A Simple Method for the Primary Isolation of *Mycobacterium* in a Carbon Dioxide Atmosphere

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SUMMARY

A simple, inexpensive method which provides increased carbon dioxide tension for the primary isolation of mycobacteria is described. Using this technique, the primary isolation of mycobacteria from 1 114 specimens of sputum, gastric washings, spinal fluids, etc. was increased by 24,2%.

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The stimulating effect of increased carbon dioxide on the growth of *Mycobacterium* has long been recognised.[1] Furthermore it has been shown[2] that many strains of tubercle bacilli from patients under continuous isoniazid treatment will not grow unless an atmosphere containing up to 10% carbon dioxide is provided.

Opinions differ as to the percentage of CO_2 that would give optimum growth. Novy and Soule[3] reported well-developed growth in concentrations ranging from 5% to 50% CO_2; Davies,[3] Corper *et al.*,[4] and Cohn *et al.*[5] added CO_2 in amounts varying between 2% and 7,5%; Gruft and Loder[6] used an atmosphere containing 8% CO_2, and Beam and Kubica[7] found that the presence of 10 - 11% of CO_2 gave the best results. It would appear that an atmosphere containing from 5% to 10% CO_2 gives good results.

At present incubation of cultures in an atmosphere of increased CO_2 tension can be carried out by one of the following methods:

1. In a CO_2 incubator—this apparatus is expensive, costly to operate and possibly beyond the reach of most small laboratories. In view of the fact that cultures must be kept for 6 - 8 weeks, such incubators must be large, or more than one must be acquired.

2. By use of gas-proof plastic bags in which the source of CO_2 is either *M. phlei*[8,9] or a gas cylinder containing, under pressure, the required mixture of CO_2 and air. This method leaves much to be desired because the observation of cultures is hampered.

3. By use of McCartney bottles containing the required medium into which a mixture of air and CO_2 is injected.[10] Disadvantages of this method include the danger of blowing viable tubercle bacilli out of the bottle during the 10 seconds in which the air-CO_2 mixture is being injected through the loosened cap, and the high cost of a special air-CO_2 mixture and the necessary flow meter and membrane filters. This last objection also applies to method 2.

A simple method which has now been used successfully in the Tygerberg Hospital laboratory is described here.

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METHODS

The medium used was Gruft's modification[11,12] of Löwenstein-Jensen medium in 1-ounce McCartney bottles. Carbon dioxide was produced by mixing 1N Na_2CO_3 and 1N HCl. The Na_2CO_3 solution was sterilised by Seitz filtration.

The following types of specimens were used in this investigation: sputum, gastric fluid, urine, menstrual blood, uterine scrapings, pleural fluid, spinal fluid and faeces. These were treated and concentrated as laid down by Kleeberg *et al.*[13] Three drops of the concentrated deposit were placed on each of two Löwenstein-Jensen slopes. These were incubated overnight at 37°C in a horizontal position. One bottle was then marked CO_2, any fluid decanted into a jar of disinfectant, and 0,25 ml of sterile 1N Na_2CO_3 dropped into the bottle on the side away from the medium. The addition of 1N HCl (0,25 ml) in the same manner resulted in effervescence and CO_2 release. The screw cap was then firmly tightened. The air-CO_2 mixture in a series of 30 uninoculated bottles was analysed by means of a Beckman gas analyser. The CO_2 content was found to vary between 6% and 10%. The liquid which remained was an approximately 3% solution of NaCl with traces of Na_2CO_3 and a pH of approximately 7,4. Both inoculated bottles were incubated at 37°C in an upright position and examined for growth at weekly intervals. They were regarded as negative if no growth appeared after 8 weeks' incubation. All growths were checked for acid-fast bacilli.

RESULTS AND DISCUSSION

This method was applied to 1 114 consecutive specimens of all types received for the diagnosis of tuberculosis. Cultures of acid-fast bacilli, which were subsequently shown to be *Mycobacterium tuberculosis*, were obtained from 149 of these specimens. The effects of an increased

TABLE I. EFFECT OF A CO_2 ATMOSPHERE

	Number positive	Percentage positive
In air and in air-CO_2 mixture	101	67,8
Air only	12	8,0
Air-CO_2 mixture only	36	24,2
Total	149	100,0

CO_2 atmosphere on the primary isolation of myobacteria is shown in Table I.

Of the 149 positive specimens, 36 grew in an air-CO_2 mixture only. Without incubation in this atmosphere, 24,2% of positives would therefore have been missed. In addition, 12 cultures (8%) were positive in air only.

Since there was no difference between the distribution of the colonies on the surface of the medium in either atmosphere, the residual liquid appeared to have no effect on the growth of mycobacteria.

In spite of the variation in CO_2 concentration used here, these results agree with the findings of the other workers mentioned above.[1-10] They also found that carbon dioxide was essential for the growth of many strains of *Mycobacterium* and that its presence increased the number of primary isolations of mycobacteria from routine specimens. To obtain optimal results, however, it is essential to incubate a duplicate culture in air.

The method described is one that can be used by any small laboratory. Its main advantages are simplicity of execution, negligible cost, and the ease and safety with which cultures can be handled and examined without disturbing the CO_2 tension.

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