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RADIOMETRIC SCREENING TEST FOR CHRONIC GRANULOMATOUS DISEASE

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A method has been developed to assay radiometrically leucocyte $^{14}CO_2$ production during phagocytosis of latex particles for the detection of chronic granulomatous disease, associated with an hereditable defect in bactericidal activity of leukocytes. Patients studied were from two affected families, with demonstration of the defect both by the radiometric assay and other methods used to measure oxidative metabolism.

Radiometric detectors for $^{14}CO_2$ such as the BacTec (Johnson Laboratories, Cockeysville, Md. 21030) have been used successfully to screen blood cultures for bacterial growth (*1*). The method, based on measurement of $^{14}CO_2$ produced by the multiplying bacteria from metabolism of ^{14}C-glucose, has been automated to allow processing of large numbers of cultures in the microbiology laboratory.

Leukocytes in the inoculum of whole blood being cultured also produce considerable CO_2 during phagocytosis (*2*) from glucose by the hexose monophosphate shunt (HMS). An exception to this is the leukocyte from patients with chronic granulomatous disease (CGD)—an hereditable defect of intracellular bactericidal activity of phagocytic cells that fail to show stimulation of HMS activity with particle ingestion (*3*). We previously reported that this reaction may be used to screen for CGD using whole blood as a source of leukocytes, latex spherules as a phagocytic stimulus, and liquid scintillation spectrometry for measurement of $^{14}CO_2$ that had been trapped in hyamine hydroxide (*4*).

Because most investigations of leukocyte function are time consuming and frequently require separation of the phagocytic cells first, only limited numbers of samples can reasonably be studied. Research interest in the disorders of phagocytic cells in no way reflects the number of known cases (*5–7*). In fact, there are no estimates of the incidence of CGD or similar disorders in the general population because mass screening is precluded by current methodology. It occurred to us that adaptation of this isotopic study assessing HMS activity of leukocytes during phagocytosis (*4*) to the simplified instrumentation of the BacTec might allow automation and thereby remove a major impediment to such investigations. We herein report the successful use of the BacTec to study leukocyte function. An additional objective of the study was to determine whether false-positive bacteremia might be suggested by leukocyte metabolism of the labeled glucose in the blood culture system.

MATERIALS AND METHODS

Flasks containing 0.25 μCi of 1-^{14}C-glucose, 0.2 cc latex particles (Bacto-latex, 0.81 μ diam, Difco, Detroit) or an equal volume of Hank's balanced salt solution, were capped with a rubber serum stopper and stored in the refrigerator. To perform the test, 1 cc of heparinized (10 μ/ml) whole blood was injected through the stopper into each of a pair of flasks, one with latex (phagocytizing) and one without (resting). Following 1 hr of incubation with shaking at 37°C, $^{14}CO_2$ production was measured directly on a BacTec-301, a smaller manual version of the automated instrument. The flask was connected to the detector by a needle assembly inserted through the serum stopper into the gas phase. By activating the instrument, the circuitry was automatically balanced—the $^{14}CO_2$ containing air phase was flushed from the flask into an ionization chamber, and the flow of current due to β disintegration was displayed on a meter scaled from 0 to 100. The process was completed in less than 5 min for a pair of flasks.

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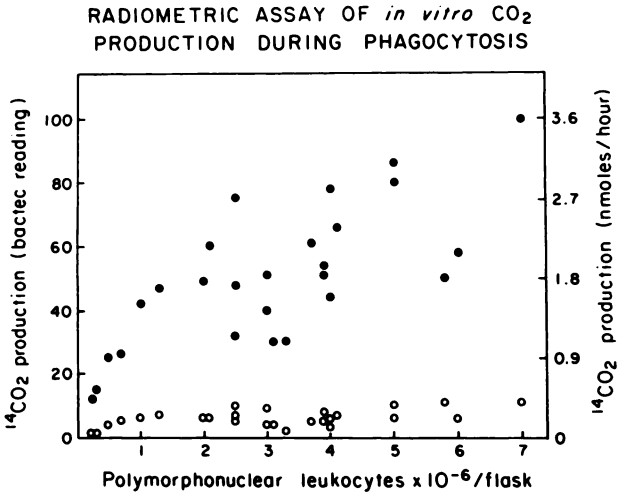

FIG. 1. Carbon-14 O_2 production by leukocytes in whole blood under resting (open circles) and phagocytizing (closed circles) conditions measured with BacTec radiometric detector.

The phagocytizing/resting (P/R) ratio of $^{14}CO_2$ production determined by liquid scintillation spectrometry, the quantitative nitrobluetetrazolium (NBT) dye reduction test, and assays of intracellular killing were determined as previously reported (*4,8*).

RESULTS

Little $^{14}CO_2$ production was detected in the resting samples, irrespective of the number of phagocytic cells contained in the 1 cc inoculum of whole blood over a wide range (Fig. 1). In the companion flask containing latex, readings were higher and dependent on the number of cells added. When at least 10^6 phagocytes were present, BacTec readings were all above 30, and the stimulation due to phagocytosis was readily discernible. Most CO_2 production occurred during the first hour of incubation and markedly diminished over the subsequent 2 hr (Fig. 2). Uniformly labeled glucose could be substituted for specific 1-^{14}C-glucose; however, more isotope was required, thus offsetting the advantage of lesser cost for the former isotope.

BacTec blood culture flasks contain media and, in place of heparin, polyanethol sulfonate (PAS), a polyanionic anticoagulant. This material is reported to inhibit phagocytosis thereby reducing intracellular killing of bacteria and increasing the chances for positive cultures (*9*). PAS is also reported to affect leukocyte HMS activity (*10*). Varying concentrations of PAS were therefore added to heparinized whole blood, and HMS metabolism was assessed as described previously (Table 1). There was a dose related effect with obliteration of the phagocytic HMS stimulation at high concentrations of PAS and elevated resting levels at lower concentrations. At the level of PAS in blood culture bottles, 0.05%, white cell activity was significantly impaired.

Two families with three known male CGD children were screened with the BacTec (Fig. 3). The diagnosis was established in all cases by means of the quantitative NBT test and assays of intracellular bactericidal activity. Results of the radiometric (BacTec) assay compared favorably with the liquid scintillation (P/R) assay of HMS activity and effectively discriminated CGD from normal samples. Presumed heterozygote mothers and one clinically well sister of a CGD boy were not clearly segregated by the BacTec, although there was a suggestion of this in one family. However, neither NBT nor bactericidal assays can consistently discriminate these subjects, and the problem of screening for such subjects remains unsolved.

One subject with severe Mediterranean type glu-

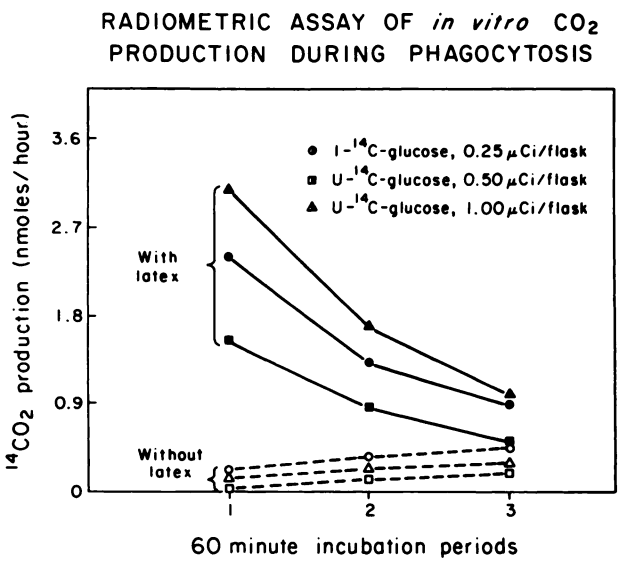

FIG. 2. Hourly increments in $^{14}CO_2$ production by leukocytes in whole blood under resting (open symbols) and phagocytizing (closed symbols) conditions. Uniformly labeled ^{14}C-glucose is metabolized to CO_2 during phagocytosis similar to 1-^{14}C-glucose; however, considerably more of uniformly labeled isotope is required for comparable results.

TABLE 1. EFFECT OF POLYANETHOL SULFONATE ON PHAGOCYTOSIS STIMULATED HMS ACTIVITY

Percent polyanethol sulfonate (v/v)	BacTec readings*	
	Resting	Phagocytizing
0	7	49
0.0005	9	40
0.005	14	41
0.05	13	15
0.5	7	11

* Mean of three experiments.

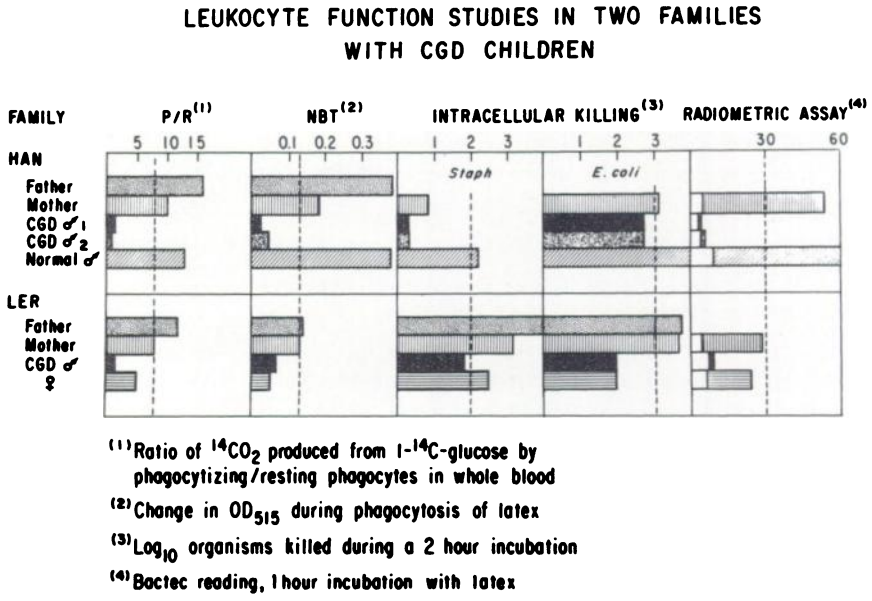

FIG. 3. Leukocyte function studies in two families with three CGD male children. BacTec assay (extreme right) compares favorably with other methods to measure oxidative metabolism directly (P/R) or indirectly (NBT dye reduction test) as screen for CGD cells.

cose-6-phosphate dehydrogenase deficiency was studied to be certain that leukocyte enzyme in these subjects would not be rate limiting. There was a marked increase in CO_2 production during phagocytosis in this sample that indicates that the more common erythrocyte G6-PD deficiency would not be confused with the rare total leukocyte G6-PD deficiency state (*11*), in which HMS activity during phagocytosis is markedly impaired.

Finally, cord blood samples were obtained from 20 full-term apparently healthy newborns and HMS studied by the radiometric method. Total leukocyte counts were elevated (mean count 14,800/mm^3) with a predominance of polymorphonuclear leukocytes (83%). The CO_2 production increased during phagocytosis, similar to normal adults.

DISCUSSION

This study shows that CO_2 production by phagocytizing leukocytes can be assayed with the BacTec radiometric detector. The technique described for measurement of this single parameter of leukocyte function is extremely simple, requiring only injection of 1 cc of whole blood into each of a pair of prepared vials. Results are obtained 1 hr later without further manipulation. Thus far, only samples from three children with proven CGD have failed to show increased CO_2 production with addition of latex particles as a phagocytic stimulus.

Because PAS inhibits phagocytosis and its metabolic concomitants, including HMS activity measured with the BacTec, use of this anticoagulant in the blood culture system prevents leukocyte release of CO_2, which might falsely signify the presence of bacteremia. The early rise and decline of leukocyte CO_2 production during phagocytosis is also quite different from the delayed, sustained metabolic activity during bacterial growth.

The assay system also works with newborn leukocytes present in cord blood. Because of the neutrophilia in these samples, less than 1 cc per assay vial appears to be adequate. Thus, with a small cord blood sample, newborns can easily be screened for CGD-like defects. We do not now know that the CGD defect will be present at birth or what effect fetal maturity or infection will have on the newborns' leukocytes. Prospective studies will be necessary to answer these questions. If, however, the cord leukocytes of newborns with CGD are indeed abnormal, then the simplicity of the present method will permit continuing prospective analysis of the incidence of this inherited disease in the general population. More importantly it will allow initiation of treatment soon after birth.

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REFERENCES

1. DELAND F, WAGNER HN: Automated radiometric detection of bacterial growth in blood cultures. *J Lab Clin Med* 75: 529–534, 1970

2. SKEEL RT, YANKEE RA, HENDERSON ES: Hexose monophosphate shunt activity of circulating phagocytes in acute lymphocytic leukemia. *J Lab Clin Med* 77: 975–984, 1971

3. HOLMES B, PAGE AR, GOOD RA: Studies of the metabolic activity of leukocytes from patients with a genetic abnormality of phagocyte function. *J Clin Invest* 46: 1422–1432, 1967

4. KEUSCH GT, DOUGLAS SD, MILDVAN D, et al: ^{14}C-glucose oxidation in whole blood: a clinical assay for phagocyte dysfunction. *Infect Immunity* 5: 414–415, 1972

5. DOUGLAS SD: Analytic Review. Disorders of phagocyte function. *Blood* 35: 851–866, 1970

6. NATHAN DG, BAEHNER RL: Disorders of phagocytic cell function. *Progr Hemat* 7: 235–254, 1971

7. QUIE PG, WHITE JG, HOLMES B, et al: In vitro bactericidal capacity of human polymorphonuclear leukocytes: Diminished activity in chronic granulomatous disease of childhood. *J Clin Invest* 46: 668–679, 1969

8. DOUGLAS SD, DAVIS WC, FUDENBERG HH: Granulocytopathies: pleomorphism of neutrophil dysfunction. *Amer J Med* 46: 901–909, 1969

9. MINKUS R, MOFFET HL: Detection of bacteremia in children with sodium polyanethol sulfonate: a prospective clinical study. *Appl Microbiol* 22: 805–808, 1971

10. BELDING ME, KLEBANOFF SJ: Effect of sodium polyanethol sulfonate on antimicrobial systems in blood. *Appl Microbiol* 24: 691–698, 1972

11. COOPER MR, DECHATELET LR, MCCALL CR, et al: Complete deficiency of leukocyte glucose-6-phosphate dehydrogenase with defective bactericidal activity. *J Clin Invest* 51: 769–778, 1972