A RAPID AUTOMATED SYSTEM FOR MEASUREMENT OF ANTIBODY TITERS

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An automated radiometric system originally developed for detection of microbial growth has been adapted for measurement of antibody titers in sera. Immune sera could be detected in dilutions of 1:10,000, which is well below the range of complement fixation techniques and above the usual range for radioimmunoassay. The technique is based on this principle—that polymorphonuclear leukocyte metabolism is altered by the end products of immunoreactions, and that these changes in metabolism are quantitatively related to the concentration of antigen and antibody in the medium. Our results support the concept that induced changes in leukocyte metabolism can be used as a rapid and sensitive technique for making serologic measurements on biologic fluids.

Many of the standard techniques in current use for measurement of antibody titers in body fluids are cumbersome and lengthy. It has been stated recently that ". . . the volume of tests for the detection of syphilis, for rheumatoid factor, for coagulation, for agglutination of viruses and for blood compatibility tests in the blood bank more than justifies an intense effort toward automation of these procedures" (*1*).

We have reported previously that measurement of glucose-1-^{14}C conversion to $^{14}CO_2$ through the hexosemonophosphate shunt in leukocytes (*2*) can serve as the basis of an automated system for serologic assays. In the presence of constant amounts of antibody, the LOG of the antigen concentration present resulted in a linear increase in the amount of $^{14}CO_2$ released. The minimum detectable antigen concentration was 2.5×10^6, 0.6×10^6 and 1.2×10^6 Ag/ml for Salmonella 0 types A, C, and E, respectively. In the experiments described in the present paper, the radiometric method was extended to the detection of specific antibody concentrations.

MATERIALS AND METHODS

Preparation of leukocytes. Dog leukocytes were isolated by sedimentation, hypotonic lysis of red blood cells, centrifugation and resuspension in Hank's balanced salt solution modified by addition of 10 mg/100 ml glucose (MHBSS) (*2*). Leukocyte concentrations were between 10 and 20×10^6/ml.

Antigens and antibody. Phenol-killed Salmonella 0 types A, C, and E antigen and specific antisera obtained from BioQuest (Div. of Becton-Dickinson, Cockeysville, Md.) were studied. Antisera was decomplemented by heating at 56°C for 30 min. Varying concentrations of Ag and Ab were prepared by serial dilution with MHBSS. One milliliter of Ag and 1 ml of Ab solution were allowed to incubate at room temperature in the siliconized incubation bottles (*2*) for up to 30 min before 1.8 ml MHBSS, 1.0 ml leukocytes, and 0.2 ml glucose-1-^{14}C were added to start the incubation for $^{14}CO_2$ production.

Incubation with leukocytes. Each 5-ml incubation mixture consisted of: 1 ml of WBC suspension, 0.2 ml, (1.0 μCi) of glucose-1-^{14}C [Amersham/Searle, Glucose-1-^{14}C (57 mCi/m*M* specific activity)], and 3.8 ml of Ag–Ab in MHBSS with or without fetal calf serum. Incubation was at 37°C for 1.5 hr with occasional (two to three times) stirring. Incubation was stopped with the addition of 1 ml of 62.5% citric acid. Liberated $^{14}CO_2$ was measured 30 min after the addition of the citric acid in a radioactivity detector which is capable of making automatic sequential measurement (Bactec 225, Johnston Laboratories, Inc., Cockeysville, Md.).

Phagocytosis. Wright-stained smears of the incubation mixtures were prepared as previously de-

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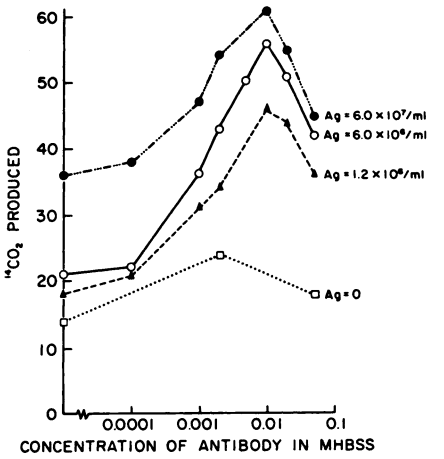

FIG. 1. Leukocyte metabolism as function of antibody concentration. Salmonella group E antigen and antibody incubated with leukocytes.

scribed (*2*) with observation of Ag–Ab clumps and calculation of a phagocytic index in units of interiorized bacteria per cell after 25 consecutive cells were counted. Bacteria seen to be totally within the border of the cell membrane were considered interiorized. Photomicrographs of appropriate slides were prepared.

RESULTS

Effect of Ab concentration on $^{14}CO_2$ liberation. In all three Salmonella systems, A, C, and E, a biphasic curve relating Ab concentration and $^{14}CO_2$ released was observed. The progressive increase at low Ab concentrations was linearly related to the LOG of the Ab concentration (Fig. 1). At higher Ab concentration, the $^{14}CO_2$ produced fell linearly as the LOG of the Ab concentration increased. An identical pattern of $^{14}CO_2$ release was observed with Salmonella A, E, and C although for Salmonella A the biphasic relationship was observed only at low levels of antigen. The minimum detectable concentration of Ab in MHBSS was 1:10,000 for Salmonella A and E, and 1:5,000 for Salmonella C.

Effect of Ab concentration on phagocytic index, clumping, and lysis. Phagocytosis of Salmonella A and E was well visualized by light microscopy although Salmonella C was not. Representative photomicrographs are shown in Fig. 2 (A–D). Phagocytic index was closely related to $^{14}CO_2$ liberation with both Salmonella A and E, the relationship being distinctly biphasic with respect to Ab concentration (Fig. 3A–B).

As can be seen in the photomicrographs, clumping of Salmonella group A did not occur until the highest concentration of Ab, about 1:10. Salmonella group E clumped at much lower Ab concentrations: aggregates of 3–10 bacteria were seen at about 1:200 Ab concentration, and clumps of greater than 100 bacteria at Ab concentrations of 1:10 (Fig. 2D).

DISCUSSION

Our goal is to develop an automated system for measurement of levels of specific antibody in sera. In 1973 Mantovani, et al (*3*) showed that phagocytosis of erythrocytes by macrophages and monocytes was proportional to the Ab concentration of the media. Phagocytosis did not occur until 10^3 to

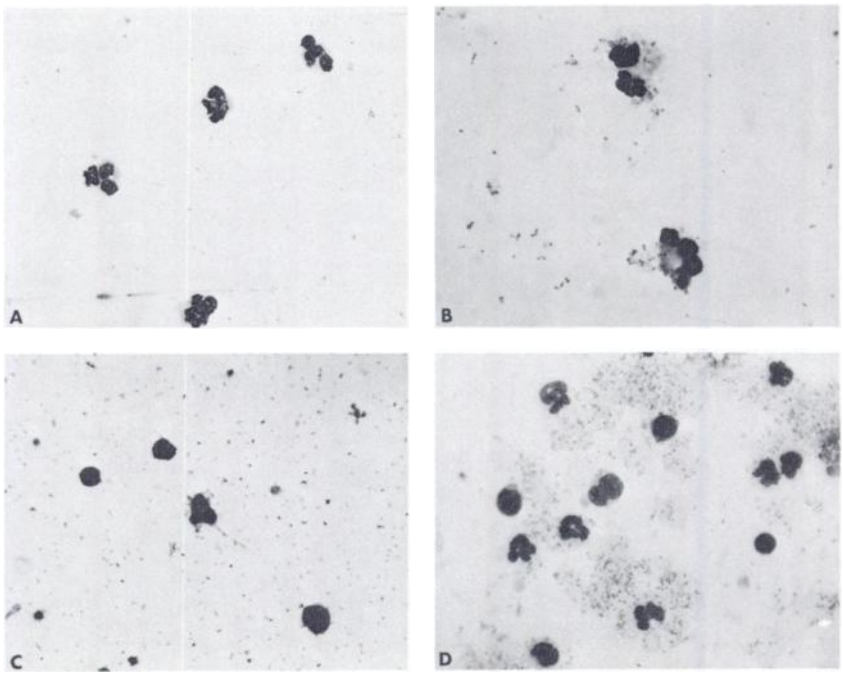

FIG. 2. (A) Four dog PMNs with Salmonella A antigen evenly spaced due to absence of antibody. Note absence of phagocytosis. (B) Six dog leukocytes in process of phagocytosis of Salmonella A antigen opsonized by high (1:10) concentration of specific antibody. Note antigen clumps of four to eight bacteria. (C) Four dog PMNs with Salmonella E antigen evenly spaced due to absence of antibody. (D) Many dog leukocytes in process of phagocytosis of Salmonella E antigen opsonized by specific antibody. Note very large antigen clumps of 50–100 bacteria.

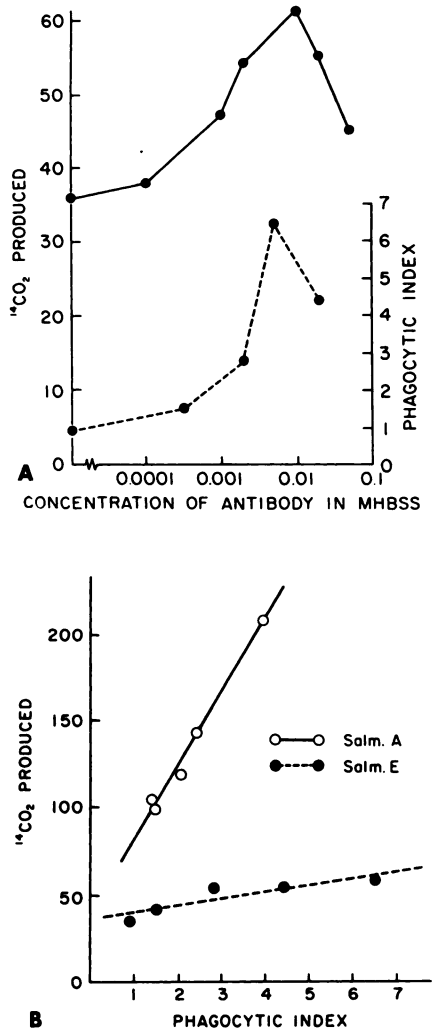

FIG. 3. (A) Leukocyte metabolism (——) and phagocytic index (- - -) as function of antibody concentration using Salmonella group E antigen (6.0×10^7/ml) and antibody. Incubation (1 hr) used for phagocytic index values. (B) Leukocytic metabolism compared with phagocytic index in tests of phagocytosis. Salmonella group A antigen (1.3×10^9/ml) and Salmonella group E antigen (6.0×10^7/ml) incubated with leukocytes at various antibody concentrations.

10^4 molecules became bound to the erythrocyte's surface. At these and higher concentrations, there was a linear relationship between the number of erythrocytes phagocytized and the number of IgG molecules bound to the erythrocyte. Their observations did not go beyond 4.0×10^4 IgG/cell although erythrocytes can accommodate up to 6.0×10^5 IgG/cell (*3*). They quantified phagocytosis using ^{51}Cr-labeled erythrocytes associated with macrophage monolayers. In our system, as we have reported previously, $^{14}CO_2$ release from leukocytes was proportional to the LOG of the Ag concentration at fixed Ab concentration. In the present experiment, we found a biphasic response of leukocyte metabolism to increasing Ab concentrations, $^{14}CO_2$ release increased as a function of the LOG of the Ab concentration. At higher concentrations there was a decrease in the amount of $^{14}CO_2$ released. The decrease was also a function of the LOG of the higher Ab concentrations. Our data suggest that the system could form the basis of an assay system at antibody levels as low as 1:10,000 for Salmonella A and E and 1:5,000 for Salmonella C levels. These are below those detectable by conventional complement fixation methods. High concentrations of Ab can be identified and assayed by testing several dilutions of the unknown.

The explanation for the biphasic relationship of $^{14}CO_2$ release and Ab concentration curve was that high Ab concentration caused clumping of Ag, effectively sequestering Ag in the center of the clumps. This was apparent from inspection of the photomicrographs. With Salmonella A, clumping began (4–6 bacteria per clump) only at the highest Ab concentration. Up to this level, there was a progressive increase in $^{14}CO_2$ release. In contrast, the Salmonella E curve peaked much earlier at an Ab concentration of 1:100. The clumping of Ag began in this region (Ab = 1:200) and progressed from 3–6 bacteria per clump to greater than 100 bacteria per clump at the highest Ab concentration. This finding is strong evidence in favor of clumping as the basis of the fall of $^{14}CO_2$ release. The fact that the phagocytic index was also biphasic, i.e., lower at higher Ab concentrations also suggests that the biphasic curves were caused by an inhibition of phagocytosis itself and not by inhibition of a later metabolic step after interiorization.

Although the wide range and sensitivity of this effect are encouraging, much further work needs to be done before this system can be applied on a large scale to clinical laboratory testing. These studies will include a detailed evaluation of cross-reactivity with normal control serum including the role of complement and systematic observations of the effect of acute and convalescent serum in Salmonella infections.

In summary, our results suggest that the automated radiometric system may be capable of fast (within 4–6 hr) analysis of the functional activity of leukocytes, specific antibody levels, and the presence of specific bacteria. Factors that must be controlled in the system include the siliconization of glass containers.

The technique is based on changes in leukocyte respiration in the presence of immune complex and has a sensitivity great enough to detect the presence of specific antibodies from immune serum in dilutions of 1:10,000.

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