INFECTION AND IMMUNITY, Mar. 1972, p. 414-415
Vol. 5, No. 3

Printed in U.S.A.

^{14}C-Glucose Oxidation in Whole Blood: a Clinical Assay for Phagocyte Dysfunction

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Received for publication 29 October 1971

A screening test for chronic granulomatous disease is described; it is based on abnormal oxidation of glucose-*1*-^{14}C to $^{14}CO_2$ during phagocytosis by leukocytes in whole blood.

Leukocytes from patients with chronic granulomatous disease (CGD) ingest bacteria, form phagocytic vacuoles (2, 6), and degranulate (3) but do not increase oxygen uptake or direct oxidation of glucose via the hexose monophosphate shunt (HMS) or peroxide formation, and the leukocyte fails to kill the microorganism (7). Several screening tests have been proposed to aid in patient identification (1, 5, 9). We report here development of a quantitative test that reliably discriminates CGD leukocytes with small samples of heparinized blood up to 48 hr after collection.

Heparinized (20 units/ml) venous blood was collected from 30 normal adults, 15 hospitalized patients with a variety of disorders, and 4 families with 5 CGD male children diagnosed on the basis of recurrent infections, an abnormal nitroblue tetrazolium test (1), and defective intracellular bacterial killing (3). Isolated phagocytes (polymorphonuclears plus monocytes), when studied, were obtained by dextran sedimentation and NH_4Cl lysis (3) and were resuspended in Hanks balanced salt solution (HBSS). Decarboxylation of glucose-*1*-^{14}C was measured as reported (8). Briefly, 0.1 ml of saline or saline-dialyzed Difco polystyrene-latex spherules (PSL; 0.8/μm in diameter, 2×10^9/ml), 0.7 ml of HBSS containing 0.0625 μCi of ^{14}C-glucose (4.6 μCi/μmole), and 0.2 ml of whole blood or isolated leukocytes (2×10^6 cells) were placed in 25-ml centerwell Erlenmeyer flasks containing a removable glass cup (Wilbur Scientific, Boston, Mass.). Flasks were stoppered and incubated with shaking at 37 C under room air for 30 min when 1 ml of 2 N HCl was injected through the stopper into the flask and 0.4 ml of hyamine hydroxide was injected into the cup. After 30 min of additional incubation for trapping of $^{14}CO_2$ in the hyamine, the cup was transferred to a vial containing 10 ml of Fluoralloy-TLA (Beckman Instruments) in toluene, and radioactivity was determined in a liquid scintillation spectrometer. Background counts from incubated flasks without cells were subtracted from experimental values.

TABLE 1. *Ratio (P/R) of $^{14}CO_2$ production from glucose-1-^{14}C by phagocytizing (P) compared to resting (R) blood cells*

Source of blood	No. of subjects	No. of tests	P/R (counts/min)
Normal subjects	30	40	11.4 ± 0.8[a]
Non-CGD hospital patients[b]	15	22	7.8 ± 0.6
CGD patients	4	8	1.1 ± 0.1
Erythrocytes	3	3	1.6 ± 0.1
Lymphocytes[c]	2	2	2.8 ± 0.3
Severely neutropenic leukemia patients	3	4	1.0 ± 0.1
Swiss white mice[d]	10	10	1.4 ± 0.1

[a] Mean ± 1 standard error of the mean.

[b] Diagnoses: cystic fibrosis without infection (2), treated bacterial pneumonia (2), treated diverticulitis (1), vasculitis (3), pulmonary aspergillosis (1), polycystic renal disease (1), subacute bacterial endocarditis (1), viral pericarditis (1), Hodgkins disease (1), and fever of undetermined etiology (2).

[c] 85-95% purity as isolated from a nylon wool column.

[d] Mean peripheral white blood cell count, 6,400/mm^3; 75 to 90% lymphocytes; 10 to 25% polymorphonuclear neutrophils plus monocytes.

In normal subjects, release of $^{14}CO_2$ from glucose-*1*-^{14}C by whole blood was linear for 240 min [resting (*R*) values]; addition of PSL resulted in a sharp increase [phagocytizing (*P*) values]. The ratio of $^{14}CO_2$ release in the presence of PSL to that in its absence (*P/R*) was maximal at 30 min. Resting $^{14}CO_2$ production was similar in whole blood and phagocytes isolated from the

TABLE 2. *Effect of storage at 4 C on P/R determined in whole blood*

Subjects	*P/R* after various time of storage at 4 C				
	0 hr	24 hr	48 hr	72 hr	96 hr
Controls.....	13.8 ± 1.5[a] (12)[b]	6.3 ± 0.4 (8)	5.0 ± 0.3 (5)	4.3 ± 1.0 (6)	3.3 ± 0.8 (4)
CGD mothers...	9.9 ± 1.2 (6)	5.5 ± 1.1 (6)	4.9 ± 1.2 (3)	2.7 ± 0.5 (2)	2.0 ± 0.1 (2)
CGD patients...	1.1 ± 0.2 (8)	1.3 ± 0.1 (7)	1.3 ± 0.3 (3)	1.2 ± 0.1 (3)	1.3 ± 0.2 (3)
P value[c].....	<0.001	<0.001	<0.001	>0.05	>0.05

[a] Mean ± standard error of the mean.
[b] Number of tests in parentheses.
[c] Significance of the difference between mean control and CGD values by Student's *t* test.

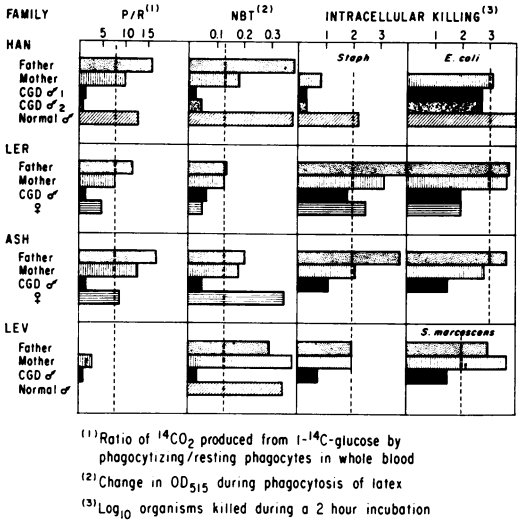

FIG. 1. *In vitro leukocyte function studies in four families with CGD children. Dotted line indicates normal values for each test.*

same specimen (114 ± 4 versus 84 ± 5 counts per min per 10^6 cells) and rose sharply during phagocytosis (1,242 ± 83 and 492 ± 62 counts per min per 10^6 cells, respectively), whereas neutropenic samples failed to increase $^{14}CO_2$ production (Table 1). These data indicate that phagocytes are the prime source of glucose oxidation in whole blood, as recently observed by Skeel et al. (10).

P/R values in whole blood and isolated cells from CGD patients were identical (1.0 ± 0.1 versus 1.3 ± 0.2). All five CGD boys were clearly identified by NBT or *P/R* tests (Fig. 1). Additionally, the *P/R* reliably discriminated CGD samples stored at 4 C for as long as 48 hr (Table 2; reference 11), thus permitting testing of transported specimens. Determination of *P/R* ratio in whole blood thus meets many of the requirements for an ideal screening test for CGD.

We are indebted to David L. Jonas for assistance and to J. M. Brush, A. Rausen, S. Stein, and D. Tuman for making their patients available for these studies.

This investigation was supported by contract DADA 71-C-1042, U.S. Army Research and Development Command, and by Public Health Service training grant AI-0041 and research grant A1-09338 from the National Institute of Allergy and Infectious Disease. Steven D. Douglas is the recipient of a Research Career Development Award (1K04 HE-42475-02) from the National Heart and Lung Institute.

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