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# Radiometric Method for the Detection of Coliform Organisms in Water

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A new radiometric method for the detection of coliform bacteria in water has been described. The method is based on the release of $^{14}CO_2$ from [$^{14}$C]lactose by bacteria suspended in growth medium and incubated at 37 C. The evolved $^{14}CO_2$ is trapped by hyamine hydroxide and counted in a liquid scintillation spectrometer. The method permits the detection of 1 to 10 organisms within 6 h of incubation. Coliform bacteria suspended in water for several days recover from starvation and may be quantitated by the proposed method. Bacteria from water samples may also be concentrated by filtration through membrane filters and detected by the radiometric assay.

Identification of bacterial species from natural environments or clinical specimens requires isolation of organisms followed by morphological, serological, and biochemical tests. These conventional methods provide the answer, at the earliest, 24 h after the receipt of the sample. To speed up the bacterial identification, efforts have been directed towards the development of more sensitive procedures. The use of radioisotopes, which serve as substrates to specific organisms, may provide a useful tool for the rapid identification of bacteria, provided that the metabolic end product can be identified with certainty. This approach was first used by Levin et al. (8-12) who added $^{14}$C-labeled carbohydrates to bacterial suspensions and trapped the radioactive $CO_2$, evolved. In the early experiments Geiger Müller detectors were used for the determination of radioactivities; these were replaced by ionization chambers in recently described automatic assays (3-6, 13, 14).

The bacteriological control of public water is usually based on the quantitation of *Escherichia coli* which serves as a marker for fecal contamination. With the increase in size and complexity of municipal water supply systems, the public health hazards and consequences of contamination became of major concern. Therefore, the rapid identification of *E. coli* in water is of utmost importance because of medical, ecological, and economic considerations. The present report describes the use of [$^{14}$C]lactose for the detection of *E. coli* in water. This carbohydrate has been selected since it permits the differentiation between *E. coli* and most of the other *Enterobacteriaceae*. It will be shown that a single organism may be detected within 6 h of incubation.

## MATERIALS AND METHODS

*E. coli* B was grown in synthetic M9 medium (1) to a turbidity corresponding to $10^9$ cells per ml. A sample of this culture was diluted (1:50) in sterile tap water and tested immediately or after keeping at room temperature for several days. The standard test was carried out as follows. To a sterile test tube (9.5 by 1.5 cm) the following materials were added: [$^{14}$C]lactose, 0.1 ml (1 $\mu$Ci/ml; 20 $\mu$Ci/$\mu$mol, The Radiochemical Centre, Amersham, England), water sample (or membrane filters), 0.1 ml; nutrient broth (Difco) supplemented with $2 \times 10^{-5}$ M lactose; and 0.001% bromophenol blue (pH 6.0 to 6.2), 0.2 ml. Tubes were immediately sealed with a rubber stopper supporting a polyethylene center well (Kontes Glass Co. no. 88230) and shaken at 37 C in a water bath. At desired times, 0.2-ml quantities of hyamine hydroxide (Packard) were injected into the plastic center wells and incubation was continued for another 15 min. The reaction was finally stopped by injecting 0.2 ml of 0.5 N HCl into the reaction mixture; samples were agitated for an additional 15 min, to allow complete absorption of the evolved $CO_2$. The center well was removed, placed in a vial containing 10 ml of scintillation fluid (2), and assayed for radioactivity in a liquid scintillation spectrometer.

Bacteria were usually diluted in nutrient broth (Difco) containing $2 \times 10^{-5}$ M lactose and counted by plating on nutrient agar (Difco). Bacteria from natural environments and from artificial systems were also collected by filtration through membrane filters (HAWG, 047A0, HA 0.45 $\mu$m pore size, 47 mm, Millipore Corp.) and analyzed.

## RESULTS

Preliminary studies indicated that bacteria suspended in buffer metabolized [$^{14}$C]lactose very slowly. This finding can easily be explained by the low concentration of [$^{14}$C]lactose in the reaction mixture, which did not permit induc-

tion of β-galactosidase. It has been well established that the uptake and metabolism of lactose by *E. coli* are subject to stringent regulatory mechanisms and that β-galactosidase can also be induced by IPTG (isopropyl-β-D-thiogalactopyranoside). We therefore tested the effect of this inducer on the metabolism of [$^{14}$C]lactose by *E. coli* and showed that the fermentation of this compound increased markedly when cells were first incubated with $5 \times 10^{-4}$ M IPTG. Best results were obtained when the incubation mixture contained nutrient broth instead of buffer; this facilitated the multiplication of bacteria and rendered the test more sensitive. In subsequent experiments β-galactosidase was also induced by increasing the molar concentration of lactose in the nutrient broth medium. It has been shown that the addition of unlabeled lactose to the nutrient broth (at a final concentration of $2 \times 10^{-5}$ M) caused β-galactosidase induction and the subsequent release of $^{14}CO_2$ from the radioactive lactose. In some experiments $CO_2$ was trapped by 15% KOH, but finally hyamine hydroxide was used because of better recoveries and reproducibilities. Since vapors of hyamine hydroxide may be toxic for bacteria, this trapping agent was injected into the center well at the end of the incubation period. In similar experiments, we found that best results were obtained when the pH of the nutrient broth was brought to 6.0 to 6.2. This was usually done by adding bromophenol blue as an indicator and adjusting the pH to the indicator inversion point. After finding the optimal conditions for the assay, we tested the effect of incubation time on the formation of $^{14}CO_2$ from radioactive lactose by using a constant number of *E. coli* cells ($8.6 \times 10^6$ cells per test tube). It may be seen (Fig. 1) that *E. coli* cells, at that concentration, released significant amounts of radioactive $CO_2$, even after 15 min of incubation. The accumulation of $^{14}CO_2$ progressed with incubation time in a linear fashion (Fig. 1).

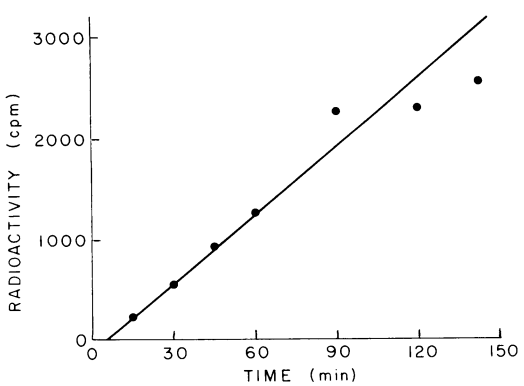


FIG. 1. *Effect of incubation time on the release of $^{14}CO_2$ from radioactive lactose. ($8.6 \times 10^6$ coliform organisms were used per test tube.)*

To test the sensitivity of the method, a suspension of *E. coli* cells was diluted in lactose-nutrient broth and the various dilutions were assayed as described in the preceding paragraph, at various times. Figure 2 shows that $10^3$ coliform cells may be detected after incubation with [$^{14}$C]lactose for at least 2 h. At least 3 h of incubation are required for the detection of $10^2$ cells, whereas 1 to 10 coliform bacteria form significant amounts of $^{14}CO_2$ after 6 h of incubation. In the aforementioned experiment, freshly grown bacteria were employed. It is to be expected that in natural water sources starved *E. coli* cells may be encountered. This is mainly due to the lapse of time between the excretion of the organisms in the feces and their recovery from the water sample which obviously lacks nutrients. It was therefore decided to mimic the conditions which prevail in nature, and to test the activity of the bacteria after keeping in tap water for several days. The results of this experiment are illustrated in Fig. 3, which shows that *E. coli* cells starved at room temperature for 3 and 6 days were still capable of fermenting [$^{14}$C]lactose—similar to freshly grown bacteria. Moreover, when the various preparations were incubated with [$^{14}$C]lactose for 60 min, starved cells caused the release of slightly higher amounts of $^{14}CO_2$, although the number of viable cells decreased by a factor of 0.5 to 1.0 log unit.

Because of the scarcity of coliform organisms in water, they are usually recovered by filtration of large volumes of fluid through membrane

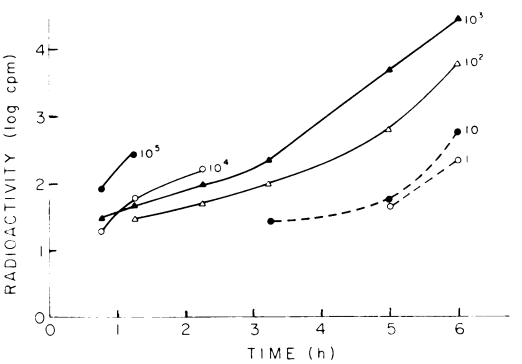


FIG. 2. *Release of $^{14}CO_2$ from radioactive lactose after incubation with various numbers of coliform organisms. Coliform organisms—$10^5$ per test (●——●); $10^4$ per test (○——○); $10^3$ per test (▲——▲); $10^2$ per test (△——△); 10 per test (●- - -●); 1 organism per test (○- - -○).*

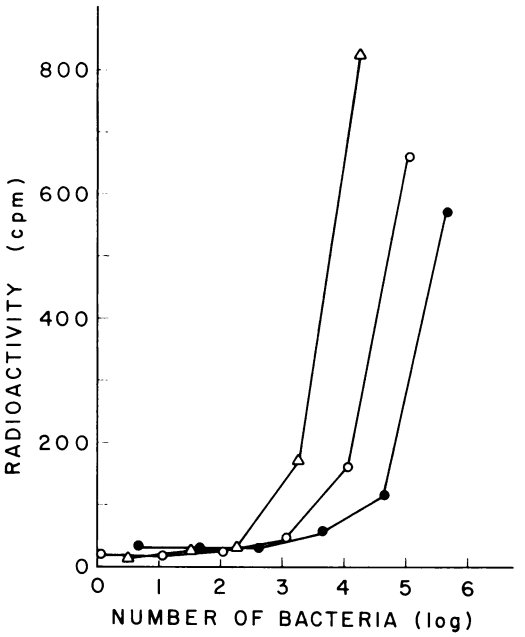


FIG. 3. *Effect of aging on the release of $^{14}CO_2$ from radioactive lactose by coliform organisms. Freshly grown bacteria (●), bacteria kept in water for 3 days (○) or 6 days (△). Incubation time, 60 min.*

filters. This method may also be used for the radioassay, and filters containing bacteria were used instead of bacterial suspensions. As expected, 1 to 10 coliform bacteria were readily detectable after incubation for 6 h under the experimental conditions that we just described.

## DISCUSSION

The radiometric method that we described may provide an important tool for the early detection of fecal organisms in water. Since radioactive lactose is used in the test, a differentiation between coliform organisms and other bacteria is possible. Furthermore, the presence of other bacteria, which originate from plants and soil, does not interfere with the assay. Coliform bacteria, maintained in water for a number of days, recover during their incubation in lactose-nutrient broth medium and ferment [$^{14}C$]lactose at the expected rate. The proposed method, which may eventually be automated, may be applied for monitoring the quality of municipal water supplies and controlling the purity of streams and rivers.

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