 million normal human lymphocytes in Hank's solution with 5.0 mM Mg^{++} and without any cold glucose were inoculated into a series of sterilized vials containing, respectively, 1.0 μCi [1-^{14}C] D-glucose (specific activity 60 mCi/mmole), [6-^{14}C] D-glucose (53.7 mCi/mmole), or [U-^{14}C] D-glucose (26.8 mCi/mmole), with and without various amounts of PHA M and Con.A. The total volume was made up to 2.0 ml by addition of modified Hank's solution. The vials containing the mix-

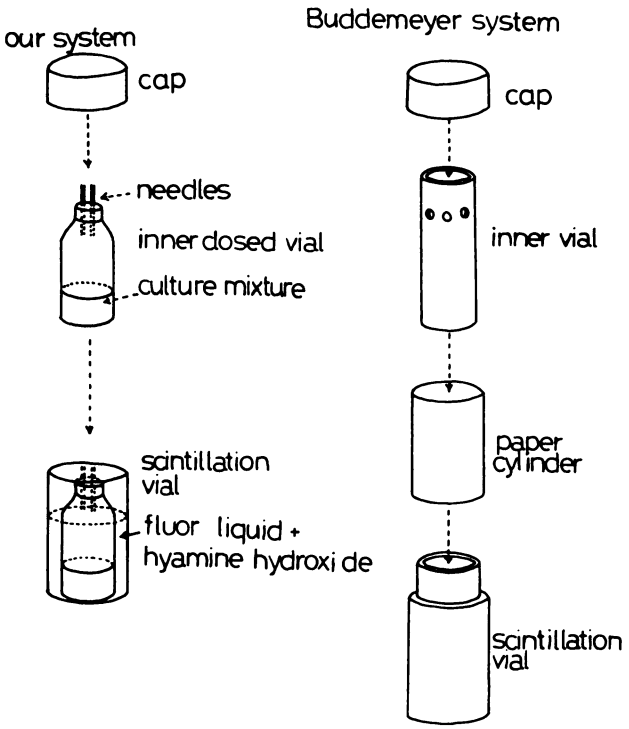

FIG. 1. Components of metabolism-detecting scintillation vial of Buddemeyer's method and our radiometric system.

Received May 20, 1977; revision accepted Sept. 6, 1977.

For reprints contact: Ngo M. Tran, Dept. of Radiology, University of California, Irvine Medical Ctr., 101 City Dr. S., Orange, CA 92668.

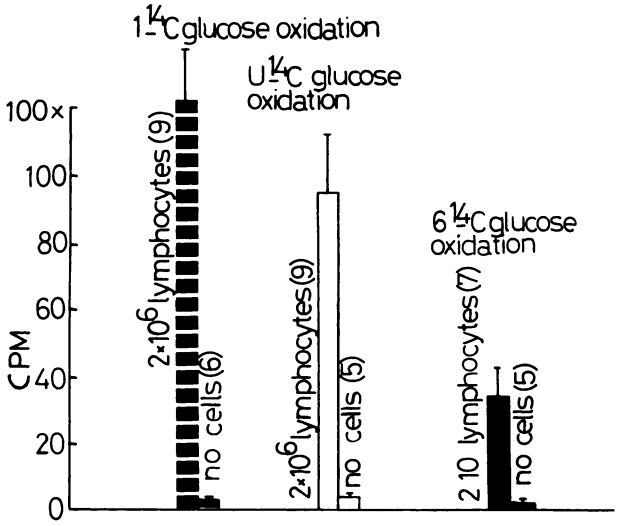

FIG. 2. Detected activity from oxidation of [1-^{14}C], [U-^{14}C], and [6-^{14}C] D-glucose in presence or absence of 2 million lymphocytes. Each bar represents ±1 s.e. of mean. Numbers of experiments are noted in parentheses.

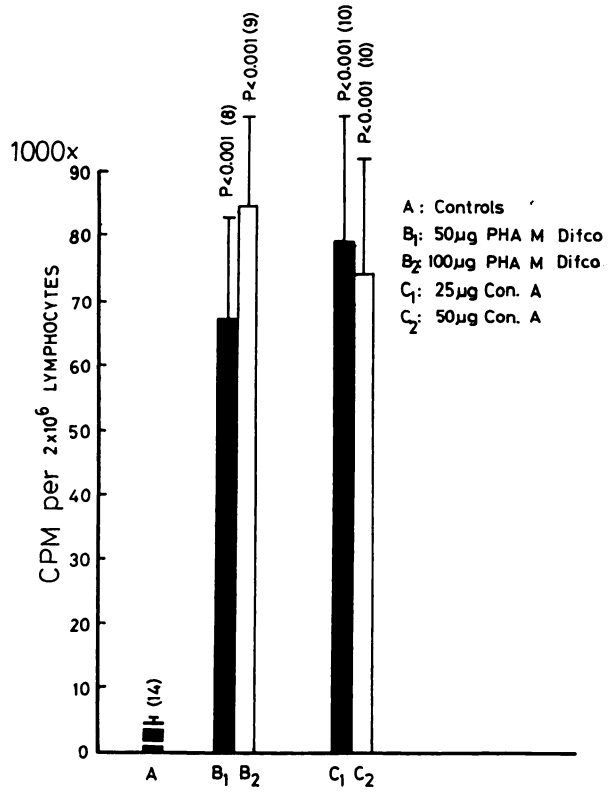

FIG. 3. Effect of 50–100 µg PHA M and 25–50 µg Con. A on the oxidation of [1-^{14}C] D-glucose by lymphocytes, respectively.

ture were then incubated at 37°C for 120 min. After the incubation, culture vials were inserted into scintillation vials containing 2 cc scintillation liquid plus hyamine. The scintillation liquid consisted of 100 cc toluene, 4.2 cc liquifluor and 2 cc hyamine hydroxide. The reaction in all culture vials was terminated by 0.5 cc 0.1 N HCL and two #19 needles were driven into the culture vials. The scintillation vials were carefully closed and sealed with cellophane tape and were then left for at least 2 hr in the room atmosphere to permit complete absorption of CO_2 by the scintillation-liquid mixture. Scintillation vials thus prepared were counted in a scintillation liquid counter* at 40% gain with a base level setting of 50 pulse height units and an upper level of 1,000, as in the experimental setting described previously (*3*).

RESULTS

Figure 2 shows that there is oxidation of [1-^{14}C], [6-^{14}C], and [U-^{14}C] glucose in the presence of 2 million lymphocytes. There is, however, very little spontaneous oxidation of C-14-labeled glucose without lymphocytes. Lymphocyte $^{14}CO_2$ production from C-14-labeled glucose at 0–2 hr in unstimulated lymphocytes was greatest from [1-^{14}C] D-glucose, intermediate from [U-^{14}C] D-glucose and least from [6-^{14}C] D-glucose. The addition of 50–100 µg PHA M to the reaction mixture containing 2 million lymphocytes and [1-^{14}C] D-glucose caused a significant increase in the production of $^{14}CO_2$ at 0–2 hr ($n = 14$ for controls, $n = 8$ for cells plus 50 µg PHA M, and $n = 9$ for cells plus 100 µg PHA M; $p < 0.001$) (Student's t test). Similarly, there is significantly increased $^{14}CO_2$ production from [1-^{14}C] D-glucose at 0–2 hr in the presence of 2 million lymphocytes and 25–50 µg Con.A ($n = 10$, $p < 0.001$), as shown in Fig. 3. The stimulation of lymphocyte carbohydrate metabolism in the presence of Con.A appears to be of the same degree as that obtained from PHA M. These overall results show that both PHA M and Con.A stimulate lymphocyte carbohydrate metabolism and that the liquid-scintillation vial with conventional liquid-scintillation detectors can be useful for the development of in-vitro tests of lymphocytes immune responsiveness. These data are preliminary and uncorrected for quench and recovery. We feel, however, that once the system is standardized for rapid quantification of cell-mediated immunity, the results will be available much more rapidly than at present—namely in approximately 6–8 hr total time, as contrasted with the 72-hr culture period required by the H-3-thymidine incorporation methods (*5*).

We note finally that this enclosed vial system is of potential importance for radiometric detection of bacterial growth in both aerobic (*3*) and anaerobic conditions, as well as in other gaseous atmospheres.

FOOTNOTE

* Packard model 3003.

REFERENCES

1. LARSON SM, MERZ T, WAGNER HN: Radiometric screening test of cell mediated immunity based on Phytohemagglutinin (PHA) induced changes in lymphocyte carbohydrate metabolism. *J Nucl Med* 15: 510, 1974

2. TRAN N, CHEN M, MCINTYRE P, et al: Radiometric assays of lymphocyte carbohydrate metabolism in response to mitogens. *J Nucl Med* 16: 576, 1975

3. BUDDEMEYER EV: Liquid scintillation vial for cumulative and continuous radiometric measurement of in vitro metabolism. *Appl Microbiol* 28: 177, 1974

4. BOYUM A: Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 21 (Suppl 97): 77, 1968

5. JUNGE U, HOEKSTRA J, WOLFE L, et al: Microtechnique for quantitative evaluation of in vitro lymphocyte transformation. *Clin Exp Immunol* 7: 431–437, 1970